

UNIVERSIDAD COMPLUTENSE DE MADRID
FACULTAD DE CIENCIAS BIOLÓGICAS



TESIS DOCTORAL

Una nueva aproximación para conseguir el acceso al diagnóstico de la enfermedad fúngica en países de renta media y baja para las personas que viven con el VIH

MEMORIA PARA OPTAR AL GRADO DE DOCTOR

PRESENTADA POR

Narda Gabriela Medina Samayoa

Directores

Ana Alastruey-Izquierdo
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Memoria para optar al grado de doctora presentada por:

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Madrid, 2023

Esta tesis está dedicada a todas las personas que viven con VIH, a nuestros pacientes. Este trabajo es por ustedes, para intentar que algún día nadie más muera por una infección fúngica.

AGRADECIMIENTOS

Escribir palabras para expresar sentimientos no es mi mayor cualidad. Sin embargo, hoy, en el momento en el que escribo estas últimas páginas o al menos eso espero, me hace reflexionar de la buena suerte que he tenido en la vida. Sé que algunos dirán que no es suerte, pero como le llamas al hecho de encontrar situaciones y personas que creen en ti, que te apoyan y que te hacen ser mejor... Pues para mí, eso es suerte y hoy, en estas páginas, quiero agradecerles.

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Vivir en otro país te cambia para siempre. Es conocer cosas nuevas, es el reto de entender las situaciones y tratar de adaptarte. Solo puedo intentar, con estas palabras agradecer a todos los que conocí en Madrid. A Clara, gracias por hacerme sentir como en casa. Quien diría que pasaríamos una pandemia juntas y que la sobreviviríamos felizmente (el vino siempre ayudó), por las tardes de noticias, las comidas, las charlas y los consejos. A todos los amigos de Clara, a Ana, Pucha, Rosa, Esperanza, Margarita, Luis y Juan y todos los que me faltan, gracias por haberme abierto su corazón. A Renata, por las tardes de los sábados. A María, Fermín y los niños, por todo su cariño.

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Abreviaturas

A

ADN Ácido Desoxirribonucleico

C

CD4 Receptor ubicado en células T del sistema inmunológico

CDC Centros para el Control y Prevención de Enfermedades (Centers for Disease Control and Prevention)

CrAg Antígeno de Cryptococcus

D

DLH Laboratorio central de Diagnóstico (por sus siglas en inglés: Diagnostic Laboratory Hub)

E

ELISA Ensayo por inmunoabsorción ligado a enzimas (por sus siglas en inglés: enzyme-linked immunosorbent assay)

F

FDA Agencia de Alimentos y Medicamentos (por sus siglas en inglés: Food and Drug Administration)

G

GAFFI Acción Global para las Infecciones Fúngicas

H

HSH Hombre que tiene Sexo con Hombres

L

LCR Líquido Ceforraquídeo

LFA Dispositivo de Flujo Lateral (por sus siglas en inglés: Lateral Flow Assay)

M

MSPAS Ministerio de Salud Pública y Asistencia Social de Guatemala

O

OMS Organización Mundial de la Salud

OPS Organización Panamericana de Salud

P

PAHO Organización Panamericana de Salud (por sus siglas en inglés: Pan American Health Organization)

PCR Reacción en Cadena de la Polimerasa (por sus siglas en inglés: Polymerase Chain Reaction)

POCT Prueba de punto de Atención al Paciente (por sus siglas en inglés: Point of Care Test)

T

TARV Terapia Antirretroviral

U

UAI Unidad de Atención Integral

UNAIDS Programa Conjunto de las Naciones Unidas sobre el VIH/Sida

V

VIH Virus de la Inmunodeficiencia Humana

W

WHO Organización Mundial de la Salud
(por sus siglas en inglés: World HEalth Organization)

Resumen

Las infecciones fúngicas son una de las principales causas de muerte entre las personas que viven con VIH, especialmente en países de renta media y baja. Los agentes etiológicos más frecuentes son *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Pneumocystis jirovecii* y *Talaromyces marneffeii*, aunque otras especies también afectan a este grupo de pacientes. Su frecuencia, depende de varios factores, entre ellos aspectos ambientales que favorecen su desarrollo. Sin embargo, uno de los principales determinantes de la infección fúngica en este grupo de pacientes es la carga de la enfermedad avanzada por VIH.

La elevada mortalidad que ocasionan las infecciones fúngicas entre las personas que viven con VIH subraya la importancia del diagnóstico rápido. La única técnica que puede proveer rapidez es la histopatología y el uso de tinciones especiales, sin embargo, su sensibilidad es baja y dependiente del observador. La técnica de referencia, el cultivo, tiene una baja sensibilidad y ofrece resultados lentos, por tanto, con escasa utilidad práctica. Actualmente existen pruebas rápidas para la detección de antígenos, específicamente para *H. capsulatum* y *C. neoformans* con una alta sensibilidad y especificidad. A pesar de estos avances y del enorme impacto que tiene la enfermedad fúngica, esta continúa siendo un problema de salud pública desentendido, sin financiación y sin estrategias específicas para su contención.

El principal objetivo de este trabajo fue analizar si el acceso al diagnóstico de la enfermedad fúngica en países de renta media y baja reduciría la mortalidad de las personas que viven con el VIH. Para ello se implantó, en Guatemala, un programa de tamizaje para infecciones oportunistas que integró el diagnóstico de: i) histoplasmosis, ii) criptococosis, iii) tuberculosis y iv) micobacterias no-tuberculosas, en tres grupos de pacientes: i) pacientes de reciente diagnóstico de VIH, ii) pacientes que abandonaron la TARV y retornaron a la atención (retornos) y iii) pacientes en TARV con sospecha clínica de una infección oportunista (pacientes en TARV). El tamizaje se realizó con independencia del número de células CD4 al momento de la evaluación.

De los resultados obtenidos se puede concluir que: (1) El acceso al diagnóstico rápido disminuye la mortalidad de los pacientes que viven con el VIH. (2) La implantación de un programa de tamizaje para infecciones oportunistas basado en un sistema de Laboratorio central de Diagnóstico «DLH-Diagnostic Laboratory Hub» es una forma eficaz y ágil de brindar resultados a

centros o clínicas que no cuentan con capacidades diagnósticas. Para el desarrollo de este modelo es esencial disponer de un sistema eficiente para el transporte de muestras. La centralización o descentralización del modelo debe evaluarse con relación a la capacidad de respuesta que tengan los centros en donde se integra el programa. (3) El tamizaje diagnostica pacientes con infecciones múltiples y permite iniciar de forma segura la terapia antirretroviral (4) las técnicas de detección del antígeno de *Histoplasma* y *Cryptococcus* deberían ser consideradas de referencia para el diagnóstico de estas infecciones en las personas que viven con VIH (5) La carga de histoplasmosis y criptococosis ha sido subestimada en Guatemala y posiblemente en otros países de la región. (7) El número de pacientes con enfermedad avanzada al diagnóstico del VIH en Guatemala es una de las más elevadas a nivel mundial con especial impacto en las poblaciones Mayas.

Abstract

Opportunistic infections remain a major cause of death in HIV-infected patients, especially in low- and middle-income countries (LMICs). The most frequent etiological agents are *Cryptococcus neoformans*, *Histoplasma capsulatum*, *Pneumocystis jirovecii*, and *Talaromyces marneffeii*, although other species can also affect this group of patients. The burden of the fungal infections relies on several factors, including environmental characteristics that make an ecological niche for certain fungi. One of the main determinants of fungal infection among HIV patients is the frequency of advanced HIV disease.

The high mortality related with fungal infections among people with HIV is clinically relevant and require a rapid diagnosis. The only technique that can provide speed is the histopathology, and special stains; however, its sensitivity is low and requires laboratory staff with mycology training. The gold standard based on the culture has a low sensitivity and long turnaround time for diagnosis, which can lead to a delayed treatment. In recent years, rapid antigen detection tests have been developed, especially for *H. capsulatum* and *C. neoformans*, which have shown a high specificity and specificity. Although recent advances have improved the diagnosis of fungal infections it continues to be a neglected issue in public health, without funding and specific strategies for its containment.

The main purpose of this study was to analyze if a screening program which provided access to diagnosis of fungal disease in low- and middle-income countries would reduce the mortality of people living with HIV. We developed this program in Guatemala. Patients enrolled were screened for: (i) histoplasmosis (ii) cryptococcal disease, (iii) tuberculosis and (iv) nontuberculous mycobacteria (NTM). The program included three groups of HIV patients: (i) newly diagnosed; (ii) patients who have not been receiving antiretroviral treatment (ART) for >90 days but who returned to care (Return/Restart); and (iii) patients on ART with symptoms of opportunistic infections (ARV treatment). The OI screening was performed regardless of the CD4 cell count.

Taking into account our findings, we shown that (1) A rapid screening program for opportunistic infections reduces mortality in HIV patients. (2) the implementation of the screening program based on a Centralized DLH is an effective way to provide results to health care facilities that have limited capabilities. Successful implementation through a central laboratory must integrate a good system for delivering samples from the peripheral centers to the DLH. Centralization or decentralization of this model must be evaluated in terms of the number of samples those local

laboratories would run per week and the capacity of response. (3) Screening for opportunistic infections allows identifying cases with multiple infections, furthermore, in those who are eligible, antiretroviral therapy can be initiated safely. (4) The antigen detection techniques should be considered the current gold standard for diagnosing cryptococcosis and histoplasmosis and should be available in every single laboratory dealing with opportunistic infections in people with HIV. (5) The burden of histoplasmosis and cryptococcosis has been underestimated in Guatemala and possibly in other countries of the region. (7) The burden of advanced disease at diagnosis of HIV in Guatemala is one of the highest in the world, with a special impact on the Mayan populations.

1. [INTRODUCCIÓN]

1. Introducción

Las infecciones oportunistas continúan siendo una de las principales causas de muerte entre las personas que viven con VIH, especialmente en países de renta media y baja (Limper et al., 2017; Low et al., 2016; Walker et al., 2012). Aunque cualquier hongo puede causar una infección en este grupo de pacientes, los más habituales son: *Cryptococcus neoformans*, *Pneumocystis jirovecii*, *Candida spp.*, *Histoplasma capsulatum* y *Aspergillus spp.* (Limper et al., 2017; Low et al., 2016). El diagnóstico temprano de las infecciones fúngicas es esencial. Sin embargo, en muchos países, el acceso al mismo es limitado y cuando existe, suele tener una respuesta lenta.

En Guatemala, el acceso al diagnóstico de las infecciones fúngicas era restringido hasta que se desarrolló la propuesta «Reducción de la mortalidad asociada a VIH a través del diagnóstico rápido y una mejora en el cuidado de las infecciones fúngicas», financiada por «Acción Global para las Infecciones Fúngicas» (GAFFI, por sus siglas en inglés) y la fundación JYLAG. A través de esta propuesta se estableció un programa de tamizaje diagnóstico. Los resultados obtenidos, dan lugar a esta tesis doctoral que define la situación de la histoplasmosis y criptococosis, dos patógenos fúngicos prioritarios según la Organización Mundial de la Salud (OMS), en pacientes que viven con VIH en Guatemala y su comparación con la tuberculosis, considerada la infección oportunista más frecuente. En el siguiente apartado se describe de forma resumida la importancia de las infecciones fúngicas relacionadas con el VIH, sus métodos de diagnóstico y el contexto existente en Guatemala.

1.1. Importancia de las infecciones fúngicas

Las micosis son una de las causas más frecuentes de infecciones (Casadevall 2018). Más de 70,000 especies han sido documentadas y de ellas, 300 se consideran patógenos humanos (García-Solache y Casadevall, 2010). La enfermedad fúngica comprende un amplio grupo de patologías que abarca desde infecciones cutáneas superficiales hasta micosis invasoras (Brown et al., 2012). En las últimas décadas, el impacto ha sido notorio, principalmente como consecuencia de otras enfermedades como el VIH, la tuberculosis, el cáncer, la enfermedad pulmonar obstructiva (EPOC), la diabetes, la gripe y COVID-19. Además, situaciones específicas como trasplantes o terapias inmunosupresoras también han tenido un impacto significativo en el aumento de su frecuencia (Bongomin et al., 2017, 2019; M. L. Rodrigues y Nosanchuk, 2020). En la actualidad, se estima que cada año 1,5 millones de personas padecen una infección fúngica invasora (**Figura 1**). En los últimos años, GAFFI ha estimado la carga de la enfermedad fúngica

en más de 85 países observando diferencias en la incidencia y prevalencia entre regiones, países y poblaciones a riesgo (Bongomin et al., 2017). Las características geográficas, socioeconómicas y la frecuencia de otras comorbilidades son factores determinantes en la epidemiología de estas patologías (Bongomin et al., 2017; Low et al., 2016). Para lograr acciones estratégicas La OMS publicó en 2022 un informe que incluye la primera lista «patógenos fúngicos prioritarios». Entre se consideran como de prioridad crítica la criptococosis y alta prioridad a la histoplasmosis (WHO, 2022). Ambas abordadas en esta tesis.

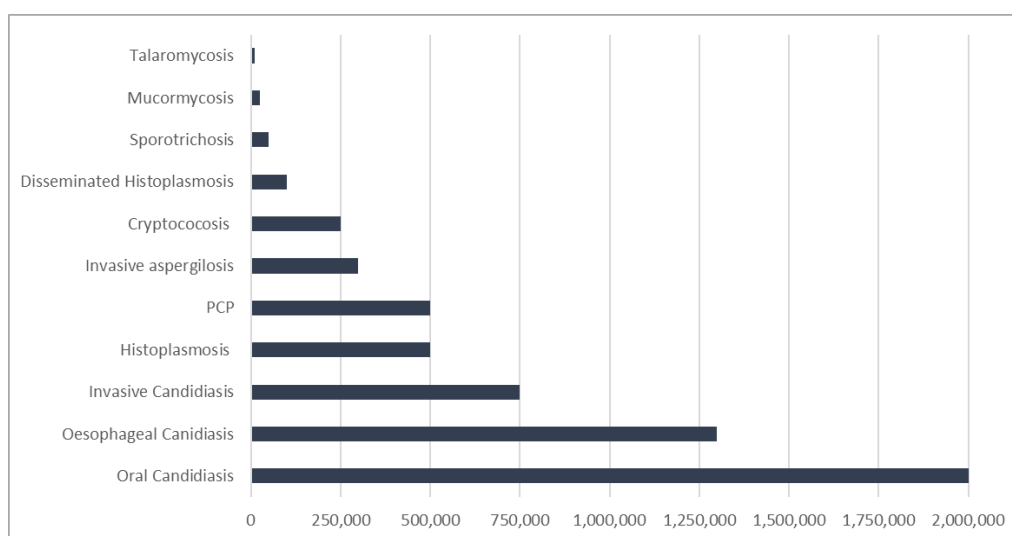


Figura 1. Estimación de la incidencia anual de las principales infecciones fúngicas invasivas. (Adaptado de: Bongomin et al., 2017).

El acceso al diagnóstico es esencial para tratar a los pacientes de forma adecuada. Aunque, desde el 2018, la Organización Mundial de la Salud (OMS) ha incluido en la lista de diagnósticos esenciales todas las pruebas esenciales para diagnosticar las infecciones fúngicas (WHO, 2018, 2019a), muchos países continúan sin disponer ellas. En muchos sitios el acceso se basa solo en el cultivo de muestras clínicas, que además de tener una baja sensibilidad es un procedimiento lento que contrapone la necesidad de una respuesta rápida que mitigue las consecuencias de enfermedades tan graves. De igual forma, la disponibilidad global de los antifúngicos es limitada. En una encuesta del 2016 realizada en 159 países, la anfotericina B solo estaba disponible en 117 (73%) (**Figura 2**) por lo que 481 millones de personas no tienen acceso a la misma. La disponibilidad de otros antifúngicos, como la 5-Fluorocitosina, es aún menor con una cobertura en 34 países (21,7%). En el caso del fluconazol y del itraconazol la disponibilidad fue prácticamente del 100% aunque con un coste variable (Kneale et al., 2016) y en muchos casos inasumible. De la misma forma sucede para la Anfotericina B liposomal, cuyo coste es extremadamente elevado (M. L. Rodrigues y Nosanchuk, 2020). Es importante destacar que

todos estos antifúngicos están incluidos en la «Lista de medicamentos esenciales de la OMS» (WHO, 2019b), lo que permite, en algunos casos, acceder a ellos a través de distintos mecanismos como el fondo estratégico de la OPS.

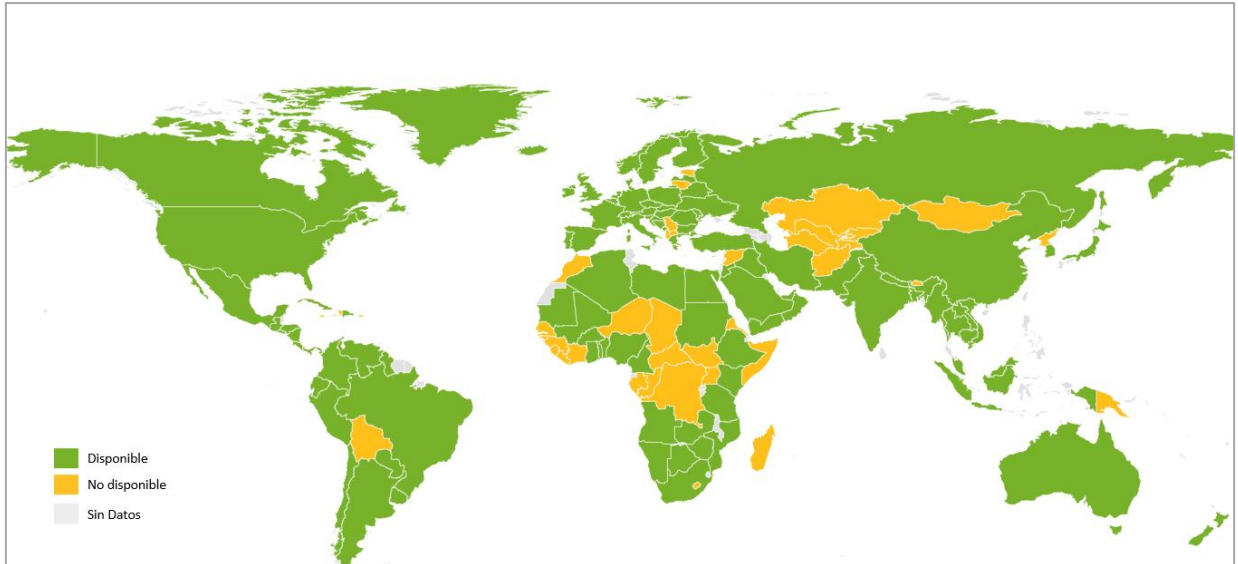


Figura 2. Disponibilidad de Anfotericina B por país (Adaptado de: Kneale *et al.*, 2017).

Anualmente mueren 1.350.000 personas por infecciones fúngicas lo que es equiparable a las muertes reportadas por tuberculosis (1.300.000) y más del doble que para el paludismo (627.000) (<http://www.gaffi.org>), enfermedades reconocidas y apoyadas por numerosas instituciones internacionales, a diferencia de las infecciones fúngicas, cuya inversión es significativamente menor (M. L. Rodrigues y Nosanchuk, 2020). Además, para combatir las micosis no existen planes estratégicos internacionales como los de la tuberculosis o el paludismo. En consecuencia, la enfermedad fúngica es un problema grave de salud pública globalmente desatendido.

1.2. Impacto del VIH en las micosis

El VIH ha impactado directamente en la epidemiología de las micosis. El deterioro que ocasiona en el sistema inmune lleva a la aparición de un gran número de huéspedes susceptibles a las infecciones fúngicas (Arturo Casadevall, 2017). Actualmente se estima que 37 millones de personas viven con VIH y se reportan 1,7 millones de nuevos casos al año. Mientras que en la mayoría de las regiones se ha observado una paulatina reducción del número de nuevos casos, en Latinoamérica entre el 2010 al 2018 se observó un aumento del 7% (UNAIDS, 2019). A escala global, se estima que el 16% de las personas desconoce su estado de portador, entre el 56% al

88% VIH tienen acceso a la terapia antirretroviral (TARV) (UNAIDS, 2021), pero solamente un 44% tiene una carga viral suprimida (Han et al., 2021), lo que significa que sigue habiendo un gran número de pacientes con un sistema inmunológico comprometido y en riesgo de padecer una infección oportunista. En 2018 se reportaron un total de 770.000 muertes asociadas con el VIH (UNAIDS, 2019).

Aunque las personas que viven con el VIH pueden padecer cualquier micosis, algunas tienen una mayor prevalencia. Por ejemplo, la criptococosis es la causa más frecuente de meningitis con una incidencia global de 223.100 casos anuales causando el 15% de las muertes relacionadas con el VIH (Rajasingham et al., 2017). *Pneumocystis jirovecii* ocasiona unos 400.000 episodios anuales (Limper et al., 2017) mientras que los casos de histoplasmosis oscilan entre los 100.000 y 300.000 (Denning, 2016). Se ha estimado que la implantación de una estrategia global para detectar y tratar estas micosis podría salvar la vida a unas 200.000 personas que viven con el VIH al año (Denning, 2016). Otras patologías como la paracoccidioidomicosis, la coccidioidomicosis o la talaromicosis también afectan a las personas que viven con VIH, pero su incidencia depende, en gran medida, de la epidemiología local. Las infecciones por *Candida* también son frecuentes. Hasta el 90% de las personas que no reciben TARV pueden padecer una candidosis oral y/o esofágica lo que se traduce en más de dos millones de casos (Bongomin et al., 2017).

1.3. VIH en Guatemala: datos previos sobre las micosis

Guatemala se encuentra ubicada en Centroamérica, tiene una extensión territorial de 108.889 km². El censo del 2018 estima una población superior a los 14 millones de habitantes, siendo el 52% mujeres (Ávila et al., 2015; *Instituto Nacional de Estadística-Censo*, 2018). El 50% de la población vive en zonas rurales y tiene menos de 24 años. El territorio está dividido en 22 departamentos, cada uno de los cuales consta de varios municipios. El 40% de sus habitantes se identifica como indígenas. El número de nacimientos ha disminuido durante los últimos 15 años y actualmente la esperanza de vida es de 66 años (Ávila et al., 2015). El sistema de salud del país es precario. La inversión aproximada, en 2016, fue del 2% del producto interior bruto (Ministerio de Salud Pública y Asistencia Social, 2017), una de las menores de la región.

Al igual que en otros países de Latinoamérica, el VIH y la enfermedad fúngica son un problema de salud pública. Según ONUSIDA, en 2017 había aproximadamente 46.000 personas viviendo con VIH, el mayor número de Centroamérica (UNAIDS, 2018). En 1960 ya existía preocupación por las micosis en la región. Así, se realizó una campaña para determinar la proporción de

habitantes expuestos a las esporas de *Histoplasma capsulatum* mediante la prueba intradérmica de la histoplasmina, detectándose que el 57% de los evaluados habían tenido una primoinfección (Taylor et al., 1960). Entre los años 2011 a 2015, un estudio realizado en Guatemala identificó que el 19,8% de los pacientes tenía una infección oportunista al momento del diagnóstico del VIH. Se reportaron doscientas cuarenta y nueve infecciones oportunistas; de ellas, el 53,8% eran micosis (Reinhardt et al., 2017). En 2015, se estimó la carga de las infecciones fúngicas invasoras en personas que vivían con VIH en el país, determinándose una frecuencia del 4,7% para la neumonía por *Pneumocystis*, 3,8% para histoplasmosis y 2,2% para la meningitis criptocócica, siendo estas las principales micosis y representando aproximadamente 1.644 casos anuales (Medina et al., 2017). Entre 2005 y 2009, se evaluaron a 263 pacientes con VIH y sospecha clínica de histoplasmosis, se diagnosticaron 101 casos (38,4%), la mortalidad a los 30 días fue del 43% (Samayoa et al., 2017). Además, en este estudio se documentó que 26 (9,9%) casos tenían una coinfección con histoplasmosis y tuberculosis (Samayoa et al., 2017).

En el caso de la criptococosis, la información disponible es limitada. Entre julio 2012 a diciembre 2014, un análisis de Guatemala y El Salvador identificó únicamente 62 casos en pacientes con síntomas y signos meníngeos (García Vázquez et al., 2003).

A continuación, se detallarán algunos aspectos generales y de diagnóstico de las infecciones fúngicas objeto de estudio en esta tesis.

1.4. Histoplasmosis

La histoplasmosis es una infección endémica en la Latinoamérica (Colombo et al., 2011; Sifuentes-Osornio et al., 2012). Es causada por el hongo dimórfico *Histoplasma capsulatum* (Afeltra et al., 2017; Erwig & Gow, 2016). El nicho natural son suelos enriquecidos con materia orgánica que contienen excretas de aves o murciélagos y temperaturas que oscilan entre los 8°C y los 25°C (Afeltra et al., 2017; Sarah Kidd, Catriona Halliday, Helen Alexiou, 2016). Produce micro y macroconidias (Sarah Kidd, Catriona Halliday, Helen Alexiou, 2016). La inhalación de las microconidias provoca la infección pulmonar (Mittal et al., 201 C.E.) que, en pacientes con factores de riesgo, logra diseminarse utilizando los macrófagos y el sistema reticuloendotelial alcanzando el bazo, el hígado, la médula ósea y los ganglios linfáticos (Erwig & Gow, 2016; Romani, 2004). Para eliminarlo, el huésped debe desarrollar una respuesta inmune adecuada, pero en ausencia de esta, el hongo es capaz de perpetuarse manteniéndose de forma intracelular (Erwig & Gow, 2016; Romani, 2004). Clásicamente la especie se ha dividido en dos variedades que afectan a humanos, *var. capsulatum* y *var. duboisii* (Oladele et al., 2018; Valero

et al., 2018); sin embargo, análisis moleculares más recientes han documentado una amplia diversidad genética (A. M. Rodrigues et al., 2020).

La presentación clínica de la histoplasmosis depende, en gran medida, del estado inmunológico del paciente, así como de otros factores de riesgo, incluyendo el tamaño del inóculo inhalado. En pacientes con VIH, la histoplasmosis diseminada es la forma clínica más frecuente (Afeltra et al., 2017; Kauffman, 2007) y es considerada una enfermedad definitoria de SIDA. Los síntomas son inespecíficos como fiebre, malestar general y pérdida de peso (Afeltra et al., 2017; Kauffman, 2007). Esto dificulta el diagnóstico diferencial con otras enfermedades como la tuberculosis (A. Adenis et al., 2014; Nacher et al., 2018; R et al., 2012). La mortalidad sin tratamiento es de al menos del 80% (Wheat et al., 2002).

1.4.1. Diagnóstico de la histoplasmosis

Entre las técnicas para diagnosticar la histoplasmosis se encuentran la histopatología y el cultivo y más recientemente la detección del antígeno circulante. La **Figura 3** presenta una comparación de la sensibilidad de los diferentes métodos de diagnóstico para la histoplasmosis.

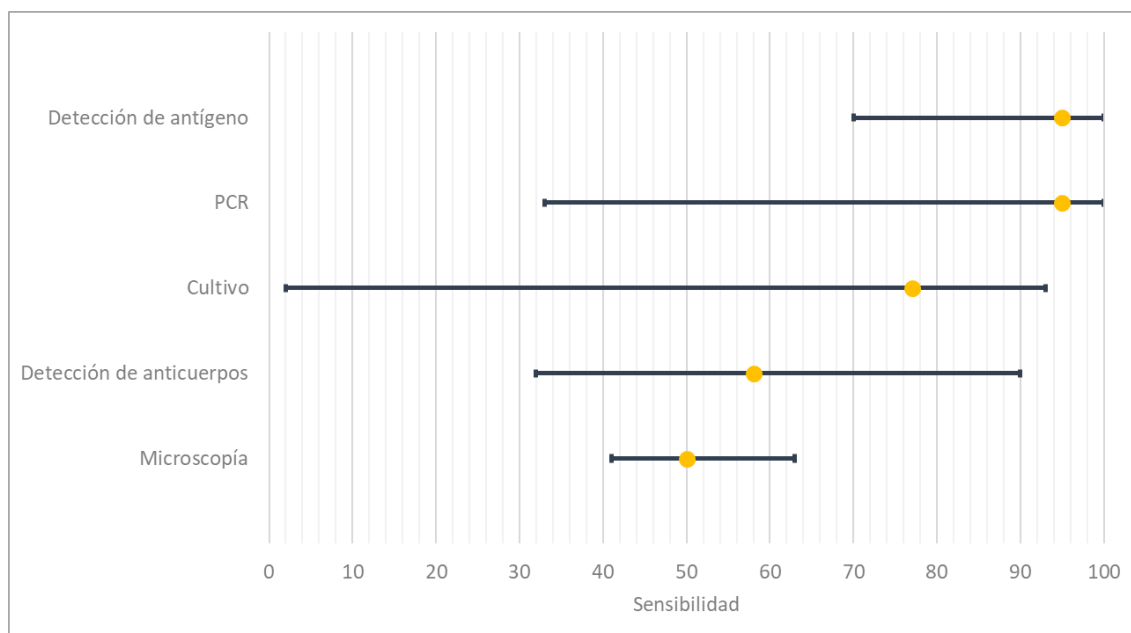


Figura 3. Comparación de la sensibilidad de los métodos de diagnóstico para la histoplasmosis (Adaptado de: PAHO., 2020).

- **Cultivo e histopatología:** El aislamiento de *H. capsulatum* a partir de muestras clínicas ha sido considerado el método de referencia para su diagnóstico (Azar & Hage, 2017; Kauffman, 2007). Sin embargo, su sensibilidad es baja. Así en pacientes con formas leves

es, en general es negativo y aunque en la forma diseminada, la sensibilidad puede alcanzar el 77%, ésta depende del tipo de muestra y su correcto procesamiento (Kauffman, 2007; PAHO, 2020). Su mayor limitación es el tiempo de respuesta ya que suele tardar varias semanas en crecer, por lo que su utilidad clínica es muy limitada. Las muestras con mayor rendimiento hay que obtenerlas mediante procedimientos invasores, como la punción de médula ósea, o biopsias de otros órganos (Azar & Hage, 2017; Kauffman, 2007; Wheat, 2003). Asimismo, el cultivo de estas muestras requiere de condiciones de bioseguridad nivel III (Buitrago & Martín-Gómez, 2020). Aunque la histopatología puede ayudar al diagnóstico de la infección (Kauffman, 2007; Wheat, 2003) ya que es un método más rápido que el cultivo, la sensibilidad es del 50% (Azar & Hage, 2017; Wheat, 2003). Las coloraciones especiales como plata metenamina o ácido peryódico de Schiff (PAS) son las recomendadas (Sarah Kidd, Catriona Halliday, Helen Alexiou, 2016). Otros microorganismos pueden ser confundidos con *H. capsulatum* en el examen histopatológico, entre ellos: *Sepedonimum* spp., *Leishmania* spp., *Pneumocystis jirovecii* y *Blastomyces dermatitidis* (Sarah Kidd, Catriona Halliday, Helen Alexiou 2016; Azar and Hage 2017). Además, para la identificación final hay que emplear métodos moleculares como sondas de ADN o secuenciación (Sarah Kidd, Catriona Halliday, Helen Alexiou 2016; Buitrago and Martín-Gómez 2020).

- **Detección de anticuerpos:** Este tipo de pruebas identifican a aquellos pacientes que han desarrollado una respuesta inmune frente al microorganismo (Azar & Hage, 2017; Guimarães et al., 2006; Wheat, 2003). Tienen una limitada utilidad para el diagnóstico rápido ya que tiene que haber pasado un cierto tiempo hasta que los anticuerpos sean detectables. La sensibilidad oscila entre el 38 y el 70% (PAHO, 2020). Asimismo, los pacientes inmunocomprometidos pueden tener concentraciones bajas de anticuerpos lo que disminuye drásticamente la sensibilidad de la prueba (Azar & Hage, 2017; Wheat, 2003). Otra limitante son las reacciones cruzadas con otras enfermedades como la paracoccidioidomicosis, la blastomicosis y la aspergilosis que pueden darse hasta en un 40% de los casos (Azar & Hage, 2017; Wheat, 2003). Por tanto, la detección de anticuerpos no es utilizada en el diagnóstico de la histoplasmosis en pacientes con VIH.
- **Detección de antígeno:** Actualmente la detección del antígeno de *H. capsulatum* se considera el método de elección para diagnosticar la histoplasmosis en pacientes con VIH. Tiene una sensibilidad del 95% y una especificidad del 97% (Cáceres et al., 2018; PAHO, 2020). Los resultados pueden obtenerse en el mismo día tras la llegada de la

muestra clínica y, además, es útil para la monitorización del tratamiento (Centers for Disease Control and Prevention, 2019). La detección del antígeno para *Histoplasma* está basada en un enzimo inmunoensayo (ELISA) (Cáceres et al., 2014; Scheel et al., 2009; Wheat, 2003; Wheat et al., 2002) que puede realizarse a partir de muestras de suero y orina, pero con una mayor sensibilidad en esta última (Azar & Hage, 2017; D. H. Cáceres et al., 2020; Kauffman, 2007). Entre las limitantes generales de la detección de antígeno de *Histoplasma* puede encontrarse la reactividad cruzada con otros hongos patógenos como *Paracoccidioides brasiliensis*, *Blastomyces dermatitidis*, y *Talaromyces marneffe* (Hage et al., 2011; Scheel et al., 2009). Sin embargo, esto tiene poco valor en Guatemala ya que un estudio reportó únicamente 5 casos anuales de paracoccidioidomicosis en un periodo de 4 años entre 1968 y 1972 (Greer & Restrepo, 1977), mientras que para las otras dos infecciones no existen casos documentados en el país. La prueba para detección del antígeno de *Histoplasma* fue incluida en 2019, en la lista de diagnósticos esenciales de la OMS (WHO, 2019a) e incluida en la guía para el diagnóstico y manejo de la histoplasmosis diseminada en pacientes viviendo con VIH publicada por la Organización Panamericana de la Salud (OPS) en 2020 (PAHO, 2020).

Aunque la prueba de detección de antígeno ha demostrado una gran utilidad, el acceso continúa siendo limitado. En el 2019, un estudio realizado en América reveló que en solo 10 países (43%) de los 23 encuestados estaba disponible (Cáceres et al., 2019). En 2020, evaluó una nueva prueba de POCT para la detección del antígeno de *Histoplasma* que ha demostrado una sensibilidad y especificidad del 96% y 92% en suero (Cáceres et al., 2020). Esta prueba comercial aún no se encuentra disponible en Latinoamérica.

- **Métodos basados en la PCR:** Existen técnicas de PCR que detectan *H. capsulatum* con un tiempo rápido de respuesta, pero de momento no existen métodos comerciales validados. Las publicaciones revisadas informan de una sensibilidad variable entre 67% al 100% y una especificidad entre el 96% al 100% (Azar & Hage, 2017; Buitrago & Martín-Gómez, 2020).

1.5. Criptococosis

La criptococosis es una micosis causada por levaduras del género *Cryptococcus* (Afeltra et al., 2017; Erwig & Gow, 2016; Sarah Kidd, Catriona Halliday, Helen Alexiou, 2016). Esta especie se encuentra en múltiples nichos ambientales como en las excretas de aves, el guano, ciertos árboles, los estanques y el suelo. La inhalación del hongo inicia el proceso infeccioso (Casadevall

& Perfect, 1998). Los individuos inmunocompetentes controlan la infección, pero en condiciones de inmunosupresión, como la de los pacientes que viven con el VIH, el hongo se disemina (Bovers et al., 2006; Casadevall & Perfect, 1998). Tiene especial predilección por el sistema nervioso central y tras cruzar la barrera hematoencefálica causa una meningitis en el 80 - 90% de los pacientes (Afeltra et al., 2017; Denham & Brown, 2018). El género *Cryptococcus* contiene más de 40 especies, siendo *Cryptococcus neoformans* y *Cryptococcus gattii* las más frecuentes y dentro de ellas existen diferentes serotipos (Afeltra et al., 2017; Casadevall & Perfect, 1998). Los pacientes con meningitis padecen cefalea, fiebre, náuseas, vómitos y/o alteración del estado de la conciencia. Durante los últimos años, se ha desarrollado una estrategia para su detección temprana en individuos asintomáticos, lo que permite un tratamiento anticipado para evitar la meningitis. Se estima que el 6% de los pacientes con un recuento de células CD4 menor de 100/mm³ tienen antigenemia, lo que representa unos 278.000 casos a escala mundial (Rajasingham et al., 2017). Además, del 50 al 70% de los pacientes con menos de 100 CD4/mm³ tienen hemocultivos positivos (Afeltra et al., 2017).

1.5.1. Diagnóstico de la criptococosis

La **Figura 4** presenta una comparación de la sensibilidad reportada de los diferentes métodos de diagnóstico para la criptococosis

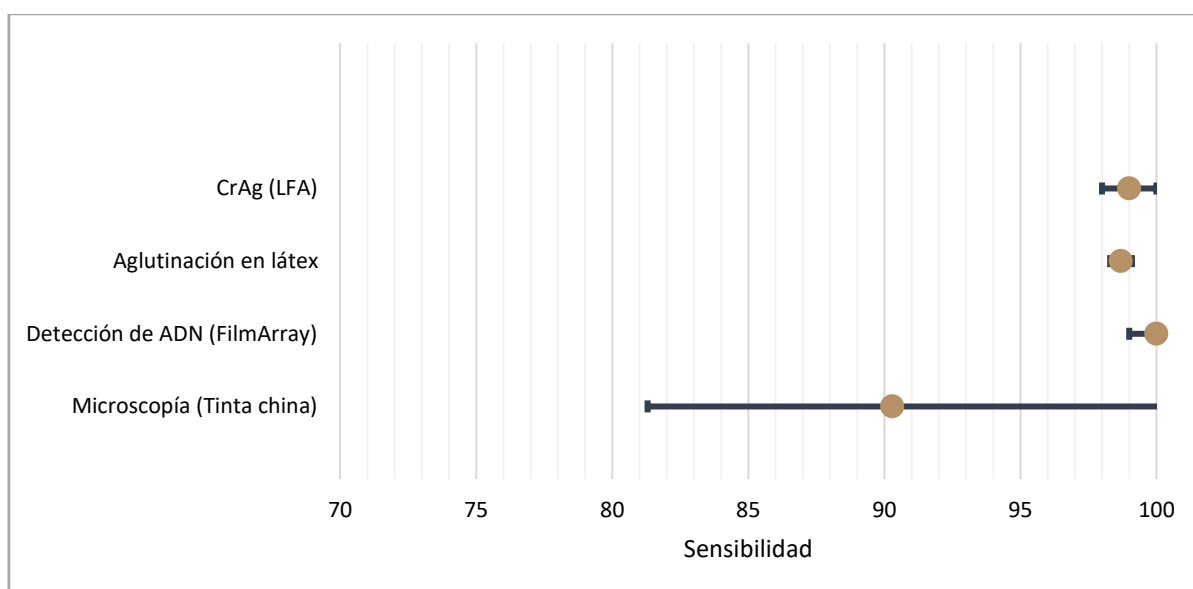


Figura 4. Comparación de la sensibilidad de los métodos de diagnóstico para la criptococosis meníngea (Adaptado de: WHO., 2018, Leber, 2016).

- **Microscopía y cultivo.** El cultivo de líquido cefalorraquídeo (LCR) ha sido considerado el método de referencia para diagnosticar la meningitis criptocócica (Afeltra et al., 2017; Casadevall & Perfect, 1998; Rajasingham et al., 2019). Sin embargo, el crecimiento del microorganismo tarda desde días a semanas (Cryptococcal Meningitis: Diagnosis and Management Update, 2015; Boulware et al., 2014). Aunque el cultivo de LCR tiene una alta sensibilidad, algunos estudios han comunicado falsos negativos. En Uganda, el 10% de los pacientes tenían un cultivo estéril a pesar de que el antígeno era positivo en el LCR (Tenforde et al., 2019). Sin embargo, la mortalidad de los pacientes con cultivo negativo y CrAg positivo fue similar a la de aquellos con cultivo positivo por lo que se podrían interpretar como falsos negativos (Ssebambulidde et al., 2019). Otra técnica sencilla, rápida y económica es la tinta china pero su sensibilidad es menor al 86% (Rajasingham et al., 2019), lo que indica que 1 de cada 7 casos es un falso negativo.
- **Detección de antígeno:** Actualmente, el antígeno es el método de elección para el diagnóstico de la criptococosis. Existen dos ensayos para su detección: la prueba de aglutinación de látex o, más recientemente, la prueba de flujo lateral (LFA) (Rajasingham et al., 2019). Ambas detectan el polisacárido capsular. La prueba de aglutinación en látex ha sido muy utilizada, y tiene una sensibilidad del 97 al 98% y una especificidad del 86 al 100%, dependiendo del método comercial (Rajasingham et al., 2019). Los resultados pueden ser cualitativos y semicuantitativos. Sin embargo, la necesidad de inactivar la muestra hace que este ensayo solo se puede realizar en un laboratorio. Además, los reactivos para la detección del antígeno por aglutinación en látex requieren de cadena de frío para su transporte y almacenamiento (Rajasingham et al., 2019). Esto es una gran limitante en lugares con recursos limitados. Más recientemente, se ha desarrollado una prueba de tipo POCT que detecta al antígeno (Kabanda et al., 2014). Este ensayo fue aprobado por la FDA para usos médicos en 2011 y ha supuesto un gran avance en el diagnóstico de la criptococosis. Se puede realizar en muestras de suero, plasma o LCR. Además, es estable a temperatura ambiente y los resultados están disponibles en diez minutos (Boulware et al. 2014; Skipper et al. 2020) lo que facilita su uso. La OMS, en la guía para el diagnóstico, prevención y manejo de la criptococosis para pacientes viviendo con VIH publicada en 2018 recomienda esta prueba (WHO, 2018). Además, también se ha desarrollado una versión semicuantitativa (CrAgSQ LFA). Este ensayo brinda resultados en 15 minutos (Jarvis et al., 2020; Skipper et al., 2020) y tiene una

sensibilidad y especificidad de 93% y 98%, respectivamente (Jarvis et al., 2020). La correlación de los resultados positivos entre la prueba cualitativa y semicuantitativa ha sido del 98% (Jarvis et al., 2020).

- **Detección de ADN:** Los métodos de amplificación de ADN para *Cryptococcus* no se utilizan rutinariamente. Sin embargo, entre los métodos moleculares actuales se encuentra el sistema FilmArray (BioFire Diagnostics, Salt Lake City, UT) que cuenta con un panel de 14 microorganismos para meningitis y encefalitis y requiere un tiempo aproximado de procesamiento de una hora. Este sistema tiene una sensibilidad del 100% y es capaz de diferenciar entre *C. neoformans* y *C. gattii* (Abbasi et al. 2015). Sin embargo, su coste es de 214 USD por ensayo (Soucek et al. 2017).

1.5.2. Detección temprana de la meningitis criptocócica

Meses antes del desarrollo de los síntomas meníngeos el antígeno de *Cryptococcus* es detectable en sangre. Un resultado positivo es un predictor independiente de la meningitis criptocócica (Liechty et al., 2007). La OMS, en las guías para el manejo y tratamiento de la criptococosis y la guía sobre manejo de la enfermedad avanzada por VIH recomiendan realizar el tamizaje sérico de la infección criptocócica en pacientes de reciente diagnóstico con un recuento de células CD4 $<200 /\text{mm}^3$, antes del inicio de la TARV (WHO 2017, 2018). En pacientes con antigenemia positiva se debe administrar un tratamiento anticipado con dosis altas de fluconazol. Además, en estos casos se aconseja realizar una punción lumbar para descartar la existencia de meningitis asintomática o paucisintomática (WHO 2017, 2018).

El diagnóstico y tratamiento anticipado de la infección criptocócica aumenta la supervivencia (Ford et al., 2018; Jarvis et al., 2009). Un estudio sobre la carga global de la criptococosis evidenció que en pacientes con un recuento de células CD4 $<100/\text{mm}^3$ la frecuencia media de la antigenemia es del 6% (2%—16%) (Rajasingham et al., 2017). En Perú y Argentina, identificaron frecuencias del 3,6% y 8,8%, respectivamente (Vidal, 2016). Además, la estrategia de diagnóstico y tratamiento anticipado ha demostrado ser costo beneficioso (WHO, 2018). En Uganda, el coste estimado del paquete de tamizaje, incluyendo el curso de tratamiento anticipado con fluconazol es de USD 30, mientras que el coste de un tratamiento de meningitis es de unos USD 400 (Vidal, 2016). De modo que, un programa de tamizaje apropiado, que prevenga la meningitis es más barato que el tratamiento y manejo intrahospitalario de los casos.

1.6. Estrategias para el abordaje de las infecciones fúngicas

Aunque la enfermedad fúngica está desatendida, algunos países han comenzado a reconocer su importancia y a integrar algunos aspectos en los programas de salud pública. Por ejemplo, en Tanzania y Zambia, se ha desarrollado un programa de tamizaje de la criptococosis y un seguimiento comunitario de los casos (Mfinanga et al., 2015). Al año de seguimiento de los pacientes se ha conseguido una disminución de la mortalidad. En Colombia, un curso de formación continuada sobre histoplasmosis consiguió que un mayor número de médicos enviará muestras a un laboratorio de referencia para diagnosticar la enfermedad, por lo que aumentó el número de casos diagnosticados (Cáceres et al., 2015). Otro estudio realizado en tres países de Centroamérica para diagnosticar mediante pruebas rápidas la histoplasmosis y criptococosis encontró una incidencia del 20% y 11%, respectivamente (Cáceres et al., 2021). Por tanto, el diagnóstico rápido de las micosis es esencial para la reducción global de su mortalidad. En este sentido, se estima que el impacto del acceso al diagnóstico y al tratamiento de las infecciones fúngicas en los programas de VIH supondría una reducción global del 30% en la mortalidad. Esto representaría una reducción de más de 200.000 muertes anuales (Denning, 2016) lo que lograría alcanzar la meta propuesta por UNAIDS de menos de 500.000, (Denning, 2016; UNAIDS, 2019).

1.7. Atención clínica a pacientes que viven con VIH en Guatemala y programa de tamizaje para infecciones oportunistas

En Guatemala, el ministerio de Salud Pública y Asistencia Social ha organizado la atención clínica a los pacientes que viven con VIH mediante unidades de atención integral. Estas unidades pertenecen a la red pública hospitalaria y se encargan de: i) Realizar pruebas de VIH y confirmar su diagnóstico; ii) Manejar exposiciones ocupacionales al VIH, virus de hepatitis B y C; iii) Atender a las personas con VIH y administrar la TARV. Además, en la última guía oficial sobre «Uso de los antirretrovirales en personas con VIH y su aplicación profiláctica» se establece que las unidades de atención integral pueden diagnosticar y tratar las infecciones oportunistas (Ministerio de Salud Pública y Asistencia Social, 2019).

Antes de la introducción del proyecto «Reducción de la mortalidad asociada a VIH a través del diagnóstico rápido y una mejora en el cuidado de las infecciones fúngicas» que dio origen al programa de tamizaje para infecciones oportunistas, el único diagnóstico disponible en algunas de las unidades era el de la tuberculosis. El programa de tamizaje ofreció participar a las 16

unidades de atención integral existentes; de ellas, 13 aceptaron participar estableciendo un consorcio denominado FungiRed™. La **Figura 5** señala la distribución geográfica de las unidades de atención integral que forman parte de esta red. Para este programa se estableció un «Laboratorio Centralizado de Diagnóstico» (DLH, “Diagnostic Laboratory Hub”) ubicado en una de las unidades de atención integral que es considerada de referencia y ubicada en la ciudad de Guatemala. El programa y sus componentes se describen con mayor detalle en la sección de resultados.

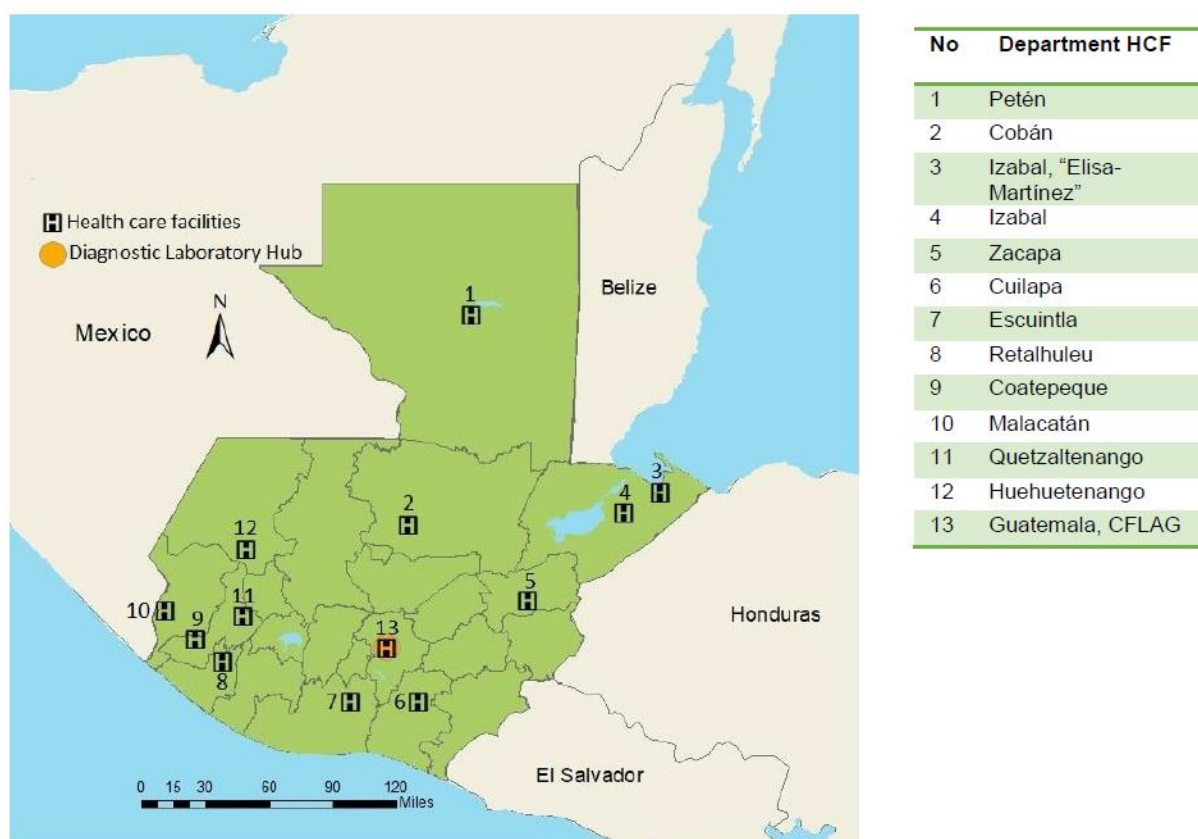


Figura 5. Distribución geográfica de las unidades de atención integral que forman parte de FungiRed™

2. [JUSTIFICACIÓN Y OBJETIVOS]

2. Justificación y objetivos

Desde el comienzo de la epidemia por VIH 76,1 millones de personas han contraído la infección y 35 millones han fallecido (UNAIDS, 2019). Las personas con VIH tienen un mayor riesgo de padecer infecciones oportunistas, estimándose que el 47,5% de las muertes asociadas al VIH se deben a una infección fúngica (Denning, 2016). El diagnóstico tardío de la infección por VIH y el abandono de la TARV son de los principales determinantes implicados en la aparición de estas infecciones (4). A pesar de su enorme impacto, la enfermedad fúngica se encuentra desatendida, sin programas de formación, sin apenas financiación y sin estrategias para su contención, lo que ha originado un círculo vicioso, en el que la falta de sospecha clínica hace que no se soliciten o dispongan de las pruebas necesarias para su diagnóstico y por tanto el tratamiento adecuado no se administre o ni siquiera esté disponible. En consecuencia, la carga real de la enfermedad fúngica permanece invisible, y como no se sospecha el círculo gira indefinidamente. Además, el diagnóstico de las infecciones fúngicas es complejo, los síntomas son inespecíficos y hay coinfecciones con otros microorganismos.

Se ha planteado que el acceso al diagnóstico de la enfermedad fúngica podría reducir la mortalidad asociada al VIH, pero esta pregunta seguía sin respuesta. Para intentar responderla, se desarrolló la propuesta: «Reducción de la mortalidad asociada a VIH a través del diagnóstico rápido y una mejora en el cuidado de las infecciones fúngicas» que conllevó las siguientes acciones: (i) establecimiento de un programa de educación continuada, (ii) introducción de un programa de tamizaje diagnóstico de las principales infecciones oportunistas fúngicas, y (iii) seguimiento de los casos.

Objetivo principal

Determinar si el acceso al diagnóstico de las infecciones oportunistas podría disminuir la mortalidad relacionada al VIH.

Objetivos secundarios

- Describir los componentes del programa para el tamizaje de las infecciones oportunistas fúngicas.
- Conocer la epidemiología real de las infecciones oportunistas en las personas que viven con el VIH en Guatemala.
- Determinar el impacto de la inesperada pandemia por COVID-19 en el proyecto.

3. [CAPÍTULOS]

[CAPÍTULO 1]

3. Capítulos. Artículos

Artículo 1. Capítulo 1. Descripción del programa para el tamizaje de las principales infecciones oportunistas en pacientes que viven con VIH en Guatemala

Publicación original:

B. Samayoa, L. Aguirre, O. Bonilla, **N. Medina**, D. Lau-Bonilla, D. Mercado, A. Moller, J.C. Pérez, A. Alastruey-Izquierdo, E. Arathoon, D.W. Denning, J.L. Rodriguez-Tudela on behalf of Fungired. The Diagnostic Laboratory Hub: A New Health Care System Reveals the Incidence and Mortality of Tuberculosis, Histoplasmosis and Cryptococcosis of PWH in Guatemala. *Open Forum Infect Dis.* 2020 Jan; 7(1): ofz534. doi: 10.1093/ofid/ofz534

El diagnóstico de las infecciones oportunistas fúngicas, especialmente de la histoplasmosis, es complejo debido a que los síntomas son inespecíficos, lo que impide distinguirla de otras infecciones oportunistas. En Guatemala se inició un programa para tamizaje para infecciones oportunistas que incluyó el diagnóstico de: i) histoplasmosis, ii) criptococosis, iii) tuberculosis y iv) micobacterias no-tuberculosas. Se incluyeron a tres grupos de pacientes, independientemente de su estado de inmunodepresión celular: i) pacientes de reciente diagnóstico de VIH, ii) pacientes que abandonaron la TARV y retornaron a la atención (retornos) y iii) pacientes en TARV con sospecha clínica de una infección oportunista (pacientes en ARV). El programa lo desarrolló y coordinó un Laboratorio central de Diagnóstico (Diagnostic Laboratory Hub «DLH») para una red de unidades que atienden a pacientes con VIH. En este trabajo se describen los componentes y la metodología del programa, así como los resultados obtenidos en el primer año de funcionamiento.

Trece unidades de atención integral conformaron la red de centros que se denominó Fungired™ (<http://fungired.gt/>) y se les brindó los servicios diagnósticos a través del Laboratorio Central. De estas unidades, doce se encuentran ubicadas en departamentos fuera de la capital mientras que el decimotercero corresponde a la Clínica Familiar «Luis Ángel García» que es una de las clínicas de referencia a nivel nacional y con mayor número de pacientes. En este artículo se describe: i) el desarrollo de actividades formativas, talleres regionales, discusiones de casos y cursos virtuales; ii) el sistema de envío de muestras desde las unidades de la red hacia el laboratorio central de diagnóstico a través de una compañía de transporte local; iii) el desarrollo

de un sistema electrónico para la solicitud de pruebas y seguimiento a pacientes; y iv) la descentralización de la prueba para la detección de antígeno de *Cryptococcus* en las unidades de atención integral y el desarrollo de un programa de control de calidad para esta. Las actividades del programa se iniciaron en 2016 con un plan de formación para los distintos centros, mediante un curso virtual para el manejo de las infecciones oportunistas. Asimismo, se realizaron las gestiones necesarias para la contratación del servicio de envíos de muestras clínicas a la par que se desarrolló el sistema electrónico para la solicitud de pruebas y seguimiento de los pacientes. El sistema permite organizar la carga de trabajo en el laboratorio, así como brindar seguimiento a los casos. El programa de tamizaje se inició en enero de 2017. La conclusión principal de este artículo es que el establecimiento de un Laboratorio central de Diagnóstico unido a un envío muestras clínicas coordinado permite acceder al diagnóstico de estas infecciones, con una calidad y un coste aceptable de los servicios prestados. Asimismo, un servicio centralizado permite la transferencia de tecnología a los laboratorios periféricos, así como el establecimiento de programas de control de calidad que garantizan la fiabilidad de los resultados y la eficiencia del sistema. En entornos con escasez de personal entrenado, la implantación de este tipo de sistemas es coste-efectivo.

The Diagnostic Laboratory Hub: A New Health Care System Reveals the Incidence and Mortality of Tuberculosis, Histoplasmosis, and Cryptococcosis of PWH in Guatemala

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Background. A Diagnostic Laboratory Hub (DLH) was set up in Guatemala to provide opportunistic infection (OI) diagnosis for people with HIV (PWH).

Methods. Patients newly presenting for HIV, PWH not receiving antiretrovirals (ARVs) for >90 days but returned to care (Return/Restart), and PWH on ARVs with symptoms of OIs (ARV treatment) were prospectively included. Screening for tuberculosis, nontuberculous mycobacteria (NTM), histoplasmosis, and cryptococcosis was done. Samples were couriered to the DLH, and results were transmitted electronically. Demographic, diagnostic results, disease burden, treatment, and follow-up to 180 days were analyzed.

Results. In 2017, 1953 patients were included, 923 new HIV infections (an estimated 44% of all new HIV infections in Guatemala), 701 on ARV treatment, and 315 Return/Restart. Three hundred seventeen (16.2%) had an OI: 35.9% tuberculosis, 31.2% histoplasmosis, 18.6% cryptococcosis, 4.4% NTM, and 9.8% coinfections. Histoplasmosis was the most frequent AIDS-defining illness; 51.2% of new patients had <200 CD4 cells/mm³ with a 29.4% OI incidence; 14.3% of OIs in new HIV infections occurred with CD4 counts of 200–350 cells/mm³. OIs were the main risk factor for premature death for new HIV infections. At 180 days, patients with OIs and advanced HIV had 73-fold greater risk of death than those without advanced disease who were OI-free.

Conclusions. The DLH OI screening approach provides adequate diagnostic services and obtains relevant data. We propose a CD4 screening threshold of <350 cells/mm³. Mortality remains high, and improved interventions are required, including expansion of the DLH and access to antifungal drugs, especially liposomal amphotericin B and flucytosine.

Keywords. cryptococcosis; histoplasmosis; HIV; opportunistic infections; tuberculosis.

Globally, there are 37 million people with HIV (PWH), and nearly 1 million died from AIDS-related diseases in 2017 [1]. Guatemala had an estimated number of 46 000 PWH in 2017, with 2100 new HIV infections in adults and 200 children [1]. Antiretroviral (ARV) coverage encompasses 39% of PWH, and 28% have a suppressed viral load. It is estimated that 50% receive a diagnosis when they have advanced disease (CD4 cell count <200 cells/mm³) [1].

Public health care for HIV in Guatemala is organized in HIV units, most with limited diagnostic capability. We decided to set up a Diagnostic Laboratory Hub (DLH) to provide diagnostics for OIs in a timely manner and at acceptable cost. We expected accurate OI diagnosis to provide data on relative incidence. Here, we describe the DLH, the network, and the results from 2017, the first year of operation.

METHODS

Participants

In November of 2015, all HIV units were invited to a kick-off meeting. The objectives were to (i) organize a fast and reliable system to request diagnosis and deliver results; (ii) provide learning about diagnosis, treatment, and management of OIs, and (iii) prospectively screen a cohort of HIV patients for OIs. Thirteen out of the 16 HIV units agreed to participate in the network, which was called FUNGIRED. A secure website

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(<http://fungired.gt/>) was set up with the following design: (i) information module about the network and an epidemiology section with statistics; (ii) continuous learning module through which e-learning courses were delivered; (iii) electronic laboratory system to request diagnostic tests online from HIV units and provide results. Once entered, the system warns the DLH of sample delivery, which smooths laboratory workload and reduces the turnaround time.

Patients

We prospectively included patients (i) with newly diagnosed HIV infection, (ii) who abandoned ARVs for >90 days but returned to care (Return/Restart), and (iii) on ARVs with symptoms compatible with an OI (ARV treatment). In 2017, all patients had to be screened for tuberculosis (TB), NTM, histoplasmosis, and cryptococcosis, irrespective of their CD4 counts.

All ages were tested, but only patients >13 years old were analyzed.

Clinical Samples

The following samples were requested from all patients: whole blood in an isolator tube (Abbott Diagnostics, IL, USA; 10 mL), serum (5 mL), urine, and sputum. At the discretion of the physician, other clinical samples were taken. From Monday to Friday, samples were delivered to DLH by a courier. Personnel at each HIV unit were trained in sample packaging using a box with a portable cooler. The parcel was picked up the same day and delivered to the DLH in <24 hours.

Diagnostic Follow-up at the DLH and Treatment

The microbiological, immunological, and molecular tests as well as the treatment of the OIs are described in the Supplementary Data.

Statistical Analysis

Variables collected include demographic data, opportunistic infection diagnostic results, treatment, and follow-up to 180 days. Statistical Package for the Social Sciences, version 25.0 (SPSS Inc., Chicago, IL, USA), was used for analysis. The chi-square test or Fisher exact test and Mann-Whitney *U* test were used to compare the groups. Kaplan-Meier survival methodology was employed, and differences were assessed by log-rank test. Cox regression was used to establish hazard ratios (HRs). A *P* value <.05 was considered statistically significant.

RESULTS

Cohort of Patients

During 2017, 2394 patients were included, but 441 (18.42%) were excluded for different reasons (Figure 1). A total of 1953 PWH were available for analysis. Full screening was done in 1541 (79%) patients and partial in 412. Out of 1953 patients, 95% were tested

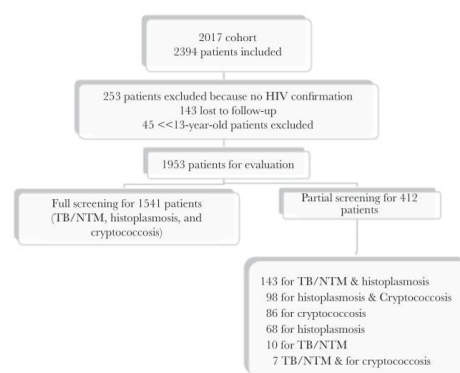


Figure 1. Description of the 2017 cohort. Abbreviations: NTM, nontuberculous mycobacteria; TB, tuberculosis.

for histoplasmosis, 87% for TB/NTM, and 89% for cryptococcosis (Figure 1).

Table 1 shows the sociodemographic data for the whole cohort of patients. There were 923 patients with new HIV infection, 701 on ARVs, and 315 Return/Restart. According to Guatemala UNAIDS estimates for 2017 [1], this study encompassed 44% (923 out of 2100 adults) of new adult HIV infections, 4% (701 out of 17 153) of those on ARVs, and 1.1% (315 out of 28 000) of Return/Restart.

For 630 patients (32.3%), CD4 cell counts were not available (Table 1). For 1323 with CD4 cell counts, 28.3% had <100 cells/mm³, 47.6% had <200 cells/mm³, 70.7% <350 cells/mm³, and 29.3% ≥350 cells/mm³. Women and men had similar numbers of CD4 cells (median women vs men, 220 vs 207; *P* = .478). This comparison between ethnic groups was significant (median Ladino vs Maya, 221 vs 176.5; *P* = .028).

Opportunistic Infections

Three hundred seventeen (16.2%) patients had an OI, and these PWH had lower counts of CD4 (median OI, 63.5; vs median non-OI, 246; *P* < .001) and higher viral loads (*P* < .001) (Table 1) than those without an OI.

Although CD4 counts were known for only 218 of 317 (68.8%) patients, there was a strong association between CD4 count and OI occurrence (Table 1). Overall, 198 (90.8%) OIs occurred in patients with CD4 counts <350 cells/mm³. Only 20 OIs (9.2%) were found in patients with ≥350 CD4 cells/mm³ (11 TB, 3 NTM, 4 cryptococcosis, and 2 histoplasmosis). Most coinfections occurred in those with very low CD4 cell counts; 17 (80.9%) were <100 cells/mm³, 3 (14.3%) 100–199 cells/mm³, and 1 (4.8%) 200–350 cells/mm³.

Table 1. Sociodemographic Data of Patients With and Without an OI in 2017 Cohort

	Total 1953	With OI 317 (16.2%)	Without OI 1636 (83.8%)
	No. (%)	No. (%)	No. (%)
Sex			
Male	1261 (64.6)	211 (66.6)	1050 (64.2)
Female	679 (34.8)	104 (32.8)	575 (35.1)
Transsexual	13 (0.7)	2 (0.6)	11 (0.7)
Age, y			
No.	1934 (99)	310 (97.8)	1624 (99.3)
Median	34	36	34
IQR	17	15	17
Unknown	19 (1)	7 (2.2)	12 (0.7)
Sexual orientation			
Heterosexual	1502 (76.9)	260 (82.0)	1242 (75.9)
Homosexual	292 (15.0)	29 (9.1)	263 (16.1)
Bisexual	86 (4.4)	11 (3.5)	75 (4.6)
Unknown	73 (3.7)	17 (5.4)	56 (3.4)
Ethnic group			
Ladino	1331 (68.2)	224 (70.7)	1107 (67.7)
Mayan	280 (14.3)	30 (9.5)	250 (15.3)
Other	9 (0.5)	2 (0.6)	7 (0.4)
Unknown	333 (17.1)	61 (19.2)	272 (16.6)
Type of patient			
Newly HIV infections	923 (47.3)	147 (46.4)	776 (47.4)
On ART	701 (35.9)	100 (31.5)	601 (36.7)
Restart/return	315 (16.1)	68 (21.5)	247 (15.1)
Unknown	14 (0.7)	2 (0.6)	12 (0.7)
CD4, cells/mm³			
No.	1323 (67.7)	218 (68.8)	1105 (67.5)
Median	210	63.5	246
IQR	307	153	297
Unknown	630 (32.3)	99 (31.2)	531 (32.5)
Viral load, copies/mL			
No.	1072 (54.9)	191 (60.3)	881 (53.9)
Median	50 477	138 585	42 322
IQR	154 376	375 132	123 730
Unknown	881 (45.1)	126 (39.7)	755 (46.1)

Abbreviations: ART, antiretroviral therapy; IQR, interquartile range; OI, opportunistic infection.

Table 2 shows the sociodemographic data of 317 patients classified by the OIs identified. There were 114 (35.9%) patients with TB, 14 (4.4%) with NTM, 99 (31.2%) with histoplasmosis, 59 (18.6%) with cryptococcosis, and 31 (9.8%) with coinfections. Coinfection incidence was 2%, and there were 13 histoplasmosis and TB, 8 cryptococcosis and TB, 7 cryptococcosis and histoplasmosis, 1 cryptococcosis and NTM, 1 TB and NTM, and 1 cryptococcosis and histoplasmosis and TB.

In total, there were 137 TB, 16 NTM, 120 histoplasmosis, and 76 cryptococcosis. These numbers were used to calculate the incidences of these infections in the cohort (Table 3).

OI incidence was 19.9% (Table 3). TB incidence was 8.1%, followed by histoplasmosis with 6.5%. OI incidence in patients with a CD4 count ≥ 350 cells/mm³ was 5.7%, with TB being the most frequent (11 cases), followed by cryptococcosis. In these patients, CD4 ranged from 358 to 1142 cells/mm³, with only 4 having CD4 counts < 400 cells/mm³.

New HIV Infections

We describe a group of 923 newly diagnosed HIV infections in detail because they represent 44% of the cases seen in Guatemala in 2017 [1]. Women and men arrived at HIV diagnosis with a similar median CD4 count (192 vs 197 cells/mm³; $P = .711$). The CD4 cell count was unknown for 23.2% of patients. Of the remainder, 31.7% had < 100 CD4 cells/mm³, 19.5% 100–199 cells/mm³, 23.8% 200–350 cells/mm³, and 25% > 350 cells/mm³. Thus, 51.2% of patients had advanced HIV disease (< 200 CD4 cells/mm³).

The incidence of OIs was 18.8% but rose to 29.4% and 39.4% for patients with < 200 CD4 cells/mm³ and < 100 CD4 cells/mm³, respectively (Table 3). In this group, TB had an incidence of 6.7% and histoplasmosis of 7.5%—the most frequent AIDS-defining illness in all groups except in those with CD4 counts ≥ 350 cells/mm³ (Table 3). There were 12 coinfections (5 histoplasmosis and cryptococcosis, 4 histoplasmosis and TB, 2

Table 2. Sociodemographic Data of Patients per OI

	TB	NTM	H	C	Coinfections
	No. (%)	No. (%)	No. (%)	No. (%)	No. (%)
	114 (35.9)	14 (4.4)	99 (31.2)	59 (18.6)	31 (9.77)
Sex					
Male	77 (67.5)	10 (71.4)	65 (65.7)	41 (69.5)	18 (58)
Female	37 (32.5)	4 (28.6)	33 (33.3)	17 (28.8)	13 (42)
Transsexual			1 (1)	1 (1.7)	
Age, y					
No.	111 (97.4)	14 (100)	99 (100)	55 (93.2)	31 (100)
Median	36	38	35	35	39
IQR	17	17	13	14	10
Unknown	3 (3.6)			4 (6.8)	
Sexual orientation					
Heterosexual	86 (75.4)	14 (100)	88 (88.9)	44 (74.6)	28 (90.3)
Homosexual	14 (12.3)		6 (6.1)	7 (11.9)	2 (6.5)
Bisexual	8 (7)		1 (1.0)	2 (3.4)	
Unknown	6 (5.3)		4 (4.0)	6 (10.2)	1 (3.2)
Ethnic group					
Ladino	79 (69.3)	11 (78.6)	69 (69.7)	44 (74.6)	21 (67.7)
Mayan	13 (11.4)	2 (14.3)	11 (11.1)	2 (3.4)	2 (6.5)
Other	2 (1.8)				
Unknown	20 (17.5)	1 (7.1)	19 (19.2)	13 (22.0)	8 (25.8)
Type of patient					
Newly HIV infections	48 (42.1)	4 (28.6)	56 (56.6)	27 (45.8)	12 (38.7)
On ART	44 (38.6)	6 (42.9)	23 (23.2)	19 (32.2)	8 (25.8)
Restart/return	21 (18.4)	4 (28.6)	20 (20.2)	12 (20.3)	11 (35.5)
Unknown	1 (0.9)			1 (1.7)	
CD4, cells/mm ³					
No.	76 (66.7)	12 (85.7)	62 (62.6)	47 (79.7)	21 (67.7)
Median	94	213	36	56	27
IQR	174	297	102	144	279
Unknown	38 (33.3)	2 (14.3)	37 (37.4)	12 (20.3)	10 (32.3)
Viral load, copies/mL					
No.	67 (58.8)	8 (57.1)	59 (59.6)	39 (66.1)	18 (58.1)
Median	123 405	122 261	234 601	96 010	128 305
IQR	397 379	162 029	739 693	294 306	271 726
Unknown	47 (41.2)	6 (42.9)	40 (40.4)	20 (33.9)	13 (41.9)

Abbreviations: C, cryptococcosis; H, histoplasmosis; NTM, nontuberculous mycobacteria; OI, opportunistic infection; TB, tuberculosis.

cryptococcosis and TB, and 1 TB and NTM), 10 of which were in patients with <350 CD4 cells/mm³. For the remaining 2 patients, the CD4 count was unknown.

Performance of Diagnostic Techniques

Cases diagnosed by direct microscopy and culture were limited (Table 4). Polymerase chain reaction (PCR) in sputum was positive for 91.6% of the cases of TB but only 56.5% for histoplasmosis. However, 75% of histoplasmosis cases were diagnosed by urine antigen test. In those considered to have disseminated histoplasmosis diagnosed by a positive antigen and/or a positive Isolator blood culture, the urine antigen was positive in 94.4% of cases. Cryptococcal antigen in serum was positive in all cases, whereas culture was only positive for 39.6%. Except for cryptococcosis, >1 technique was required to diagnose 100% of cases (Table 4).

Treatment of OIs and Survival

Twenty-one patients were lost to follow-up, 10 with OIs (3 TB, 1 NTM, 2 histoplasmosis, and 4 cryptococcosis) and 11 without OIs. Thirteen patients died immediately after admission and were not treated, 15 patients refused any treatment (death was confirmed by the National Registry), and 5 did not receive treatment for other reasons. The actual therapies delivered are shown in Table 5.

Figure 2 shows survival curves for patients with and without OIs. Mortality at 180 days of patients with OIs was 29.6%. The highest mortality at 180 days was caused by coinfections, at 48.4% (HR, 7.9), followed by cryptococcosis, 32.7% (HR, 4.9), TB, 29.7% (HR, 4.7), and histoplasmosis, 24.7% (HR, 3.7) (Figure 3).

Mortality at 180 days in patients with nonadvanced disease without an OI was 0.5%; it was 11.6% in those with OIs (HR,

Table 3. Incidence of OIs in the Global Cohort and in the New HIV Infections Subset

		Global Cohort					
		All Cases	Unknown CD4 ^a	<100 CD4 ^a	<200 CD4 ^a	<350 CD4 ^a	≥350 CD4 ^a
TB	Cases/No. screened	137/1701	46/518	51/343	70/572	80/843	11/340
	Incidence, %	8.1	8.9	14.9	12.2	9.5	3.2
NTM	Cases/No. screened	16/1701	2/518	6/343	8/572	11/843	3/340
	Incidence, %	0.9	0.4	1.7	1.4	1.3	0.9
H	Cases/No. screened	120/1850	45/569	55/368	65/615	73/910	2/371
	Incidence, %	6.5	7.9	15.0	10.6	8.0	0.5
C	Cases/No. screened	76/1732	16/530	38/336	52/568	56/853	4/349
	Incidence, %	4.4	3.0	11.3	9.2	6.6	1.1
Total incidence, %		19.9	20.2	42.9	33.4	25.4	5.7
Newly HIV infections							
		All Cases	Unknown CD4 ^a	<100 CD4 ^a	<200 CD4 ^a	<350 CD4 ^a	≥350 CD4 ^a
TB	Cases/No. screened	55/825	15/180	25/207	31/332	36/485	4/160
	Incidence	6.7	8.3	12.1	9.3	7.4	2.5
NTM	Cases/No. screened	5/825	1/180	2/207	4/332	4/485	0/160
	Incidence	0.6	0.6	1.0	1.2	0.8	0.0
H	Cases/No. screened	65/872	21/186	36/220	41/352	44/516	0/170
	Incidence	7.5	11.3	16.4	11.6	8.5	0.0
C	Cases/No. screened	34/840	7/187	20/201	24/328	26/489	1/164
	Incidence	4.0	3.7	9.9	7.3	5.3	0.6
Total incidence		18.8	24.0	39.4	29.4	22	3.1

Abbreviations: C, cryptococcosis; H, histoplasmosis; NTM, nontuberculous mycobacteria; OI, opportunistic infection; TB, tuberculosis.

^aNumber of CD4 cells/mm³.**Table 4. Diagnostic Performance for OIs**

		Tuberculosis ^{a,b}	NMT ^c	Histoplasmosis ^{d,e}	Cryptococcosis ^f
Direct exam	No. cases	137	16	120	76
	No. tested	133	ND	ND	ND ^g
	Positive, %	18	—	—	—
Conventional culture	No. tested	135	16	110	34 ^h
	Positive, %	38.5	100	10.9	56
Isolator Blood culture	No. tested	82	ND	69	59
	Positive, %	19.5	—	30.4	17
Ag detection in serum	No. tested	ND	ND	ND	76
	Positive, %	—	—	—	100
Ag detection in CSF	No. tested	ND	ND	ND	34
	Positive, %	—	—	—	79.4
Ag detection in urine	No. tested	ND	ND	112	ND
	Positive, %	—	—	75	—
PCR	No. tested	131	ND	92	ND
	Positive, %	91.6	—	56.5	—

Abbreviations: CSF, cerebrospinal fluid; ND, not done; NMT, nontuberculous mycobacteria; OI, opportunistic infection; PCR, polymerase chain reaction.

^aThere were 114 single tuberculosis and 23 coinfections.^bOne hundred percent tuberculosis diagnosis was reached with a combination of culture and PCR.^cThere were 14 single nontuberculous mycobacteria and 2 coinfections.^dThere were 99 single histoplasmosis and 21 coinfections.^eOne hundred percent histoplasmosis diagnosis was reached with a combination of Ag detection and polymerase chain reaction.^fThere were 59 single cryptococcosis and 17 coinfections.^gND: not done. For Cerebrospinal fluid, Indian ink was performed, but the results are not shown because of the 100% sensitivity of Ag detection.^hOnly cerebrospinal fluid culture.

26.5). For patients with advanced disease without OIs, the mortality was 7.3% (HR, 16.2); it was 28.7% in those with OIs (HR, 72.9) (Figure 4).

Log-rank testing was statistically significant for all survival comparisons ($P < .0001$) except for NTM. In the Cox regression model, age, gender, sexual orientation, ethnic group, and

Table 5. Opportunistic Infection Treatment for 286 Patients Followed up to 180 Days

	114 TB	14 NTM	99 Histoplasmosis	59 Cryptococcosis
	No. (%)	No. (%)	No. (%)	No. (%)
AmB	—	—	21 (21.2)	1 (1.7)
AmB followed by FZ	—	—	4 (4.0)	23 (39.0)
AmB followed by ITZ	—	—	31 (31.3)	—
FZ	—	—	3 (3.0)	22 (37.3)
ITZ	—	—	21 (21.2)	—
Antibiotics	—	5 (35.7)	—	—
Anti-TB drugs	98 (86)	5 (35.7)	—	—
No treatment or unknown ^a	16 (14)	4 (28.6)	19 (19.2)	13 (22.0)

Abbreviations: AmB, conventional amphotericin B; FZ, fluconazole; ITZ, itraconazole; NTM, nontuberculous mycobacteria; TB, tuberculosis.

^aThirteen patients died immediately after admission; 19 were lost to follow-up, and there is no evidence that they received treatment; 15 refused the treatment (death was confirmed using the National Registry), and 5 did not receive treatment for other reasons.

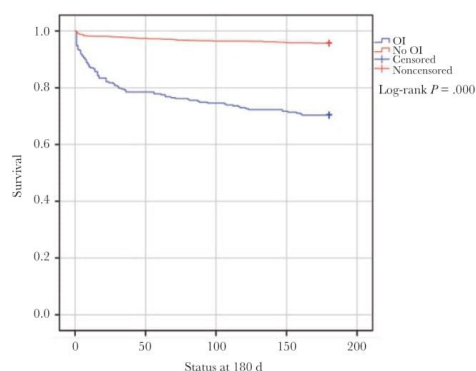


Figure 2. Kaplan-Meier survival curves of patients with and without opportunistic infections. Abbreviation: OI, opportunistic infection.

type of patient were included to ascertain interactions. None of these comparisons were statistically significant except age. When age and OIs were analyzed together, age was not statistically significant and thus was discarded as an interactive variable in survival.

DISCUSSION

Here we show that a national DLH can provide a high-quality diagnostic service for fungal disease and TB/NTM for an LMIC. This demonstration is the first of its kind in terms of population reach, timeliness, and clinical impact for a large population, as opposed to a local district. Not only did this new health care diagnostic system improve the diagnosis and management of OIs; it also provided crucial epidemiology data for these life-threatening infections in Guatemala.

A common current diagnostic approach is to have laboratories close to the patients. However, in many LMICs such laboratories are not well equipped, the staff are undertrained, and

the budget is severely constrained. On the other hand, in many countries there are reference laboratories, but they are focused on public health and development of surveillance programs, diagnosis of imported or infrequent diseases, analysis of local or national outbreaks, identification of rare species and new mechanisms of resistance, etc. However, the main objective of a DLH is to provide quick diagnostic services of high quality to a network of clinics or hospitals with a limited diagnostic capability. The implementation of DLH has (i) improved the training of staff utilizing several different diagnostic technologies; (ii) enhanced the quality of the results obtained; and (iii) reduced the cost per test for those that are formatted for multiple samples. Time to result is already fast but would be enhanced further with a 24-hour/7-day working pattern, but this is not currently feasible. A DLH that knows 24 hours in advance how many samples it will receive the next day can organize its workload appropriately, minimizing the turnaround time and cost per test while at the same time reaching a quality standard similar to high-income countries' (HICs) laboratories.

The main bottleneck was the time to deliver the samples to the DLH, which was solved with a frequent and rapid courier service. Currently, 70% of the HIV units are at 5 hours' driving distance from the DHL, whereas the remote HIV units are at a maximum of 9 hours. In the near future, drones could be used to deliver parcels in a shorter time [10, 11].

The performance of the DLH has been analyzed with its 2017 activity providing diagnostics to a network of 13 HIV clinics. There were 2141 PWH originally included, with 45 children excluded and only 143 (6.8%) lost to follow-up. From the 3 groups of HIV patients evaluated, 923 were new presentations of HIV infection, a remarkable 44% of the estimated total of first PWH presentations in the country in 2017 [1]. For the first time, we reliably describe what the most frequent AIDS-defining illnesses are in Guatemala, with disseminated histoplasmosis taking top place. We have not formally documented other less common presentations such as *Pneumocystis pneumonia*, bacterial sepsis, toxoplasmosis, pneumonia, cytomegalovirus

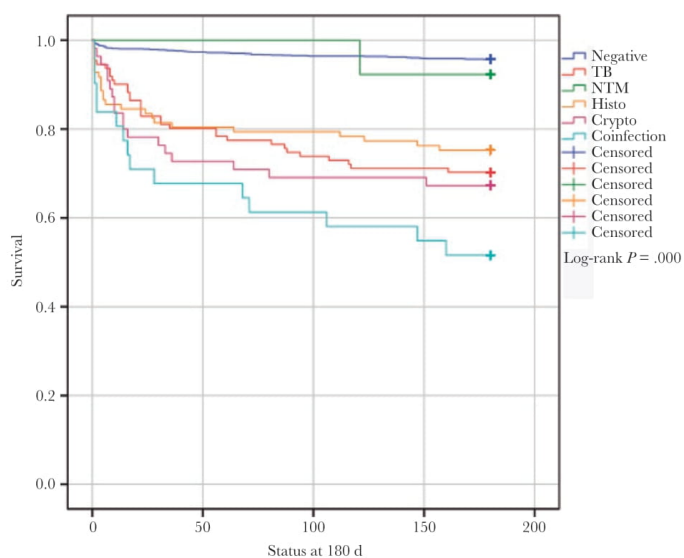


Figure 3. Kaplan-Meier survival curves of opportunistic infection cases. Abbreviations: NTM, nontuberculous mycobacteria; TB, tuberculosis.

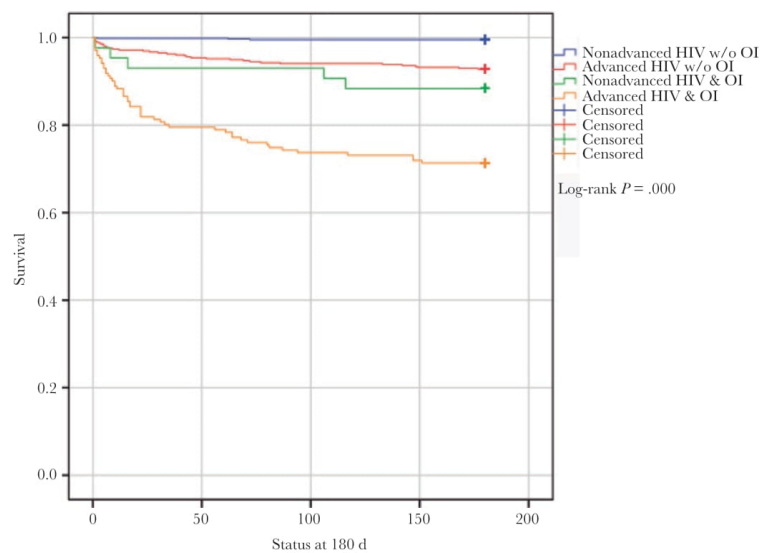


Figure 4. Survival curves for advanced vs nonadvanced HIV and opportunistic infections. Abbreviation: OI, opportunistic infection.

infections, and others, but our clinical experience places all these OIs at lower frequencies. Reliable data on the burden of different OIs, before and after ART initiation, are critical for

planning of health services, including procurement of drugs and diagnostics, which will provide better care to PWH. In this group of patients, the epidemiological data are robust, giving a

clear picture about their sociodemographic characteristics and the burden of OIs (Tables 1 and 2).

For the last 30 years of the HIV epidemic, CD4 count has been a key parameter to assess risk of opportunistic infections and define advanced HIV infection in adults. Although the CD4 cell count was unknown for 32.3%, for the 1323 PWH with known CD4 cell count, 47.6% had <200 cells/mm³ and 29.3% >350 cells/mm³. Specifically, for the 923 new HIV infections, 51.2% had a CD4 cell count <200 cells/mm³, which confirms prior data that PWH in Guatemala present very late, with a high mortality risk and cost of care, the worst in the region [12]. HIV awareness campaigns in Guatemala have clearly not achieved their intended objectives. A relevant finding is that women and men arrive with similar CD4 cell counts.

Recent World Health Organization (WHO) guidelines recommend specific care to patients with advanced disease (<200 CD4 cells/mm³ or WHO clinical stage 3 or 4 disease) [13]. Here, we have analyzed all patients, irrespective of CD4 count, but CD4 cell count provides a critical tool for stratifying the OI risk for PWH to reduce the cost of screening. It should be available in every single HIV clinic, or the DLH should offer it. Knowing the CD4 cell count in real time allows decisions on which patients should be screened.

Following the WHO's advice to screen only patients with <200 CD4 cells/mm³, we would have missed 20.2% and 14.3% of OIs in the whole cohort and in the new HIV infection group, respectively. Therefore, our results support an OI screening threshold of <350 CD4 cells/mm³. We cannot make any recommendation for OIs other than TB, NTM, histoplasmosis, and cryptococcosis, because others were not addressed in this study.

In Guatemala, fungal infections are more prevalent than mycobacterial infections, 10.9% vs 9%, respectively. For newly presenting HIV infection, the situation is similar, 11.5% fungal infections vs 7.3% mycobacterial. These figures did not include *Pneumocystis pneumonia*, and molecular diagnosis for this infection will be included in the network in 2018. The resources devoted to diagnosis and management of mycobacterial diseases dwarf those dedicated to fungal infections—still neglected after >35 years of AIDS. For instance, in Guatemala flucytosine, an essential antifungal for the treatment of cryptococcal meningitis, is not available. The optimal timing of introducing ARVs in PWH with histoplasmosis has not been addressed at all, as another example.

Histoplasmosis and TB had similar incidence, although for new PWH, histoplasmosis was the most frequent OI associated with AIDS illness. Differentiating between histoplasmosis and TB by clinical means is difficult, and some patients have both infections. Until the implementation of the DLH in Guatemala, the epidemiology of both infections was unknown, and presumably many of the histoplasmosis cases were treated as TB, with a fatal outcome and a high cost of care. Using a screening strategy, independent of the physician's requests, has proven to be useful

for diagnosing OIs, especially the 31 coinfections that would not have been diagnosed without this approach. Concomitant TB and histoplasmosis are problematic to treat because of the interactions of rifampicin and itraconazole [9]. There were few NTM infections, but their diagnosis was important because the treatment is different from that of tuberculosis [9, 10].

Antigen detection was the best means of diagnosis of cryptococcosis and histoplasmosis. For TB, culture had a low sensitivity, whereas PCR was more useful. Xpert MTB/RIF and TB urinary lateral flow LAM assay were not available at the DLH. Despite limited sensitivity and slow response, culture should be performed because it is important for identification and antimicrobial susceptibility testing. The positive rate of histoplasmosis culture found in this study is low when compared with other studies [14, 15]. The reasons are unknown, but we can speculate about the length of time to deliver the samples from the HIV units to the DLH and the introduction of urine Ag detection, which could reduce the need for invasive samples like bone marrow, which is well known to increase the culture positivity rate. In addition, recovery of *Histoplasma* spp. in culture is a slow and risky procedure; resistant strains have not been detected so far, and antifungal susceptibility testing is not standardized for this species. Nowadays and after the introduction of Ag detection in urine, this diagnostic approach has limited clinical value.

For diagnosis of histoplasmosis by antigen, urine is required, and the ease of acquiring urine explains why 95% of the patients were antigen tested. But the timing of urine sampling for histoplasmosis diagnosis could be important. An early morning urine collection improves the sensitivity of TB lateral flow LAM assay [16]. Another report shows an increased sensitivity after urine concentration [17]. The impact of these parameters on the sensitivity and specificity of *Histoplasma* antigen testing require research. Further improving the performance of the test would be valuable because culture is so slow and has a low sensitivity and there is no commercial PCR. The in-house PCR used in this work had a limited sensitivity (Table 4), although it was essential in the diagnosis of 26 cases (21.7%).

In addition to the diagnostic techniques employed in this work, the first WHO Model List of Essential In Vitro Diagnostics List (EDL) include Gen Xpert, LPA resistance, and LAM antigen [18], which we believe should be included in the portfolio of the DLH. In 2019, *Histoplasma* antigen detection was added to the EDL [19].

Cryptococcosis mortality of 32.7% (HR, 4.9) is similar to that of sub-Saharan Africa [20], where flucytosine is also not available and amphotericin B not routinely used. For histoplasmosis, we found a mortality of 24.7% at 180 days; 2 previous studies from that Guatemala and French Guyana showed mortality rates of 24.8% and 16.8%, respectively, but at 30 days of follow-up [14, 21]. An international cohort study evaluating TB-related mortality in PWH from Mexico, Chile, and

Argentina found an 11% mortality at 1 year of follow-up, better than the 29.7% mortality at 6 months described here. Mortality in Eastern Europe (27%) is similar [22]. Notably, 9.8% of PWH here had lethal co-infections because only 51.6% were alive at 180 days (HR, 7.9).

As 52% of patients in Guatemala with HIV present with advanced disease, it is critical to establish a nationwide screening strategy with 2 main objectives, to diagnose and treat patients with OIs and to start safe and rapid HIV ART within 7 days of the day of HIV diagnosis, with the documented exception of cryptococcal meningitis (5 weeks' delay) and tuberculosis (2 weeks' delay) [11, 23]. As we show, OIs are the main causes of premature death of PWH, regardless of the CD4 count. Besides, with this screening strategy, 1636 patients who tested negative for the OIs screened were candidates for immediate ARVs. Several recent randomized trials have indicated that rapid ART initiation, including same-day start, can improve patients' outcomes, especially by reducing loss to care in the pre-ART period [13].

Now validated in terms of clinical and survival impact, we propose a new health care diagnostic system, the diagnostic laboratory hub. This kind of organization is simpler, cheaper, and of higher quality than setting-up laboratories in every medical center. Courier companies and current Internet technology minimize the delays for delivery of samples and results, although there is room for improvement. In addition, the continuously high workload of a DLH ensures high-quality results, easier quality control, low cost per sample and diagnostic assay, and simpler continuing education for the staff. Even with full 24-hour/7-day operations, the DLH laboratory would be cheaper than any other kind of diagnostic system. In addition, an economic evaluation of the DLH is planned for 2020; conversations with the Ministry of Health have also started to analyze the sustainability of the project in the near future. For the time being, tuberculosis/NTM diagnosis is fully covered by the government, as well as the patient's treatment for all OIs.

Strong technical and clinical leadership is a key feature of the DLH and other successful diagnostic laboratories, and this is easier to deliver with a focused program compared with a distributed one. With a good configuration and organization, a DLH, in limited-resource setting, can deliver similar performance as a laboratory in an HIC but at a lower cost. Depending on the size of the country, careful planning is required to ensure DLH coverage of the whole territory, taking into consideration the main bottleneck of this structure, the elapsed time clinical samples spend in transit to the DLH. Network communication is now possible through different software systems, allowing online meetings, conference rooms, and video webinars at affordable prices. This facilitates continuing education, discussions of clinical cases, and a permanent update of the DLH and its network of clinics. Other countries may choose to adopt this model of care to reduce deaths in PWH on a national or regional basis.

Supplementary Data

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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Artículo 2. Capítulo 1. Evaluación del desempeño de las pruebas para diagnóstico de infecciones oportunistas utilizadas en el programa de tamizaje

Publicación original:

Narda Medina, Ana Alastruey-Izquierdo, Danicela Mercado, Oscar Bonilla, Juan C. Pérez, Luis Aguirre, Blanca Samayoa, Eduardo Arathoon, David W. Denning, Juan Luis Rodriguez-Tudela, on behalf of Fungired. Comparative performance of the laboratory assays used by a Diagnostic Laboratory Hub for opportunistic infections in people living with VIH. *AIDS*, 2020, 34:1625–1632. doi:10.1097/QAD.0000000000002631

El diagnóstico de las infecciones oportunistas fúngicas ha evolucionado en las últimas décadas y actualmente existen varios ensayos para su detección. Este trabajo evaluó la rentabilidad comparativa de las técnicas diagnósticas utilizadas en el programa de tamizaje durante los años 2017 y 2018. Los métodos evaluados incluyeron hemocultivos en tubos Isolator, cultivo de muestras de esputo o líquido cefalorraquídeo, ensayos caseros de PCR para la detección de *M. tuberculosis* e *H. capsulatum*, así como la detección de los antígenos circulantes de *H. capsulatum* y *C. neoformans* mediante técnicas comerciales.

Se diagnosticaron 716 (16,2%) infecciones oportunistas de un total de 2.245 pacientes tamizados; de ellas, 227 (31,7%) fueron histoplasmosis y 138 (19,3%) criptococosis. Se detectaron 62 (8,6%) casos de coinfecciones causadas por más de un patógeno. En este análisis se observó que: i) En la histoplasmosis, el hemocultivo mediante el sistema Isolator tuvo una sensibilidad del 36,3% (IC 95% 29,8—43,3), ii) Las pruebas comerciales para la detección de antígeno de *H. capsulatum* y *C. neoformans* tuvieron el mayor rendimiento, con una sensibilidad del 94% y 100%, en los casos de histoplasmosis diseminada y criptococosis meníngea. En ausencia de estas pruebas, el 35% de los casos de histoplasmosis diseminada y el 32,7% de los casos de criptococosis meníngea no se hubieran diagnosticado con dos consecuencias significativas: la casi certera muerte de los pacientes al no ser diagnosticados y la infravaloración de la carga de enfermedad fúngica, iii) La PCR casera para la detección de *Histoplasma* diagnosticó el 62,7% de los casos.

Las principales conclusiones de este artículo fueron: (i) A pesar de su baja sensibilidad hay que continuar cultivando las muestras clínicas para recuperar las cepas lo que permite la detección de la resistencia a los antifúngicos y su tipificación molecular; (ii) Las pruebas comerciales para la detección de antígeno para *C. neoformans* y el antígeno urinario de *H. capsulatum* deben ser consideradas de «referencia» para el diagnóstico de estas infecciones por lo que deben de estar disponibles en la asistencia rutinaria de los pacientes que viven con el VIH; (iii) El elevado número de pacientes con coinfecciones (8,6%) resalta la importancia de un programa de tamizaje completo.

Comparative performance of the laboratory assays used by a Diagnostic Laboratory Hub for opportunistic infections in people living with HIV

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Objectives: We evaluated the comparative performance of different assays used in a Diagnostic Laboratory Hub that linked 13 HIV healthcare facilities for the diagnosis of tuberculosis (TB), histoplasmosis, and cryptococcosis, and describing its functions in Guatemala compared with other National Reference Laboratories.

Methods: The following diagnostic techniques were analyzed in 24 months (2017–2018) in a cohort of patients with HIV: smear microscopy, mycobacterial and fungal cultures, isolator blood culture, PCR assays, and antigen detection tests.

Results: A total of 4245 patients were included, 716 (16.2%) had an opportunistic infection: 249 (34.7%) TB, 40 (5.6%) nontuberculous mycobacteria, 227 (31.7%) histoplasmosis, 138 (19.3%) cryptococcosis, and 62 (8.6%) had multiple opportunistic infections. Two hundred sixty-three [92.6%; 95% confidence interval (CI), 89–95.1] of TB cases were diagnosed by PCR. Urine antigen assay detected 94% (95% CI, 89–96) of the disseminated histoplasmosis cases. A lateral flow assay to detect cryptococcal antigen diagnosed 97% (95% CI, 93.3–98.7%) of the cryptococcal cases. In 85 patients (51.5%) with a cerebrospinal fluid sample, cryptococcal meningitis was diagnosed in 55 (64.7%), of which 18 (32.7%) were only detected by cryptococcal antigen.

Conclusion: Validated commercial antigen tests, as used in this program, should be the new gold standard for histoplasmosis and cryptococcosis diagnosis. In their absence, 35% of disseminated histoplasmosis and 32.7% of cryptococcal meningitis cases would have been missed. Patients with multiple opportunistic infections were frequently diagnosed and strategies should be designed to screen patients irrespective of their clinical presentation. In low resource settings, Diagnostic Laboratory Hubs can deliver quality diagnostics services in record time at affordable prices.

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Keywords: cryptococcosis, histoplasmosis, laboratory diagnosis, opportunistic infections, tuberculosis

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Introduction

In 2017, the WHO published their first guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy (ART), which were aimed to reduce global HIV morbidity and mortality [1]. These guidelines recommend a package of interventions to diagnose and treat major opportunistic infections, which continue to be an important cause of AIDS-related mortality [1,2]. Hence, identifying people who are eligible for elements of the package of care, requires quality diagnostic services which have a crucial role to improve clinical outcomes [3–5].

According to UNAIDS, Guatemala has the largest number of people living with HIV (PLWH) in Central America, the highest proportion presenting with advanced HIV disease and high viral loads which are factors that increase the risk of opportunistic infections [6,7]. Despite the development of the HIV healthcare program and increasing coverage of ART, access to accurate testing for opportunistic infections was limited. In 2017, to provide diagnostic services for mycobacterial and fungal infections, a Diagnostic Laboratory Hub (DLH) was implemented linked with a national network of HIV healthcare facilities (HCFs). Here, we evaluate the comparative performance of different diagnostic assays employed in the DLH and describe the functions of this Health System in comparison with those provided by National Reference Laboratories (NRLs).

Materials and methods

Setting and population

From January 2017 through December 2018, diagnostic services for tuberculosis (TB), nontuberculous mycobacteria (NTM), histoplasmosis and cryptococcosis were provided by the DLH to a national network of 13 HCFs. Screening for these opportunistic infections was performed regardless of CD4⁺ cell count for newly diagnosed patients; patients who had abandoned ART (>90 days) and returned to care, and those on ART when an opportunistic infection was suspected. Clinical specimens were obtained in the HCFs and delivered to the DLH by means of a courier service. In addition, an electronic system was set up to capture patient data, request diagnostic services and report results.

Laboratory procedures

HCFs were requested to send sputum samples, urine, serum, and 10 ml of blood in an Isolator tube (Wampole Laboratories, Cranbury, New Jersey, USA). Additional clinical samples were also received depending on the clinical criteria. Diagnostic methods included smear for mycobacteria; cultures for fungi and mycobacteria; isolator blood culture; *in-house* PCR for the detection

of *Mycobacterium tuberculosis* and *Histoplasma capsulatum* [8,9]; ELISA (IMMY, Norman, Oklahoma, USA) to detect *Histoplasma* antigen in urine and; lateral flow assay (IMMY) to detect cryptococcal antigen (CrAg). Detailed methods are described in the Supplementary material, <http://links.lww.com/QAD/B790>.

Role of the National Reference Laboratories and the Diagnostic Laboratory Hub

To compare the role and functions of the NRLs with the DLH, the official websites of the Center for Disease Control and Prevention (USA), the National Administrations of Laboratories and Health Institutes 'Dr Carlos G. Malbrán' (Argentina), Institute Gorgas Reference Central Laboratory (Panama), and the NRL (Guatemala) were searched.

Data analysis

The percentage of the diagnostic performance of each technique, with their corresponding 95% confidence intervals (CIs), was calculated against the number of total cases diagnosed. Chi-squared test was used to compare the positive proportions and significance was set at $P = 0.05$.

Geographic information system and Google maps tools were used to describe the location of the DLH and HCFs. Statistical analysis was performed using SPSS 19.0 software (IBM Iberica, Madrid, Spain).

Results

Healthcare network

Guatemala has an area of 108 889 km², with an asphalted road system of 7342 km. The national network included 13 (81.2%) of the 16 available HCFs in the country, which are mainly located in rural areas (12 out of 13; 92.3%). Nine (69.3%) of these facilities are located within an average time of 5 h to the DLH. Regarding the distribution, five (38.5%) HCFs are located in the west, four (30.7%) in the east, two (15.3%) in the north, one (7.6%) in south, and one (7.6%) in the metropolitan area.

Patients characteristic and opportunistic infections diagnosed

During 2017 and 2018, a total of 4245 PLWH were screened for opportunistic infections. Baseline characteristics are shown in Table 1. A total of 2677 (63.1%) patients were male with a mean age of 36 years (range 13–89), and 2127 (50.1%) patients were newly diagnosed with HIV infection. Patients with opportunistic infections were slightly older than without opportunistic infections (38 vs. 36 years, $P = 0.002$), and had a significantly higher HIV viral load (median log₁₀ 5.1 vs. 4.6 copies/ml, $P < 0.0001$). Only 71% of patients had CD4⁺ cell counts done (Table 1).

Table 1. Baseline characteristics of the patients with opportunistic infections screening.

Characteristics	OIs cases		Non-OI cases		Total	
	<i>n</i> = 716 (16.8%)		<i>n</i> = 3529 (83.2%)		<i>n</i> = 4245 (100%)	
Sex	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Male	476	66.5	2201	62.4	2677	63.1
Female	235	32.8	1291	36.6	1526	35.9
Transsexual	5	0.7	37	1.0	42	1.0
Age, mean (range)	<i>n</i>		<i>n</i>		<i>n</i>	
Mean (range)	709	99.0	3517	99.6	4226	99.5
	38	(14–78)	36	(13–89)	36	(13–89)
Sexual orientation						
Heterosexual	592	82.7	2642	74.9	3234	76.2
Homosexual	68	9.5	602	17.1	670	15.8
Bisexual	30	4.2	207	5.9	237	5.6
Unknown	26	3.6	78	2.2	104	2.4
Category of patient						
Newly HIV infection	385	53.8	1742	49.4	2127	50.1
On ARV	191	26.7	1134	32.1	1325	31.2
Return/Restart	138	19.3	641	18.2	779	18.4
Unknown	2	0.3	12	0.3	14	0.3
CD4 ⁺ cell count						
<i>n</i>	477	66.6	2537	71.9	3014	71.0
<200	376	78.8	1104	43.5	1480	34.8
Viral load						
<i>n</i>	470	65.6	2355	67.1	2825	66.5
Median (IQR)	5.1	(4.4–5.6)	4.6	(3.5–5.1)	4.7	(3.6–5.2)

ARV, antiretroviral therapy; IQR, interquartile range; OI, opportunistic infection.

Seven hundred sixteen (16.9%) patients had opportunistic infections: 249 (34.7%) TB, 40 (5.6%) NTM, 227 (31.7%) histoplasmosis, 138 (19.3%) cryptococcosis, and 62 (8.6%) had multiple opportunistic infections. Among patients with multiple opportunistic infections, there were 24 (38.7%) histoplasmosis and TB, 15 (24.1%) cryptococcosis and histoplasmosis, 11 (17.7%) cryptococcosis and TB, three (4.8%) cryptococcosis and NTM, three (4.8%) TB and NTM, three (4.8%) histoplasmosis and NTM, and three (4.8%) triple infections. Almost 79% of opportunistic infections patients had less than 200 CD4⁺/μl defined as advanced HIV disease by the WHO (Table 1) [1].

Comparative diagnostic performance among techniques

Full screening for mycobacteria, histoplasmosis and cryptococcosis was performed in 3448 (81.2%) patients. Partial screening was done in 797 (18.8%) patients; of those 626 (78.5%) were tested for histoplasmosis, 371 (46.5%) for cryptococcosis and 272 (34.1%) for mycobacteria. The diagnostic performance results were similar between those with full and partial screening. Therefore, all cases were included to have more statistical power (Table 2).

A total of 290 cases of TB, 50 of NTM, 271 of histoplasmosis and 170 of cryptococcosis were diagnosed.

Table 2. Comparative performance of the techniques employed for the opportunistic infections diagnosis.

Diagnostic modality	Tuberculosis ^a , <i>n</i> = 290			Histoplasmosis, <i>n</i> = 271			Cryptococcosis, <i>n</i> = 170		
	No. (%)	+ve	(95% CI)	No. (%)	+ve	(95% CI)	No. (%)	+ve	(95% CI)
Single test									
Smear	284 (97.9)	40	14.1 (10.5–18.6)	–	–	–	–	–	–
Culture ^b	286 (98.6)	109	38.1 (32.7–43.9)	211 (77.8)	18	8.5 (5.4–13.0)	85 (51.5)	37	–
Isolator ^c	207 (71.4)	18	8.7 (5.6–13.3)	190 (70.1)	69	36.3 (29.8–43.3)	129 (75.8)	19	14.7 (9.6–21.8)
PCR	284 (97.9)	263	92.6 (89–95.1)	215 (79.3)	135	62.7 (56.6–69.4)	–	–	–
Urine Ag	–	–	–	260 (95.9)	188	72.3 (66.5–77.3)	–	–	–
Serum Ag	–	–	–	–	–	–	165 (97.0)	165	100 (99.7–100)
CSF Ag	–	–	–	–	–	–	93 (54.7)	64	68.8 (58.8–77.3)
Combined test									
Smear + culture	284 (97.9)	119	41.9 (36.3–47.7)	–	–	–	–	–	–
Smear + PCR	278 (95.8)	261	93.9 (90.4–96.1)	–	–	–	–	–	–
PCR + culture	280 (96.5)	280	100 (98.5–100)	207 (76.4)	138	66.7 (60–72.7)	–	–	–
PCR + isolator	–	–	–	157 (57.6)	119	75.8 (68.5–81.8)	–	–	–
Urine Ag + isolator	–	–	–	186 (68.6)	136	73.1 (66.3–79.0)	–	–	–
Urine Ag + PCR	–	–	–	206 (76.0)	198	96.1 (92.5–98.0)	–	–	–
Urine Ag + isolator	–	–	–	153 (56.4)	153	100 (99.7–100)	–	–	–
PCR + culture	–	–	–	–	–	–	–	–	–

^aNontuberculous mycobacterium were excluded because the unique diagnostic technique employed was culture.

^bCulture was performed from sputum for histoplasmosis and tuberculosis, and cerebrospinal fluid (CSF) for cryptococcal meningitis.

^cIsolator blood culture.

These numbers encompass single infections and cases with multiple opportunistic infections; thus, total cases were used to assess the comparative performance of the diagnostic techniques (Table 2).

Out of the cases tested for TB (Table 2), direct microscopy was positive in 14.1% (95% CI, 10.5–18.6), culture in 38.1% (95% CI, 32.7–43.9), isolator blood culture in 8.7% (95% CI, 5.6–13.3), and PCR in 92.6% (95% CI, 89.0–95.1). One hundred and sixty-one (55.5%) were only detected by PCR. All TB cases were diagnosed by a combination of PCR and conventional culture. NTM cases were diagnosed by culture.

Of those tested for histoplasmosis (Table 2), isolator blood culture, a longstanding high volume blood culture technique employed before the appearance of urine antigen for histoplasmosis, was positive in 36.3% (95% CI, 29.8–43.3), urine antigen in 72.3% (95% CI, 66.5–77.3), culture of respiratory samples in 8.5% (95% CI, 5.4–13.0), and PCR in 62.7% (95% CI, 56.6–69.4), sputum was the sample used for PCR in 92.6% of the samples tested. Urine antigen and PCR were the only positive test in 95 (35.1%) and 64 (23.6%) cases, respectively. Combining urine antigen and PCR assay the positive rate increased by 23.8% (72.3–96.1%). The median CD4⁺ cell count of those diagnosed by urine antigen testing or isolator was 33 and 20 cells/ μ l, respectively ($P=0.667$).

For cryptococcal disease, 165 out of 170 cases were diagnosed by serum CrAg. The remaining five cases were diagnosed by cerebrospinal fluid (CSF) CrAg (two cases) and isolator blood culture (three cases). A lumbar puncture was done to 85 (51.5%) of 165 CrAg serum positive patients. A total of 55 (64.7%) cases had cryptococcal meningitis. Out of these cases, 37 (67.3%) were CrAg and culture positive, and 18 (32.7%) were only CrAg positive. Of those, 35 and 16 cases had CD4⁺ cell counts, with a median of 36 and 47 cells/ μ l, respectively ($P=0.692$).

Functions of the National Reference Laboratories and the Diagnostic Laboratory Hub

A review of the main activities of NRL is summarized in Table 3. The functions listed are consistent with the role of assessment, policy development, and assurance, which contribute to the public health system. Main activities included: confirmatory/reference diagnostic testing, typing of microorganisms, assessment of antibiotic resistance, and standardization of methodologies. In Guatemala, the NRL perform most of these activities; however, do not include a Reference Laboratory for fungal infections. For the DLH the main role is to provide rapid diagnostic services and to coordinate activities to improve the management of opportunistic infections in the network. Indirectly, the DLH collects epidemiological data as a result of the diagnostic activities. This

information is shared with the HCF network as well as with the Ministry of Health.

Discussion

Access to accurate diagnosis services is crucial to guide therapy and to improve clinical outcomes. In this study, a DLH provided rapid diagnostic services for opportunistic infections to a national network of HCFs. We used a courier service for specimen transportation and a similar strategy implemented in Uganda showed an eight-fold increase in referral samples and TB case detection [10]. In Guatemala, this system was able to provide diagnostic access to 81.2% of the HCFs in the country including the most remote ones.

Regarding TB, a low positivity rate (14.1%) of smears was found. Lower performance of sputum microscopy in HIV patients has been widely described because they frequently have paucibacillary infections [11]. However, our results were lower than those found by previous studies, where sensitivity ranged from 30 to 48% [11,12]. A low positivity rate was also found for TB culture with 38.1 vs. 62.6% in the literature [13]. Nevertheless, culture is essential to isolate the microorganism, and determine drug susceptibility, and monitor resistance rates. To increase the detection rate, it would be necessary to explore the use of different transportation systems that could improve mycobacteria recovery. TB PCR detected 92.6% of the cases, which is similar to other reports (90–100%) [14,15]. Xpert MTB/RIF and lateral flow lipoarabinomannan (LAM) assay were not available at the DLH.

Concerning histoplasmosis, the sensitivity of the urine antigen (72.3%) and the isolator blood culture (36.3%) were lower than previously reported 81–98% and 66.7–74.2%, respectively [16–19]. It is well known that urine antigen is designed to diagnose disseminated histoplasmosis and thus, it is expected to be negative in localized histoplasmosis. On the contrary, in this study clinical symptoms and signs that could have helped to classify the histoplasmosis cases were not recorded by the DLH. Therefore, we assumed that a patient with a positive urine antigen, and/or isolator blood culture had disseminated histoplasmosis. Considering this definition, the number of disseminated histoplasmosis was 205 (77.3%) out of 265 patients. Of those, 94% had a urine antigen positive. In patients with isolator and urine antigen test, seven cases had a positive isolator blood culture and negative result in urine antigen, which means that the use of both techniques increases the positivity rate by 3.5% but at a substantially higher cost. The cost of one determination of *Histoplasma* antigen in Guatemala is \$13 vs. 22 for the isolator blood culture. Other study found that histoplasmosis may cause proteinuria [20] and other kidney

Table 3. Main activities of the National Reference Centers and the Diagnostic Laboratory Hub in Guatemala.

General area of action	National Reference Laboratories			
	CDC United States of America	ANLIS Dr G. Malbran, Argentina	Institute Gorgas Reference Central Laboratory (Panama)	National Reference Laboratory Guatemala
Health services	Detect infectious organisms, food-borne outbreaks, and biosecurity threats Identify environmental and workplace hazards Monitor the health of communities Invent new ways to rapidly test for infectious disease in the field Arm state, county, and local public health laboratories with the expertise and data they need to protect their citizens	Coordinates the national network of laboratories and provides technical assistance Perform diagnostic confirmation Contributes to surveillance activities Provides scientific advice	Design, promote, coordinate, and execute research programs Provides scientific advice to the Ministry of Health and other institutions Evaluate new health technology and research policies Promote cooperation agreements, for the development of health knowledge Provide services such as the Central Laboratory of Public Health	DLH Guatemala Provides high-quality diagnosis services for fungal infections and tuberculosis for a network of HCFs Coordinates communication activities for the network Implements and monitor the delivery of specimens between the HCF and the DLH Develops informatics tools to strengthening communication of the network Collects epidemiological data
Early warning and response	Deploy diagnostic tests and tools, such as advanced molecular detection technology that helps scientists detect health threats more quickly	Description of possible warnings to detect outbreaks and communication to the Ministry of Health	Develop surveillance and public health programs	Participates in research in health surveillance and public health
Training	Sponsors and hosts new initiatives and training to encourage laboratory scientist networking and promote cross-cutting concepts	Provides educational programs	Offer health information and documentation services	Supports training activities within the network

Centers for Disease Control and Prevention: <https://www.cdc.gov/labs/protecting-america.html>; ANLIS Dr G. Malbran, Argentina: <http://www.anlis.gov.ar/mision-vision-y-objetivos/>; Institute Gorgas Reference Central Laboratory: <http://www.gorgas.gob.pa/objetivos/>; National Reference Laboratory of Guatemala: <http://portal.ins.gob.gt/>; ANLIS, National Administration of Laboratories and Institutes of Health; CDC, Centers for Disease Control and Prevention; DLH, Diagnostic Laboratory Hub; HCF, HIV healthcare facility.

damage, that in some cases, could render a false negative antigen result. An assay designed for TB in urine samples, LAM have shown that early morning urine and sample concentration can increase the sensitivity [21]. Further studies are needed to evaluate the influence of these factors. The urine antigen test has resulted in a higher detection of the histoplasmosis cases. Global access to these diagnostic tests, especially in Latin America, would increase awareness as well as the availability of an early diagnosis and treatment which will certainly increase the patient survival.

For cryptococcosis, we found that the positivity rate of the CrAg test in serum was consistent with previous reports (98–100%) [22,23]. Eighteen meningitis cases were diagnosed only by CrAg detection, highlighting the importance of this assay in the rapid diagnosis of this severe infection. CSF culture alone would have only diagnosed 67% of cryptococcal meningitis cases and at a slower response rate. Similar results were reported in other study with 10% of patients with a positive CSF CrAg having a negative culture [24]. The DLH used CrAg CSF results as India ink microscopy is less sensitive and time consuming [25].

Taking into account our findings, we recommend the following approaches. First, the sensitivity of direct microscopy for acid-fast bacilli is very low. It is a demanding technique that requires patience and expertise to get good results but provides a quick answer. Laboratories should evaluate the usefulness of this technique considering their available workforce. Second, culture is an insensitive technique for both, *Mycobacteria* and fungi, but in our opinion, should be maintained to recover the microorganisms for proper identification, susceptibility testing, and typing. Third, isolator blood culture has a low sensitivity and we do not recommend its use as a diagnostic tool. However, it could be useful for the recovery of *Histoplasma* isolates when the urine antigen is positive. Therefore, if a patient had a positive urine antigen, we would recommend asking for an urgent isolator blood culture. Fourth, TB PCR should be used because it has a good sensitivity and delivers results in a short time. However, we recommend the implementation of standardized commercial techniques, already available, such as the Xpert MTB/RIF assays. For histoplasmosis there is no commercial PCR system and, although our *in-house* PCR has an acceptable performance, we cannot give a clear recommendation about its use until further analysis about its comparative diagnostic performance in the diagnosis of localized histoplasmosis is done. Fifth, antigen detection techniques are the current gold standard for diagnosing cryptococcosis and disseminated histoplasmosis and should be available in every single laboratory dealing with opportunistic infections in PLWH. Without these tests, 95 (35%) disseminated histoplasmosis and 18 (32.7%) cryptococcal meningitis

cases would have been missed. Currently, these methods are included in the WHO Essential Diagnostics List [26]. Sixth, the rate of patients with multiple opportunistic infections in advanced HIV in Guatemala is substantial. Therefore, optimal case-finding strategies should include screening for potential opportunistic infections irrespective of their clinical presentation, and the local prevalence of different opportunistic infections. Similar diagnostic interventions should be considered in other low resource settings.

This study also demonstrates that the DLH does not interfere with other healthcare systems. The European Center for Diseases Control (ECDC) classifies the main activities of microbiology reference laboratories into five core functions: first, diagnostic confirmation services, which include reference methods for specific pathogens with a low prevalence or not covered by the usual commercial portfolio; second, scientific advice; third, collaboration and research; fourth, provision of reference materials; and fifth, monitoring alert and response [27]. The functions of the NRLs are similar to the core activities outlined by the ECDC, which reflect the homogeneity of the Reference Laboratory activities. Guatemala NRL aims are similar but opportunistic infections diagnosis for PLWH is not included in its activity's portfolio. On the other hand, the main goal of the DLH is to provide quick diagnostics services for improving the care and management of patients as well as to aggregate the epidemiology data obtained from the diagnostic analysis. Besides, with more automation, the DLH can manage thousands of samples without a huge increase in labor costs.

The current study has several limitations. The transport time was not measured and thus we cannot determine their impact. In addition, we did not collect data of patients' symptoms and the added value of it was not determined. Despite this, a large number of patients and events were analyzed allowing us to make clear diagnostic recommendations for other countries and states that want to improve the diagnosis of opportunistic infections in PLWH. The DLH is a promising approach to provide diagnostic services to a large community of HCFs at an affordable cost.

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Conflicts of interest

A.A.-I. has received research grants or honoraria as a speaker or advisor from Astellas, Gilead Sciences, MSD, Pfizer, F2G, Amplyx, and Scynexis outside the submitted work. E.A. has received honoraria from GILEAD for educational conferences and participation in Advisory board meeting. D.W.D. holds Founder shares in F2G Ltd, a University of Manchester spin-out antifungal discovery company, in Novocyt, which markets the Myconostica real-time molecular assays and has current grant support from the National Institute of Allergy and Infectious Diseases, National Institute of Health Research, North West Lung Centre Charity, Medical Research Council, Global Action Fund for Fungal Infections and the Fungal Infection Trust. He acts or has recently acted as a consultant to Astellas, Sigma Tau, Basilea, Biosergen, Cidara, and Pulmocide. In the past 3 years, he has been paid for talks on behalf of Astellas, Dynamiker, Gilead, Merck, and Pfizer. He is also a member of the Infectious Disease Society of America Aspergillosis Guidelines and European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines groups. All other authors declare no conflicts of interest.

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[CAPÍTULO 2]

Capítulo 2. Carga de las infecciones oportunistas fúngicas en la cohorte de pacientes de reciente diagnóstico de VIH e impacto del acceso al diagnóstico

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La ausencia de estudios prospectivos que analicen la situación de las personas al diagnóstico del VIH impide establecer estrategias dirigidas a la contención de la pandemia y sus consecuencias. Asimismo, se desconoce la verdadera cara de las infecciones oportunistas lo que complica que los limitados recursos se distribuyan a las patologías que más afectan a estos pacientes. Con el fin de determinar la incidencia de las principales infecciones oportunistas fúngicas y el impacto de la estrategia de tamizaje, se analizaron los resultados de la cohorte de pacientes de reciente diagnóstico durante 2017 y 2018.

Un total de 2.127 pacientes fueron tamizados y se estableció que: i) Al diagnóstico del VIH, el 52% de los pacientes tenían una enfermedad avanzada, definida como un recuento de células CD4 $<200/\text{mm}^3$; ii) El 65,8% de los pacientes mayas tenían una enfermedad avanzada en comparación con un 49,7% de la población ladina; iii) La incidencia de las infecciones oportunistas tamizadas fue del 21%, siendo la histoplasmosis la infección más frecuente (7,9%), seguida de la tuberculosis (7,1%) y de la criptococosis (4,8%). En conjunto la incidencia de las infecciones fúngicas fue del 12,7%, superando en un 5,6% a la tuberculosis; iv) El 95% de los pacientes con una infección oportunista tenía un recuento de células CD4 menor a $350/\text{mm}^3$; v) En el 2017 la mortalidad de los pacientes con una infección oportunista fue del 34%, y en el 2018 del 27%, un 7% menos.

Los datos indican que la frecuencia de la enfermedad avanzada por VIH en Guatemala es una de las más altas a nivel mundial, siendo la población maya la más desfavorecida. La histoplasmosis es la infección oportunista fúngica definitoria de SIDA más frecuente en el país, y en conjunto con la criptococosis, superan en más de un 5% la incidencia de la tuberculosis. La conclusión más relevante de este trabajo es que la mortalidad asociada de las infecciones oportunistas se puede

reducir cuando los pacientes tienen acceso al diagnóstico y especialmente si se emplea un programa de tamizaje, que sea capaz de brindar resultados en un tiempo corto para que el tratamiento específico se pueda administrar rápidamente. Además, los resultados de este trabajo sugieren que se debe realizar el tamizaje de las infecciones oportunistas a todos los pacientes de reciente diagnóstico de VIH con menos de 350 células CD4. La determinación de la carga real de estas infecciones permite estimar con mayor precisión los recursos necesarios para el diagnóstico y tratamiento de estas lo que además lo convierte en una herramienta de política sanitaria esencial en entornos de recursos limitados.

Article

A Rapid Screening Program for Histoplasmosis, Tuberculosis, and Cryptococcosis Reduces Mortality in HIV Patients from Guatemala

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Abstract: Opportunistic infections (OIs) and advanced HIV disease (AHD) contribute to HIV-related mortality. Here, we analyzed the situation of AHD and OIs in a cohort of newly diagnosed HIV patients from Guatemala. We included 2127 adult patients from 13 facilities across the country during 2017 to 2018. Patients were screened for tuberculosis (TB), nontuberculous mycobacteria (NTM), histoplasmosis, and cryptococcal disease, independently of their CD4 cell count. Of the 2127 enrolled patients, 1682 (79.1%) had a CD4 cell count available; of which 52% presented with AHD. Of the Mayan population, 65% had AHD. The overall OI incidence was 21%. Histoplasmosis was the most frequent OI (7.9%), followed by TB (7.1%); 94.4% of these infections occurred in patients with a CD4 < 350 cells/mm³. Mortality at 180 days was significantly higher in those with OIs than without OIs (29.7% vs. 5.9%, $p < 0.0001$). In one year, this program decreased the OI mortality by 7% and increased the OI treatment by 5.1%. Early OI diagnosis and appropriate therapy reduced OI mortality among newly diagnosed HIV patients in Guatemala. Screening for OIs should be considered in all newly diagnosed HIV patients who have a CD4 cell count < 350 cells/mm³ or those without a CD4 cell count available. To improve results, interventions such as early HIV detection and access to flucytosine and liposomal amphotericin B are required.

Keywords: laboratory diagnosis; opportunistic infections; tuberculosis; histoplasmosis; cryptococcosis

1. Introduction

Opportunistic infections (OIs) remain a major cause of death in HIV-infected patients, especially in low- and middle-income countries (LMICs) [1]. Despite the increasing coverage of antiretroviral therapy (ART), 770,000 people died in 2018 [2]. In Latin America (LATAM), these deaths have decreased by 14% since 2010; however, other regions such as eastern and southern Africa have shown a 44% reduction [2]. National and regional data on specific causes of HIV mortality are crucial to develop strategies and to allocate resources effectively.

In 2017, the World Health Organization (WHO) published the first guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy [3]. These

guidelines recommended a package of interventions oriented to reduce AIDS-related mortality while advising rapid diagnosis and treatment of major OIs. Although these recommendations are focused on people with advanced HIV disease (AHD), there may be benefits of screening strategies that reach all patients at higher risk, such as those newly diagnosed with HIV. Since 2010, the number of new HIV infections has increased by 7% in LATAM [2]. Differences in the burden of OIs have also been described, especially for histoplasmosis, which is an important OI in the region [4,5]. Therefore, we developed a prospective screening study to assess the burden of AHD, the incidence of four OIs, and the mortality at 180 days in a large cohort of newly diagnosed HIV patients in Guatemala.

2. Materials and Methods

2.1. Study Design and Participants

Patients were enrolled in the OIs program, which was established in Guatemala in 2017. This program encompasses a network of 13 health care facilities (HCFs) and a central Diagnostic Laboratory Hub (DLH) [6]. This work analyzes all newly diagnosed HIV patients enrolled in this program between January 2017 to December 2018 who were over 13 years of age.

2.2. Procedures and Definitions

Demographic data were collected in the HCFs using a standard electronic form. Patients were screened for the following OIs: tuberculosis (TB), nontuberculous mycobacteria (NTM), histoplasmosis, and cryptococcosis. Screening was performed independently of the CD4 cell count. The following samples were requested: whole blood in an isolator tube (Abbott Diagnostics, Illinois, Chicago, USA), serum, urine, and sputum. Additional samples were also received based on clinical criteria. Specimens were referred to the DLH to be processed as previously described [6,7]. Laboratory assays included: (i) Smear microscopy; (ii) Sputum culture; (iii) Isolator blood culture; (iv) in-house PCR for *M. tuberculosis* and *H. capsulatum*; (v) Detection of urine antigen of *H. capsulatum* by IgG monoclonal antibodies (Immuno-Mycologics (IMMY), Norman, Oklahoma, USA); and (vi) the cryptococcal antigen lateral flow assay (CrAg) (LFA, IMMY, Norman, Oklahoma, USA). If serum CrAg was positive, a lumbar puncture was advised. The relative diagnostic performance of these tests has been described by Medina et al. [7]. A full screening was considered when the four OIs were analyzed. OI diagnosis required a positive result of at least one of the following techniques: antigen detection, culture, and/or PCR test.

Disseminated histoplasmosis was considered when an Isolator blood culture and/or urine antigen was positive, and cryptococcal meningitis when we detected a positive CrAg test or isolated *C. neoformans* in cerebrospinal fluid (CSF). AHD was defined as having a CD4 count < 200 cells/mm³. CD4 cell counts were obtained by the different HCFs. An available CD4 cell count was defined as a cell count obtained 90 days before or after the moment of the OI screening. Patients from Guatemala City and other cities were categorized as urban while other areas were defined as rural.

Patients were treated in the HCFs and antiretroviral therapy was started in accordance with the national guidelines [8,9].

2.3. Ethics Statement

Because this study analyzed data obtained during the implementation of a routine program that provided diagnostic services to newly diagnosed HIV patients, informed consent was not requested for the OI screening; however, a written informed consent for HIV testing was obtained by the HCFs of the network. For the analysis, no personally identifiable data information was collected, and confidentiality of patients was assured.

2.4. Data Analysis

The participants were categorized according to the OI diagnosis. Baseline characteristics were compared with chi-square or Fisher's exact test for categorical variables and

Mann-Whitney U-test for continuous variables. We performed univariate and multivariate analyses to identify demographic factors associated with AHD. Receiver operator curves (ROCs) were carried out to evaluate different CD4 thresholds among the OI cases for screening purposes. Sensitivity, specificity, and their two-sided 95% confidence intervals (95% CI) were determined.

3. Results

3.1. Study Population

A total of 2127 newly diagnosed HIV patients were enrolled between January 2017 and December 2018. This study encompassed 58.3% (2127 out of 3646) of the new HIV infections reported by the HIV national program. Table 1 summarizes the patients' characteristics. The median age at HIV diagnosis was 31 years (IQR: 25–41) and 70.3% of the patients were male. CD4 cell counts were available for 1682 patients (79.1%), of those, 877 (52.1%) had AHD. Women and men had similar CD4 cell counts (median, 244 vs. 228; $p = 0.623$); however, heterosexual men had significantly lower CD4 counts than men who have sex with men (MSM) (median CD4, 124 cells/mm³ vs. 264 cells/mm³; $p < 0.0001$). The difference of AHD between the Mayan and Ladino population was statistically significant (65.8% vs. 49.7%, $p < 0.0001$), but no difference was observed in OI rate (18% vs. 17.5%, $p = 0.282$). Mayan women and men had similar CD4 cell counts (median CD4, 160 cells/mm³ vs. 125 cells/mm³; $p = 0.411$).

Table 1. Baseline characteristic of newly diagnosed HIV patients.

Characteristic	Total 2127 (100%)		With OI 385 (18%)		Without OI 1742 (82%)	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Sex						
Male	1495	70.3	280	72.7	1215	69.7
Female	608	28.6	104	27.0	504	28.9
Transsexual	24	1.1	1	0.3	23	1.3
Age (years)						
<i>n</i>	2121	99.7	380	98.7	1741	99.9
Median, IQR	31	(25–41)	35	(28–45)	30	(24–40)
Sexual orientation						
Heterosexual	1420	66.8	306	79.5	1114	63.9
Homosexual	486	22.8	39	10.1	447	25.7
Bisexual	163	7.7	19	4.9	144	8.3
Unknown	58	2.7	21	5.5	37	2.1
Ethnic group						
Ladino	1524	71.7	266	69.1	1258	72.2
Mayan	334	15.7	60	15.6	274	15.7
Other	13	0.6	4	1.0	9	0.5
Unknown	256	12.0	55	14.3	201	11.5
Residence	2077	97.6	371	96.3	1706	97.9
Urban	995	47.9	159	42.9	836	49
Rural	1082	52.1	212	57.1	870	51
BMI						
<i>n</i>	1198	56.3	185	48.0	1013	58.1
Underweight	148	12.4	45	24.5	103	10.2
Normal	695	58.1	98	53.3	597	58.9
Overweight	275	23.0	33	17.9	242	23.9
Obese	79	6.6	8	4.3	71	7.0
CD4 (cells/mm ³)						
<i>n</i>	1682	79.1	268	69.6	1414	81.2
Median, IQR	187	(73–326)	53	(19–137)	220	(102–359)
<200	877	52.1	227	84.7	650	46.0
<350	1298	77.2	253	94.4	1045	73.9
≥350	384	22.8	15	5.6	369	26.1
Viral load (copies/mL)						
<i>n</i>	1668	78.4	277	72.0	1391	79.8
Log ₁₀ Median, IQR	4.8	(4.2–5.3)	5.2	(4.7–5.7)	4.7	(4.1–5.2)

Among 877 patients with AHD, 322 (36.7%) had CD4 cell counts between 0–49 cells/mm³, 202 (23%) between 50–99 cells/mm³, and 353 (40.2%) between 100–199 cells/mm³. In the univariate analysis, risk factors associated with AHD were older age at the moment of diagnosis, belonging to a Mayan ethnic group, heterosexual category, and patients who live in rural areas. In the multivariate analysis, the risk factors significantly associated with AHD were: (i) age 30–50 years (aOR 1.5, 95% CI (1.2–1.9), $p < 0.0001$), (ii) >50 years (aOR 2.3, 95% CI (1.6–1.9), $p < 0.0001$), and (iii) Mayan ethnic group (aOR 1.5, 95% CI (1.1–2.0), $p = 0.009$). Compared with heterosexuals, MSM were less associated with AHD (aOR 0.48, 95% CI (0.37–0.62), $p < 0.0001$).

3.2. Opportunistic Infections

Of the 2127 participants, 1821 (85.6%) had full screening done. Three hundred eighty-five patients (18.1%) had OIs: 140 (36.4%) histoplasmosis, 121 (31.4%) TB, 78 (20.3%) cryptococcal disease, 31 (8.1%) multiple OIs, and 15 (3.9%) NTM infection. In patients with multiple OIs, we found histoplasmosis/cryptococcosis (35.5%), histoplasmosis/tuberculosis (32.3%), and cryptococcosis/tuberculosis (12.9%) as the most frequent. In 2018, the incidence of OIs increased 3.9% (from 15.9% to 19.8%). Patients with OIs were significantly older (median, 35 years vs. 30 years; $p < 0.0001$), tended to be heterosexual (79.5% vs. 63.9%; $p < 0.0001$), and had higher HIV viral load (Log₁₀ 5.2 OIs vs. 4.7 $p < 0.0001$) than those without OIs (Table 1). OIs were more frequent in patients living in rural areas than those living in urban zones (10% vs. 5.8%, $p = 0.018$). Among country regions, histoplasmosis and cryptococcal disease were frequent in the East (10.1% and 8.1%), West (10.9% and 5.7%), and Central regions (8.8% and 4.5%). In Guatemala City, TB was more frequent (8.3%).

As expected, the incidence of an OI was inversely correlated with CD4 cell count (Table 2). Histoplasmosis was the most frequent AIDS-defining illness. The difference between histoplasmosis and TB incidence was statistically significant in patients with CD4 cell counts < 50 cells/mm³ (19.7% vs. 11.5%, $p = 0.003$). Among those with AHD, the incidence of OIs was 30.3%. In patients who had a CD4 cell count ≥ 350 cells/mm³, TB was more frequent than histoplasmosis (2.9% vs. 1.1%, $p = 0.0807$) (Table 2).

Table 2. Opportunistic infections incidence stratified by CD4 cell count.

OIs	Overall Incidence	Interval CD4 Cell Count				
		<50	50–99	100–199	200–350	>350
Tuberculosis	7.1%	11.5%	10.8%	7.5%	2.7%	2.9%
NTM	1.1%	2.4%	0.5%	1.9%	0.3%	0.0%
Histoplasmosis *	7.9%	19.7%	7.0%	4.1%	2.4%	1.1%
Cryptococcosis +	4.8%	14.3%	7.4%	3.3%	1.5%	0.3%
Total	21.0%	47.9%	25.8%	16.8%	6.8%	4.3%

* All cases of histoplasmosis (disseminated and non-disseminated) were included; + All cryptococcal cases (meningitis and non-meningitis) were included.

We analyzed, by means of a ROC curve, OIs (Yes/No) against CD4 cell counts to determine the best threshold to screen patients for OIs. The AUC was 0.771 (95%CI; 0.741–0.801). Table 3 shows the sensitivity and specificity of the different thresholds. Almost 95% of the OIs investigated occurred in patients with <350 CD4/mm³. Fifteen OIs were diagnosed in patients with ≥ 350 CD4 cells/mm³: 10 TB, 4 histoplasmosis, and 1 cryptococcal disease. Eighty-one (63.3%) of 128 patients with disseminated histoplasmosis had a CD4 cell count available. If histoplasmosis screening had been limited to patients with CD4 cell counts < 100 or <200 CD4 cells/mm³, 16 (19.7%) and 6 (7.4%) cases would have been missed, respectively. For cryptococcal disease, 5 (13.8%) and 1 (2.8%) cases would have been missed, respectively. A total of 117 (30.4%) OIs were diagnosed in patients without a CD4 cell count result available.

Table 3. CD4 cell counts thresholds. Sensitivity and specificity for OIs screening.

CD4 Threshold	Sensitivity (95% CI)	Specificity (95% CI)
<100	66.6 (59.8–71.1)	75.2 (73.1–77.6)
<200	85.1 (80–88.5)	53.9 (51.4–56.6)
<350	94.4 (90.1–96.6)	26.0 (23.8–28.4)

3.3. Treatment and Outcome

A total of 324 (84.1%) patients with OIs were treated. Information was available for 36 (59%) out of the 61 patients who did not receive treatment: 18 (50%) died before initiation of treatment, 12 (33.3%) were lost to follow-up, and 6 (16.7%) refused treatment (median CD4, 88 cells/mm³). Treatment information for the OI cases is shown in Table 4.

Table 4. Opportunistic infection treatments.

Treatments	Total (<i>n</i> = 385) <i>n</i> (%)	TB (<i>n</i> = 121) <i>n</i> (%)	NTM (<i>n</i> = 15) <i>n</i> (%)	Histo (<i>n</i> = 140) <i>n</i> (%)	Crypto (<i>n</i> = 78) <i>n</i> (%)	Coinfections (<i>n</i> = 31) <i>n</i> (%)
Antifungal therapy	206 (53.5)	-	-	116 (82.9)	64 (82)	25 (80.6)
Amphotericin B	141 (36.6)	-	-	89 (63.6)	39 (50)	13 (41.9)
Itraconazole	74 (19.2)	-	-	67 (47.9)	2 (2.5)	5 (16.1)
Fluconazole	75 (19.5)	-	-	7 (5)	52 (66.7)	12 (38.7)
Anti-tuberculous drugs	127 (33)	112 (92.5)	-	-	-	12 (38.7)
Antibiotics	11 (2.9)	-	2 (13.3)	-	-	2 (6.4)

Abbreviations: Crypto, cryptococcal disease; Histo, histoplasmosis; NTM, nontuberculous mycobacteria; TB, tuberculosis.

After 180 days of enrollment, 213 (10%) patients died. The OIs investigated in this program accounted for 111 of the 213 deaths (52.1%): 18.3% were due to histoplasmosis, 12.7% to TB, 11.3% to cryptococcosis, 7.0% to multiple OIs, and 2.8% to NTM. Unknown etiology accounted for 24.9% of deaths and other causes for 23.0%. One hundred fifteen deaths (53.9%) occurred in the first month after enrollment.

Figure 1A shows the significantly higher mortality in patients with OIs than those without OIs (29.7% vs. 5.9%; $p < 0.0001$). In 2018, mortality from OIs was 7% lower than in 2017 (34% vs. 27%; $p = 0.187$). Regarding each OI, and including cases of multiple infections, patients with tuberculosis had a lower mortality in 2018 compared to 2017 (18.5% vs. 34.6%, $p = 0.039$) with a similar trend for cryptococcal disease (32.8% vs. 46.9%, $p = 0.210$); treatments for these infections increased in 2018 compared to 2017 (TB, 87.3% to 97.6% ($p = 0.022$); and cryptococcal disease 67.6% to 90.2% ($p = 0.008$)). In NTM cases, mortality increased from 17.5% in 2017 to 42.5% in 2018 ($p = 0.126$), with fewer patients receiving treatment (80% vs. 37.5%); 4 deaths (60%) occurred between 90–180 days. In patients with multiple OIs, overall mortality decreased 16.2% (from 58.3% to 42.1%, $p = 0.469$). Histoplasmosis mortality was similar in 2017 and 2018 (32.8% and 32%, $p = 0.965$), but 3% fewer patients were treated in 2018 (84.6% to 81.6%, $p = 0.392$). In patients without OIs, mortality rate was lower in MSM compared with heterosexuals (2% vs. 6.9%, $p < 0.0001$). Figure 1B shows the cumulative probability of death by each OI at 180 days. A mortality rate of 48.4% was observed in patients with multiple OIs followed by NTM (40%), cryptococcal disease (32.9%), histoplasmosis (28.3%), and TB (23.1%) ($p < 0.0001$). Of those with multiple OIs and a CD4 cell count available, 24 (95.8%) had AHD. Concerning histoplasmosis (Figure 1C), the mortality rate was higher in those with disseminated histoplasmosis than non-disseminated cases (32.7% vs. 13.3%, $p < 0.0001$). Mortality was also higher in patients with cryptococcal meningitis than those with a negative lumbar puncture (34.4% vs. 8.3%; $p = 0.141$). However, in patients with a positive CrAg in serum who did not undergo a lumbar puncture, the mortality was 37% (10 out of 27 cases).

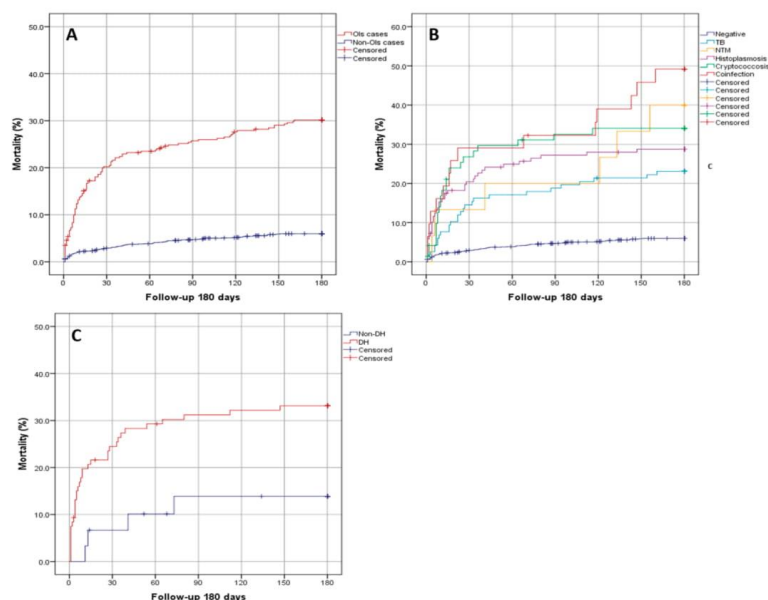


Figure 1. Kaplan–Meier survival curves. (A) Patients with and without opportunistic infections. (B) Patients without opportunistic infections, with tuberculosis, nontuberculous mycobacteria, cryptococcal disease, histoplasmosis, and with multiple opportunistic infections. (C) Patients with disseminated histoplasmosis and non-disseminated histoplasmosis.

4. Discussion

This study analyzes the situation of newly diagnosed HIV patients in Guatemala. The data are able to provide robust evidence since they account for 58.3% (2127 out of 3646) of the new HIV infections reported by the national HIV program during 2017–2018. The proportion of the newly diagnosed HIV patients presenting with AHD was 52%, which was 6% higher than the estimated frequency by UNAIDS of 46% [2]. In other LATAM countries, AHD ranged from 20 to 40% [2]. Several studies in Asia (36.3%), Ethiopia (39%), South Africa (35.6%), and Rwanda (29.4%) also showed lower rates of AHD [10–14]. Therefore, Guatemala has one of the highest rates of newly diagnosed HIV patients presenting with AHD in the world. This rate was especially high in the Mayan population with 65.8%. A previous study in Guatemala found lower levels of HIV knowledge in the Mayan population compared with Ladinos [15]. Thus, limited information and structural disadvantages could explain the late diagnosis. We also found that heterosexual patients were more likely to be associated with AHD at the moment of diagnosis compared with MSM, similar to results found in other studies [16,17]. This is possibly due to awareness campaigns and active HIV screening programs implemented in recent years that have promoted an earlier diagnosis in MSM. Overall, the high frequency of AHD underscores the urgent need for national campaigns and adapted HIV testing strategies.

To diagnose OIs in newly diagnosed HIV patients, the WHO recommends screening for cryptococcal antigen in patients with CD4 cell counts < 100 cells/mm³ and routine clinical evaluation for TB [3,18]; however, we screened all patients irrespective of their CD4 cell count. Our results showed that 27.9% of OIs occurred in patients with CD4 cell counts > 100 cells/mm³ (Table 2). The ROC analysis using CD4 cell counts < 200 cells/mm³ as the screening threshold showed a sensitivity of 85.1% and in patients with < 350 cells/mm³ a sensitivity of 94.4%. The number needed to test is six patients to diagnose one case of OI at CD4 count of 100–200 cells/mm³ and 15 at a

CD4 count of 200–350 cells/mm³. For this reason, we cannot recommend a CD4 cell count <200 cells/mm³ as the screening threshold since almost 15% of the OIs would have been missed. Considering that OIs are usually life-threatening diseases, we find it reasonable to spend \$7.5 on cryptococcal antigen tests and \$13 on histoplasmosis testing. Furthermore, TB should be investigated independently of the CD4 cell count.

Histoplasmosis was the most common OI with an overall incidence of 7.9% rising to 19.7% in patients with CD4 cell counts < 50 cells/mm³. Guatemala is known to be a hyperendemic area for histoplasmosis [4,5,19]. Here, we found almost double the incidence than the estimated in a previous study of the histoplasmosis burden in LATAM (4.1% vs. 7.9%) [4]. In June 2020, the Pan American Health Organization (PAHO) published guidelines for diagnosing and managing disseminated histoplasmosis in people living with HIV [20]. This document recommends urine antigen detection as the reference technique to diagnose disseminated histoplasmosis [20]. In Guatemala, a comparative study of the laboratory assays showed the highest rate of detection for histoplasmosis with the urine antigen test [7]. In addition, it is clinically difficult to determine whether the patient has histoplasmosis or tuberculosis. Therefore, the screening approach provides the means to differentiate both OIs. Concerning cryptococcal disease, current estimations in LATAM are limited. A previous study of the global burden of HIV-associated cryptococcal meningitis estimated the overall prevalence of cryptococcal antigen in patients with CD4 cell counts <100 cells/mm³ to be 6% [21]. Here, we found that 8.2% of AHD patients had a positive serum CrAg test. Hence, it seems that the burden of histoplasmosis and cryptococcal disease have been underestimated in the region. For TB cases, a previous report by the Pan American Health Organization (PAHO) estimated an incidence of 9.9% among HIV patients in Guatemala in 2014 [22]. This was 1.95% higher than the overall TB incidence found in this study (9.9% vs. 7.1%). In our cohort, the incidence of TB was 0.8% lower than histoplasmosis. Considering that TB is more frequent than histoplasmosis in urban areas and one of the largest HCFs, which is located in Guatemala City, did not participated in this study, we can hypothesize that the incidence of TB might be higher.

Mortality among newly diagnosed HIV patients was high with 52.1% of deaths attributed to the OIs screened. A substantial number of patients also died with an unknown diagnosis. This should be improved with the expansion of the diagnostic portfolio. It is important to highlight that the diagnosis has to be made quickly since 53.9% of deaths occurred in the first 30 days. The highest mortality rate was observed in patients with multiple OIs (48.4%). Contributing factors to this increased mortality could include severe immunosuppression (96% had AHD), complicated management, and drug–drug interactions [23]. Histoplasmosis was also associated with a high mortality, similar to previous analyses (28.3%) [24,25]. We found a 7% reduction in the OI mortality in 2018 compared to 2017, with a simultaneous increase in the OI treatment (5.1%). This finding should be attributed to the early diagnostic capacity provided by the OI program. Nevertheless, mortality of cryptococcal meningitis was 34%. It is well known that amphotericin B plus flucytosine decreases cryptococcal meningitis mortality [26,27], yet flucytosine is not available in Guatemala. Therefore, its introduction into the country should be a priority. In addition, the mortality rate in patients with cryptococcal antigenemia without a lumbar puncture (LP) was similar to those with meningitis (37% vs. 34%), suggesting that better management of these patients should be instituted. Reasons for not realizing LP in these patients was not recorded. Since the introduction of the *Histoplasma* urine antigen test, the associated mortality has decreased by 8.3% (From 36.6% in a reference HCF to 28.3% in the network) [28]. However, liposomal amphotericin B is not available in Guatemala, and mortality could be further decreased with its introduction. Concerning NTM, 15 cases were diagnosed with a high mortality rate (40%). Here, NTM identification requires the growth of the microorganism, which can take several weeks. Thus, rapid methods such as GenoType CMdirect (Hain Lifescience) that can be performed from direct specimens would be required.

The present study has its limitations. The OI status was based on the available laboratory techniques in this program, but other OIs have been missed. This will have led to an underestimation of the burden of OIs. Other factors such as ARV treatment were not determined. The integration of all HCFs in the network will improve the accuracy of the data. Despite the limitations, our findings show that Guatemala has one of the highest rates of AHD in newly diagnosed HIV patients, the burden of OIs among newly diagnosed HIV patients is substantial, the burden of fungal infections has been previously underestimated, and access to rapid diagnosis of OIs has decreased the overall mortality by 7% in a single year, showing that it is an essential tool to achieve the goal of eliminating AIDS and reducing deaths.

Author Contributions: Conceptualization, N.M., A.A.-I., and J.L.R.-T.; data curation and resources, D.M., O.B., O.G.; writing—original draft, N.M., A.A.-I., and J.L.R.-T.; writing—review and editing, N.M., A.A.I., L.R.S. and J.L.R.-T., J.C.P., E.A., and D.W.D. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki; it was not subject to IRB review because it analyzed data obtained of a routine program that provided diagnostic services, and no human experiments were performed.

Informed Consent Statement: Informed consent was not requested for the OI screening, because it was implemented as a routine service; a written informed consent for HIV testing was obtained by the HCFs of the network.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

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Conflicts of Interest: A.A.-I. has received in the last three years honoraria as a speaker from Astellas, Gilead Sciences, MSD and Pfizer outside the submitted work. E.A. has received honoraria from GILEAD for educational conferences and participation in Advisory board meeting. D.W.D. holds Founder shares in F2G Ltd., a University of Manchester spin-out antifungal discovery company, in Novocyt, which markets the Myconostica real-time molecular assays and has current grant support from the National Institute of Allergy and Infectious Diseases, National Institute of Health Research, North West Lung Centre Charity, Medical Research Council, Global Action Fund for Fungal Infections and the Fungal Infection Trust. He acts or has recently acted as a consultant to Astellas, Sigma Tau, Basilea, Biosergen, Cidara and Pulmocide. In the past 3 years, he has been paid for talks on behalf of Astellas, Dynamiker, Gilead, Merck and Pfizer. He is also a member of the Infectious Disease Society of America Aspergillosis Guidelines and European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines groups. All other authors declare no conflicts of interest.

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[CAPÍTULO 3]

Capítulo 3. Carga de la histoplasmosis comparación con estimaciones previas

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La histoplasmosis representa una infección oportunista frecuente entre las personas que viven con VIH. En Guatemala, antes del desarrollo del programa de tamizaje para infecciones oportunistas, se desconocía la verdadera carga de la histoplasmosis. Con el fin de determinar la incidencia de las histoplasmosis y el impacto del programa de tamizaje, se analizaron los resultados de los pacientes tamizados para histoplasmosis entre 2017 y 2019. Los resultados se compararon con estimaciones previas disponibles en la literatura.

El programa de tamizaje incluyó a 6.366 pacientes. Dos estudios habían reportado la incidencia de la histoplasmosis en el país. La incidencia global de histoplasmosis en este estudio fue del 7,4%, casi el doble de lo estimado en el estudio de Adenis et al. y Medina et al. con 4,16 y 3,8%, respectivamente. Entre en 2017 y 2019, el programa demostró una tendencia al alza en los casos de histoplasmosis del 6,5% al 8,8%. A los 180 días de seguimiento, la mortalidad general por histoplasmosis entre los pacientes de reciente diagnóstico de VIH disminuyó un 11,6% pasando de un 32,8% en 2017 a 21,2% en 2019.

Se detectó un exceso de 1.523 casos de histoplasmosis entre las personas que viven con VIH en comparación con las estimaciones anteriores. Por tanto, existe una subestimación de la carga de enfermedad que puede estar relacionada con el uso de pruebas con baja sensibilidad, la naturaleza de los estudios y la falta de sospecha clínica. Lo que además podría extrapolarse a otros países de la región. Existe una necesidad clara de reforzar las capacidades diagnósticas de los países de renta media y baja. Los ensayos basados en la detección del antígeno de *H. capsulatum* a través de la prueba ELISA tienen una alta sensibilidad y especificidad, lo que ha cambiado el panorama del diagnóstico de histoplasmosis, permitiendo que se pueda instaurar rápidamente.



Article

Incidence of Histoplasmosis in a Cohort of People with HIV: From Estimations to Reality

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Abstract: Among people with HIV, histoplasmosis represents an important cause of mortality. Previous studies provided estimates of the disease incidence. Here, we compared those estimates with the results obtained from a screening program implemented in Guatemala, which included histoplasmosis detection for people with HIV. To compare the results of this program with previous estimations, a literature search was performed and reports concerning histoplasmosis incidence were analyzed. The screening program enrolled 6366 patients. The overall histoplasmosis incidence in the screening program was 7.4%, which was almost double that estimated in previous studies. From 2017 to 2019, the screening program showed an upward trend in histoplasmosis cases from 6.5% to 8.8%. Histoplasmosis overall mortality among those who were newly HIV diagnosed showed a decrease at 180 days from 32.8% in 2017 to 21.2% in 2019. The screening approach using rapid diagnostic assays detects histoplasmosis cases more quickly, allowing a specific treatment to be administered, which decreases the mortality of the disease. Therefore, the use of these new techniques, especially in endemic areas of histoplasmosis, must be implemented.

Keywords: histoplasmosis; antigen; HIV; opportunistic infections

1. Introduction

In recent years, histoplasmosis has been recognized as an important opportunistic infection among people with HIV in Latin America [1,2]. However, until the development and commercialization of the antigen detection assay, diagnosis was cumbersome. It involved invasive procedures, which were unavailable in many low- and middle-income countries [3,4]. Classical diagnostic procedures, such as blood culture, were the gold standard for diagnosis of histoplasmosis. However, they require complex laboratory infrastructure (Biosafety Level 3), are time-consuming, and have a limited sensitivity to detect the disease, from 36% to 77% [1,5]. In this context, accurate diagnosis was challenging. Recently, the antigen detection assay has shown a high analytical sensitivity and specificity of 98% and 97%, respectively [1,4]. Therefore, results from this test provide a more accurate picture of the real disease burden.

In Guatemala, since 2017, a screening program for people with HIV has provided the detection of histoplasmosis, among other opportunistic infections. This program has allowed the real incidence of this disease to be quantified in people with HIV. Here, we compare histoplasmosis incidence published in the literature and the results of the screening program implemented in Guatemala from 2017 to 2019, showing the implications that the different approaches have in the care of the population with HIV.

2. Materials and Methods

2.1. Study Design and Participants

A literature search was performed in July 2021 using MEDLINE, Scielo, and Google Scholar to identify published studies in which the incidence of histoplasmosis in Guatemala was reported. Then, we compared the absolute differences in histoplasmosis incidence between these analyses and data obtained from the screening program performed from 2017–2019. A detailed description of the program was published previously [6,7]. Briefly, patients were enrolled at healthcare facilities, which are part of a national network that covers 81.2% (13 out of 16) of the sites that provide care to HIV-infected patients in the country. The program included three groups of HIV patients: (i) newly diagnosed; (ii) patients who have not been receiving antiretroviral treatment (ART) for >90 days but who returned to care (Return/Restart); and (iii) patients on ART with symptoms of opportunistic infections (ARV treatment). The clinical samples for the screening were sent from the healthcare facilities to a central diagnostic laboratory hub located in Guatemala City. Patients were screened for tuberculosis (TB), nontuberculous mycobacteria (NTM), histoplasmosis, and cryptococcal disease, independently of their CD4 cell count. A diagnosis of histoplasmosis was considered when the antigen detection, culture, or PCR test was positive. Advanced HIV disease (AHD) was defined as having a CD4 count of <200 cells/mm³. In this analysis, we describe histoplasmosis trends from 2017 to 2019 and the reported numbers of deaths at 180-day follow-up.

Demographic data were collected in the healthcare facilities using a standard electronic form. Patients received ART and treatment for opportunistic infections in accordance with the national guidelines [8]. Histoplasmosis refers to a positive result for Isolator blood culture, urine antigen, and/or a histoplasma PCR, not subacute disseminated or chronic pulmonary histoplasmosis, which were not estimated or documented.

2.2. Statistical Analysis

Baseline characteristics were compared with the chi-square or Fisher's exact test for categorical variable. The histoplasmosis incidence was assessed using the chi-square trend test. The statistical analysis was performed using SPSS (IBM Iberica, Madrid, Spain) and graphics were developed in Microsoft Excel. Incidences were calculated from the patients tested globally and for each group of patients using percentages. Differences between incidences obtained in the literature and this study were compared in absolute numbers. Histoplasmosis-related mortality was analyzed with Kaplan–Meier at 180-day follow-up. A *p* value of <0.05 was considered statistically significant.

3. Results

Two previous publications reported histoplasmosis incidence among people with HIV in Guatemala. In 2018, Adenis et al. estimated an overall histoplasmosis incidence of 1.48% for the Latin American region, with Guatemala showing an incidence of 4.16%. This accounted for 2676 cases [9]. Previously, in 2015, Medina et al. had estimated the incidence in 3.8% [10]. However, case incidence of 7.4% in the screening program is about two-fold or an additional 1523 cases. A total of 6366 patients were included in the screening program. The overall incidence per year found was 6.5% in 2017, 6.8% in 2018, and 8.8% in 2019 showing a statistically significant upward trend in the absolute number of histoplasmosis cases (*p* = 0.005). However, there were differences among the three group of patients. Thus, newly diagnosed HIV patients had a histoplasmosis incidence of 8.5%, patients

who returned to care of 8.3%, and those on ART of 4.8% (Figure 1). We observed a steady annual increase in histoplasmosis incidence within the newly diagnosed HIV patients and patients on ARV, while the other group, i.e., patients who returned to care, showed a decrease in 2018. Table 1 shows the annual incident histoplasmosis cases per type of patients. Considering histoplasmosis cases, the urine antigen was positive in 69% (311 out of 452), the PCR in 60% (249 out of 416), and the isolator blood in 35% (123 out of 353). One hundred and sixty-five cases were diagnosed by combining assays.

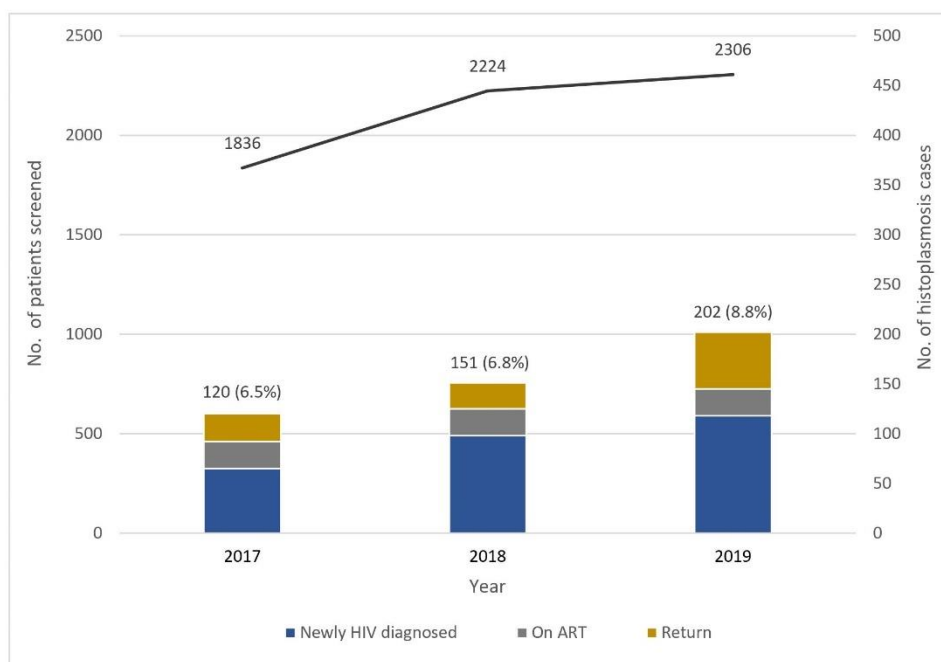


Figure 1. Annual screening patient numbers and histoplasmosis cases detected in the OI program.

Table 1. Incidence of histoplasmosis among different groups of patients, by year.

Type of Patient	Screened Patients	Overall Incidence	Year 2017	Year 2018	Year 2019
Newly diagnosed HIV	3322	8.5%	7.5%	8.3%	9.3%
On ART	1704	4.8%	4.1%	4.6%	5.9%
Return to care	1338	8.3%	9.2%	5.8%	9.8%
Overall	6366	7.4%	6.5%	6.8%	8.8%

ART = antiretroviral therapy.

At enrollment, CD4 cell counts were available for 4625 patients (72.5%); of those, 2235 (48.3%) had AHD. The incidence of histoplasmosis in AHD was 11.9% among the newly diagnosed HIV patients in comparison with 2.7% in those without AHD. In the group of patients on antiretroviral therapy and those who returned to care with AHD, the histoplasmosis incidence was 8.6% and 11.5%, respectively.

Among those who had histoplasmosis, most patients were male (65.8%) between 28 to 42 years old. Sexual orientation and area of residence were found to be associated with histoplasmosis status through the chi-squared analysis ($p < 0.005$). Incidence of histoplasmosis in rural areas was higher than in urban areas (9.4% vs. 5.4%, $p < 0.0001$) and

among heterosexual men vs. men who have sex with men (8.1% vs. 4.9%, $p < 0.0001$). Coinfection was found in 74 (15.6%) out of 473 patients diagnosed with histoplasmosis. The most common dual or triple infection was histoplasmosis plus tuberculosis with 42 cases (56.8%), followed by histoplasmosis plus cryptococcosis with 22 cases (29.7%), histoplasmosis plus NTM with 7 cases (9.5%), and histoplasmosis–cryptococcosis–tuberculosis with 3 cases (4.1%). *Pneumocystis pneumonia* and esophageal candidosis data were not systematically collected during these years. Table 2 summarizes several characteristics of the screened patients.

Table 2. Baseline characteristics of patients screened for histoplasmosis.

Characteristics	Number of Patients Screened	Histoplasmosis (n = 473)		Non-Histoplasmosis (n = 5893)	
		n	%	n	%
Sex					
Male	4049	311	65.8%	3738	63.4%
Female	2252	158	33.4%	2094	35.5%
Transsexual	65	4	0.8%	61	1.0%
Age (years), median (IQR)	6360	35	(28–42)	34	(27–43)
Sexual orientation					
Heterosexual	4796	389	82.2%	4407	74.8%
Homosexual	1083	53	11.2%	1030	17.5%
Bisexual	371	19	4.0%	352	6.0%
Unknown	116	12	2.5%	104	1.8%
Residence					
Urban	3015	163	35.2%	2852	49.8%
Rural	3180	300	64.8%	2880	50.2%
Ethnic group					
Ladino	4909	368	77.8%	4541	77.1%
Mayan	914	67	14.2%	847	14.4%
Other	45	0	0%	45	0.8%
Unknown	498	38	8.0%	460	7.8%

Four hundred and sixty-nine patients with histoplasmosis were followed up at 180 days; of these, 131 deaths (27.9%) were reported. Eighty-seven (66.4%) of these deaths occurred in the first 30 days. Figure 2 shows the overall probability of death in patients with histoplasmosis per type of patient, and Table 3 displays this mortality per year. Among newly diagnosed HIV patients, there was a decline in histoplasmosis deaths along with the overall decline in mortality (32.8% in 2017 to 21.6% in 2019; $p = 0.0626$). However, patients who returned to care and those on ARV showed a mortality increase at 180 days in 2018 and decline in 2019. The number of patients receiving histoplasmosis treatment in those newly diagnosed with HIV increased from 84.6% in 2017 to 92.4% in 2019; whereas in those who returned to care, this number increased slightly from 82.1% in 2017 to 86% in 2019, and in patients on ART, it increased from 74.1% in 2017 to 85.2% in 2019. Amphotericin B deoxycholate and itraconazole were the drugs available in the country during the study period.

Table 3. Histoplasma mortality at 180-day follow-up among HIV patients.

Type of Patient	Overall Mortality	Mortality by Year		
		2017	2018	2019
Newly diagnosed HIV	27.6	32.8	32.0	21.2
On ART	30.0	30.8	40.7	18.5
Return to care	27.3	21.4	30.8	28.6

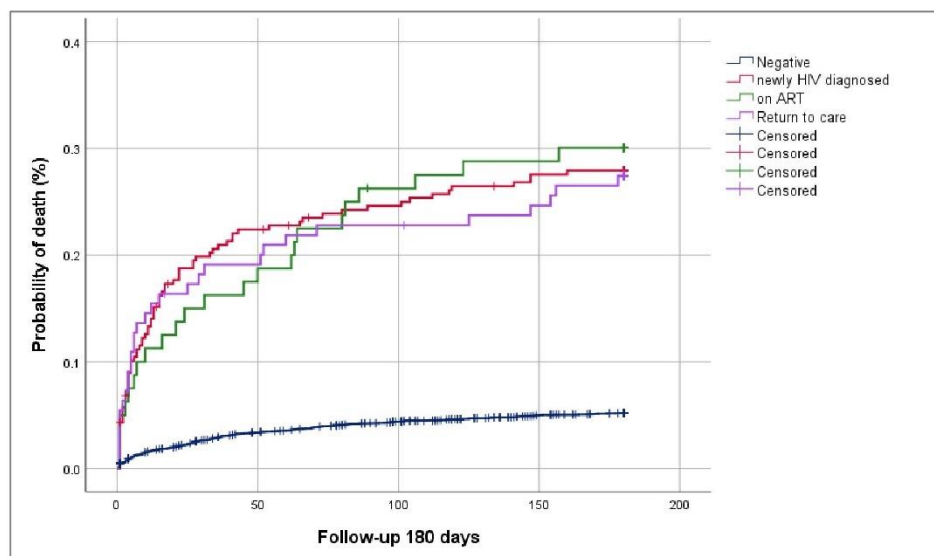


Figure 2. Probability of death in patients with histoplasmosis and HIV per type of patient.

4. Discussion

Fungal diseases are generally neglected for many reasons. Diagnosis is often complicated, although new detection methods have become available. Furthermore, the content of educational programs concerning fungal diseases is a minor topic in medical schools and even in continuous educational postgraduate programs. This means that clinical suspicion of fungal disease is limited even though many of these infections are life threatening. This low level of suspicion implies that clinicians do not obtain an appropriate diagnosis and consequently many laboratories do not have the expertise and techniques required for prompt detection or exclusion of serious fungal disease. The project performed in Guatemala illustrates this situation. Using a screening strategy in high-risk patients with HIV infection, irrespective of the clinical suspicion and immunological status, we found that histoplasmosis was routinely underestimated. Our program provides much more accurate incidence data, allowing for the correct tools to diagnose and treat this disease to be allocated in the country. Guatemala has limited resources, but appropriate rapid screening for histoplasmosis is clearly worthwhile in HIV patients.

The use of a central diagnostic laboratory hub for the screening program, as opposed to local laboratories, should be analyzed carefully. A centralized laboratory, such as that implemented in Guatemala, offers advantages such as a lower cost per sample, more expertise, improved test quality control, and centralized epidemiological data. On the other hand, local laboratories are closer to patients, making results more easily available. However, it is important to consider the number of samples that local laboratories run per week and the availability of a quality control program to ensure the correct performance of the whole diagnostic process. Successful national or regional implementation through a central laboratory must provide a good system for delivering samples from the peripheral centers linked with a structured program to facilitate point-of-care tests (when they are available) at the bedside or in clinic.

Over the study period, we observed a general upward trend in histoplasmosis detection. In 2012, the national epidemiological report only identified 32 cases of histoplasmosis in the country [10]. In the screening program, a diagnosis of histoplasmosis was reached in 158 people per year on average, from more than 1800 tests performed. Access to diag-

nosis is the key change in the care of people with HIV in Guatemala, as it provides more accurate epidemiological data and allows for better clinical care. A key component of the project was the training program. Unexpected diagnostic results from certain tests made clinicians more aware of histoplasmosis, and encouraged them to request more tests and treat patients quickly and appropriately, both for histoplasmosis alone and in cases with dual or triple infections. The increasing histoplasmosis incidence shown in this analysis should spur others to increase efforts to address all cases with rapid diagnostic testing.

As a result of comparing our findings with the previously published data, it can be seen that the introduction of new assays such as the antigen *Histoplasma* detection test should be mandatory. This assay is affordable, provides rapid results, and the clinical sample tested is urine, which is easily collected. The literature data show an underestimation of histoplasmosis cases that may be related to the use of tests with a low sensitivity, the nature of the studies, and the lack of clinical suspicion. Histoplasmosis incidence in the screening program was about two-fold higher than the previous estimate by Adenis et al. (7.4% vs. 4.16%) [9]. Guatemala is known as a hyperendemic country with one of the highest incidences of histoplasmosis in Latin America. Our study shows an excess of 1523 cases of histoplasmosis among people with HIV in Guatemala, as compared to prior estimates. Other countries such as Guyana (2.76%), Venezuela (2.90%), Argentina (1.89%), and the Central America region were also cataloged as having high incidence levels ($\geq 1.5\%$) [9], but the annual incidence is probably underestimated also. Unavailability of laboratory tests and a lack of screening strategies in patients at high risk of opportunistic infections are related to the underestimation of the burden of diseases. Moreover, in people with HIV, histoplasmosis symptoms are nonspecific and are often indistinguishable from other diseases like tuberculosis. Examples of common clinical features that can be confused with other disease entities include nonspecific respiratory complains and pulmonary infiltrates (TB, *Pneumocystis*, or bacterial pneumonia), pancytopenia (HIV, visceral leishmaniasis, lymphoma, drug toxicity), hepatosplenomegaly (leishmaniasis, lymphoma, disseminated TB), diarrhea and weight loss (HIV, disseminated NTM infection, intestinal infection), and others. As our work shows, histoplasmosis can be present with other HIV complications in the same patient. The frequency of this co-occurrence has been reported as being from 9% to 38% [11–13].

We also found that the incidence of histoplasmosis is almost double in rural as compared to urban areas of Guatemala. Many studies are conducted in urban settings only. In 1960, a histoplasmin skin survey was performed showing an overall reactivity in the general population of 57.2% [14], with the rural areas exhibiting the highest frequencies, which is in accordance with our current findings. According to the national HIV continuum report, facilities included in the screening program provide healthcare attention to approximately 60% of population with HIV in the country [15]. Therefore, our epidemiology data represent a robust estimate and indicate that diagnostic services need to reach rural areas for histoplasmosis.

The screening program provided access to diagnosis independently of the CD4 cell count. If a limit of <200 cells/mm³ had been used, 27.5% of the patients would not have been screened. In our study, 92% of the histoplasmosis cases occurred in patients with <350 CD4 cells. Our findings also showed that the overall histoplasmosis mortality was 29.6% in 2017, 33.3% in 2018, and 22.8% in 2019. Excluding the 2018 value, there was an overall reduction in mortality from 2017 to 2019 of 6.8%. The reduction in histoplasmosis mortality was observed in those with newly diagnosed HIV, from 32.8% in 2017 to 21.2% in 2019. Clearly, access to histoplasmosis diagnosis is essential to guide appropriate treatment decisions. Patients who returned to care and those on ART showed an unexpected increase in mortality for histoplasmosis in 2018. Although there is no clear explanation for this, the unavailability of rapid immunological and virological status results of patients already entered in ARV programs could have influenced the speed of diagnosis requests and treatment approaches.

This study has certain limitations. First, differences between healthcare facilities as regards the way in which they actively search for patients to return to treatment and the way in which patients on ARV are managed might have influenced the screened group. Second, we did not collect patient clinical information; therefore, we were not able to determine the number of incident cases that presented with and without symptoms, and with which clinical presentation. Third, the histoplasmosis incidence calculated in this study was compared with estimates that were extrapolated to the HIV-positive population, and therefore it is not completely comparable; however, this is the only study available and we believe that due to the high number of patients screened, the numbers gathered are quite robust and give an idea of the real incidence. Despite these limitations the program gathered a significant amount of data from active screening, providing a new perspective for estimating the burden of disease and allowing access to diagnosis for life-threatening infections in a high-risk population of patients. Therefore, we strongly recommend a screening approach in high-burden locations and countries for several reasons: (i) the high rate of coinfections in this population; (ii) the difficulties in distinguishing tuberculosis and other conditions; and (iii) as an indirect tool for continuing the education of clinicians, which forces them to make a careful evaluation of the patient to confirm unexpected laboratory diagnoses and to take treatment decisions to save patients' lives.

5. Conclusions

There is a clear need to reinforce diagnostic laboratories in low- and middle-income countries. The new diagnostic tools with a high sensitivity and specificity have changed the landscape of fungal infection diagnosis and can be implemented very quickly. Training of clinicians is also required alongside the introduction of new tests. This can take a long time if the disease is unfamiliar. By introducing screening, we can quickly improve the quality of patient care as it requires a clinical response when tests are positive. Strategic introduction of diagnostic laboratory hubs with a screening approach should accelerate clinical care improvements. In addition, regional or national diagnostic laboratory hubs can establish the burden of several diseases and develop rational screening programs according to early results. The proper delivery of clinical samples to the hub and training and quality control programs could provide global access to diagnosis and decrease the mortality of fungal diseases everywhere, as the Guatemala program has shown. Nowadays, diagnostic technique variability is minimal compared with the variability of medical practice. Diagnosis of life-threatening diseases should not rely solely on clinical training but should be reinforced by the provision of valuable diagnostic information. The implementation of diagnostic laboratory hubs with screening programs would improve patient care more quickly and with a lower variability than medical training programs alone, for most fungal diseases. Laboratory diagnostic hubs are also a cost-effective means of providing high-quality access to the diagnosis of fungal diseases.

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Conflicts of Interest: A.A.-I. has received in the last three years honoraria as a speaker from Gilead Sciences and Pfizer outside the submitted work. E.A. has received honoraria from GILEAD for educational conferences and participation in Advisory board meeting. D.W.D. holds Founder shares in F2G Ltd., a University of Manchester spin-out antifungal discovery company, in Novocyt, which markets the Myconostica real-time molecular assays and has current grant support from the National Institute of Allergy and Infectious Diseases, National Institute of Health Research, North West Lung Centre Charity, Medical Research Council, Global Action Fund for Fungal Infections and the Fungal Infection Trust. He acts or has recently acted as a consultant to Astellas, Sigma Tau, Basilea, Biosergen, Cidara and Pulmocide. In the past 3 years, he has been paid for talks on behalf of Astellas, Dynamiker, Gilead, Merck and Pfizer. He is also a member of the Infectious Disease Society of America Aspergillosis Guidelines and European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines groups. All other authors declare no conflicts of interest.

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[CAPÍTULO 4]

Capítulo 4. Impacto de la pandemia por COVID-19 en la atención a VIH y el programa para el tamizaje de infecciones oportunistas en Guatemala

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Narda Medina, Ana Alastruey-Izquierdo, Oscar Bonilla, Brenan Ortíz, Osmar Gamboa, Luis Roberto Salazar, Danicela Mercado, Juan C. Pérez, David W. Denning, Eduardo Arathoon, Juan Luis Rodriguez-Tudela, on behalf of Fungired. Impact of the COVID-19 pandemic on VIH care in Guatemala. *International Journal of Infectious Diseases* 108 (2021) 422–427. <https://doi.org/10.1016/j.ijid.2021.06.011>.

El virus del síndrome respiratorio agudo grave tipo-2 (SARS-CoV-2), agente de la COVID-19, fue declarado pandemia por la OMS, en marzo de 2020. En Guatemala, el primer caso se declaró el 13 de marzo de 2020. Más allá del impacto directo de la pandemia por COVID-19, todos los sectores de salud se han visto interrumpidos. Los servicios rutinarios de atención clínica a pacientes que necesitan seguimiento como los pacientes con VIH también han sido afectados. Este trabajo evaluó el impacto de la pandemia por COVID-19 en diferentes puntos de la atención al paciente con VIH. Para ello se analizó la atención del paciente en la Clínica Familiar «Luis Ángel García» y el registro del programa de tamizaje para infecciones oportunistas. Se compararon dos períodos de tiempo, pre-COVID-19, de marzo a agosto de 2017, 2018 y 2019 y, un periodo COVID-19, con la información recolectada de marzo a agosto de 2020. En este tiempo también se evaluó el impacto en la referencia de muestras del programa de tamizaje y la mortalidad de los pacientes en la Clínica Familiar que habían sido diagnosticados con una infección oportunista.

Respecto a la atención brindada en la Clínica Familiar, se observó: (i) Una reducción del 54,7% en el tamizaje de VIH; (ii) Una reducción del 10,7% en el número de nuevos diagnósticos; (iii) la mortalidad de los pacientes recién diagnosticados de VIH fue un 10,7% más elevada que en el periodo pre COVID-19 y (iv) el envío de muestras desde los laboratorios periféricos al Laboratorio central de Diagnóstico disminuyó, de forma global, en un 43,7%, con un mayor impacto en los centros que se encontraban más alejados de la capital.

La pandemia COVID-19 está teniendo un impacto significativo en la población que vive con VIH en Guatemala. Los resultados evidencian una reducción el número de tamizajes por VIH y de nuevos diagnósticos, que repercutirá en una mayor propagación del virus. En solo ocho meses la pandemia por COVID-19 ha revertido los logros del programa para tamizaje para infecciones

oportunistas, que había reducido un 7% la mortalidad asociada a estas infecciones regresando a los valores reportados al inicio del programa, en 2017.



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Impact of the COVID-19 pandemic on HIV care in Guatemala

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ABSTRACT

Objectives: To describe the impact of the coronavirus disease 2019 (COVID-19) pandemic on the diagnosis of human immunodeficiency virus (HIV) and deaths from opportunistic infections in Guatemala.

Methods: A retrospective study was conducted to investigate the impact of the COVID-19 pandemic on people with HIV at a referral clinic (Clinica Familiar Luis Angel García, CFLAG), as well as the disruption of services at a diagnostic laboratory hub (DLH) which provides diagnosis for opportunistic infections to a network of 13 HIV healthcare facilities. Comparative analysis was undertaken using the months March–August from two different time periods: (i) pre-COVID-19 (2017–2019); and (ii) during the COVID-19 period (2020).

Results: During the COVID-19 period, 7360 HIV tests were performed at Clinica Familiar Luis Angel García, compared with an average of 16,218 tests in the pre-COVID-19 period; a reduction of 54.7% [95% confidence interval (CI) 53.8–55.4%]. Deaths from opportunistic infections at 90 days were 10.7% higher in 2020 compared with 2019 (27.3% vs 16.6%; $P = 0.05$). Clinical samples sent to the DLH for diagnosis of opportunistic infections decreased by 43.7% in 2020 (95% CI 41.0–46.2%).

Conclusion: The COVID-19 pandemic is having a substantial impact on HIV care in Guatemala. Diagnostic services for HIV have been severely affected and deaths from opportunistic infections have increased. The lessons learnt must guide the introduction of strategies to reduce the impact of the pandemic.

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Introduction

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) emerged in China in late 2019 (Li et al., 2020; Schlottau et al., 2020). Following the first reported case of coronavirus disease 2019 (COVID-19), it spread rapidly and the World Health Organization (WHO) declared a pandemic in March 2020 (World Health Organization, 2020a). At the time of writing, there have been a total of 134,957,021 confirmed cases worldwide (World Health Organization, n.d). In Guatemala, the first case was reported on 13 March 2020, and by the end of November 2020, 74,989 cases and 3094 deaths had been reported (Gobierno de Guatemala, n.d.).

Beyond the direct impact of the COVID-19 pandemic, all sectors of the healthcare system have been affected. In this context, access to routine services has been reduced due to reassignment of clinical staff to activities related to COVID-19, disruption of medical supplies, and restrictions in transportation and personal mobility. According to a WHO survey in 155 countries, 53% of healthcare services have been partially or completely disrupted during the COVID-19 pandemic (World Health Organization, 2020b). Among people living with human immunodeficiency virus (HIV), interruptions in both healthcare attention and treatment could result in an increased mortality rate and further spread of HIV in the community. This increase in mortality would most likely be attributed to delays in the diagnosis of opportunistic infections secondary to a lack of adequate access to care. Therefore, this study evaluated the impact of the COVID-19 pandemic on the diagnosis of HIV and deaths from opportunistic infections in Guatemala.

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Methods

Study design and data sources

To understand the COVID-19 situation in Guatemala, data on the total number of monthly COVID-19 cases reported by the Ministry of Health (MOH) from March to August 2020 were collected. The MOH and the Pan American Health Organization publish an online open-access dashboard with essential information on the COVID-19 epidemic in Guatemala (Gobierno de Guatemala, n.d.). According to the MOH, a positive result on a polymerase chain reaction assay or an antigen test was considered as a laboratory-confirmed case of COVID-19. Data were organized by date. Government policies implemented to contain the COVID-19 pandemic were also collected from the Central America Diary (<https://legal.dca.gob.gt>).

A programme to improve the diagnosis of the most common opportunistic infections in Guatemala was implemented in 2017. Diagnostic services are provided to a healthcare facility network, composed of 13 HIV facilities located across the country, by a diagnostic laboratory hub (DLH) in Guatemala City that receives clinical samples daily via a courier service. The Clínica Familiar Luis Angel García (CFLAG) is the largest healthcare facility of the network, located within the Hospital General San Juan de Dios in Guatemala City, which is one of two national reference hospitals. Since 1987, CFLAG has provided inpatient and outpatient services as well as HIV testing and counselling programmes. This study included all outpatients and inpatients diagnosed with HIV, and cases of opportunistic infection detected at CFLAG. Two periods were defined for comparison. Each period encompassed 8 months: the pre-COVID-19 period from March to August of 2017–2019, and the COVID-19 period from March to August 2020. The same months were chosen for comparative purposes, and to see the impact of the COVID-19 pandemic in the first 8 months after its introduction in Guatemala. Deaths from opportunistic infections at 90 days were analysed for patients diagnosed in 2017, 2018 and 2019 compared with those diagnosed in 2020. Deaths that occurred within 90 days of patient screening were attributed to the opportunistic infection diagnosed at the DLH.

Opportunistic infection screening was performed for patients newly diagnosed with HIV, out-of-care who return, and patients on antiretroviral therapy (ART) with symptoms compatible with an

opportunistic infection. Opportunistic infection screening included testing for histoplasmosis, cryptococcosis, tuberculosis and non-tuberculous mycobacteria infections, as described by Medina et al. (2020, 2021). In addition, screening for *Pneumocystis jirovecii* pneumonia has been implemented since 2018. To analyse disruption of the opportunistic infection diagnosis programme caused by the COVID-19 pandemic, the number of samples received at the DLH during the first 8 months of the COVID-19 period were compared with the pre-COVID-19 period. DLH services functioned in accordance with government guidelines throughout the COVID-19 pandemic.

Ethics statement

This study was based on data from the national COVID-19 dashboard and the opportunistic infection system from the network. Patient informed consent was not required as overall data were collected and analysed (rather than individual data).

Statistical analysis

Statistical analysis was performed using SPSS Version 25.0 (IBM Corp., Armonk, NY, USA). Proportions, medians and a trend χ^2 test were used for descriptive analysis. Continuous variables were compared using the Mann–Whitney *U*-test. $P < 0.05$ was considered to indicate significance.

Results

Between March and August 2020, 75,004 new cases of COVID-19 were registered in Guatemala, along with 3207 deaths. In total, 244,720 COVID-19 tests were performed during this period, with a positivity rate of 31%. Figure 1 shows the number of COVID-19 cases per month and the main measures implemented by the Government of Guatemala. On 29th March 2020, several restrictions were implemented, including the closure of public transport, establishment of curfew hours, and limitations on mobility between regions. These measures remained in place until July 2020 and were then modified progressively. This led to limitations of the healthcare attention given to people with HIV during this period.

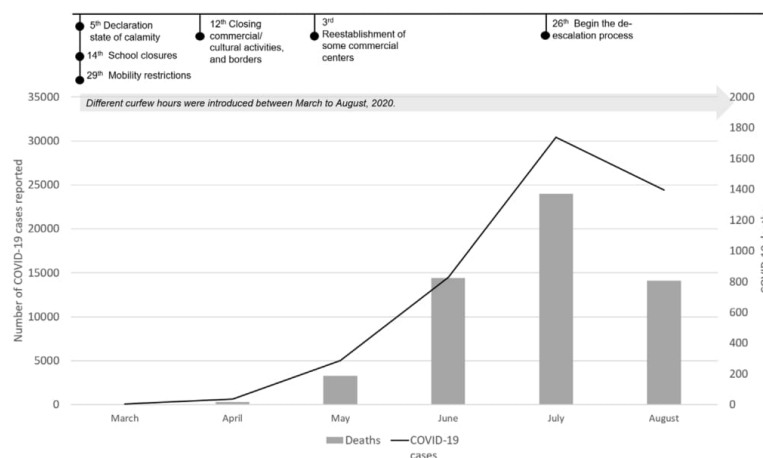


Figure 1. Number of cases of coronavirus disease 2019 (COVID-19) and timeline of the principal governmental measures in Guatemala. Total number of cases was based on the date of the result test according to the Ministry of Health.

Regarding HIV testing during the COVID-19 period, CFLAG experienced a decrease of 54.7% [95% confidence interval (CI) 53.8–55.4%; $P < 0.0001$]: 7360 HIV tests were performed in the COVID-19 period compared with 16,218 tests performed, on average, during the pre-COVID-19 period. Figure 2 shows the number of HIV tests performed and the number of new HIV cases diagnosed at CFLAG. As expected, the rate of HIV testing decreased progressively, with the lowest number of HIV tests performed in April 2020. Despite this trend, the number of patients newly diagnosed with HIV during the COVID-19 period only decreased by 10.7% (216 cases in the COVID-19 period vs 242 cases in the pre-COVID-19 period); a higher proportion of men who have sex with men (MSM) were referred to CFLAG in the COVID-19 period compared with previous years. The proportion of MSM in 2020 was 56.2% ($n = 221$), compared with 40% ($n = 203$) in 2019 and 39.6% ($n = 180$) in 2018 ($P < 0.0001$).

Concerning the HIV programme for pregnant women, which includes labour and delivery services as well as the outpatient obstetric clinic, the results were as follows. In the COVID-19 period, HIV testing decreased by 32.5% (95% CI 31.5–33.6%) with 5805 HIV tests in the COVID-19 period vs 8609 tests performed, on average, in the pre-COVID-19 period. Among pregnant women, four cases were newly diagnosed with HIV in the COVID-19 period vs eight cases in the pre-COVID-19 period, a 50% decrease.

At CFLAG, screening for opportunistic infections decreased by 31.2% (95% CI 28.7–33.9%): 836 samples were tested in the COVID-19 period vs 1216 in the pre-COVID-19 period. The number of opportunistic infections diagnosed decreased by 67.9% (95% CI 57.1–77.1%): 26 cases in the COVID-19 period vs 81 cases in the pre-COVID-19 period. Overall deaths from opportunistic infections at 90 days was 27.3% in 2020 (18/66 cases), which was 10.7% higher than the overall mortality observed in 2019 (16.6%; $P = 0.053$), 7.9% higher than in 2018 (19.4%; $P = 0.191$) and 2.3% higher than in 2017 (25%; $P = 0.650$). Figure 3 shows the number of people living with HIV screened for opportunistic infections, the total number of cases detected, and deaths from opportunistic infections identified at CFLAG by year.

By January 2020, a total of 3677 people living with HIV comprised the cohort of patients from CFLAG; by August 2020, 44 (1.2%) cases of COVID-19 had been diagnosed, 36 (81.8%) of whom were on ART. The mean age of those diagnosed with COVID-19 was 44 years, and four patients had co-infections (three cryptococcal disease and one tuberculosis). Twenty-six of these patients had a CD4 cell count available, and the median cell count was 301 cells/

mm³ (interquartile range 61–475). Mortality at 90 days for patients with COVID-19 was 27.2% (12/44 cases).

This study also determined the impact of the COVID-19 pandemic on the opportunistic infection screening programme performed at the DLH for the whole network of 13 HIV units. Table 1 shows the number of samples received by the DLH every month. In the COVID-19 period, the number of samples received by the DLH decreased to 38% (95% CI 36.1–39.8%), and this increased to 43.7% (95% CI, 41.0–46.2%) when the 12 healthcare facilities located outside of Guatemala City were analysed. Compared with the pre-COVID-19 period, the decreases observed in April and June 2020 were remarkable: 46% and 75%, respectively.

Discussion

The COVID-19 pandemic has affected healthcare services for people living with HIV. At CFLAG, a referral healthcare facility in Guatemala City, HIV testing decreased by 54.7% (95% CI 53.8–55.4%) during the COVID-19 period. HIV testing represents an entry point to identify and bring HIV-infected patients into care. In March 2020, when the first case of COVID-19 was reported in Guatemala, the number of HIV tests decreased, and this trend was maintained during the following months (Table 1). This finding is in accordance with the results from other studies (Bechini et al., 2020; Lagat et al., 2020; Mhango et al., 2020). Lagat et al. (2020) reported a 50% decline in female index enrolment in an assisted partner notification programme for HIV in Kenya. As expected, the number of HIV tests performed in pregnant women at CFLAG also decreased. As essential services were maintained in the labour and delivery ward, the difference was lower compared with the overall decrease in HIV testing (32.5% vs 54.7%).

A 10.7% decrease in the number of newly diagnosed HIV patients was noted in the COVID-19 period; this value was lower than expected considering that there was a 54.7% decrease in HIV testing. The results showed that the proportion of MSM patients who were brought into care at CFLAG was 16.2% higher in 2020 compared with 2019 and 2018 ($P < 0.0001$). This could be attributed to higher HIV awareness in this group, along with an increased number of programmes dedicated to testing this specific population. Nevertheless, an overall decrease in the number of newly diagnosed HIV patients was seen; this is of concern as many recently acquired cases of HIV are not diagnosed, especially within the heterosexual population who are more likely to progress to advanced HIV disease (Meléndez et al., 2019; Medina et al., 2021).

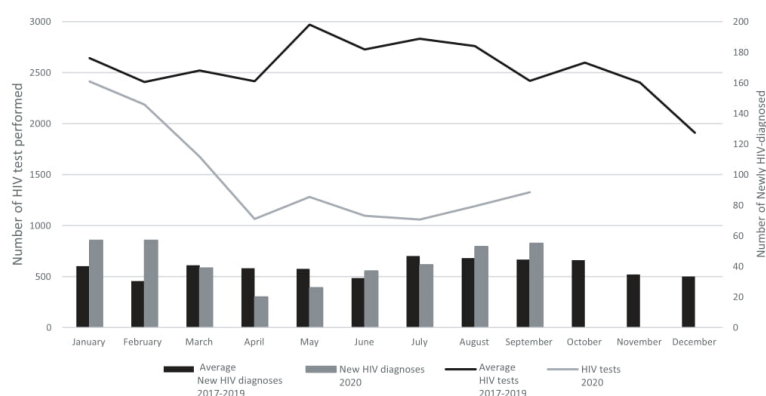


Figure 2. Number of human immunodeficiency virus (HIV) tests performed at Clínica Familiar Luis Angel García each month in 2017–2019 compared with 2020, and new HIV diagnoses.

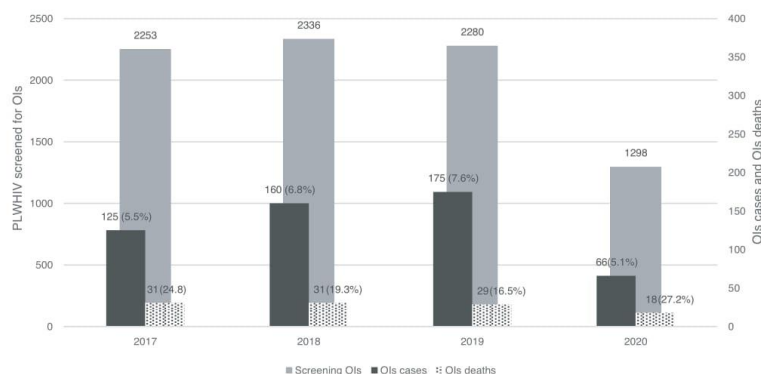


Figure 3. Number of people living with human immunodeficiency virus (PLWHIV) screened for opportunistic infections (OIs), cases of OI detected and deaths due to OIs at Clínica Familiar Luis Angel García.

Table 1
Number of samples received for the diagnosis of opportunistic infections at the diagnostic laboratory hub and overall difference in 2020.

Month	Number of samples received for OI diagnosis		Overall difference (95% CI)
	Average 2017–2019	2020	
January	376	550	+46.3% (41.3–51.3)
February	408	524	+28.5% (24.3–32.9)
March	391	327	–16.4% (13.3–20.4)
April	366	90	–75.4% (70.7–79.5)
May	481	260	–46.0% (41.5–50.4)
June	417	221	–47.0% (42.3–51.8)
July	509	383	–24.8% (21.2–28.7)
August	480	360	–25.1% (21.3–29.1)
September	448	409	–8.8% (6.4–11.7)
October	451	–	–
November	387	–	–
December	278	–	–

CI, confidence interval.

On the other hand, patients are normally able to select their preferred clinic, but in the context of the COVID-19 pandemic where HIV services have been disrupted in many places, more MSM patients may have been referred to CFLAG.

Regarding the reduced detection of newly diagnosed HIV patients during the COVID-19 period, in 2018, the national report of the HIV care continuum showed that only 63% of people living with HIV were aware of their diagnosis (*Programa Nacional de Prevención y Control de ITS VIH y SIDA, 2019*), which is much lower than the UNAIDS goal of 90%. As such, it is critical that the national HIV programme develops strategies to identify patients with HIV even in the context of the COVID-19 pandemic. An example of a possible alternative is HIV self-testing, which is now recommended by WHO as an additional method to increase HIV testing rates (*World Health Organization, 2019*).

The impact of the COVID-19 pandemic on patients with opportunistic infections was striking. The diagnostic programme for opportunistic infections at CFLAG showed a steady decrease in deaths from opportunistic infections, from 25% in 2017 to 16.6% in 2019. This represented an overall decrease of 8.4% since the programme's implementation in 2017. However, as soon as the COVID-19 pandemic hit in Guatemala, deaths from opportunistic infections increased by 10% compared with 2019, slightly surpassing the rate in 2017 (25%). A modelling study by *Hogan et al. (2020)* estimated that the potential impact of the COVID-19 pandemic on people living with HIV in low-to-middle-income countries will be reflected as an increase in deaths due to HIV of up

to 10% in the next 5 years. The contributing factors for the increased deaths include, but are not limited to: a delay in HIV diagnosis until presenting with advanced disease; inadequate diagnosis of life-threatening opportunistic infections (evidenced here by a 38% decrease in referred samples for opportunistic infection screening at the DLH); an indirect impact of COVID-19 on patients with HIV affected by opportunistic infections (27.2% mortality); and the organizational arrangements that divert healthcare workers away from caring for people living with HIV.

As expected, the number of opportunistic infection screening samples received at the DLH from the healthcare facilities located outside of Guatemala City decreased more than the samples obtained from CFLAG, which is located in Guatemala City (43.7% vs 31.2%). During the COVID-19 period, the overall delivery of clinical samples to the DLH decreased by 38% (95% CI 36.1–39.8%). Therefore, limitations to the number of opportunistic infection samples sent for diagnosis may result in underestimation of cases and deaths.

Other immediate effects of COVID-19 on survival that were not analysed include the consequences of ART interruptions and loss of virological suppression associated with increased ART resistance. In South Africa, a social media survey for people living with HIV found that 13% of the people surveyed did not have access to their ART during lockdowns (*HSRC Study on COVID-19, n.d.*). Moreover, a mathematical model estimated that these potential effects could lead to 1.63 times more deaths considering the effects of a 6-month interruption of ART supply (*Jewell et al., 2020*). Although the

Government of Guatemala started to de-escalate restrictions on mobility by the end of July 2020, public transportation remained limited and more costly, making it difficult for many patients to travel to their respective healthcare facilities to receive their appropriate medications, including ART. Due to this situation, alternatives to minimize the impact of the COVID-19 pandemic have been developed. Several studies have proposed the implementation of telemedicine platforms (Mgbako et al., 2020), while UNAIDS and WHO have called on governments to provide multi-month dispensing of ART for people living with HIV who are currently stable (UNAIDS, n.d., World Health Organization, 2020); this concept has already been implemented at CFLAG where ART is dispensed for 3-month periods. In South Africa, one project packaged and delivered medications to community-based locations (Mendelsohn and Ritchwood, 2020). In Guatemala, a similar strategy was implemented to deliver ART directly to the patients. This programme has been functioning for the network since June 2020.

Concerning the direct impact of COVID-19 on people living with HIV, a lower risk of infection was initially hypothesized due to the possible suppression of coronavirus replication by ART; however, a randomized clinical trial with patients who had severe COVID-19 showed no benefit from the ritonavir-boosted lopinavir over standard care (Cao et al., 2020). Other analysis also showed no differences in previous use of nucleoside reverse transcriptase inhibitors or non-nucleoside reverse transcriptase inhibitors (Vizcarra et al., 2020). Despite these findings, there is no additional evidence to support a higher risk of COVID-19 infection among people living with HIV (Vizcarra et al., 2020; World Health Organization, 2020d). The present study found 44 cases of COVID-19 in one clinic in Guatemala City. Considering the entire cohort of people living with HIV in this facility, the frequency of COVID-19 infection was 1.2% (95% CI 0.9–1.6%). This rate was slightly lower compared with that reported by Vizcarra et al. (2020) in Spain (1.8%, 95% CI 1.3–2.3%). The number of deaths in the present study was much higher compared with deaths reported in the Spanish cohort (4%). Other missed diagnoses, such as opportunistic infections, could contribute to this result. Overall deaths due to COVID-19 were similar to overall deaths from opportunistic infections (27.2% vs 27.3%). Considering the decrease in deaths due to opportunistic infections achieved by the programme in 2019 (16.6%), the indirect impact of the COVID-19 pandemic on the care of people living with HIV and management of opportunistic infections is remarkable.

This study has several limitations. Available data represent the situation from one clinic in Guatemala City, while effects on other HIV clinics in more remote parts of the country could be more profound. Unfortunately, it was not possible to track the effects on these clinics. Furthermore, other aspects that might increase the magnitude of the effect of the COVID-19 pandemic, such as the interruption of ART, were not determined. Although all patients diagnosed with COVID-19 at CFLAG were included in this study, it is possible that this was underestimated because of the low availability of HIV testing in patients with COVID-19 in other hospital units. The presence of comorbidities and outcomes were also not estimated. Despite its limitations, this is the first study to describe the impact of the COVID-19 pandemic on people living with HIV in the region. This study found that the COVID-19 pandemic is having a substantial impact on HIV care in Guatemala, HIV testing programmes were severely affected, and deaths due to opportunistic infections have increased. Maintaining the most essential healthcare services and implementing new strategies could substantially reduce the overall impact of the pandemic.

Author contributions

Conceptualization: JLRT, AAI, NM.
Formal analysis: NM and JLRT.

Resources: OB, BO, OG and DM.

Writing – original draft: NM.

Writing – review and editing: JLRT, AAI, OB, BO, DM, JCPS, LRS, EA, and DWD.

Conflict of interest statement

Over the last 3 years, Dra Ana Alastruey Izquierdo has received honoraria as a speaker from Astellas, Gilead Sciences, MSD and Pfizer outside the submitted work. Dr Eduardo Arathoon has received honoraria from GILEAD for educational conferences and participation in advisory board meetings. Dr. David W. Denning and family hold founder shares in F2G Ltd., a University of Manchester spin-out antifungal discovery company. Current grant support includes the National Institute for Health Research Biomedical Research Centre in Manchester, the Medical Research Council, Carigest Foundation and the Fungal Infection Trust. Dr Denning also acts or has recently acted as a consultant to Pulmatrix, Pulmocide, Zambon, iCo Therapeutics, Mayne Pharma, Biosergen, Bright Angel Therapeutics and Metis. Over the last 3 years, Dr Denning has been paid for talks on behalf of Dynamiker, Hikma, Gilead, Merck, Mylan and Pfizer. Dr Denning is a long-standing member of the Infectious Disease Society of America Aspergillosis Guidelines group, the European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines group. All other authors declare no conflicts of interest.

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4. [DISCUSIÓN INTEGRADORA]

4. Discusión integradora

Los esfuerzos dirigidos a disminuir la mortalidad asociada al VIH no han conseguido los objetivos propuestos. Desde el año 2013 a 2018, sólo ha disminuido un 15% (Denning, 2016; UNAIDS, 2019). La mayoría de las muertes asociadas al VIH, especialmente en países de renta media y baja, es consecuencia de las infecciones oportunistas, entre ellas la infección fúngica (Debroy et al., 2015; Solomon et al., 2018; Tanuma et al., 2016) que ocasiona, aproximadamente, el 50% de estas (Denning, 2016). Una realidad incuestionable es que el diagnóstico de estas infecciones es inaccesible en muchos países. En general, el médico juega un papel central en la asistencia sanitaria. Sin embargo, la formación pre y posgrado que reciben sobre la enfermedad fúngica es prácticamente inexistente. Esta carencia formativa tiene importantes implicaciones. Si en el ejercicio médico habitual del diagnóstico diferencial, el clínico no incluye a la enfermedad fúngica y no solicita las pruebas complementarias para confirmar su sospecha. Termina reduciendo la demanda de las pruebas, en consecuencia, sin demanda, las pruebas no están disponibles y sin diagnóstico, la enfermedad fúngica no existe por lo que el paciente recibe un tratamiento equivocado y la evolución natural, si es grave, es la muerte. Además, esto ocasiona que se desconozca la carga de enfermedad por lo que, como ya se ha comentado, están subestimadas y el sistema sanitario no dedica recursos para su diagnóstico y tratamiento. En los países de renta media y baja el número de médicos por habitante es mucho menor que en los países de renta y alta, y los especialistas están concentrados en núcleos urbanos y hospitales universitarios por lo que la mayoría de la población no tiene acceso a estos recursos (<https://data.worldbank.org/>). En estos entornos, las estrategias para el acceso al diagnóstico de las enfermedades graves, independientemente del criterio clínico, deben ser analizadas con esmero. En el caso del paciente que vive con el HIV, especialmente en el grupo con enfermedad avanzada, deben considerarse de máxima importancia. La sospecha clínica de las infecciones oportunistas fúngicas es compleja debido a la inespecificidad de los síntomas clínicos, especialmente de la histoplasmosis, que impide distinguirla de otras enfermedades como, por ejemplo, la tuberculosis. Además, muchos pacientes tienen varias infecciones concomitantes lo que complica aún más la situación (Debroy et al., 2015; Solomon et al., 2018; Tanuma et al., 2016).

Además del indudable valor médico, se desconoce si el acceso al diagnóstico de estas infecciones tiene una influencia «*per se*» en la mortalidad de colectivos específicos de pacientes que padecen una incidencia elevada de estos procesos (Denning, 2016). Con este contexto, se

planteó la siguiente hipótesis: «¿El acceso al diagnóstico de las infecciones oportunistas fúngicas podría reducir las muertes asociadas al VIH?»

En Guatemala, la asistencia sanitaria del paciente que vive con el VIH está organizada en unidades de atención integral que cubren la mayor parte del territorio. Estas unidades están adscritas a hospitales nacionales o regionales de la red pública. A pesar de esta organización, el desarrollo de las unidades no ha sido uniforme. Para finales del 2015, algunos centros tenían acceso únicamente al diagnóstico de la tuberculosis, por lo que la detección de otras infecciones era prácticamente inexistente. Asimismo, el recuento de células CD4 y carga viral eran referidos y la tardanza en la emisión de los resultados impedía la estratificación de los pacientes de acuerdo con su riesgo. En consecuencia, la asistencia de los pacientes estaba basada en el empirismo. Una de las unidades de atención integral, la Clínica Familiar Luis Ángel García, es considerada «Centro de Referencia». Esta clínica se encuentra asociada a un hospital nacional y recibe apoyo financiero y logístico de la Asociación de Salud Integral (ASI), que es una organización sin fines de lucro fundada junto con la clínica en 1987. Estas organizaciones, en colaboración con el CDC de Atlanta, EE.UU, desarrollaron en 2009 una prueba casera para la detección de antígeno del *Histoplasma* (Scheel et al., 2009). Esto permitió el diagnóstico rápido de la histoplasmosis y la definición preliminar de la importancia de esta enfermedad en el colectivo de personas que viven con VIH. En 2015, se estimó que había unos 5.000 casos anuales de infecciones fúngicas invasoras en pacientes con VIH en el país (Medina et al., 2017). Otros estudios, apoyaron estos hallazgos (Reinhardt et al., 2017; Samayoa et al., 2010, 2017). Con toda esta problemática, a finales de ese mismo año se propuso abordar el diagnóstico y tratamiento de las infecciones oportunistas de forma global. En una reunión presencial con las unidades de atención Integral celebrada del 24 al 28 de noviembre de 2015 en la ciudad de Guatemala se presentaron los objetivos del proyecto y se acordaron las siguientes actuaciones: (i) Desarrollo de un programa de formación continuada, (ii) implementación de un programa de tamizaje para infecciones oportunistas, y (iii) monitoreo para el seguimiento de la información. Trece de las dieciséis unidades de atención integral decidieron participar en el programa, conformando así un consorcio que se llamó FungiRed™. Se designó como Laboratorio central de Diagnóstico «DLH» a la Clínica Familiar «Luis Ángel García/ASI» en Ciudad de Guatemala. Las muestras clínicas eran referidas desde las diferentes unidades de atención integral hacia este centro. Entre 2017 y 2019, el programa de tamizaje reclutó 6.621 pacientes detectándose 1.194 (18%) infecciones oportunistas, de ellas 579 (48,5%) eran fúngicas, 448 (37,5%) tuberculosis, 98 (8,2%) coinfecciones y 69 (5,7%) micobacterias no tuberculosas.

Este nuevo sistema implantado en Guatemala debe ser analizado cuidadosamente. Un programa de diagnóstico centralizado ofrece varias ventajas como: i) un mejor coste-efectividad de las pruebas, ii) una fácil formación continuada y una mayor experiencia del personal, iii) una factible implantación de un sistema de control de calidad y, iv) datos epidemiológicos globales y fácilmente explotables. Sin embargo, el acceso al diagnóstico en los laboratorios locales, más cercanos al paciente, puede reducir, considerablemente, la emisión de los resultados lo que, probablemente, permitiría una instauración más rápida del tratamiento. No obstante, el número de muestras que los laboratorios locales procesan es una variable determinante para que el hecho de la cercanía del paciente se vea correspondido con una asistencia de calidad adecuada. No existen dudas de que es necesario disponer de personal entrenado, así como de una carga de trabajo suficiente que permita establecer un adecuado tiempo de entrega y controles de calidad que minimicen los resultados falsos positivos y negativos más allá de la inherente sensibilidad y especificidad de la prueba. Por tanto, en este contexto, el principal condicionante para que el laboratorio central sea útil en el diagnóstico y el tratamiento es un buen sistema de envío de muestras desde los centros periféricos hacia el laboratorio, así como un programa estructurado para transferir las pruebas al punto de atención cuando éstas puedan ser utilizadas en ese entorno. Para el programa de tamizaje implantado fue esencial el servicio de mensajería que se encargó de la recogida de las muestras en las unidades de atención integral y su entrega al Laboratorio central de Diagnóstico. Nueve (70%) de las unidades de atención integral de la red se encuentran a cinco horas de distancia por carretera del laboratorio. Esto ha permitido un funcionamiento adecuado, pero no óptimo, especialmente para una de las unidades que se encuentra a ocho horas de distancia. Otros programas también han utilizado estrategias similares (Joloba et al., 2016). Entre ellas se han utilizado servicios de mensajería que incluyen transporte a través de motocicletas y más recientemente se ha descrito el uso de drones (Lippi & Mattiuzzi, 2016), que en un futuro podría explorarse especialmente en áreas de difícil acceso. La organización para el acceso al diagnóstico mediante este nuevo sistema de salud debe de prestar especial atención a este aspecto, si se quieren obtener resultados similares a los ofrecidos por un laboratorio cercano. El Laboratorio central de Diagnóstico debería estar en una zona de fácil acceso y bien comunicada, lo que permitiría disponer de los resultados en el mismo día de la llegada de las muestras. Otras consideraciones, como el funcionamiento 24 horas, 7 días de la semana, deben valorarse detenidamente en función del número de muestras recibidas y sobre todo si muchas de ellas provienen de enfermedades graves en las que la instauración rápida del tratamiento es esencial para la supervivencia del paciente.

A la enfermedad fúngica se le presta poca atención en la docencia de pre y posgrado por lo que un programa específico de formación fue un componente necesario. Este englobó los siguientes aspectos: (i) la capacitación al personal de laboratorio y médico de las unidades de atención integral a través de un curso virtual sobre el diagnóstico y manejo de las principales infecciones oportunistas, con énfasis en las infecciones fúngicas, (ii) realización de cuatro talleres regionales para dar a conocer el programa de tamizaje, así como los criterios de selección, envío y transporte de muestras y (iii) celebración de un taller nacional anual con el objetivo de determinar la situación de la red. Además, esta estrategia ha demostrado que el programa de tamizaje funciona como una herramienta de formación indirecta cuando el clínico recibe los resultados del tamizaje, y analiza que la enfermedad fue causada por un patógeno diferente al que esperaba.

Para conseguir una rápida y correcta comunicación entre las unidades y el Laboratorio central de Diagnóstico se desarrolló un sistema electrónico de información. Este sistema cumple los siguientes requisitos: (i) que la solicitud de órdenes para laboratorio pueda realizarse en tiempo real, (ii) que permita añadir nuevas solicitudes a pacientes ya incluidos en el sistema, (iii) que permita el seguimiento de los resultados tanto a nivel de laboratorio como a nivel clínico, y (iv) que genere un informe que facilite la monitorización de los casos. Este programa informático se denominó «SisLab» y ha permitido estructurar la carga de trabajo en el laboratorio y el desarrollo de una base de datos que ha facilitado el análisis epidemiológico de la cohorte. En el país, existe un sistema para el manejo de la información sobre VIH, pero no se utiliza en la mayoría de las unidades. Es más, antes de la instauración del programa de tamizaje muchas de las unidades no tenían información digital sobre los pacientes o su seguimiento. Una de las principales brechas señaladas por cooperantes externos ha sido la falta de información y de un sistema integrado para analizar la situación de los pacientes que viven con el VIH (Ministerio de Salud y Asistencia Social Guatemala, 2018). Con esta herramienta informática se han analizado las características demográficas, de laboratorio, incluyendo el recuento de células CD4 y la carga viral, así como el seguimiento y tratamiento de más de 6.000 pacientes.

La cohorte de pacientes de reciente diagnóstico fue el punto de inicio para conocer la situación de la atención del paciente con VIH en Guatemala. Entre 2017 y 2018, se tamizaron a 2.127 pacientes recientemente diagnosticados de VIH, lo que representó el 58,3% de los casos anualmente notificados por el programa nacional, por lo que los resultados son estadísticamente robustos y describen la situación en Guatemala. Se ha constatado que el 52% de los pacientes llegan al diagnóstico de VIH con enfermedad avanzada. Esto sitúa al país en los

primeros puestos a nivel mundial de diagnóstico tardío (Burden et al., 2018; Kiertiburanakul et al., 2014; Melaku et al., 2010; UNAIDS, 2019). Además, en la población Maya la frecuencia de enfermedad avanzada es del 65%, significativamente más elevada. En este grupo de pacientes, las desventajas socioeconómicas y de salud son contribuyentes importantes. Es necesario el desarrollo de acciones diferenciadas para su identificación.

Se recomienda que la búsqueda de las infecciones oportunistas se realice en pacientes con enfermedad avanzada (<200 células CD4/mm³). Para ello es necesario estratificar al paciente mediante un recuento urgente de células CD4. Debido a que un elevado número de pacientes carecía de recuento de CD4 — 21%, en el análisis final — el tamizaje se realizó con independencia del recuento de CD4. Esto permitió realizar un análisis más global de la realidad de la infección oportunista. En primer lugar, se hizo un análisis mediante curvas ROC para averiguar qué relación existe entre el recuento de CD4 y el diagnóstico de las infecciones oportunistas. Así, si hubiéramos estratificado y tamizado por enfermedad avanzada (<200 células/mm³) hubiéramos detectado el 85,1% de las infecciones oportunistas. Una estratificación de los pacientes con <350 células/mm³ hubiera diagnosticado al 94,4% de estos, lo que significa casi un 10% de diferencia. Teniendo en cuenta que las infecciones oportunistas tienen una rápida y elevada mortalidad parece más razonable tamizar a todos los pacientes que tengan un recuento de células CD4 <350 /mm³. Además, si no se dispone de un recuento urgente de CD4, habría que valorar el coste efectividad de un programa global de tamizaje, con independencia de este parámetro. Los resultados finales apoyan esta estrategia ya que el 52% de los pacientes alcanzan el diagnóstico con enfermedad avanzada y el 77% con menos de 350/mm³. Parece evidente que el conocimiento de la situación epidemiológica real ayuda a los pacientes y a la política sanitaria.

Con respecto a la carga de enfermedad de las infecciones oportunistas en los pacientes recién diagnosticados de VIH podemos destacar una incidencia global del 21% con una relación inversa al recuento de células CD4. A diferencia de la creencia instaurada de que la tuberculosis es la infección oportunista más frecuente en el VIH, este programa demostró que, en los pacientes de reciente diagnóstico, la histoplasmosis es más frecuente. La diferencia es de 0,8% (7,9% vs 7,1) pero tiene una gran importancia clínica ya que el tratamiento es completamente diferente pero la presentación clínica es similar. Hasta que se alcanzó el acceso al diagnóstico de la histoplasmosis mediante una prueba rápida de ELISA, muchos de los pacientes recibían un tratamiento equivocado. Al revisar la literatura encontramos, que la realidad supera las estimaciones: La incidencia de la histoplasmosis en el programa de tamizaje (7,9%) es casi del

doble a la anteriormente estimada por Adenis (4,16%) y Medina (3,8%) (Adenis et al., 2018; Medina et al., 2017). Esto significa unos 1.523 casos adicionales. Además, esta subestimación probablemente sea generalizada en la región. Durante el período de estudio, se ha demostrado que el acceso al diagnóstico se asocia con un aumento paulatino del número de casos de histoplasmosis. En 2012, se diagnosticaron 32 casos de histoplasmosis en el país, mientras que el promedio del programa de tamizaje es de 158 casos anuales, lo que representa un aumento del 80%. En el caso de la criptococosis, las estimaciones globales indican que un 6% de los pacientes con <100 células CD4/mm³ tienen antigenemia (Rajasingham et al., 2017). En nuestro estudio es un 8,2%, nuevamente una subestimación.

El diagnóstico de las infecciones oportunistas fúngicas mediante los métodos clásicos es una tarea lenta, difícil y con una baja sensibilidad (Azar & Hage, 2017; Kauffman, 2007; PAHO, 2020; Rajasingham et al., 2019; Wheat, 2003). La introducción de técnicas basadas en la detección del antígeno circulante para *Histoplasma* y *Cryptococcus*, tiene la ventaja de su rapidez y su elevada sensibilidad y especificidad (Cáceres et al., 2018; Ford et al., 2018; Loyse et al., 2013). En este trabajo se ha evaluado su empleo rutinario con muestras clínicas provenientes de 4.245 pacientes.

La sensibilidad global del antígeno urinario de Histoplasmosis fue del 72,3%. La imposibilidad de analizar individualmente las características clínicas de los pacientes con un resultado positivo para algunas de las pruebas empleadas para el diagnóstico de la histoplasmosis impidió una diferenciación más certera de la histoplasmosis diseminada frente a la localizada. Como dato informativo, en el 90% los pacientes en los que *Histoplasma* creció en el hemocultivo, el antígeno urinario fue positivo. Al definir como histoplasmosis diseminada los casos con un antígeno o un hemocultivo positivo, encontramos que el antígeno urinario fue positivo en el 94% de los casos. Asimismo, un 35% de los casos fueron diagnosticados gracias al antígeno urinario, siendo el resto de las pruebas negativas. La sensibilidad del hemocultivo Isolator fue del 36%. En conjunto el uso del antígeno y de la prueba PCR, detectaron el 96,1% de los casos de histoplasmosis. Este hallazgo ha sido similar a lo identificado recientemente en Brasil en un programa de tamizaje a pacientes hospitalizados y con enfermedad avanzada por VIH (Vidal et al., 2021). Aunque los métodos moleculares basados en la técnica de PCR han tomado relevancia, también presentan limitaciones para su uso rutinario. Desafortunadamente, la transferencia de técnicas caseras (*"in house"*) a otros laboratorios es complicada. Diferencias entre los reactivos utilizados obligan a una validación desde cero, lo que en muchos entornos es difícil. Una técnica comercial es la solución, pero, en la actualidad, no existen métodos moleculares aprobados para el diagnóstico de la histoplasmosis (Buitrago et al., 2013; Buitrago

& Martín-Gómez, 2020). Finalmente, la disponibilidad de programas de control de calidad y competencia para el diagnóstico de la enfermedad fúngica a un coste asumible facilitaría la acreditación de los laboratorios con el consiguiente acceso al diagnóstico de calidad.

La criptococosis se diagnosticó mediante la prueba de flujo lateral (LFA) (Jarvis et al., 2020; WHO 2017). El antígeno de *Cryptococcus* puede estar circulante en suero meses antes del desarrollo de la meningitis. En los casos en los que la prueba es positiva se debe realizar una punción lumbar para confirmar o descartar que el paciente tiene meningitis, la cual no se realiza siempre. En Guatemala, de los 165 casos con un antígeno sérico positivo, solo en 81 (51,5%) se hizo la punción lumbar, lo que resalta la importancia de guías locales, lo que muchas veces no es sencillo. En estos pacientes, se detectaron 55 casos de meningitis, de las cuales 18 (32,7%) fueron solo diagnosticados mediante la detección del antígeno en LCR. Hay mas estudios que reportan una mayor sensibilidad de la prueba de antígeno que la del cultivo en muestras de LCR (Ssebambulidde et al., 2019). La titulación sérica del antígeno de *Cryptococcus*, también es importante para establecer el grado de la enfermedad. Se ha demostrado que altas titulaciones se asocian con meningitis (Wake et al., 2018). En todos los casos, el cultivo del LCR de los pacientes con antígeno negativo fue estéril.

La introducción de las pruebas de detección de antígeno para histoplasmosis y criptococosis ha permitido diagnosticar un 35% y un 32,7% más de casos respectivamente. Por su capacidad diagnóstica, sencillez tecnológica, y rapidez en la obtención de resultados, estas pruebas deben estar disponibles en los centros de atención al paciente con VIH. Tras el análisis de los resultados del programa, las recomendaciones adicionales son las siguientes: (i) el cultivo de muestras clínicas debe realizarse principalmente para recuperar el hongo, lo que permite realizar análisis de resistencia y tipificación de las cepas; (ii) en el caso de la histoplasmosis, tras un antígeno urinario positivo recomendamos la obtención de hemocultivos Isolator antes del inicio del tratamiento específico para aumentar las posibilidades de la recuperación de la cepa; (iii) En el caso de la detección de *Histoplasma* mediante PCR, no existen recomendaciones claras. Solo existen PCRs caseras que si se emplean deberían estar validadas. Pueden ser útiles en combinación con la prueba de antígeno y considerando las características clínicas del paciente para definir si la histoplasmosis es localizada o diseminada.

Paralelamente a la publicación de este estudio, en julio de 2020, se lanzaron las guías de la OPS para el diagnóstico y manejo de la histoplasmosis en pacientes que viven con VIH (PAHO, 2020). Esta guía refuerza nuestras recomendaciones para que la prueba de detección de antígeno sea

utilizada para el diagnóstico de la histoplasmosis diseminada en pacientes que viven con VIH y sea un componente esencial en la atención. Además, está recogido en la lista de diagnósticos esenciales de la OMS (WHO, 2019a) donde también se incluye la prueba de antígeno para *Cryptococcus* y otra serie de técnicas diagnósticas de la enfermedad fúngica.

Las infecciones oportunistas se acompañan de una elevada mortalidad relacionada con el diagnóstico y tratamiento tardío. En nuestro estudio, la mortalidad a los 180 días de seguimiento de los pacientes con una de las infecciones oportunistas investigadas fue significativamente mayor (29% vs 5,9%, $P<0.0001$). El 53,9% de las muertes ocurrieron en los primeros 30 días tras la inclusión del paciente en el estudio. El impacto del programa de tamizaje se evaluó mediante la comparación de la mortalidad entre el 2017 y 2018, en el grupo de pacientes de reciente diagnóstico de VIH, encontrándose una disminución del 7%. Además, los hallazgos preliminares del 2019 han mantenido esta tendencia con una disminución del 14,2% en comparación con el 2017. Esto demuestra que el acceso al diagnóstico reduce las muertes asociadas al VIH. En 2018, el número de pacientes que recibió tratamiento para la infección oportunista fue un 5% más que en el 2017. La mortalidad disminuyó un 16,8% en la criptococosis, 16,1% en la tuberculosis y un 16% en las coinfecciones. En los casos de criptococosis se debe tomar en cuenta que la prueba se realiza en el punto de atención primaria, lo que favorece la rapidez de las decisiones. Para la tuberculosis, es posible que la familiarización de los médicos con su manejo y tratamiento, aunado a un diagnóstico rápido, haya influido en la supervivencia. Además, esto también podría explicar la mejora en los casos de coinfecciones ya que más del 50% de los pacientes con una infección múltiple tenían tuberculosis. En el caso de los pacientes con histoplasmosis, la mortalidad fue similar (+0,8% en 2018), mientras que para los 15 pacientes con micobacterias no tuberculosas aumentó un 25%. Las razones de este aumento son desconocidas, pero valdría la pena comprobar si la introducción de métodos rápidos de detección e identificación de las micobacterias no tuberculosas en muestras clínicas como GenoType CMdirect (Hain Lifescience) ofrecería ventajas frente a los métodos clásicos de cultivo que son lentos y obligan a tratar al paciente empíricamente.

La anfotericina B liposomal y la 5-Fluorocitosina, son los tratamientos óptimos recomendados en las guías de manejo de la histoplasmosis y la criptococosis (PAHO, 2020; WHO, 2018), pero no están disponibles en Guatemala, por lo que, a pesar de los problemas de toxicidad y manejo, se utiliza la anfotericina B deoxicolato. La introducción de los tratamientos recomendados es indispensable. Además, como se encuentran en la lista de medicamentos esenciales de la OMS (WHO, 2018, 2019a), su adquisición se puede realizar a través de mecanismos como el fondo estratégico de la OPS. Gracias a los resultados del programa de tamizaje, en 2021, se han iniciado

gestiones para la compra de anfotericina B liposomal, que será utilizada en el tratamiento de ambas infecciones fúngicas. En el caso de la 5-Fluorocitosina se necesitan más gestiones incluyendo su registro en el país.

Para terminar, es importante tener en cuenta la fragilidad de este tipo de actuaciones si no se incorporan a los programas de atención a largo plazo y se sustentan con presupuestos que permitan mantenerlas, incluyendo los recursos humanos. Desafortunadamente, la pandemia por la COVID-19 ha afectado a todo el sistema de salud global. En este programa hemos evaluado su impacto en algunos puntos de la atención al paciente con VIH mediante la actividad del Laboratorio central de Diagnóstico. Para este análisis se compararon ocho meses de resultados, de enero a agosto, en promedio, de los años 2017 a 2019, denominado prepandemia y los hallazgos de los mismos meses del 2020 durante la pandemia en una de las unidades, la Clínica Familiar «Luis Ángel García». El análisis demostró que el número de pruebas de VIH y los casos de reciente diagnóstico se redujeron un 54,7 y 10,7%, respectivamente. Aunque se esperaba que ambos indicadores presentaran una reducción similar, el número de casos nuevos fue menor. Esto podría estar relacionado al hecho de que la Clínica Familiar mantuvo en gran parte los servicios de atención, al igual que otras organizaciones que apoyan la búsqueda del VIH en ciertos grupos como los hombres que tienen sexo con hombres (HSH). Esta idea podría estar reforzada por el aumento que se observó de pacientes HSH vinculados a esta clínica en 2020, un 16,2% más de lo reportado en la cohorte en 2019.

El conocimiento sobre el estado de portador del VIH es el primer pilar de la cascada de atención. En Guatemala antes de la pandemia por COVID-19, se estimaba que el 62% de los pacientes con HIV conocían su diagnóstico (Programa Nacional de Prevención y Control de ITS VIH y SIDA, 2019). Sin embargo, los resultados de este estudio indican una reducción del 10,7% lo que significaría que aproximadamente solo un 50% conocería su estado. Esto tiene graves consecuencias. Por un lado, estos pacientes se presentarán tardíamente lo que aumenta el riesgo de padecer una o varias infecciones oportunistas. En segundo lugar, estos pacientes, que no conocen su estado de portador, suelen tener una carga viral alta lo que facilita la transmisión del VIH a otras personas. Esto tendrá consecuencias a medio y largo plazo con un aumento de la incidencia de las infecciones por VIH. El Fondo global en su último reporte sobre el impacto de la COVID-19 incide en esta problemática (Global Fund, 2021).

La mortalidad asociada a las infecciones oportunistas durante la epidemia COVID-19 en la Clínica Familiar aumentó un 10,7% en comparación al 2019. Otros autores describen situaciones similares (Global Fund, 2020; Jewell et al., 2020; Mhango et al., 2020). Al comparar esta mortalidad con la encontrada al inicio del desarrollo del programa de tamizaje, se observa un

aumento del 2,4%, lo que representa un retroceso tras cuatro años de programa en tan solo 8 meses. La Clínica Familiar representa aproximadamente el 30% de la cohorte de la red y es factible que la situación en el resto de las unidades de la red sea aún más complicada. La reducción en el número de muestras para tamizaje fue más pronunciada en unidades de atención integral que se encuentran a nivel departamental. Aunque este programa ha logrado determinar el impacto de la COVID-19 dentro de algunos componentes de atención en VIH, principalmente en una de las unidades de atención integral, sería importante determinar el impacto global en la red implantando un plan de restablecimiento y promoción de los servicios.

4.1. Aplicabilidad y perspectivas futuras

A través de este proyecto se ha desarrollado una estrategia eficaz de acceso al diagnóstico de cuatro infecciones oportunistas, lo que representa una herramienta robusta y confiable para que los pacientes sean diagnosticados y tratados rápidamente, mejorando así la supervivencia relacionada al VIH. Con los resultados provistos otros países que busquen reducir la mortalidad relacionada al VIH pueden integrar esta estrategia.

A partir de los resultados presentados en esta tesis, las perspectivas de trabajo futuro se orientan en tres direcciones. En primer lugar, se deberían expandir las acciones necesarias para fortalecer el programa de tamizaje de infecciones oportunistas. Esto incluye: (i) el desarrollo de un programa de control de calidad dirigido por el Laboratorio central de Diagnóstico, (ii) la implantación de otros Laboratorios de Diagnóstico Centralizado que permitan mantener un sistema de referencia entre las distintas unidades tomando en cuenta la capacidad de respuesta y el número de muestras a procesar, (iii) la expansión del portafolio de diagnósticos ofertados a la red y (iv) la integración de la prueba de flujo lateral para el diagnóstico de la histoplasmosis. En segundo plano, las acciones deben estar orientadas hacia la mejora del diagnóstico del VIH en el país. Estas actuaciones deben de: (i) aumentar el diagnóstico temprano de la infección por VIH en el país, (ii) desarrollar estrategias diferenciadas para aumentar el diagnóstico temprano en la población Maya. En tercer lugar, las acciones futuras deberían asegurar la disponibilidad de los tratamientos de elección para el manejo de las infecciones oportunistas.

5. [CONCLUSIONES]

5. Conclusiones

1. La implantación de un programa de tamizaje para infecciones oportunistas a través de sistema de Laboratorio central de Diagnóstico «DLH-Diagnostic Laboratory Hub» es una forma eficaz y ágil de brindar acceso al diagnóstico,
2. Los métodos de detección del antígeno para *Histoplasma capsulatum* y *Cryptococcus neoformans* deben ser establecidos como la técnica de referencia para el diagnóstico de estas infecciones en las personas que viven con VIH y deben de ser integrados en los programas de atención para este grupo de pacientes,
3. La carga de enfermedad avanzada por VIH en Guatemala es una de las más altas a nivel mundial siendo el grupo más desfavorecido la población Maya con una frecuencia aun mayor,
4. El tamizaje de infecciones oportunistas debería realizarse en todos los pacientes de reciente diagnóstico de VIH que tengan un recuento de células CD4 menor a 350/mm³
5. La carga de histoplasmosis y criptococosis ha sido subestimada en Guatemala y posiblemente en otros países de la región,
6. El acceso al diagnóstico de las infecciones oportunistas a través de un programa de tamizaje reduce la mortalidad asociada al VIH.

6. [REFERENCIAS]

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