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The Cycle of Threshold Value of Cartridge Based Nucleic Acid Amplification as a Measure of Sputum Bacillary Burden

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Abstract:
Background: *To determine the correlation of cycle of threshold (CT) values of Cartridge based nucleic acid amplification (CBNAAT) with Time to culture positivity(TTCP).*
Materials and Methods*: We analyzed data retrospectively from Intermediate reference laboratory (IRL), Visakhapatnam, that tested 149 sputum samples from January to October 2019, which were Xpert positive cases. Paired sputum samples were collected and tested using Xpert and BACTEC MGIT 960 liquid culture. Analyzed an association between CT value and growth at weeks by using Chi-square test (p value<0.05).*
Results: *out of 149 Xpert positive samples, 32.21% (n=48) samples were culture positive whereas 60.40% (n=90) were negative and 7.4% (n=11) were contaminated samples. The mean CT value and mean TTCP among culture positives were 22.18 and 30.87, respectively. There was a significant association between CT values and growth at weeks (p-value <0.05). The samples with high and medium detected CT values mostly shown earlier culture positivity. Spearman correlation of mean CT value with TTCP showed a moderate positive correlation (r=0.47). Linear regression analysis found the association between MGIT TTCP and CT value of CBNAAT (R^2=0.22).*
Conclusion: *CBNAAT is an accurate test for the diagnosis of Tuberculosis. Although CT values of CBNAAT are correlating with TTCP, further studies are needed to suggest as an independent predictor of a bacillary load.*
Key Word: *Tuberculosis; Diagnostics; CBNAAT.*

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I. Introduction

Tuberculosis is one of the leading causes of mortality in the world. Despite the significant progress, Tuberculosis continues to be a global health problem. Globally, notified 7.0 million new cases of Tuberculosis in 2018- an increase from 6.4 million in 2017. In India, notifications of new cases rose from 1.2 million to 2 million between 2013 and 2018[1]. India shares nearly one-fourth of the global tuberculosis burden.

The significant primary measure in reducing tuberculosis burden is to control the transmission of Tuberculosis because before the patient gets diagnosed and treated, the transmission has already occurred to many susceptible contacts. Community settings account for the majority of tuberculosis transmission and need increased focus[2]. So, contact tracing of high transmitters helps in early diagnosis of Tuberculosis among contacts and hence, breaking the chain of transmission[3]. The bacillary burden is assessed as grades of smear positivity to evaluate the infectiousness of the patients. But, it is less sensitive and requires high bacillary counts[4]. And also to know the risk of transmission, to quantify the bacillary burden and even to predict patient treatment outcomes, culture methods are used[5]. Culture, however, is not readily available to know about the risk of transmission due to the long turnaround time.

The Cepheid Xpert assay is developed for rapid diagnosis of Tuberculosis (short turnaround time). A semi-quantitative measure of the bacillary load in sputum is given by the assay cycle threshold (CT) value, which is the number of polymerase chain reaction cycles during which each of the five probes is considered positive[6,7].

Aims and Objectives

To determine the correlation of cycle of threshold (CT) values of CBNAAT with Time to culture positivity (TTCP).

II. Materials and Methods

Study design and setting- We analyzed data retrospectively from Intermediate reference laboratory (IRL), Visakhapatnam that tested 149 sputum samples from January to October 2019, which were Xpert positive cases.

Inclusion criteria:
1.Valid Xpert results are those providing a usable result (i.e., not an invalid or error result)
2.Valid culture results.

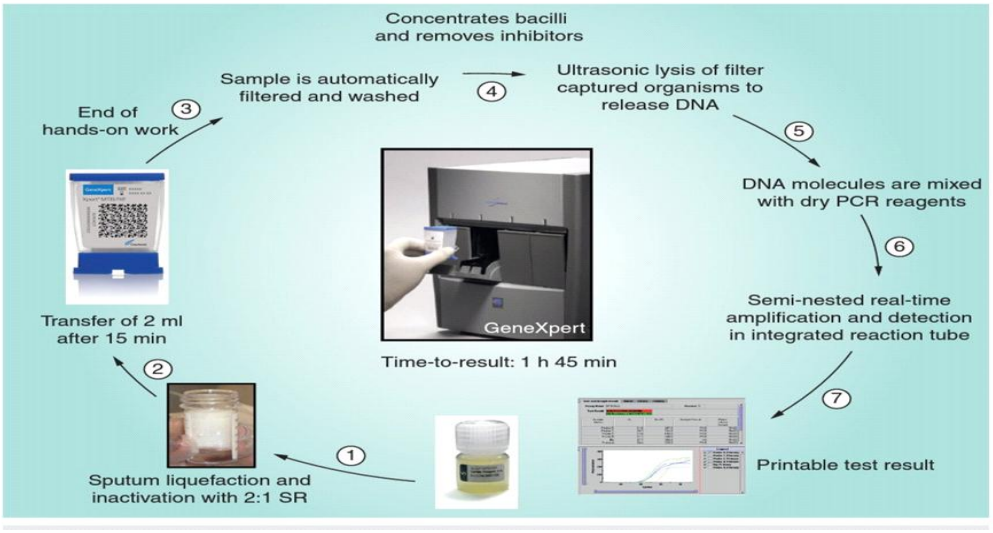
Exclusion criteria:
Xpert results with internal control value greater than 34[6].

Microbiology and measures of bacillary burden:

Paired sputum samples were collected from all patients and after decontamination of sputum with sample reagent, concentrated for 15 minutes by centrifugation. Then, the sample was tested using Xpert and BACTEC MGIT 960 liquid culture.

III. Methodology

FIG 1: VARIOUS STEPS WITHIN THE XPERT MTB/RIF ASSAY PROCEDURE[4,8]



*SR- sample reagent (sodium hydroxide and isopropanol containing reagent)

The five molecular probes which overlap (probes A-E) that are collectively complementary to the entire 81 bp rpoB core region detect Mycobacterium Tuberculosis in Xpert[7,9]. The mean of the five probes was used to quantify bacilli by Xpert, and the latter reported as mean Ct value. A semi-quantitative estimate of bacilli concentration as defined by the range of CT- High (<16 cycles), Medium (16-22 cycles), Low (22-28 cycles) and Very low (>28 cycles)[9].

M.tuberculosis was cultured in liqud culture MGIT 960. The duration between sample inoculation into the MGIT tube and the time it becomes positive was reported as days to positivity (and also in weeks). All positive cultures were cultured on blood agar to rule-out contamination.

Statistical analysis

Descriptive statistics were analyzed using proportions for categorical variables, a mean or median and interquartile range for continuous variables. Analyzed an association between CT value and growth at weeks by using Chi-square test (p value<0.05). The correlation of Xpert CT values with time to culture positivity was calculated by the Spearman correlation.Linear regression analysis was done to assess the association between Liquid culture time to positivity and CBNAAT CT values. The analysis excluded patients who were rifampicin-resistant because rifampicin resistance has shown to have delayed Polymerase Chain Reaction amplification and therefore will not have accurate CT values[10].

IV. Result

A total of 149 Xpert positive sputum samples which subjected to liquid Culture were collected. Out of 149 Xpert positive sputum samples, 32.21% (n=48) samples were culture positive whereas 60.40% (n=90) were negative and 7.4% (n=11) were contaminated samples.

Among 48 samples of Xpert and liquid Culture positive, 66.67% were males (n=32), and 33.33% were females (n=16) with a male: female ratio of 2:1. The mean age distribution was 39.12 years, while the mean age among males and females too, 39 years. Despite the gender difference, the most common presentation of Xpert

positive sputum was in young adult age groups. 89.58% (n=43) were HIV negative whereas 10.42% (n=5) were HIV positive.

The mean CT value among Xpert positives (48 samples) was 22.18±6.69 cycles, and the range was 10.16 to 33.22 cycles (**fig 2).** The mean TTCP among culture-positive was 30.87±15.47 days, and the range was 3 to 66 days **(fig 3).**

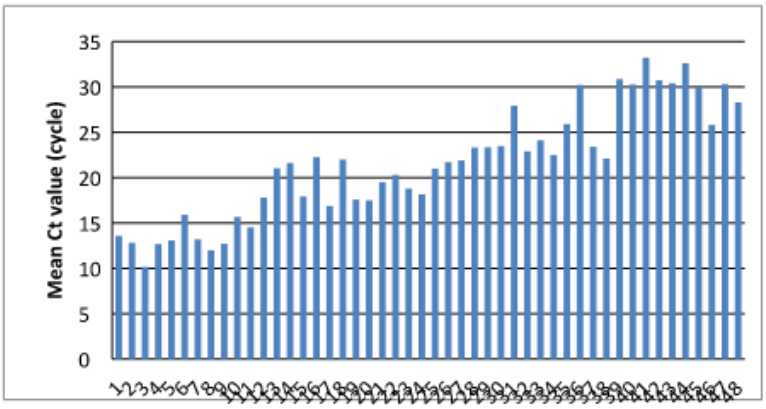


FIG 2: INDIVIDUAL'S MEAN CT VALUES AMONG XPERT POSITIVE

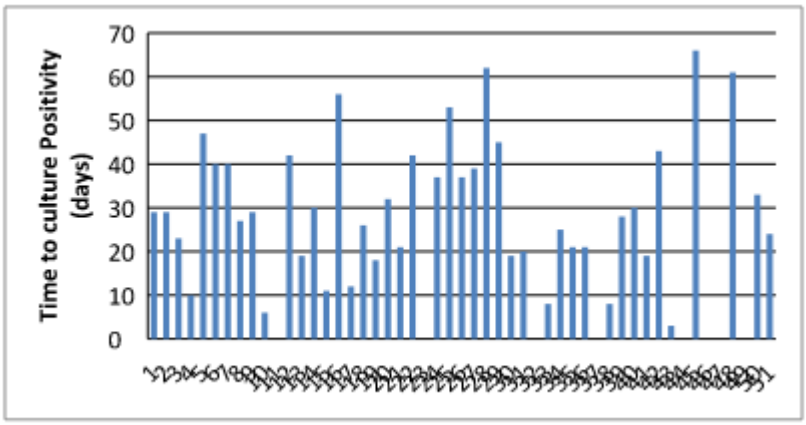


FIG 3:INDIVIDUAL'S TTCP AMONG CULTURE POSITIVE SAMPLES

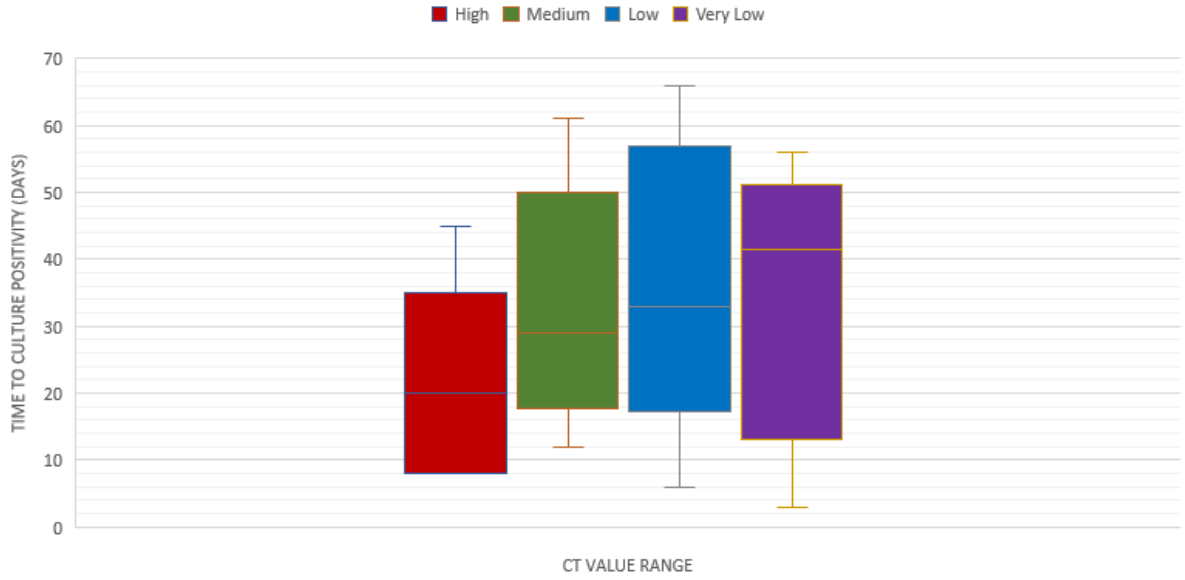
The mean CT value was similar by gender, whereas mean TTCP was slightly higher among women. The mean CT value and mean TTCP were similar among HIV uninfected and HIV infected individuals. Distribution of mean CT values and TTCP by gender and HIV status failed to show significance on using Student t-test mean comparison **(Table 1).**

	Mean CT value	p-value	Mean days to culture positivity	p-value
Sex		0.9342		0.9534
Male	22.18±6.69		30.87±15.47	
Female	22.01±6.70		31.15±15.72	
HIV		0.9574		0.9795
Negative	22.01±6.70		31.10±15.55	
Positive	22.18±6.69		30.91±15.63	

TABLE 1: Mean CT value and Mean Time to Culture positivity by Cohort Characteristics

A semi-quantitative estimate of bacilli concentration as defined by the range of CT- High (<16 cycles), Medium (16-22 cycles), Low (22-28 cycles) and Very low (>28 cycles)[9]. Among 48 samples, 23% (n=11) were high whereas medium, low and very low were 33.33% (n=16), 23% (n=11) and 20.83% (n=10) respectively (**fig 4**). Maximum of high (n=9; 81.81%) and medium (n=9; 56.25%) CT range had liquid culture positive at 1 to 4 weeks of inoculation whereas maximum of low (n=8; 72.72%) and very low (n=7; 70%) had liquid culture positive at 5 to 8 weeks of inoculation **(table 2).**

FIG 4: BOX PLOTS OF XPERT CT VALUE RANGE WITH TTCP



Weeks	High	Medium	Low	Very Low	Total
Growth at 1-4 wks	9	9	3	3	24
Growth at 5-8 wks	2	7	8	7	24
	11 (23%)	16 (33.33%)	11 (23%)	10 (20.83%)	48

TABLE 2: Significance of the association between CT value and Time to Culture positivity

There was a significant association between CT values and growth at weeks (Chi-square value = 8.58, p-value = 0.03). The samples with high and medium detected CT values mostly shown earlier culture positivity.

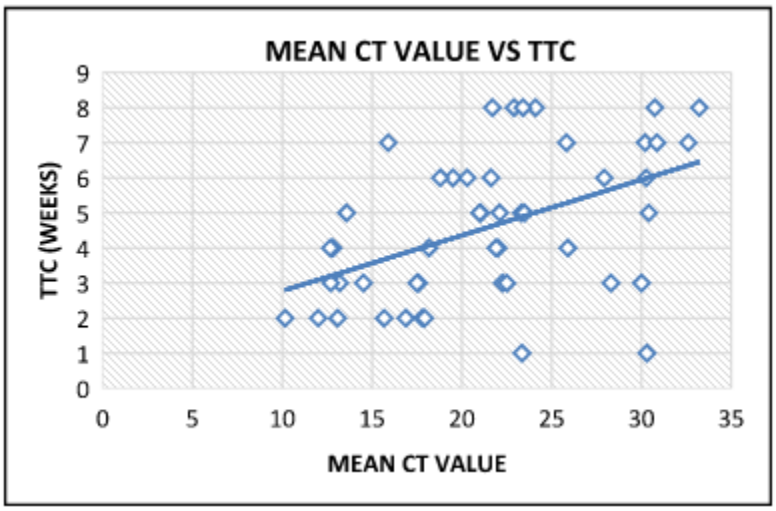


FIG 5: Correlation of mean CT value with time to Culture positivity

Spearman correlation of mean CT value with TTCP showed a moderate positive correlation (r=0.46) and linear regression analysis showed each cycle increase in Xpert, 0.1585 times changes will be in TTCP. (R^2=0.22) (p-value <0.01) (**fig 5**).

On subgroup analysis of CT range, Spearman correlation showed a moderate positive correlation between Xpert CT value and TTCP in high CT range samples (r=0.47). Linear regression analysis showed an R^2 of 0.22 (p-value >0.05). (**fig 6)**

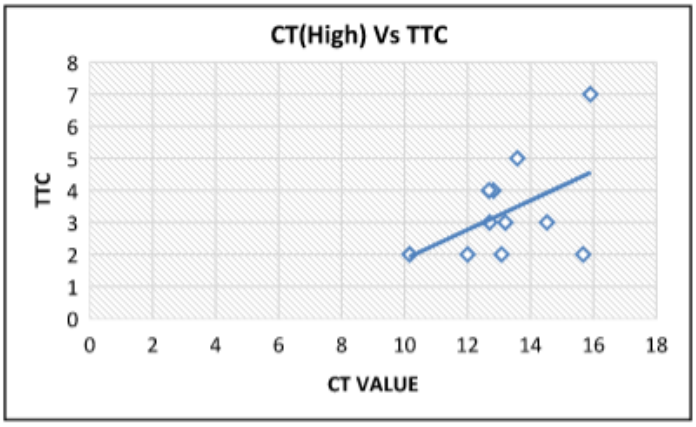


FIG 6: Correlation of mean CT value (High) with time to Culture positivity

Spearman correlation showed a moderate positive correlation between Xpert CT value and TTCP in medium CT range samples (r=0.54). Linear regression analysis showed R^2 of 0.29 (p value= 0.02) (**fig 7)**

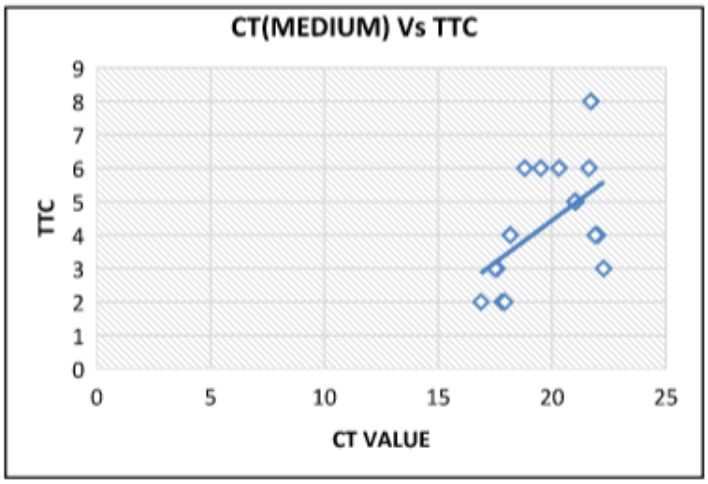


FIG 7: Correlation of mean CT value (Medium) with time to Culture positivity

Spearman correlation showed a weaker correlation between Xpert CT value and TTCP in low CT range samples (r= 0.22). Linear regression analysis showed an R^2 of 0.05 (p-value >0.05). **(fig, 8).**

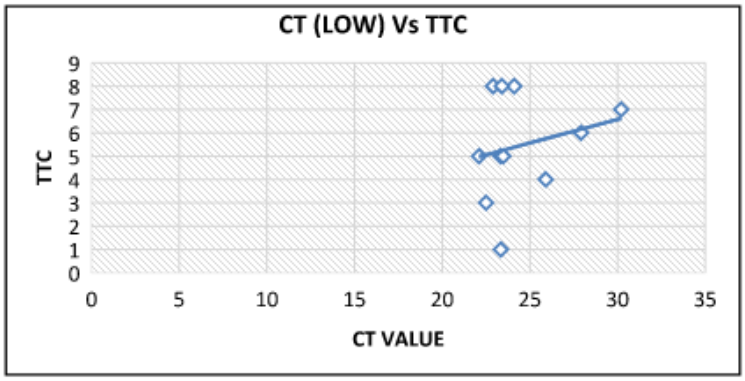


FIG 8: Correlation of mean CT value (Low) with time to Culture positivity

Spearman correlation showed a weaker correlation between Xpert CT value and TTCP in very low CT range samples (r=0.25). Linear regression analysis showed R^2 OF 0.06 (p-value >0.05) (**fig 9).**

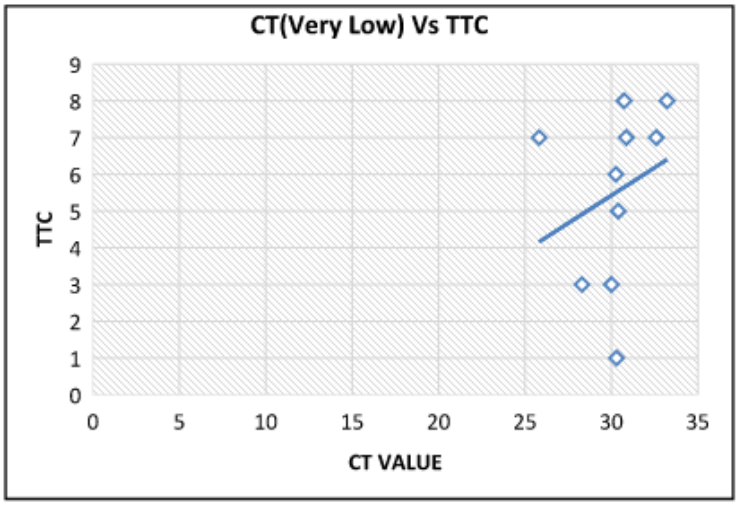


FIG 9: Correlation of mean CT value (Very Low) with time to Culture positivity

V. Discussion

This is a retrospective study on the correlation of cycle threshold values of CBNAAT with time to culture positivity in predicting bacterial load. This study included 48 samples positive for CBNAAT and MGIT culture. The cycle threshold values of CBNAAT have an inverse relationship with a bacillary burden in sputum. The result is interpreted based on CT value as High (<16 cycles), Medium (16-22), Low (23-28) and Very low (>28 cycles)[9]. The present study demonstrated a significant association between the CT category and TTCP, which is a measure of the bacillary burden.

We found a linear relationship between the CT of CBNAAT and TTCP with moderate correlation (r=0.46) and also statistically significant (p-value <0.01). On subgroup analysis based on CT category, also found a linear relationship between CT categories (High, medium, low and very low) and TTCP. But, high and medium CT category only showed moderate correlation whereas low and very low CT category showed a weak correlation between CT of CBNAAT and TTCP. Although high and medium category CT were showing moderate correlation, only a medium category of CT was statistically significant (p <0.05).

Linear regression analysis found the association between MGIT culture time to positivity and CT of CBNAAT (as well with categories of CT of CBNAAT).

The present study showed CT of CBNAAT and TTCP of HIV infected and HIV uninfected individuals were similar. This study lacks information about CD4 count and ART treatment and also sample insufficient to discuss immunosuppression.

On the contrary, Najjingo et al. study showed a weaker correlation between the CT of CBNAAT and TTCP (r=0.37)[10]. Hanrahan et al. study revealed correlation of 0.41 to 0.64 which varied by level of immunosuppression[11] and also Blakemore et al. study showed a correlation of 0.68 which are comparable to the present study[6]. A study by Prakash et al. showed that a correlation between Xpert and Culture would help predict the load of bacilli; however, the reduced sensitivity was noted, especially for samples with a very low load of bacilli[12]. The present study observed there was a moderate correlation between CT values (especially high and medium category) and MGIT culture time to positivity.

Limitations

1. Small sample size.
2. Xpert and Culture were performed on different samples.
3. Limited HIV infected samples.

VI. Conclusion

To conclude our study, measuring sputum bacillary burden from CT of CBNAAT instead of TTCP (MGIT), we can trace the contacts and break the chain of transmission. Cycle threshold values of a high and medium category are correlating with time to culture positivity (MGIT). Due to moderate to a weak correlation between Xpert CT values with TTCP, CT values cannot be used as an independent predictor of a bacillary burden. To evaluate this relationship, studies with an appropriate sample size should, therefore, be performed.

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