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Predictive value of anaemia for tuberculosis in HIV-infected patients in sub-Saharan Africa: an indication for routine microbiological investigation using new rapid assays

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Abstract

Background—The relationship between anaemia and undiagnosed tuberculosis (TB) in patients living with HIV in sub-Saharan Africa is incompletely defined. We assessed the prevalence of TB among those with HIV-related anaemia and evaluated new means of rapid TB diagnosis.

Methodology—Blood haemoglobin levels were measured in unselected antiretroviral treatment (ART)-naïve patients in Cape Town, South Africa, and anaemia was classified according to WHO criteria. All patients were screened for TB by testing paired sputum samples using liquid culture (reference standard), fluorescence microscopy and Xpert MTB/RIF. Urine samples were tested for lipoarabinomannan (LAM) using the Determine TB-LAM diagnostic assay.

Results—Of 602 adults screened, 485 had complete results. Normal haemoglobin levels were found in 44.5% (n=216) of patients and mild, moderate or severe anaemia were present in 24.9% (n=121), 25.4% (n=123) and 5.2% (n=25) of patients, respectively. Culture-confirmed pulmonary TB was diagnosed in 8.8% (19/216) of those without anaemia compared to 16.5% (20/121), 26.0% (32/123) and 40.0% (10/25) with mild, moderate or severe anaemia, respectively ($p<0.001$). Anaemia was a strong independent predictor of TB. The sensitivities of diagnostic assays were much higher among those with moderate/severe anaemia compared to those with no/mild anaemia using sputum microscopy (42.9% vs 15.4%); urine LAM (54.8% vs 0%); sputum microscopy plus urine LAM (71.4% vs 15.4%) and sputum Xpert (73.8% vs 41.0%) ($P<0.01$ for all).

Conclusions—A very high prevalence of undiagnosed TB was found in patients with moderate or severe anaemia. Such patients should be prioritized for routine microbiological investigation using rapid diagnostic assays.

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Conflicts of Interest

None to declare.

Authors' Contributions

SDL and ADK initiated and planned the study. SDL, RW and MV collected the data. SDL and MV ran laboratory assays. ADK did the data analysis. ADK and SDL wrote the paper with input from RW. All authors approved the final version of the manuscript prior to submission.

Keywords

HIV; AIDS; TB; tuberculosis; anaemia; haemoglobin; Africa; diagnosis; Xpert; LAM

Background

Anaemia is the most common haematologic abnormality associated with HIV.[1–3] It is frequently observed among people living with HIV (PLWH), especially among those with evidence of advanced HIV disease.[4] Clinical consequences associated with HIV-associated anaemia include severe fatigue,[5] poorer quality of life for PLWH [6,7] and possibly an increased rate of HIV disease progression.[2,8,9] Anaemia is independently associated with increased mortality risk [10,11] and remains a common problem among ART-naïve patients in sub-Saharan Africa.[12]

Anaemia may also be related to HIV-associated tuberculosis (TB), which remains the leading cause of death among PLWH worldwide.[13] Anaemia is an independent predictor of early incident TB among those initiating ART in sub-Saharan Africa [12,14] and is also associated with increased mortality in those with HIV-associated TB.[15–18] For these reasons, some have recommended routine TB investigations among PLWH with low haemoglobin levels living in high TB incidence settings, before starting ART.[19]

We conducted this study to further assess whether there is a need to investigate for TB in PLWH who are enrolling for ART and are found to be anaemic. In this study of ART-naïve patients enrolling in a large community-based clinic in Cape Town, South Africa, we report on the prevalence of anaemia, the prevalence of active TB and the relationship between the two. We also evaluated novel tools and approaches to diagnosis of TB in those with anaemia to identify effective means of systematic screening in this patient group.

Methods

Patient characteristics

We have previously described in detail the ART service in Gugulethu township in Cape Town, and the high TB burden of TB among its patients.[20–23] Those eligible for this study were ART-naïve, HIV-infected adults, who were without a current TB diagnosis and were consecutively recruited from among patients newly referred to the clinic for initiation of ART between March 2010 and April 2011. Written informed consent was provided by all patients and the study was approved by the research ethics committees of the University of Cape Town and the London School of Hygiene & Tropical Medicine, UK.

At the first visit to the clinic, patients completed standard symptom screening that included the World Health Organization (WHO) symptom screen (the presence of more than one of the following symptoms: cough of any duration, fever, weight loss or night sweats) for HIV-associated TB.[24] They were clinically characterized and clinical samples were obtained. Whenever possible, two sputum samples were obtained; the first sample was a spot specimen followed by a second sample.[25] Urine samples were collected and stored at −20ºC. Blood CD4 cell counts and plasma viral load was measured. Haemoglobin levels were determined using ADVIA 2120 hematology analyzer (Siemens Healthcare Diagnostics, Erlangen, Germany). Chest radiographs were obtained when possible and were reported by a reader experienced in using the Chest Radiographic Reading and Recording System (CRRS).[26]

Laboratory procedures

Sputum samples were processed in an accredited laboratory according to standardized protocols with external quality assurance procedures. Decontaminated and centrifuged sputum samples were examined for acid-fast bacilli (AFB) using auramine O fluorescent stain, tested using the Xpert MTB/RIF assay and cultured in liquid media as previously described.[27–29] Positive cultures were speciated. Determine TB-LAM lateral-flow assay was used to retrospectively analyse defrosted frozen urine samples for the presence of lipoarabinomannan (LAM). A test band equal to or greater than the intensity of the weakest band on the reference card (grade 1) defined a positive LAM result. Technologists blinded to the outcomes of the other assays read the results of all tests.

Patient outcomes

Patients were followed up within the routine ART service. Those diagnosed as having TB were referred to local TB treatment clinics. ART service patient records were reviewed to determine vital status at 90 days.

Definitions and analysis

Mycobacterium tuberculosis cultured from one or more sputum samples was used to define TB cases. Using haemoglobin values prior to ART initiation, all patients were categorized according to WHO criteria [30] as having: no anaemia (≥13.0 g/dL for men, ≥12.0 g/dL for non-pregnant females and ≥11.0 g/dL for pregnant females), mild anaemia (11.0–12.9 g/dL for men, 11.0–11.9 g/dL for non-pregnant females, 10.0–10.9 g/dL for pregnant females), moderate anaemia (8.0–10.9 g/dL for males and non-pregnant females and 7.0–9.9 g/dL for pregnant females) or severe anaemia (<8.0 g/dL for males and non-pregnant females and <7.0 g/dL for pregnant females).

Medians were compared using either Wilcoxon rank-sum tests or Kruskal-Wallis tests where appropriate. Chi-squared tests or Fisher's exact tests were used as indicated to compare proportions. The sensitivity, specificity, and predictive values of the different TB diagnostic assays were calculated (with corresponding 95% confidence intervals) for patient groups stratified by severity of anaemia. Logistic regression analyses were used to identify factors independently associated with HIV-associated anaemia. All variables in the univariable model meeting a cut-off of $p \leq 0.1$ were included in the multivariable model. Statistical tests were 2-sided at $\alpha = 0.05$.

Results

Anaemia diagnoses

Of 602 patients recruited, 485 had complete results of tests done on blood, sputum and urine samples. Among those included in the analysis (n=485), the median age was 33.6 (IQR, 27.9–40.7), 63.5% were female and the median CD4 count was 169 cells/uL (IQR, 96–231). The median blood haemoglobin level was 12.0 g/dL (IQR, 10.6–13.4).

Anaemia was diagnosed in 269 patients (prevalence, 55.5%; 95% CI, 50.9–60.0) and the remaining 216 (44.5%) had normal haemoglobin levels. Anaemia was classified as mild in 121 (24.9%), moderate in 123 (25.4%) and severe in 25 (5.2%). Patients with greater severity of anaemia were more likely to be female, have lower BMI's and have higher white cell counts and absolute neutrophil counts (Table 1). Such patients also tended to have lower CD4 cell counts, higher HIV viral load, pulmonary TB and either stage 3 or 4 HIV disease at programme enrolment. However, pregnancy was not associated with degree of anaemia.

Prevalence of TB among patients with HIV-associated anaemia

Overall, 81 patients had culture-positive TB diagnosed (prevalence, 16.7%; 95% CI, 13.5–20.3) and 404 (83.3%) patients had negative sputum cultures. Among the 269 patients with any degree of anaemia, 62 had TB (prevalence, 23.0%; 95% CI, 18.2–28.5). The prevalence of TB was strongly and directly correlated with degree of anaemia (Figure 1). However, the prevalence of TB did not differ by gender, as 33.3% (95%CI, 15.6–55.3) of men with moderate or severe anaemia had TB compared to 27.4% (95%CI, 19.8–36.2) among females with moderate or severe anaemia (p=0.556). A positive WHO symptom screen was found in 66 (81.5%) of overall patients with TB and in 88.1% of the sub-set of TB patients with either moderate or severe anaemia compared to 75.0% among those with no or mild anaemia (p=0.0094).

Risk factors for HIV-associated anaemia

We next used logistic regression to define whether TB was an independent risk factor associated with anaemia. Both univariable and multivariable analyses demonstrated that a number of variables were associated with moderate or severe anaemia (Table 2) TB remained strongly associated with moderate and severe anaemia after adjustment for all other variables.

Characteristics and outcomes of patients with HIV-associated TB and anaemia

Among patients with HIV-associated TB (n=81), those with lower haemoglobin levels were more likely to be female, have higher absolute neutrophil counts, lower CD4 cell counts and higher HIV viral loads (data not shown). Radiological abnormalities were generally not associated with anaemia with the exception that those with more severe anaemia were more likely to have mediastinal lymphadenopathy. Patients with a greater severity of anaemia were much less likely to be retained in programme after 90 days due to death or loss to follow-up (Figure 2). Patients with TB who died during follow-up (n=5) all had moderate or severe anaemia (p=0.026).

Diagnosis of TB among patients with HIV-associated anaemia

We next assessed the diagnostic accuracy of a range of microbiological assays for TB used during routine systematic screening (Table 3). It was striking that for each diagnostic assay, the sensitivities were significantly greater among those with moderate or severe anaemia compared to those with mild or no anaemia (Table 3). This increment ranged from 27.5% for sputum microscopy to 54.8% for Determine TB-LAM. Sputum Xpert MTB/RIF and Determine TB-LAM each detected a majority of TB cases among those with moderate or severe anaemia, whereas sputum smear microscopy did not. Combining Determine TB-LAM with either sputum smear microscopy or Xpert MTB/RIF increased the diagnostic sensitivity among those with moderate or severe anaemia to more than 70% and 80%, respectively.

Both sputum Xpert MTB/RIF and Determine TB-LAM correctly identified TB in all of those who died in the first 90 days of clinical follow-up (n=5) whereas sputum microscopy only diagnosed TB in two of the five cases. The positive predictive value was maintained above 90% for all assays among those with moderate or severe anaemia (Table 3). The specificity of all assays either in isolation or in combination was greater than 98% and 97%, respectively, and did not differ according to the severity of anaemia (Table 3).

Discussion

This study found a high prevalence of anaemia among treatment-naïve patients enrolling to start ART and that a substantial proportion of those with anaemia had underlying TB, which

was a strong independent risk factor for anaemia. The prevalence of TB among those with moderate or severe anaemia was so high and their clinical outcomes were so poor that it suggests the need for routine microbiological investigations for TB in this patient group. A majority of TB among those with moderate or severe anaemia could be rapidly diagnosed using Determine TB-LAM and/or Xpert MTB/RIF.

The prevalence of TB among ART-naive patients with anaemia in developing countries is poorly defined and is likely to vary greatly between settings. We carefully documented this relationship in a South African cohort, showing that a higher prevalence of TB was directly associated with lower haemoglobin levels (reaching as high as 40% among those with severe anaemia) and that TB was a strong independent risk factor for HIV-associated anaemia. These data are consistent with the findings of previous studies from Malawi,[31] Rwanda [32] and India.[33] Multivariable analysis additionally found that while low CD4 cell count, high viral load and low body mass index also demonstrated associations, female sex was the factor most strongly associated with moderate or severe anaemia. This may be explained, for example, by menstrual blood loss and low dietary iron intake.

The mechanisms underlying anaemia in patients with HIV-associated TB remain incompletely defined and are likely to be multiple. The most common mechanism is likely anaemia of chronic disease.[34,35] Iron-deficiency anaemia [35,36] may also occur as a result of insufficient dietary intake or blood loss from the gastrointestinal (GI) tract due to mucosal involvement with TB.[37] TB may also disseminate to the bone marrow [38,39] and impair all hematopoietic cell lines, including red blood cells. Other reported mechanisms may include autoimmune hemolysis [40,41] and nutritional deficiencies of folate, selenium [42] and rarely vitamin B12 secondary to malabsorption caused by ileal TB involvement.[43] Additionally, HIV-associated anaemia is associated with worsening HIV disease parameters, [44] including higher HIV viral load and low CD4 counts [2,45] and the risk for active TB disease increases with greater severity of immunosuppression.

Among those with HIV-associated TB, all deaths occurred among those with moderate or severe anaemia and patients were overall less likely to be retained in care after 3 months in programme. While the outcomes of those lost to follow-up are unknown, it is likely that those not retained in care were at high risk for mortality.[46,47] Our findings are consistent with other studies which have demonstrated that lower haemoglobin levels are associated with decreased survival and may independently predict mortality among patients with HIV-associated TB.[15–18] Therefore, we suggest that routine investigation for TB among HIV patients with anaemia may not only yield a large number of TB cases, but may identify many of those at greatest risk for significant TB-related morbidity and mortality.

In resource-limited settings, the WHO symptom screen for HIV-associated TB [24] is used to identify those who require further evaluation with a view to possible microbiological screening for TB. However, the sensitivity of this screen is incomplete [24] (as found in the present study). Moreover, due to its very low specificity, symptom screening identifies very large numbers of patients for whom it is simply not feasible to conduct microbiological investigations. However, the association between anaemia and TB was so strong that this might reasonably be used as an absolute indication for microbiological screening. Thus, the presence of anaemia readily identifies a sub-set of patients for whom investigations should be prioritized regardless of the presence or absence of symptoms.

We have previously shown that sputum Xpert MTB/RIF and urine Determine TB-LAM assays have higher diagnostic sensitivity in those with poorer prognostic characteristics, including anaemia.[48] In the present study, we have now demonstrated the sensitivities of these assays used alone, together or in combination with smear microscopy among those

with anaemia classified according to WHO criteria. The observation that the sensitivities of these diagnostic assays were much higher among those with greater severity of anaemia is likely to be related to the probability that both these factors are associated with more advanced and disseminated disease and mycobacterial load.[48] Anaemia may be particularly severe in patients with disseminated HIV-associated TB due to several factors. These include high levels of systemic inflammation (with up-regulation of myelosuppressive pro-inflammatory cytokines) direct involvement of the bone marrow (leading to suppression of haematopoiesis) and gastrointestinal tract (leading to blood loss).

Determine-TB LAM is best prioritized for use in screening for TB among HIV-infected patients with CD4 cell counts <200 cells/μL.[49,50] However, in many resource-limited settings, CD4 counts may not be available. Low haemoglobin levels may, however, represent an alternative simple trigger for appropriate TB testing among HIV-infected patients using LAM point-of-care assay. Further studies to assess the diagnostic accuracy and impact of TB screening using this and other rapid diagnostic assays in patients with moderate-to-severe HIV-associated anaemia in resource-limited settings are prudent.

Among patients with moderate or severe anaemia, the sensitivity of LAM point-of-care assay combined with sputum microscopy was very similar to that of a single sputum Xpert cartridge. This has important implications for resource limited settings. While Xpert is being scaled up and is becoming available in several sub-Saharan African countries, its cost and technical requirements will prohibit its implementation in some settings with high HIV-associated TB burdens.[51] Microscopy is already widely available in most resource-limited settings and continues to be first line for TB diagnosis in most of these settings. LAM point-of-care assay is rapid, easy to use and inexpensive and may be a very useful add-on test in resource-limited settings to increase the yield of TB diagnoses.

Strengths of this study include consecutive enrollment of patients who were well characterized. Sputum induction was used to obtain quality samples and liquid culture was used as the reference gold standard and processed in an accredited laboratory according to standardized protocols and quality assurance procedures. A limitation of this study includes the lack of available red blood cell (RBC) indices or iron studies. With only haemoglobin levels available, we could only classify patients according to the degree of anaemia without being able to further investigate possible underlying mechanisms, such as iron-deficiency or chronic inflammation. An additional limitation is that it was not possible to determine whether anaemia in patients with TB was directly related to their TB disease, attributable to their HIV infection, or was simply a prevalent co-morbidity unrelated to either their TB or HIV disease. Finally, the reference standard was determined by testing paired sputum samples using liquid culture. As extrapulmonary TB is more common in patients with advanced immunodeficiency, this may have underestimated the prevalence of active disease. Sampling multiple sites of disease for extrapulmonary TB may have therefore enhanced the reference standard.

In conclusion, HIV-associated anaemia was common and there was a very high prevalence of undiagnosed TB among those with moderate or severe anaemia that was associated with very poor clinical outcomes. Sputum Xpert MTB/RIF and urine Determine TB-LAM were able to rapidly diagnose TB with useful sensitivity among such patients. PLWH with moderate or severe anaemia in high burden settings should be investigated for TB using rapid microbiological assays regardless of symptoms.

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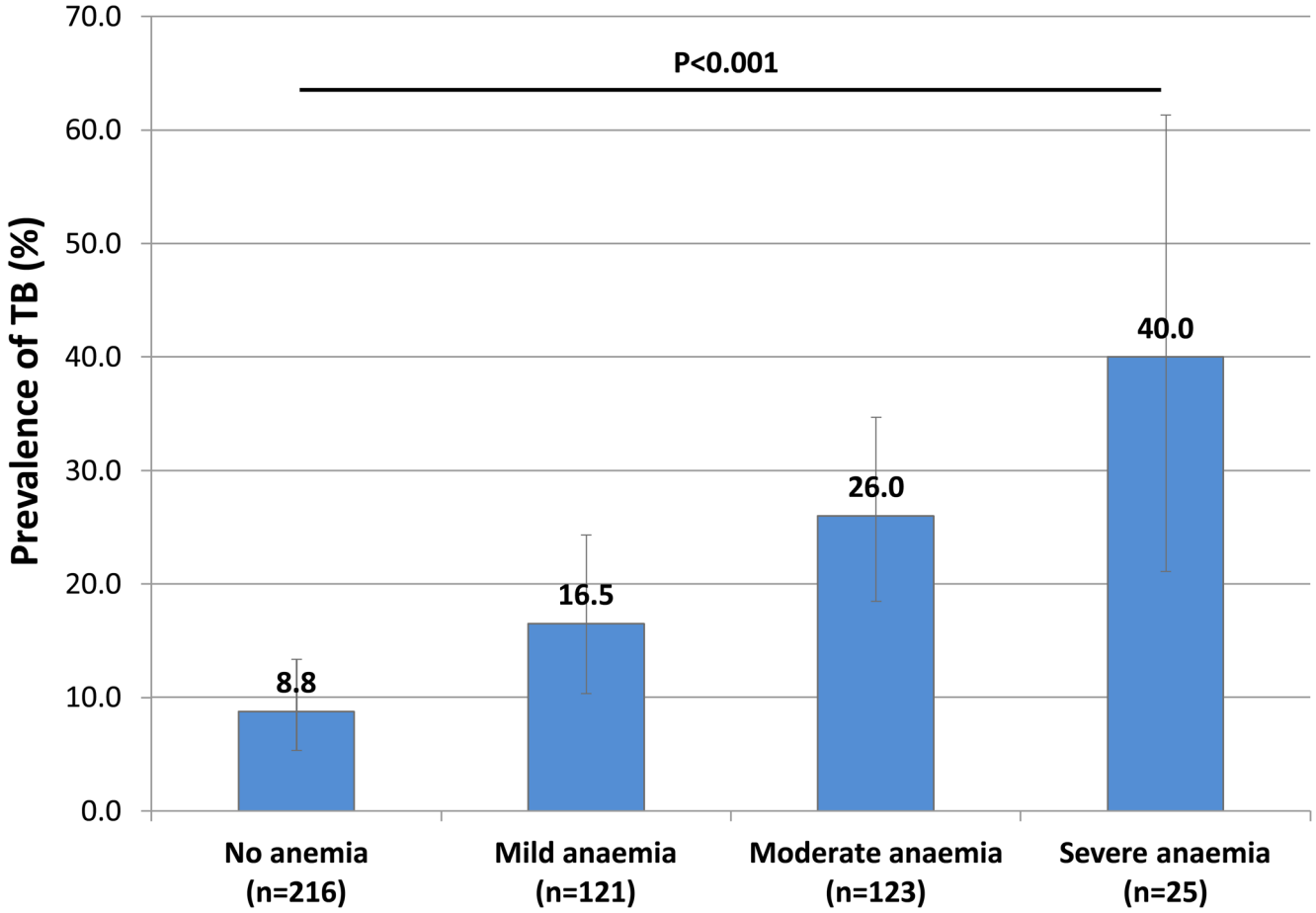

Figure 1.
The proportion (with 95% confidence intervals) of HIV-infected patients (n=485) who have TB, stratified by degree of anaemia.

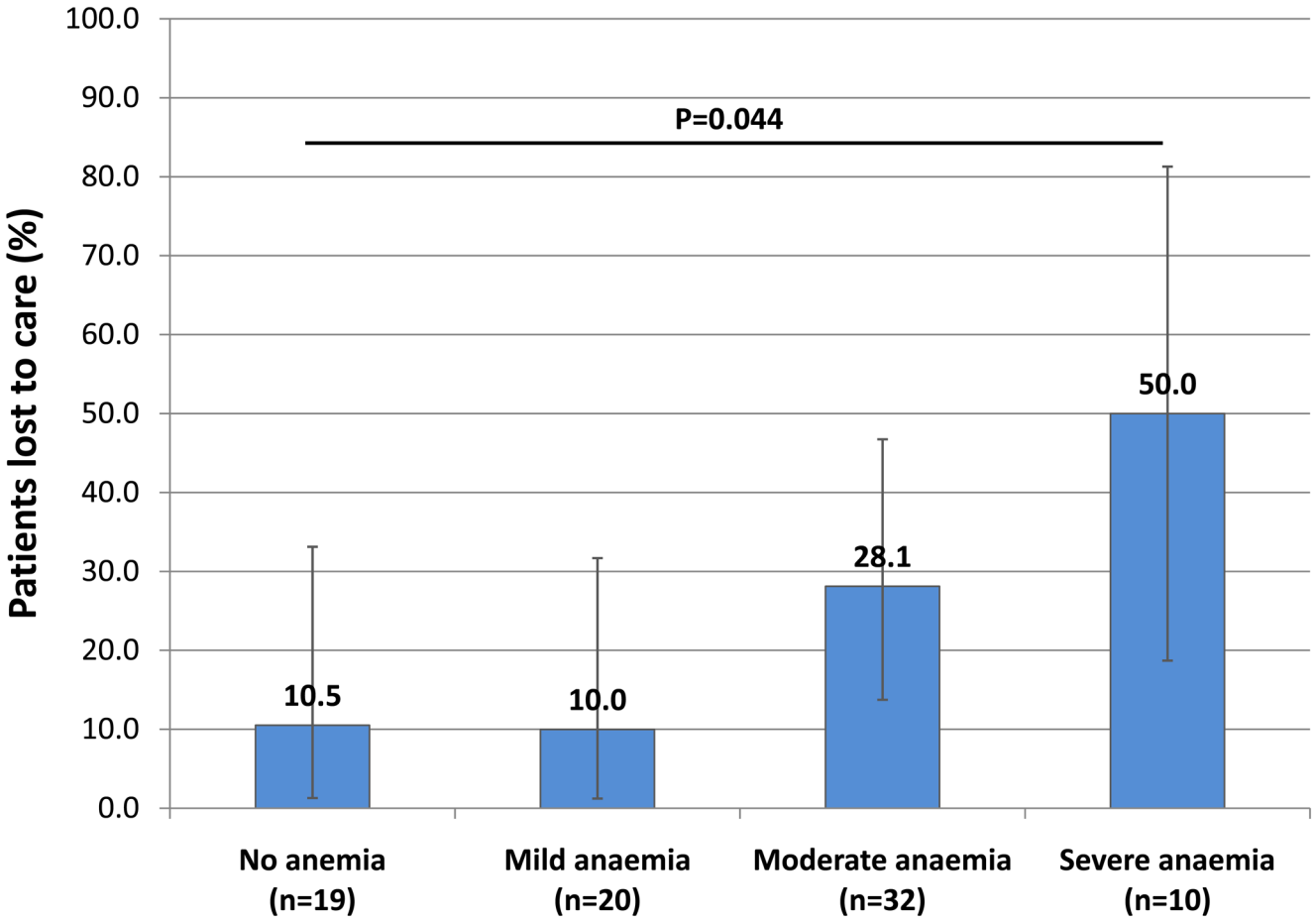

Figure 2.
The proportion (with 95% confidence intervals) of patients with HIV-associated TB (n=81) who either died or were lost to follow-up within 90 days of ART initiation, stratified by degree of anaemia.

TABLE 1

Patient characteristics among all HIV patients independent of TB status (n=485)

	All patients n=485 (100%)	No anaemia n=216 (44.5%)	Mild anaemia n=121 (24.9%)	Moderate anaemia n=123 (25.4%)	Severe anaemia n=25 (5.2%)	p-value
Patient characteristics						
Age, median (IQR)	33.6 (27.9–40.7)	34.1 (28.5–41.1)	33.8 (27.9–40.7)	33.2 (27.7–41.2)	29.4 (25.8–33.7)	0.244
Female (%)	308 (63.5)	115 (53.2)	69 (57.0)	102 (82.9)	22 (88.0)	<0.001
Pregnant (%)	20 (4.1)	6 (2.8)	8 (6.7)	6 (4.9)	0	0.293
BMI, median (IQR)	23.6 (20.9–27.1)	23.8 (21.3–28.5)	23.1 (20.7–26.1)	23.8 (19.8–26.9)	21.4 (19.7–24.6)	0.004
Haemotological tests[a]						
Hemoglobin (g/dL)	12.0 (10.6–13.4)	13.5 (12.6–14.4)	11.7 (11.3–12.2)	10.0 (9.1–10.6)	7.5 (7.0–7.7)	<0.001
White blood cell count (cells/μL)	4.9 (3.9–6.1)	4.8 (3.9–5.8)	4.4 (3.6–5.8)	5.3 (4.1–6.6)	6.5 (4.9–8.2)	0.003
Absolute neutrophil count (cells/μL)	2.6 (1.9–3.6)	2.6 (1.8–3.5)	2.3 (1.8–3.1)	3.0 (2.1–4.0)	3.7 (2.1–5.7)	<0.001
Absolute lymphocyte count (cells/μL)	1.6 (1.2–2.1)	1.6 (1.3–2.0)	1.5 (1.2–2.0)	1.5 (1.2–2.1)	1.4 (0.7–2.2)	0.343
Platelets (platelets/μL)	265 (211–335)	257 (203–319)	260 (210–328)	303 (232–382)	273 (178–414)	0.002
CD4 cell count (cells/μL)[b]						
Median (IQR)	169 (96–231)	192 (126–248)	157 (86–227)	143 (60–201)	129 (35–184)	<0.001
CD4 0–99	123 (25.5)	38 (17.7)	34 (28.1)	43 (35.3)	8 (32.0)	0.002
CD4 100–199	181 (37.5)	77 (35.8)	46 (38.0)	47 (38.5)	11 (44.0)	
CD4 ≥200	179 (37.1)	100 (46.5)	41 (33.9)	32 (26.2)	6 (24.0)	
Log viral load (copies/ml), Median (IQR)	4.6 (4.0–5.0)	4.4 (3.8–4.8)	4.6 (4.1–5.0)	4.8 (4.3–5.3)	5.2 (4.4–5.6)	<0.001
WHO stage at enrolment						
1 or 2	325 (67.0)	167 (77.3)	84 (69.4)	65 (52.9)	9 (36.0)	<0.001
3 or 4	160 (33.0)	49 (22.7)	37 (30.6)	58 (47.2)	16 (64.0)	
Tuberculosis						
History of previous TB (%)	130 (26.8)	55 (25.5)	35 (28.9)	33 (26.8)	7 (28.0)	0.920
Positive WHO symptom screen (%)	333 (68.7)	142 (67.0)	83 (70.9)	87 (66.4)	21 (84.0)	0.312
Prevalence of current culture-confirmed TB (%)	81 (16.7)	19 (8.8)	20 (16.5)	32 (26.0)	10 (40.0)	<0.001

[a] Median (IQR)

[b] n=483

TABLE 2

Univariable and multivariable analysis of factors associated with moderate or severe anemia among HIV-infected patients (n=485)

Risk factor	Unadjusted OR (95%CI)	P-value	Adjusted OR (95%CI)	P-value
Age (years) -for every 1 unit increase	1.00 (0.97–1.02)	0.6804	-	-
Female gender	4.30 (2.64–6.99)	<0.0001	8.00 (4.56–14.05)	<0.0001
Pregnancy	0.97 (0.37–2.58)	0.9542	-	-
Tuberculosis	3.03 (1.86–4.94)	<0.0001	2.62 (1.49–4.64)	0.0009
BMI (kg/m^2)- for every 1 unit decrease	1.06 (1.02–1.10)	0.0017	1.11 (1.06–1.16)	<0.0001
CD4 cell counts (cells/uL)- for every 50 unit decrease	1.20 (1.09–1.33)	0.0002	1.14 (1.01–1.29)	0.0242
Log viral load (log copies/mL)- for every 1 unit increase	2.07 (1.53–2.81)	<0.0001	1.65 (1.17–2.33)	0.0030

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TABLE 3

Diagnostic utility of novel diagnostics for the detection of TB among patients with HIV-associated anaemia; **(a)** sensitivity among patients with culture-confirmed TB **(b)** PPV and NPV among the overall cohort **(c)** specificity among patients without culture confirmed TB

a)

	TB Prevalence % (95% CI)	Sputum microscopy		Sputum Xpert x1		LAM		LAM+sputum microscopy		LAM+ SputumXpert	
TB patients		**Number**	**Sensitivity (95% CI)**	**Number**	**Sensitivity (95% CI)**	**Number**	**Sensitivity (95% CI)**	**Number**	**Sensitivity (95% CI)**	**Number**	**Sensitivity (95% CI)**
Overall (n=81)	-	24	30 (20–41)	47	58 (47–69)	23	28 (19–39)	36	44 (33–56)	50	61 (50–72)
No anaemia or mild anaemia (n=39)	-	6	15 (6–31)	16	41 (26–58)	0	0 (0–9)	6	15 (6–31)	16	41 (25–57)
Moderate or severe anaemia (n=42)	-	18	43 (28–59)	31	74 (58–86)	23	55 (39–70)	30	71 (55–84)	34	81 (66–91)
P-value[1]	-	-	0.007	-	0.003	-	<0.001	-	<0.001	-	<0.001

b)

Overall cohort	**TB Prevalence % (95% CI)**	**Sputum microscopy**		**Sputum Xpert x1**		**LAM**		**LAM+sputum microscopy**		**LAM+ SputumXpert**	
		PPV (95% CI)	**NPV (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**	**PPV (95% CI)**	**NPV (95% CI)**
Overall (n=485)	17 (13–20)	96 (78–100)	88 (84–90)	92 (80–98)	92 (89–94)	79 (60–91)	87 (84–90)	83.7 (69–93)	90 (87–92)	83 (71–91)	93 (90–95)
No anaemia or mild anaemia (n=337)	12 (8–15)	100 (52–100)	90 (86–93)	84 (60–96)	93 (89–95)	0 (0–60)	88 (84–91)	60 (27–86)	90 (86–93)	70 (47–86)	93 (89–95)
Moderate or severe anaemia (n=148)	28 (21–36)	95 (72–100)	81.4 (73.4–87.5)	97 (82–100)	91 (83–95)	92 (72–98)	85 (77–90)	91 (75–98)	90 (82–94)	92 (77–98)	93 (86–97)

c)

	TB Prevalence % (95% CI)	Sputum microscopy		Sputum Xpert x1		LAM		LAM+sputum microscopy		LAM+ SputumXpert	
		Number	**Specificity (95% CI)**	**Number**	**Specificity (95% CI)**	**Number**	**Specificity (95% CI)**	**Number**	**Specificity (95% CI)**	**Number**	**Specificity (95% CI)**
Overall (n=404)	-	403	100 (98–100)	400	99 (97–100)	398	99 (97–99)	403	100 (98–100)	400	99 (97–100)
No anaemia or mild anemia (n=298)	-	298	100 (99–100)	295	99 (97–100)	294	99 (97–100)	294	99 (97–100)	291	98 (95–99)
Moderate or severe anaemia (n=106)	-	105	99 (95–100)	105	99 (95–100)	104	98 (93–100)	103	97 (92–99)	103	97 (92–99)

[1]P value for comparison of sensitivity of diagnostic assays in those with no anaemia/mild anaemia versus those with moderate/severe anaemia.