

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

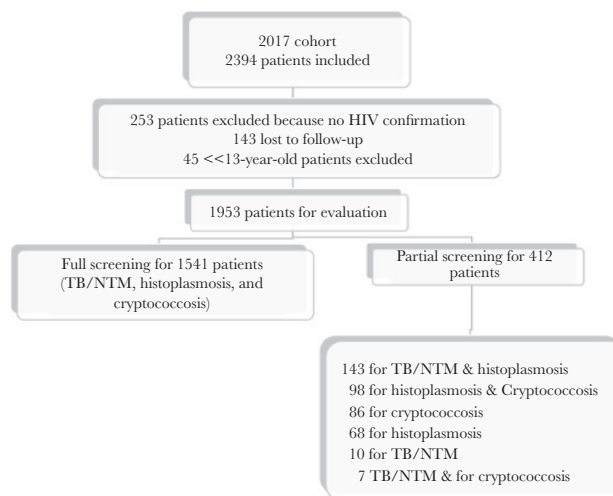


Figure 1. Description of the 2017 cohort. Abbreviations: NTM, nontuberculous mycobacteria; TB, tuberculosis.

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/mm³					
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.

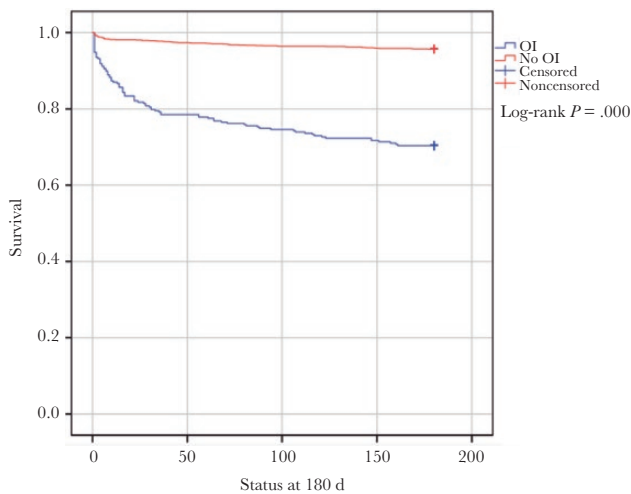


Figure 2. Kaplan-Meier survival curves of patients with and without opportunistic infections. Abbreviation: OI, opportunistic infection.

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

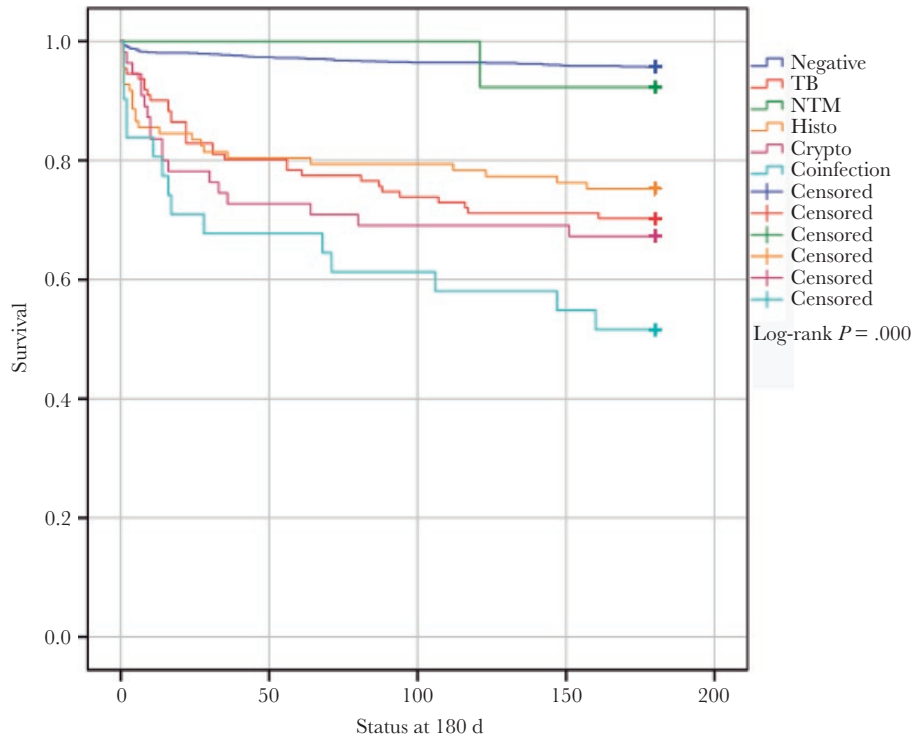


Figure 3. Kaplan-Meier survival curves of opportunistic infection cases. Abbreviations: NTM, nontuberculous mycobacteria; TB, tuberculosis.

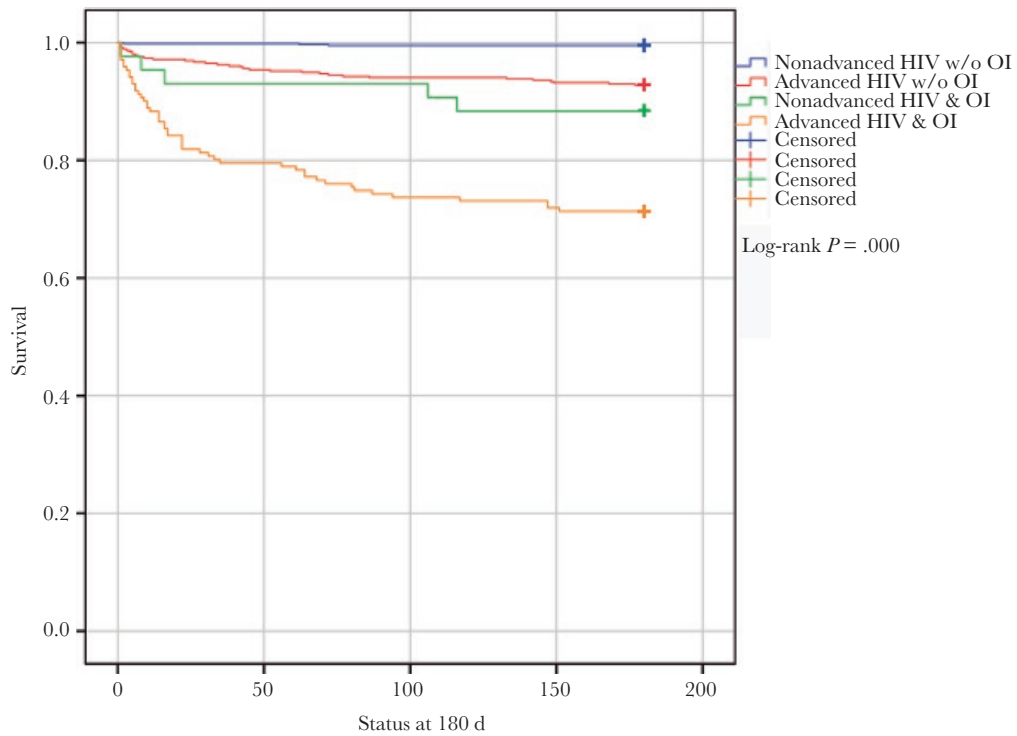


Figure 4. Survival curves for advanced vs nonadvanced HIV and opportunistic infections. Abbreviation: OI, opportunistic infection.

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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References

1. UNAIDS. Fact sheet - latest global and regional statistics on the status of the AIDS epidemic. 2018. Available at: https://www.unaids.org/sites/default/files/media_asset/UNAIDS_FactSheet_en.pdf. Accessed May 19, 2019.
2. Global Laboratory Initiative Advancing TB Diagnosis. A publication of the Global Laboratory Initiative a Working Group of the Stop TB Partnership mycobacteriology laboratory manual. 2014. Available at: <https://www.who.int/tb/laboratory/mycobacteriology-laboratory-manual.pdf>. Accessed November 30, 2019.

3. Montenegro SH, Gilman RH, Sheen P, et al. Improved detection of *Mycobacterium tuberculosis* in Peruvian children by use of a heminested IS6110 polymerase chain reaction assay. *Clin Infect Dis* **2003**; 36:16–23.
4. Muñoz C, Gómez BL, Tobón A, et al. Validation and clinical application of a molecular method for identification of *Histoplasma capsulatum* in human specimens in Colombia, South America. *Clin Vaccine Immunol* **2010**; 17(1):62–7.
5. Bialek R, Feucht A, Aepinus C, et al. Evaluation of two nested PCR assays for detection of *Histoplasma capsulatum* DNA in human tissue. *J Clin Microbiol* **2002**; 40:1644–7.
6. World Health Organization. Treatment of tuberculosis: guidelines for treatment of drug-susceptible tuberculosis and patient care. **2017**. Available at: <https://apps.who.int/iris/bitstream/handle/10665/255052/9789241550000-eng.pdf;jsessionid=8B82E1F8D678BD6DA19310B13848EC62?sequence=1>. Accessed May 19, 2019.
7. Ministerio de Salud Pública y Asistencia Social de Guatemala. Manual de Atención Para el Manejo del Paciente con Tuberculosis. Guatemala: Ministerio de Salud Pública y Asistencia Social Guatemala; **2018**.
8. Griffith DE, Aksamit T, Brown-Elliott BA, et al; ATS Mycobacterial Diseases Subcommittee; American Thoracic Society; Infectious Disease Society of America. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* **2007**; 175:367–416.
9. Centers for Disease Control and Prevention. Guidelines for Prevention and Treatment of Opportunistic Infections in HIV-Infected Adults and Adolescents. Available at: <https://aidsinfo.nih.gov/e-news>. Accessed June 27, 2019.
10. Lippi G, Mattiuzzi C. Biological samples transportation by drones: ready for prime time? *Ann Transl Med* **2016**; 4:92–96.
11. Amukele TK, Sokoll LJ, Pepper D, et al. Can unmanned aerial systems (drones) be used for the routine transport of chemistry, hematology, and coagulation laboratory specimens? *PLoS One* **2015**; 10:e0134020.
12. UNAIDS. AIDSinfo | UNAIDS. **2017**. Available at: <https://aidsinfo.unaids.org/>. Accessed May 19, 2019.
13. World Health Organization. Guidelines for managing advanced HIV disease and rapid initiation of antiretroviral therapy. **2017**. Available at: <https://apps.who.int/iris/bitstream/handle/10665/255884/9789241550062-eng.pdf?sequence=1>. Accessed May 19, 2019.
14. Adenis A, Nacher M, Hanf M, et al. HIV-associated histoplasmosis early mortality and incidence trends: from neglect to priority. Hamill RJ, editor. *PLoS Negl Trop Dis* **2014**; 8(8):e3100.
15. Arango-Bustamante K, Restrepo A, Cano LE, De Bedout C, Tobón AM, González A. Diagnostic value of culture and serological tests in the diagnosis of histoplasmosis in HIV and non-HIV Colombian patients. *Am J Trop Med Hyg* **2013**; 89(5):937–942.
16. Gina P, Randall PJ, Muchinga TE, et al. Early morning urine collection to improve urinary lateral flow LAM assay sensitivity in hospitalised patients with HIV-TB co-infection. *BMC Infect Dis* **2017**; 17:339–344.
17. Savolainen L, Kantele A, Sandboge B, et al. Modification of clear view tuberculosis (TB) enzyme-linked immunosorbent assay for TB patients not infected with HIV. *Clin Vaccine Immunol* **2013**; 20(9):1479–1482.
18. Nacher M, Blanchet D, Bongomin F, et al. *Histoplasma capsulatum* antigen detection tests as an essential diagnostic tool for patients with advanced HIV disease in low- and middle-income countries: a systematic review of diagnostic accuracy studies. *PLoS Negl Trop Dis* **2018**; 12(10):e0006802.
19. World Health Organization. Second WHO Model List of Essential In Vitro Diagnostics. Geneva: World Health Organization; **2019**.
20. Limper AH, Adenis A, Le T, Harrison TS. Fungal infections in HIV/AIDS. *Lancet Infect Dis* **2017**; 17(11):e334–e343.
21. Samayoa B, Roy M, Cleveland AA, et al. High mortality and coinfection in a prospective cohort of human immunodeficiency virus/acquired immune deficiency syndrome patients with histoplasmosis in Guatemala. *Am J Trop Med Hyg* **2017**; 97(1):42–48.
22. Podlekareva DN, Efsen AMW, Schultze A, et al. Tuberculosis-related mortality in people living with HIV in Europe and Latin America: an international cohort study. *Lancet HIV* **2016**; 3(3):e120–31.
23. Boulware DR, Meya DB, Muzoora C, et al. Timing of antiretroviral therapy after diagnosis of cryptococcal meningitis. *N Engl J Med* **2014**; 370(26):2487–98.