Exposure and Clinical Features Associated With Culture Positivity Among Patients Presumed To Have TB Disease in the EAPHLNP - OR Project Sites, Kenya

*Wanzala P[1], Mwangi M[1,] Sang WK[2,] Kiptoo M[3], Githui W. A[4]

1. *Kenya Medical Research Institute(KEMRI)-Centre for Public Health Research*
2. *KEMRI-Centre for Microbiology Research*
3. *South Eastern Kenya University*
4. *KEMRI-Centre for Respiratory Disease Research*

Corresponding Author: Wanzala P. Email: wanzap2003@yahoo.com

Summary

INTRODUCTION

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. Triage is the initial stage that persons presumed to have TB will be directed to for proper disease management procedures. Diagnosis of Pulmonary TB (PTB) in adults is usually done using clinical cardinal signs and symptoms followed by direct smear microscopy and sometimes with molecular techniques such GeneXpert where available.

Although Lowenstein-Jensen (LJ) Culture is a gold standard for diagnostic of TB, it is not routinely performed. There is limited information on the association between exposure factors and other clinical features apart from the cardinal signs with culture positivity.

OBJECTIVE

The aim of this study was to determine Socio-demographic factors, Clinical features and Laboratory diagnostic results associated with culture on confirmed diagnosis of Pulmonary Tuberculosis (PTB) .

METHODOLOGY

A total of 1869 participants presumed to have TB in Health facilities were enrolled in the East Africa Public Health Laboratories Network Project - Operational Research TB (EAPHLNP - OR TB) study.

During the triage, clinical history and physical examination was done. TB clinical symptoms and signs were recorded in a structured medical form and that included the following: productive cough, weight loss, night sweats, low grade fever (classical cardinal signs and symptoms), general patient condition, body temperature, Body Mass Index (BMI), blood pressure, pulse, respiratory rate, heart sounds (vital signs and symptoms), pallor conjunctival presentation(aneamia), neck swellings(*lymphadenopathy*), difficulty in breathing, ease of air entry, percussion and auscultation findings(respiratory signs and symptoms).

Sputum smear microscopy with Ziehl-Neelsen (ZN) microscopy and Light Emitting Diodes (LEDs) for Fluorescent Microscopy (FM) , GeneXpert and Culture. Lowenstein-Jensen solid media was used as a gold standard for isolation of mycobacteria. *Capilia* Immuno-Chromagraphic Assay (ICA) was used to identify Mycobacterium Tuberculosis Complex(MTBC). Data was analysed with SPSS software and presented as frequency and percentages. The Odd Ratio (OR)

and 95% Confidence Intervals (CI) was performed using a multivariate logistic regression model and Adjusted Odds Ratios (AOR) was performed for a final parsimonious multivariate logistic regression model .

Using a systematic backward approach, non-significant variables were removed from the model until no further variables were eligible for removal to arrive at the final parsimonious model. A p-value of <0.05 in the final model was considered statistically significant.

RESULTS

Significant results from the final parsimonious multivariate logistic regression model included sociodemographic factors : Only one study site AOR = 3.91 (95% CI = 1.76 - 8.71) and age group (20-29 years) Adjusted Odds Rates (AOR) = 2.13 (95% CI = 1.10 - 4.10). Male gender showed marginal association 1.39 (95% CI = 0.96 - 2.01). Physical examination finding significant were weight loss AOR = 1.94 (95% CI = 1.16-3.25), Hepatomegaly AOR = 0.22 (95% CI = 0.05 - 0.92). Other cardinal signs and symptoms of TB were not significantly associated.

CONCLUSION

The clinical signs and symptoms associated with TB in the parsimonious multivariate logistic regression model may be indicative of health seeking behavior of participants presumed to be having TB. However, these clinical findings provides an opportunity for consideration of which signs and symptoms to be inclusive in improving early TB clinical diagnosis

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Introduction

Tuberculosis (TB) remains a major cause of morbidity and mortality worldwide. Systematic screening for active TB is defined as the systematic identification of people with suspected active TB, in a predetermined target group, using tests, examinations or other procedures that can be applied rapidly [1].

Options for the initial screening include Screening for symptoms (screening either for cough lasting for longer than 2 weeks), or Screening for any symptom compatible with TB, including cough of any duration, haemoptysis, weight loss, fever or night sweats.

Diagnosis of pulmonary TB (PTB) in adults is usually done using clinical cardinal signs and symptoms followed by direct smear microscopy in (ZN and LED), and sometimes with molecular techniques such GeneExpert where available [2,3,4].

Globally the diagnosis of TB has been hampered by HIV pandemic [5,6] . HIV increases the rate of recurrent TB in this setting by increasing the rate of re-infection disease, not relapse [7].

Culture is mostly performed at designated specific centres (WHO-algorithm for diagnosis of and drug susceptibility testing), but it has a long turn around time [1].

TB was the leading cause of morbidity and mortality in Kenya [8]. Sputum culture and newer diagnostic techniques, although useful, were expensive and not widely available in resource-poor settings.

In Kenya, sputum culture is not usually performed for routine diagnosis due to several reasons. These include lack of adequate infrastructure focussing on safety issues, and skilled manpower in several health facilities.

Case detection in Kenya was mainly based on clinical triaging, microscopy and use of signs alone (which have high concordance). There was a possibility for the use of exposure factors, signs and symptoms, in settings with the absence or inadequate laboratory diagnostics.

There was likelly limited information on the association between exposure factors and other clinical

features apart from the cardinal signs with culture positivity.

During the validation phase of the new TB diagnostics in the EAPHLNP, there arose an opportunity to perform culture on the specimens from people presumed to have TB who enrolled in the EAPHLNP project.

The aim of this study was to determine Socio-demographic factors, Clinical features and Laboratory diagnostic results associated with culture-confirmed PTB .

Methodology

In this cross-sectional study patients for intra-examiner and patients for inter-examiner calibrations were selected from eligible respondents with symptoms or signs suggestive of TB. During the implementation of the East Africa Public Health laboratories Network Project (EAPHLNP) in nine satelite sites in Kenya.

TB clinical symptoms and signs recorded in a structured medical form include the following:

> Productive cough, Weight loss, Night sweats, Low grade fever(Classical Cardinal Signs and Symptoms), general patient condition, Body temperature, Body Mass Index(BMI), Blood Pressure, Pulse, Respiratory rate, Heart - Sounds (Vital Signs and Symptoms), Conjunctival presentation (Aneamia), Neck swellings *(lymphadenopathy*), Difficulty in breathing, Ease of air entry, Respiratory Signs and Symptoms) Percussion and auscultation findings.

These were obtained by the history of mor-bidities and co-morbidities and physical examination of the enrolled patients.

Using quality assurance sampling for a total population of ten thousand people with symptoms or signs suggestive of TB from the satellite sites. The minimum defective sample acceptable was 0 with a probability of defect accepted at 1% and an alpha of 5% . The sample size of repeatable samples was 262 patients in total for the satellite sites per year.

This gave a sampling interval of approximately 50 patients per site per year. This translated to 1 randomly selected patient per site per week.

Matching was done by the site clinical supervisor to ensure that every examiner was paired with another examiner so that the recommended number of matched results were obtained for each examiner.

The results were in custody of the site clinical supervisor and released quarterly to the study team. The serial number of which patient to selected was computer generated.

These randomly selected recruited eligible patient suspects in the study then underwent intra-examiner (same clinician making two independent examinations on same patient at recruitment and at delivery of morning sample) and between clinicians or inter-examiner (two clinicians making two independent examinations on the same patient) the same day. Calibration of the weight, height and temperature tools for consistency were also done at the different sites.

TB diagnosis was first done by sputum smear microscopy Ziehl–Neelsen stain(ZN), secondly by optimized sputum smear microscopy with a Light Emitting Diodes microscope (LED) or fluorescent microscopy(FM), and thirdly by GeneXpert technique (GeneXpert or Gx).

The results from the clinicians and reference laboratory findings for these patients were entered in a computer, verified and analyzed in SPSS for reliability statistics of the screening performance of all combinations and separate (Signs and Symptoms vs TB laboratory diagnosis) variables of interest.

These unweighted Cohen Kappa scores were interpreted as follows:

Poor	0.01	–	0.20
Moderate	0.21	–	0.40
Fair	0.41	–	0.60
Good	0.61	–	0.80
Excellent	0.81	–	1.0

based on the agreement between the intra-examiner and inter-examiner findings in different TB laboratory diagnosis / HIV categories

Bivariate analysis was done to determine the association between culture positivity and socio-demographic clinical features of patients and results of ZN, FM and GeneExpert. Multivariate binary logistic regression was also performed on these variables to determine the effects of covariate adjustment.

Table 1: *Socio-Demographic Factors*

Variables	Cultue Positive (n=265)		Culture Negative (n=1424)		Bivariate Analysis				Multivariate Analysis			
					OR	95% CI		p value	AOR	95% CI		p value
	n	%	n	%		L	U			L	U	
Overall	265	15.7%	1424	84.3%								
Study site												
Busia	42	13.7%	265	86.3%	1.14	0.75	1.72	0.541	0.79	0.43	1.45	0.443
Kitale	73	19.4%	304	80.6%	1.72	1.20	2.47	**0.003**	1.51	0.76	3.01	0.239
Machakos	24	19.8%	97	80.2%	1.78	1.06	2.97	**0.029**	1.49	0.70	3.15	0.299
Kisii-Keroka	16	21.9%	57	78.1%	2.01	1.10	3.71	**0.024**	3.91	1.76	8.71	**0.001**
Nyahururu	17	20.5%	66	79.5%	1.85	1.02	3.34	**0.041**	2.14	0.95	4.83	0.067
Narok*	7	16.7%	35	83.3%	1.44	0.61	3.36	0.405	2.37	0.81	6.95	0.116
Lamu*	3	30.0%	7	70.0%	3.08	0.78	12.18	0.110	7.57	1.50	38.22	**0.014**
Wajir*	15	12.5%	105	87.5%	1.03	0.56	1.86	0.935	0.76	0.33	1.74	0.514
Malindi	68	12.2%	488	87.8%	1.00				1.00			
Gender												
Male	165	19.1%	701	80.9%	1.70	1.30	2.23	**<0.001**	1.39	0.96	2.01	0.078
Female	100	12.2%	723	87.8%	1.00				1.00			
Age in years												
<20 years	22	15.6%	119	84.4%	2.40	1.24	4.65	**0.009**	0.89	0.37	2.12	0.787
20 - 29 years	81	22.2%	284	77.8%	3.71	2.16	6.36	**<0.001**	2.13	1.10	4.10	**0.024**
30 - 39 years	76	19.2%	319	80.8%	3.10	1.80	5.32	**<0.001**	1.55	0.80	2.99	0.197
40 - 49 years	36	12.5%	251	87.5%	1.87	1.03	3.37	**0.040**	1.16	0.56	2.39	0.684
50 - 59 years	20	10.6%	168	89.4%	1.55	0.79	3.02	0.199	0.97	0.43	2.19	0.936
Missing	12	19.7%	49	80.3%	3.18	1.44	7.03	**0.004**	1.17	0.41	3.31	0.766
60 and above years	18	7.1%	234	92.9%	1.00				1.00			

** sample size inadequate* **OR** : Odd Ratio **CI** : Confidence Interval **AOR** : Adjusted Ratio

Results

The extended Mantei-Haenszel chi-square for trend between sites was 5.83 (p =0.02) showing significant differences in occurrence of culture positivity. Significant results from the final parsimonious multivariate logistic regression model include socio-demographic factors: Only one study site AOR = 3.91 (95% CI = 1.76 - 8.71) and age group (20 - 29 years) AOR = 2.13 (95% CI = 1.10 - 4.10). Male gender showed marginal association 1.39 (95% CI = 0.96 - 2.01). In a multivariate logistic regression parsimonious model indicated that confounding, occurred at Kitale, Machakos and Nyahururu sites.

The odds ratios for Keroka and Nyahururu doubled after showing confounding adjustment for gender and all ages in the reproductive age except 20-25 years was indicative of confounding.

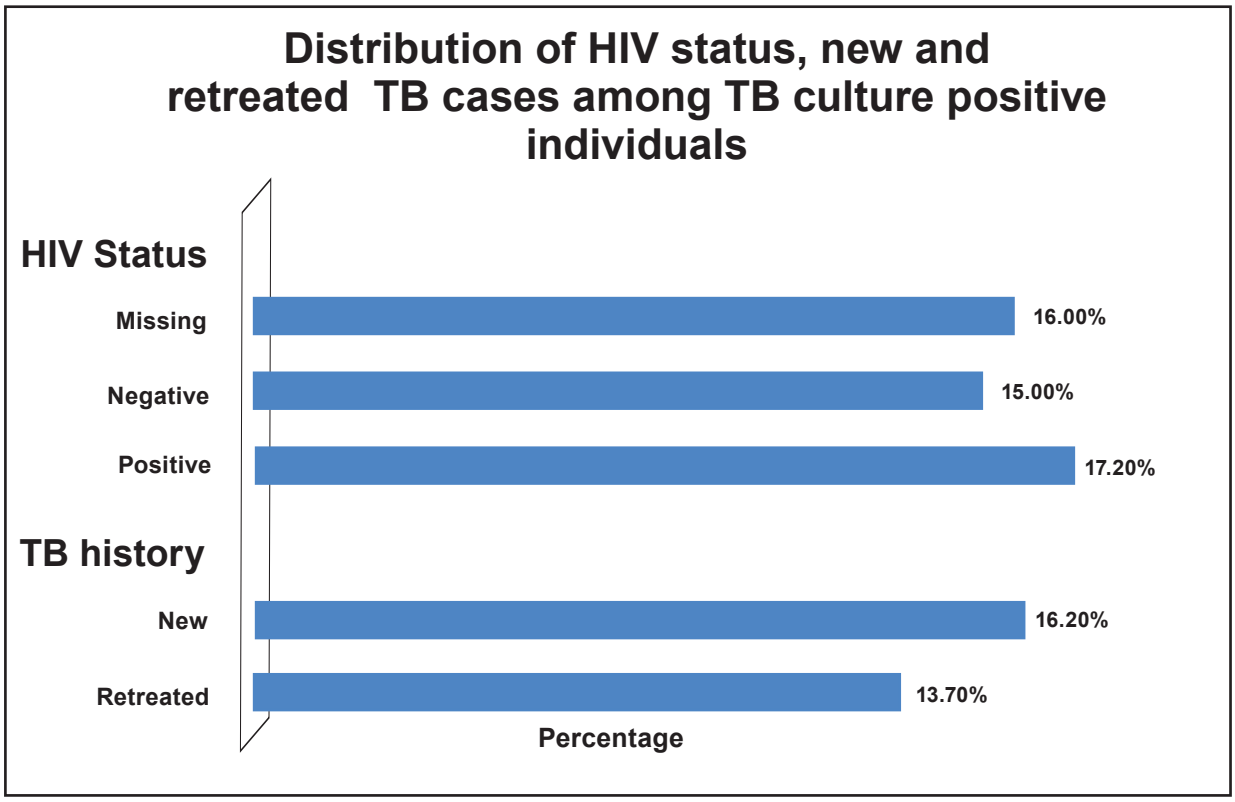


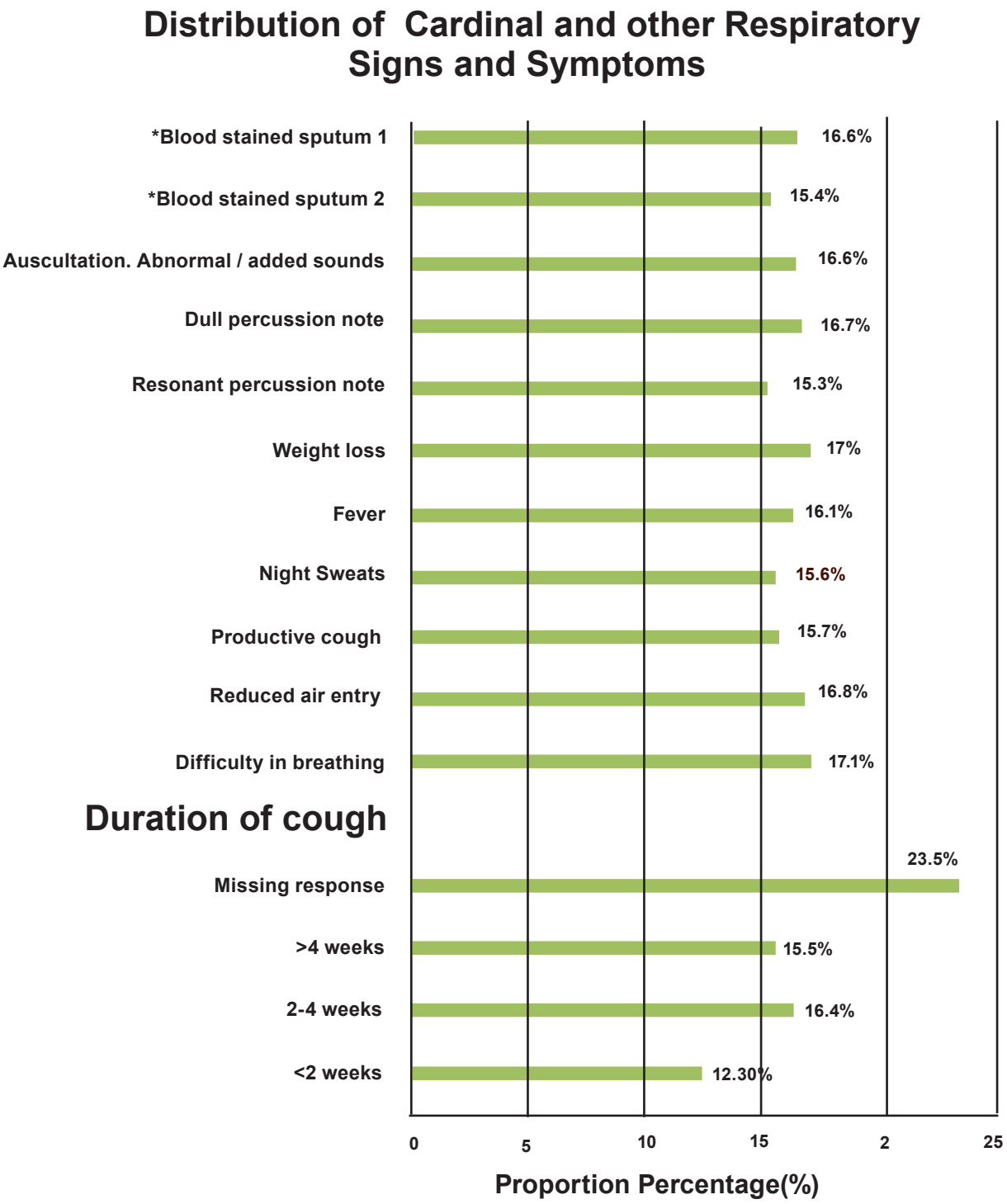
Figure 1: *Distribution Of HIV Status, New and Retreated TB Cases Among TB Culture Positive Individuals*

Table 2: *HIV Status, New and Retreated TB Cases Among TB Culture Positive Individuals*

			Bivariate Analysis			
Variables	**Positive (n=265)**	**Negative (n=1424)**	**OR**	**95% CI**		**p value**
				L	**U**	
TB History						
Retreated	34	215	1			
New	231	1191	1.23	0.83	1.81	0.302
Missing	0	18	ND	ND	ND	0.998
HIV status						
Positive	61	293	1.18	0.85	1.65	0.316
Negative	135	768	1			
Missing	69	363	1.081	0.789	1.483	0.627

Key: ***95% CI:*** *Confidence Interval,* ***OR:*** *Odds Ratio,* ***L:*** *Lower limit,* ***U:*** *Upper limit*

There was no significant difference between participants with TB retreated and newly TB diagnosed individuals with TB culture positivity. HIV status was not significantly associated with TB culture positivity.



* *Repeat red herring question for reliability of response*

Figure 2: *Distribution Of Cardinal and Other Respiratory Signs and Symptoms*

Table 3: Cardinal and Other Respiratory Signs and Symptoms.

Variables			Bivariate analysis				Multivariate Analysis			
	positive +n	Negative - n	OR	95% CI		P value	AOR	95% CI		P value
				L	U			L	U	
Duration of cough										
<2 weeks	16	114	1.00							
2- 4 weeks	99	504	1.40	0.80	2.47	0.244				
>4 weeks	146	793	1.31	0.76	2.28	0.335				
Missing	4	13	2.19	0.64	7.55	0.214				
Productive cough										
Yes	259	1392	0.99	0.41	2.40	0.986				
No	6	32	1.00							
Blood stained sputum										
Yes	63	316	1.09	0.80	1.49	0.571				
No	202	1108	1.00							
Night sweats										
Yes	228	1235	0.94	0.65	1.38	0.762				
No	37	189	1.00							
Fever										
Yes	220	1144	1.20	0.85	1.69	0.309				
No	45	280	1.00							
Weight loss										
Yes	229	1121	1.72	1.18	2.50	<0.001	1.94	1.16	3.25	0.012
No	36	303	1.00				1.00			
Blood stained sputum										
Yes	50	274	0.98	0.70	1.36	0.887				
No	215	1150	1.00							
Difficulty in breathing										
Yes	102	496	1.17	0.89	1.53	0.253				
No	163	928	1.00							
Air entry										
Reduced	112	553	1.15	0.88	1.50	0.294				
Normal	153	871	1.00							
Precussion note										
Resonant	175	966	1.01	0.56	1.82	0.975				
Dull	76	380	1.11	0.60	2.07	0.732				
Normal	14	78	1.00							
Auscultation										
Abnormal / added sounds	101	508	1.11	0.85	1.46	0.448				
Normal	164	916	1.00							

Key: *95% CI: Confidence Interval,* ***OR:*** *Odd Ratio,* ***AOR:*** *Adjusted Ratio,* ***L:*** *Lower limit,* ***U:*** *Upper lim*

Only weight loss showed significant association with culture positivity (OR =0 1.72(95% CI 1.18-2.50)p =0.012) Similar crude and adjusted odds ratios indicated that weight loss was an effect modifier. The odds ratios showed no statistical significant differences between the other signs and symptoms.

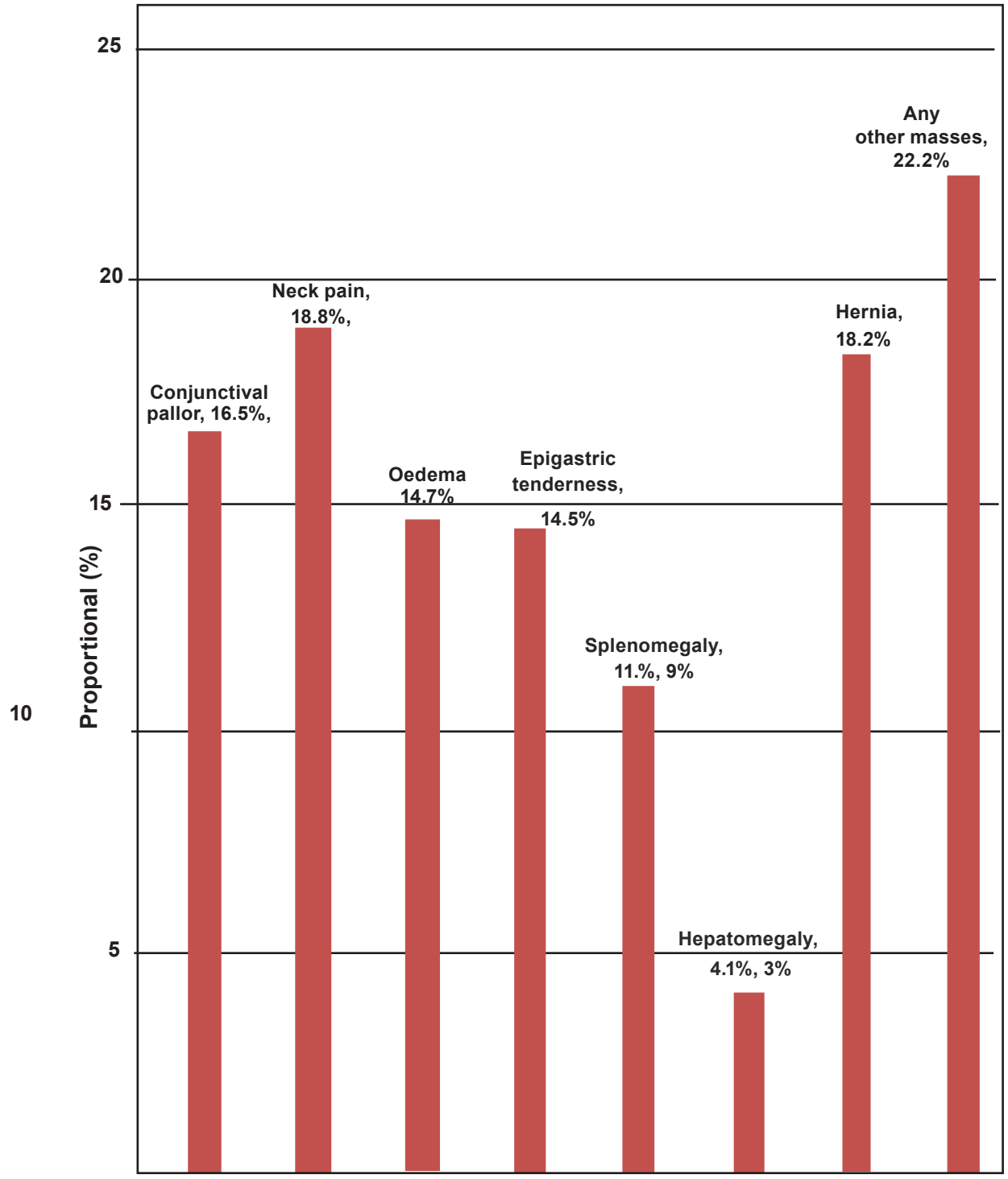


Figure 3: *Distribution of Systemic Signs and Symptoms Among TB Culture Positive Individuals*

Table 4: Distribution Of Systemic Signs and Symptoms Among TB Culture Positive Individuals

Variables	Culture		Bivariate Analysis			
	Positive (n=365)	Negative (n=1424)	OR	95% CI		p value
	n	n		L	U	
Conjuctiva						
Pallor	44	222	1.08	0.76	1.54	0.677
Normal	221	1202	1.00			
Neck pain						
Yes	21	91	1.26	0.77	2.07	0.357
No	244	1333	1.00			
Oedema						
Present	11	64	0.92	0.48	1.77	0.803
Absent	254	1360	1.00			
Epigastric pain						
Yes	42	247	0.90	0.63	1.28	0.553
No	223	1177	1.00			
Splenomegaly						
Yes	14	113	0.65	0.37	1.15	0.133
No	251	1311	1.00			
Hepatomegaly						
Yes	2	47	0.22	0.05	0.92	0.023
No	263	1377	1.00			
Hernia						
Yes	4	18	1.20	0.40	3.57	0.746
No	261	1406	1.00			
Any other masses						
Yes	2	7	1.54	0.32	7.45	0.639
No	263	1417	1.00			

Key: ***95% CI:*** *Confidence Interval,* ***OR:*** *Odd Ratio,* ***AOR:*** *Adjusted Ratio,* ***L:*** *Lower limit,* ***U:*** *Upper limit*

Significant reduction of *hepatomegaly* was found in TB culture positive individuals (OR = 0.22 (95% CI = 0.05 - 0.92) p = 0.023). The odds ratios showed no statistical significant differences between these signs and symptoms

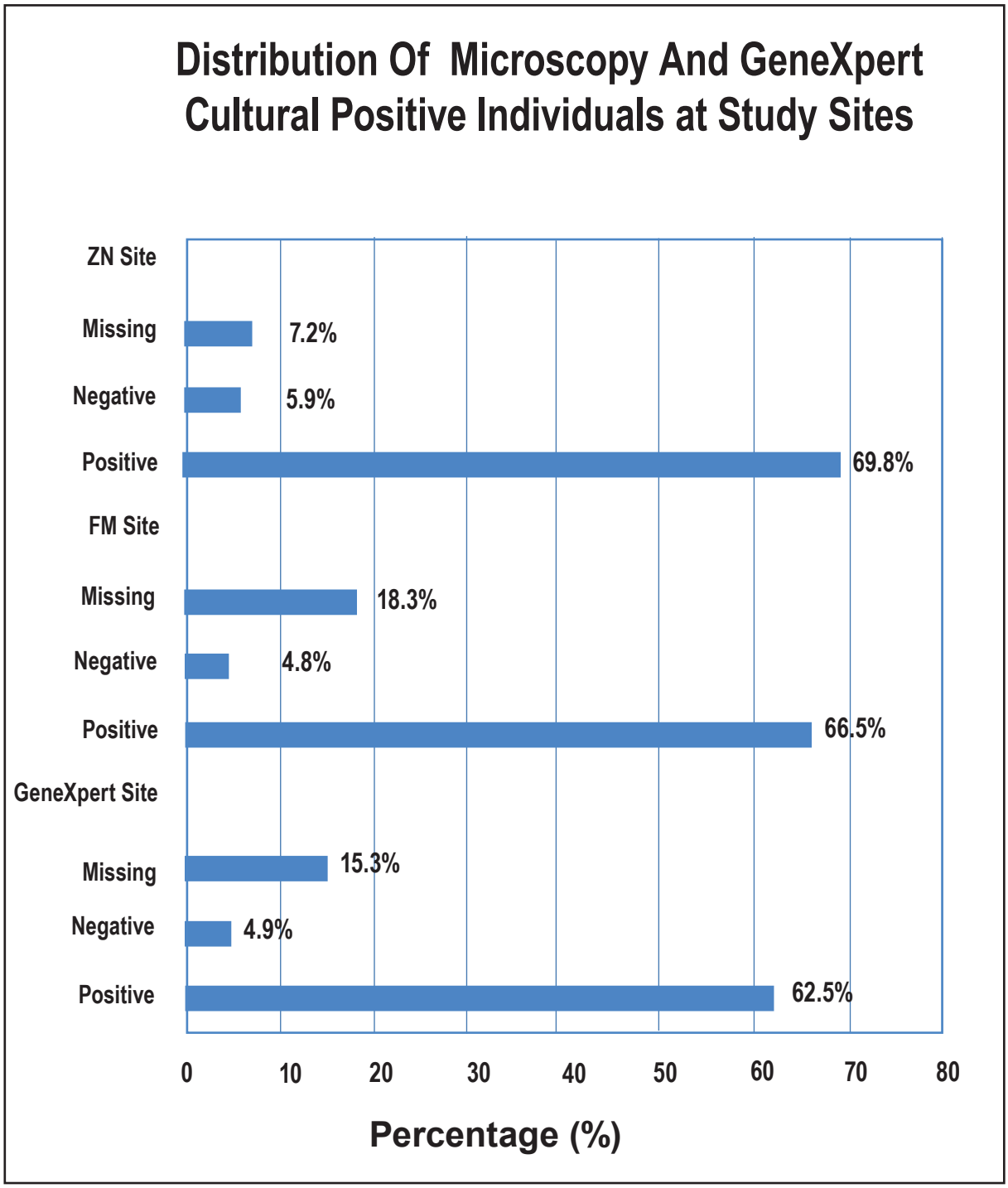


Figure 4: *Distribution Of Microscopy and GeneXpert In Culture Positive Individuals At Study Sites.*

***Table 5**: Microscopy and GeneXpert In Culture Positive Individuals at Study Sites*

Variables	Culture		Bivariate Analysis				Multivariate Analysis			
	Positive +n	Negative -n	OR	95% CI		P value	AOR	95% CI		P value
				L	U			L	U	
ZN site										
Positive	178	77	36.57	25.69	52.04	**<0.001**	7.45	4.25	13.06	**<0.001**
Negative	77	1218	1.00				1.00			
Missing	10	129	1.226	.619	2.429	0.559	0.78	0.36	1.71	0.537
FM site										
Positive	143	72	39.12	26.34	58.10	**<0.001**	7.19	3.89	13.29	**<0.001**
Negative	53	1044	1.00				1.00			
Missing	69	308	4.413	3.018	6.452	**<0.001**	1.82	1.04	3.17	**0.036**
GeneXpert site										
Positive	70	42	32.35	18.21	57.45	**<0.001**	3.89	1.86	8.14	**<0.00**
Negative	22	427	1.00				1.00			
Missing	173	955	3.516	2.224	5.559	**<0.001**	1.13	0.59	2.18	**0.716**

Key: *95% CI: Confidence Interval, **OR:** Odd Ratio, **AOR:** Adjusted Ratio, **L:** Lower limit, **U:** Upper limit*

Discussion

The socio-demographic, clinical signs and symptoms associated with TB in the parsimonious multivariate logistic regression model included the Kisii-Keroka site. Weight loss, lack of hepatomegaly and TB diagnostics. These factors may be indicative of health seeking behaviour of participants presumed to have TB.

However, this clinical information provides consideration of which additional signs and symptoms to include improving early TB clinical diagnosis. The sample size was in Lamu was small, limiting interpretation of the significant association with TB culture positivity.

The extent to which the signs and symptoms are associated with culture positivity show the extent to which damage has occurred to the various parts of the body. Systemic examination for the different signs and symptom revealed, lack of association with culture positivity except with weight los. lack of hepatomegaly. The speed of progression to clinically detectable disease depends largely on the immune competency of the host.

Apparently, there was lack of association between HIV and Culture Positivity in this study . This was also observed in a study in Luska , Zambia where there was a strong trend towards lower grade or negative sputum smear in the HIV-positive group (P = 0.003)[9]. HIV-positive cases also had lower colony counts on Culture and colonies took longer to appear.

The primary objective of screening for active TB was to ensure that, active TB was detected early and treatment initiated promptly. The ultimate aim is reducing the risk of poor treatment outcome, health sequelae, adverse social and economic consequences of TB in helping to reduce TB transmission globally.

For TB control, the highest priority was to detect at least 70% of the sputum smear positive cases and to cure at least 85% of the sputum smear positive cases. If these targets are achieved, there will be a decrease in prevalence incidences of transmission and drug resistance to TB [10,11].

Microscopy was significantly associated with culture positivity focusing on the likelihood of success of screening in resource constrained settings.

In a case control study in Southern Ethiopia smear-positive pulmonary TB was associated with HIV infections, previous episodes of TB, low body-mass index, drinking unpasteurized milk and sharing a bedroom with more than three individuals.

Even though not significant after controlling the effect of confounders; being male gender, illiteracy, low monthly income, being farmer and bad housing condition were associated with active pulmonary TB in univariate analysis [12]

.Acknowledgement

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