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Pooled Sputum for Xpert MTB/RIF Testing: A Cost Effectiveness Diagnostic Tool in Mwanza, Tanzania

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Abstract

Background: Tuberculosis (TB) is a global public health problem, with the highest burden occurring in low-income countries, wherein the use of more sensitive diagnostics, such as Xpert MTB/RIF (GeneXpert), is still limited by costs. Testing of pooled samples from various individuals has been thought and thus investigated as a cost saving strategy to diagnose some diseases including TB. Then in cases where a pool is positive, retesting of the individual samples in that pool is done to identify the positive sample. We assessed the utility of a pooled testing strategy to optimize the affordability of GeneXpert for the diagnosis of TB Mwanza Tanzania.

Methods: Remainder of samples from presumptive TB patients submitted for routine TB diagnosis were used for pooled samples (5 per pool) testing. The agreement of the results between individual sample testing against pooled samples testing and cost-effectiveness were assessed.

Results: A total of 250 individual routinely submitted samples for TB diagnosis were tested using the established protocols. The median age of study participants was 35 [IQR 27 – 40] years and 143 (57.2%) were males. Of the 250 samples, 28 (11.2%) were detected to have MTB whereas 222 (88.8%) samples, were negative. Of the 50 sputum pools made, MTB were detected in 17 (34.0%) pools. Following retesting of these 17 positive pools, all 28 (100%) individual MTB samples were detected with the overall agreement being 100% (With the sensitivity of 100% and specificity of 100%). The number of individual MTB positive ranged from 1 to 3 per pool. Using pooling of sputum samples, the technique saved 115 (46.0%) of the cartridges in running 250 samples. This is equivalent to saving US$ 1147.7.

Conclusion: The pooled sputum testing strategy reduced cartridge costs by 46.0%. The use of the pooled testing strategy reduces costs and has the potential to increase the affordability of GeneXpert testing in countries with limited resources. Pooled sputum for Xpert MTB/RIF can be used as an affordable diagnostic and/or screening tool in resource limited settings, such as Tanzania.

Introduction

Despite the reduction in Tuberculosis (TB) associated deaths of up to 47.0% since 1990, TB remains one of the deadliest infectious diseases in the world. Ranking above HIV as the world's greatest health threats, TB is on top of the list of causes of death from a single infectious agent accounting for around 1.4 million deaths in 2019 [1]. This death toll is unacceptably high and early detection and treatment is of paramount importance. Despite the decline of TB incidence from 306 per 100,000 populations in 2015 to 253 per 100,000 in 2018, Tanzania remains one of the high TB countries [2]. The introduction of the Xpert/MTB RIF in the diagnosis of TB and detection of the bacterial resistance to rifampicin in 2010, by the WHO, has been seen as a major diagnostic revolution as far as TB is concerned [3]. This is a fully automated real-time PCR assay that simultaneously detects *Mycobacterium tuberculosis* (MTB) and resistance to rifampicin in less than 2 hours [4]. The assay provides an opportunity to select treatment options and therefore serves a significant role in the detection and management of TB.

However, despite the achievements brought about by the introduction of the GeneXpert, the running costs and difficulties in supply chains are still a major challenge in low and middle-income countries like Tanzania. A very sensitive and affordable diagnostic tool must be in place to achieve the goal of TB by 2035 [5]. The GeneXpert is very sensitive but fails to address the problem of affordability in the context of low-income countries. Pooling of sputum samples to test for GeneXpert MTB/RIF has been investigated as a cost-effective strategy to be able to serve as many patients as possible with the available limited resources. This strategy has been assessed for its effectiveness in Nigeria and South Africa and there has been a high agreement between the pooled and individual samples [6, 7]. The purpose of this study was to determine the effectiveness of this approach to be able to employ it in the screening and/or diagnosis of TB. This was done by assessing the diagnostic utility of the test, cost-effectiveness as well as establishing the optimum-pooling ratio in the context of our country.

Materials And Methods

Study Design

This was a cross sectional study performed between May to July 2018 on remaining sputum samples submitted and tested routinely for TB diagnosis at Bugando Medical Centre laboratory. Bugando Medical centre is a 1000-bed tertiary hospital located in Mwanza Tanzania serving about 15.0% of the Country's population of about 60 million. A total of 250 samples from the remainder of samples from presumptive TB patients submitted for routine GeneXpert MTB/RIF testing at Bugando Medical Centre Laboratory were used for pooling analysis. A**ll methods were carried out in accordance with standard operating procedures, relevant guidelines and regulations.**

Laboratory Analyses

Residual sputum samples with sufficient volume to make up a pool were randomly selected, de-identified and used to make pools of five samples per pool as determined to be an optimum pooling ratio by Zishiri *et al.,* [7] with each sample contributing 0.5mL. These pooled samples were transferred into 5mL falcon tubes, each tube making one pool and stored at -80°C until the time of testing. The MTB/RIF testing for both individual and pooled samples were carried out following the tertiary hospital laboratory standard operating procedures adopted from the manufacturer's instructions (Cepheid, Drive, Sunnyvale, & USA, 2010) [8].

Data Analysis

Data from questionnaires and laboratory forms were entered into the computer using Microsoft excel and then imported to STATA version 13 for analysis. Continuous data were summarized using median with interquartile range [IQR] whereas categorical data were summarized using frequency and proportions (percent). To determine the utility of pooled 5 samples testing using we used 2×2 contingency tables to determine the sensitivity and specificity.

Ethics approval and consent to participate

The ethical approval to conduct this study was obtained from the joint Bugando Medical Centre/Catholic University of Health and Allied Sciences (BMC/CUHAS) ethics review board (CREC 672/2018). **A waiver of informed consent was obtained from the ethics committee/institutional review board Central Pathology Laboratory to use the samples for this study.**

Results

Patient Demographics Of the 250 samples, 143 (57.2%) were from male patients. The median age for all patients was 35 (IQR 27 – 40) years. Table 1 shows the demographic information. Pooled Sputum Samples MTB/RIF Results Of the 50 pooled sputum samples, MTB was detected in 17 (34.0%) pools whereas 33 pooled sputum samples (66.0%) had no MTB detected. Of the 17 MTB positive pools, 10 (58.8%) were detected as medium while the remaining 7 (41.2) pools were detected as low MTB. Of note, there was no pool that had a high MTB detected. Re-testing of the individual samples contained into the MTB positive pooled samples Following the re-testing of the individual samples contained into the 17 MTB positive pooled samples, a total of 85 samples were analyzed individually. Of these, 28 individual samples were positive for MTB, making the overall case detection of 11.2% (28 of the 250 samples). Each MTB detected pooled samples had a range between 1 – 3 individual MTB positive samples. Individual Sputum Samples MTB/RIF Results Finally results from the individual MTB/RIF testing were retrieved for all 250 samples and there were 28 (11.2%) samples in which MTB were detected whereas 222 (88.8%) samples had no MTB detected. Of the 28 samples in which MTB was detected, 2 (7.1%) had high MTB detected results, 13 (46.4%) medium MTB detected and the rest 13 (46.4%) had low MTB detected. These results showed an agreement of 100% between individual and pooled sputum on Xpert MTB/RIF testing. Table 2 summarizes the comparison of Cycle Thresholds Results of Pooled versus Individual sputum samples. The dilution effect after pooling samples The pooling of samples has the effect in increasing the dilution (decreasing MTB concentration) as compared to the individual sample analysis. This is shown by the increase in cycle thresholds whereby pooled samples had slightly higher cycle thresholds than the original individual sample cycle threshold. Table 3 summaries the comparison of cycle thresholds and dilutions between the individual sputum samples and pooled sputum samples. Agreement of detection between Individual and Pooled Gene Xpert Results Pooling sputum Gene Xpert detected all the positive MTB cases that were also detected from the individual Gene Xpert MTB/RIF testing. There was a 100% agreement between results from pooling sputum Gene Xpert and individual Gene Xpert results. The sensitivity and specificity were 100% respectively. Cost-Effectiveness of using Pooled Sputum for MTB/RIF Testing The technique of pooling sputum, as was used in this scenario, has saved a total of 115 cartridges by using 135 cartridges instead of 250 that would have been used for individual MTB/RIF testing.

Discussion

Pooled Sputum Diagnostic Accuracy

This study has shown a level of agreement of 100% between individual and pooled sputum on Xpert MTB/RIF testing with a sensitivity and specificity of 100% respectively. These results are in-line with other studies conducted in Nigeria and South Africa [6, 7] where a sensitivity and specificity of 96%, 100% and 100%, 100% respectively were found. The concentration of MTB for positive samples was reduced in pooled sputum samples as compared to the individual samples; this was shown by the increase in cycle thresholds whereby pooled samples had slightly higher cycle thresholds than the original individual sample cycle threshold. Among all the positive individual sputum samples there was no one with a very low MTB detected as samples were randomly selected without the knowledge of the individual results. This scenario partly explains why we have a sensitivity of 100% apart from the dilution effect observed after pooling samples together. The dilution effect has also expressed itself in this study whereby there were two samples with high MTB detected but none could be identified in pooled samples as there was no single pool with a high MTB detected. This effect was also observed in a study done by Abdurrahman and colleagues [6] whereby samples with very low MTB detected turned negative when included in pools.

Cost-Effectiveness of Pooled Sputum

The strategy has proved to be cost-effective by saving 115 GeneXpert test cartridges. This implies that if the technique is employed; 50 cartridges would be used to test the 50 pooled sputum samples and re-testing individual sputum samples making the 17 pools with detected MTB results (85 cartridges) making a total of 135 test cartridges. Taking into consideration of the concession price of US$ 9.98 per cartridge [9] the strategy would have saved a total of US$ 1147.7. Furthermore, this study has shown that performing less number of tests using this technique directly saves time as it has also been shown in a study done in Nigeria [6].

Conclusion And Recommendation

This study has shown that the strategy can be used for screening and/or diagnosing MTB due to its high diagnostic accuracy but only when an optimum-pooling ratio that accommodates the dilution effect observed has been determined. It has in addition shown to save both cost and time. It is, therefore, high time to establish the optimum-pooling ratio in our settings to be able to consider using the technique.

Study Limitations

Pooling of sputum requires a biosafety cabinet and the strategy can therefore not be adapted by lower level laboratories. This strategy is also not suitable for laboratories with low sample turnover of samples for GeneXpert. Small sample size limited this study from reaching one of its objectives which aimed at establishing the pooling ratio. Also, in this study we did not have sputum samples with very low MTB detected.

Abbreviation

BMC: Bugando Medical Centre

CREC: Catholic University of Health and Allied Sciences/Bugando Medical Centre Research and Ethics Committee

CUHAS: Catholic University of Health and Allied Sciences

DAHW: German Leprosy and Tuberculosis Relief Association (Deutsches Aussätzigen-Hilfswerk)

IQR: Interquartile Range

NTLP: National Tuberculosis and Leprosy Programme

TB: Tuberculosis

WHO: World Health Organization

Xpert MTB/RIF: Xpert *Mycobacterium tuberculosis*/Rifampicin

Declarations

Ethics approval and consent to participate

The ethical approval to conduct this study was obtained from the joint Bugando Medical Centre/Catholic University of Health and Allied Sciences (BMC/CUHAS) ethics review board (CREC 672/2018). **A waiver of informed consent was obtained from the ethics committee/institutional review board Central Pathology Laboratory to use the samples for this study.**

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing of Interests

All authors declared no competing of interests

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Author's contributions

Edited and reviewed critically the manuscript: SH RVM BCO BRK GJ LGA CK. Conceived and designed the experiments: SH BRK CK. Performed the experiments: SH RVM BCO GJ LGA. Analyzed the data: SH BRK CK. Contributed reagents/materials/analysis tools: BRK CK. Wrote the paper: SH RVM BCO. All authors read and approved the final manuscript.

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Tables

Table 1: Demographic information for patients whose samples were used in the study

Patient characteristic	Median	IQR
Median age	35	27 – 40
Sex	**Number (n)**	**Percent (%)**
Male	143	57.2
Female	107	42.8

Table 2: Comparison of Cycle Thresholds Results for positive Pooled versus Individuals samples

Sputum Pool	Number of Individual MTB Detected	Individual sample CT Value	Pooled sample CT Value
P 1	2	25.4, 16.5	23.8
P 6	1	16.8	17.4
P 7	1	23.7	24.8
P 11	1	23.4	23.7
P 19	3	24.7, 26, 26.5	24.8
P 22	3	15.5, 16.7, 17.3	16
P 23	3	9.7, 19.5, 18.8	16.8
P 28	2	16.4, 17.4	16.3
P 31	1	16.7	17.4
P 32	1	23.2	26.4
P 33	1	16.3	16.9
P 40	1	23.3	24.3
P 42	2	16.3, 16.8	17.3
P 44	1	24.7	27.8
P46	1	23.8	24.6
P 48	3	23.7, 24.6, 25.4	26.7
P 49	1	16.8	17.4
Total	**28**		

Table 3. Cycles threshold and dilution and effect observed after pooling samples

	CT Range	Individual sputum samples	Pooled Sputum samples
Very Low	<16	0	0
Low	16-22	13	7
Medium	23-28	13	10
High	>28	2	0
Total		**28**	**17**