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Xpert MTB/RIF Assay among Extra-Pulmonary and Non-Sputum Respiratory Samples: One-Year Experience at National Tuberculosis Reference Laboratory of Bangladesh

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Abstract

Background: Extra-pulmonary tuberculosis (EPTB) is a diagnostic and therapeutic challenge due to the paucity of suitable rapid diagnostic tools. **Objectives:** The aim was to assess the frequency of detection of Mycobacterium tuberculosis using Xpert MTB /RIF assay among the extra-pulmonary and non-sputum respiratory samples (NSRS) along with demographic profile, life style variables and proportion of Rifampicin resistant TB among those specimens. **Methodology:** This cross-sectional retrospective study was conducted at National Tuberculosis Reference Laboratory (NTRL) from January 2018 to December 2018 for period of 1 year. Total 3918 extra-pulmonary and non-sputum respiratory samples from suspected TB patients were enrolled. Detection of Mycobacterium tuberculosis and Rifampicin resistance was performed by Xpert MTB /RIF assay and culture in both liquid and solid media. **Results:** Among 3918 samples, 3152 were new cases and 766 were previously treated TB cases. A total of 853 (21.77%) MTB were detected by Xpert MTB /RIF assay. Among them, 713 (83.59%) were extra-pulmonary and 140 (16.41%) were non-sputum respiratory samples. Lymph node (LN) aspirates and tissues were the most frequent (37.9 and 9.9%) samples of EPTB. The proportion of RR cases among EPTB was 2.8% and 6.3% among new and previously treated cases. And 2.6% and 11.1% among NSRS new and previously treated cases respectively. **Conclusion:** This study will give an insight of EPTB and NSRS profile with a view to diagnostic and therapeutic approach in Bangladesh.

Keywords: EPTB; Non-sputum respiratory samples; Xpert MTB/RIF assay; National Tuberculosis; Reference Laboratory

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Introduction

Tuberculosis (TB) is the leading explanation for death from single infectious agent. When it spread through lymphatic or hematogenous dissemination to any organs and tissues of body, it ends up in the event of extra-pulmonary tuberculosis (EPTB). Of the 6.3

million new TB cases recognized by WHO in 2017, 16.0% were EPTB cases; incidence rates ranged from 8.0% within the Western Pacific Region to 24.0% within the Eastern Mediterranean Region[1]. In the past few decades, studies from high-income countries have shown that EPTB cases comprise an increased proportion of total TB cases[2-3]. Research on EPTB is limited, possibly because it transmits in a lesser extent than pulmonary TB[4]. According to WHO global report 2019, Bangladesh is one of the 30 high burden countries for tuberculosis where EPTB constitutes about 15.0% to 20.0% of all TB cases and it is more common in low socioeconomic groups[5]. So EPTB cannot be overlooked as it causes significant complications, lifelong sequelae, morbidity and mortality hooked in to the organs affected like central nervous system. In general, EPTB affects persons with diabetes and HIV, similarly as young children less than 15 years of age and older adults more than 65 years of age[6]. Current studies have revealed that women and persons who migrate from areas of high TB incidence are at greater risk for EPTB[7-8]. So EPTB is critical to think about as a public health priority as pulmonary TB.

Although drug resistant EPTB is uncommon, but limited available data stimulated us to detect drug resistant TB cases of extra-pulmonary and non-sputum respiratory samples of Bangladesh. In the context of the discussion, Xpert MTB/RIF assay is used in our study which is recommended by WHO for the diagnosis of pulmonary and extra-pulmonary TB in adults and children[1,9]. So, the aim of this study was to estimate the frequency of bacteriologically confirmed TB among extra-pulmonary and non-sputum respiratory samples, to explore the importance of demographics and life style variables, to determine the organs affected and evaluate Rifampicin resistance by using both Xpert MTB/RIF assay and conventional culture and drug sensitivity.

Methodology

Study Design and Data Collection: This cross-sectional retrospective study was conducted to analyze the diagnostic data of National Tuberculosis Reference Laboratory (NTRL) from January 2018 to December 2018. A total of 3918 samples were enrolled of suspected TB patients. Here 3152 were new cases and 766 were old cases with treatment failure or relapse. Extra-pulmonary samples (n = 2475) include lymph node aspirates, tissue mainly lymph node and colonoscopy tissue, pus, wound swab, bone marrow, urine and biological fluids. Non-sputum respiratory samples (n=1443) were tracheal aspirates, Broncho alveolar lavage and gastric lavage.

Sample Processing and Identification of Mycobacteria: Both conventional methods (culture and direct smear microscopy) and Xpert MTB/RIF assay were used for identification of mycobacteria. All samples were processed by standard N-acetyl-L-cyteine-NaOH (NALC-NaOH) procedure[10-11]. Fluids samples were concentrated by centrifugation and solid samples such as thick pus and tissues were homogenized by disposable homogenizer before being inoculated. Liquid media were inoculated first, followed by the egg-based solid LJ media [19]. The MGIT tubes and LJ slants showing positive growth were further subjected to Zeihl-Neelsen staining, p-nitrobenzoic Acid (PNB) stock 500 µg/ml and rapid card test for MTB complex, Bioline SD TB Ag MPT 64 Rapid (Standard Diagnostic, Inc., Republic of Korea). A 1-mL unconcentrated sample was used (without centrifuge) for Xpert MTB/RIF cartridge based nucleic acid amplification test, performed in accordance with the manufacturer's instructions described in the package insert and with Xpert MTB/RIF implementation manual/WHO/ 201412.

Drug Susceptibility Testing (DST): Drug susceptibility testing of isolates to Rifampicin was determined using the Xpert MTB/RIF assay12 and proportion method on the Lowenstein–Jensen medium. Resistance was expressed as the percentage of colonies that grew on critical concentrations of the drug (40 µ g/mL for Rifampicin). Mycobacterium tuberculosis H37Rv strain (ATCC 27294) was used for quality control.

Statistical Analysis: After collection of data, all interviewed questionnaires were checked for completeness, correctness and internal consistency to exclude missing or inconsistent data and those were discarded. Statistical analyses were performed with SPSS software, versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Continuous data were summarized in terms of the mean, standard deviation, median, minimum, maximum and number of observations. Categorical or discrete data were summarized in terms of frequency counts and percentages. For end points analysis, Fisher's exact test was used for categorical variables and an analysis of variance (Student t Test) was applied for continuous outcomes. A two-sided P value of less than 0.05 was considered to indicate statistical

significance.

Ethical Considerations: Permission was taken from the authority of National Tuberculosis Reference Laboratory and National TB Control Project of DGHS to conduct this study. All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee. Participants in the study were informed about the procedure and purpose of the study and confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods. All data were collected anonymously and analyzed using the coding system.

Results

Among 3918 samples, 3152 were new and 766 were previously treated cases. 853(21.77%) and 868 (22.15%) cases were found positive for M. tuberculosis by Xpert MTB/RIF assay and culture respectively. 31 and 33 cases were found Rifampicin resistant by Xpert MTB/RIF assay and drug susceptibility testing (DST) respectively (Table 1).

Tissues and LN aspirates were the most frequent EPTB samples with high MTB and RR detection by both conventional method (40% & 37.8%) and Xpert MTB/RIF (42.5% & 38.9%). Among the NSRS, RR was found higher in gastric lavage (11.1%) in Xpert

Table 1: Frequency of M. tuberculosis and RR M. tuberculosis among the study population

Variables	EPTB	NSRS	Total
Samples			
• New	2020(81.6%)	1132(78.45%)	3152
• Previously Treated	455(18.4%)	311(21.5%)	766
MTB detected by Xpert MTB/RIF			
• New	570(28.2%)	113(9.9%)	683
• Previously Treated	143(31.4%)	27(8.7%)	170
RR detected by Xpert MTB/RIF			
• New	16(2.8%)	3(2.6%)	19
• Previously Treated	9(6.3%)	3(11.1%)	12
MTB detected by culture			
• New	575(28.5%)	117(10.3%)	692
• Previously Treated	149(31.9%)	27(8.7%)	176
RR detected by culture & DST			
• New	17(2.9%)	4(3.4%)	21
• Previously Treated	9(6.04%)	3(11.1%)	12

EPTB = Extra-pulmonary TB samples; NSRS = Non sputum respiratory samples; RR = Rifampicin resistance

Table 2: Sample Sites Included in The Study with Frequency of MTB and RR detection

Specimen	Total	MTB by Xpert MTB/RIF	RR detected by Xpert MTB/RIF	MTB Detected by Culture	RR detected by culture & DST
LN aspirate	939(37.9%)	366(39.0%)	18(4.9%)	355(37.8%)	16(4.5%)
Pus/ wound swab	412(16.6%)	172(41.7%)	2(1.2%)	184(44.7)	03(1.6%)
Pleural Fluid	355(14.3%)	31(8.7%)	2(6.4%)	30(8.4%)	03(10.0%)
CSF	316(12.8%)	23(7.3%)	0(0.0%)	20(6.3%)	0(0.0%)
Tissue	245(9.9%)	105(42.6%)	3(2.8%)	98(40.0%)	04(4.1)
Other fluid	122(4.9%)	06(4.9%)	0(0.0%)	04(3.3%)	0(0.0%)
Urine	62 (2.5%)	05(8.1%)	0(0.0%)	03(4.8%)	0(0.0%)
Breast Lump Aspirate	13 (0.5%)	02(15.3%)	0(0.0%)	03(23.1%)	0(0.0%)
Chest Wall Swelling	6(0.2%)	03(50.0%)	0(0.0%)	04(66.7%)	0(0.0%)
Bone Marrow	5(0.2%)	0(0.0%)	0(0.0%)	01(20.0%)	0(0.0%)
BAL & Tracheal aspirate	825 (57.2%)	104(12.6%)	02(1.9%)	119(14.4%)	2(1.68)
Gastric Lavage	618(42.8%)	36(5.8%)	04(11.1%)	47(7.6%)	5(10.64)
Total	**3918**	**853**	**31**	**868**	**33**

LN, Lymph nodes; BAL, Bronco alveolar lavage; Tissue= mostly LN, colonoscopy tissue, surgical incision site etc.; CSF= Cerebrospinal Fluid

Table 3: Demographic Profile of the TB patients

Variables	New case (n=683)		Prev. treated (n=170)		Total (n=853)	
	Extra-pulmonary (n=569)	Non-Sputum (n=114)	Extra-pulmonary (n=143)	Non-putum (n=27)	Extra-pulmonary (n=712)	Non-Sputum (n=141)
Age group (years)						
1 - 10	16 (2.8)	22 (19.3)	8 (5.6)	6 (22.2)	24 (3.4)	28 (19.9)
11 - 20	180 (31.6)	11 (9.6)	36 (25.2)	6 (22.2)	216 (30.3)	17 (12.1)
21 – 30	239 (42.0)	22 (19.3)	60 (42.0)	4 (14.8)	299 (42.0)	26 (18.4)
31 – 40	75 (13.2)	12 (10.5)	28 (19.6)	2 (7.4)	103 (14.5)	14 (9.9)
41 – 50	35 (6.2)	15 (13.2)	9 (6.3)	0 (0.0)	44 (6.2)	15 (10.6)
51 – 60	13 (2.3)	14 (12.3)	2 (1.4)	5 (18.5)	15 (2.1)	19 (13.5)
61 – 70	8 (1.4)	12 (10.5)	0 (0.0)	2 (7.4)	8 (1.1)	14 (9.9)
71 – 80	3 (0.5)	6 (5.3)	0 (0.0)	2 (7.4)	3 (0.4)	8 (5.7)
Gender						
Male	238 (41.8)	74 (64.9)	57 (39.9)	17 (63.0)	295 (41.4)	91 (64.5)
Female	331 (58.2)	40 (35.1)	86 (60.1)	10 (37.0)	417 (58.6)	50 (35.5)
Residence						
Urban	463 (81.4)	75 (65.8)	4 (2.8)	23 (85.2)	467 (65.6)	98 (69.5)
Rural	106 (18.6)	39 (34.2)	139 (97.2)	4 (14.8)	245 (34.4)	43 (30.5)
DM						
Non-diabetic	419 (73.6)	76 (66.7)	110 (76.9)	19 (70.4)	529 (74.3)	95 (67.4)
Pre-diabetic	85 (14.9)	27 (23.7)	22 (15.4)	0 (0.0)	107 (15.0)	27 (19.1)
Diabetic	65 (11.4)	11 (9.6)	11 (7.7)	8 (29.6)	76 (10.7)	19 (13.5)
Wealth Index						
1st quartile (Poor)	264 (46.4)	37 (32.5)	0 (0.0)	3 (11.1)	264 (37.1)	40 (28.4)
2nd quartile (Middle class)	273 (48.0)	48 (42.1)	3 (2.1)	0 (0.0)	276 (38.8)	48 (34.0)
3rd quartile (Rich)	32 (5.6)	29 (25.4)	140 (97.9)	24 (88.9)	172 (24.2)	53 (37.6)
HIV status (+ve)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

MTB/RIF and 10.6% in culture & DST (Table 2).

About 417 (58.6%) EPTB samples were from female and 91 (64.5%) NSRS samples were from male, most affected age group were 21-30 years (299, 42.0%) and 11-20 years (216, 30.3%). Majority cases were found of 2nd quartile (276, 38.8%), residing in urban area (476, 65.6%) and were non-diabetic (529, 74.3%) (Table 3).

Distribution of RR in TB population trait shows maximum RR cases were in 11-20 years age group 12 (38.7%), in females 18 (58.1), residing in urban area 18 (58.1%), among the non-diabetic 23 (74.2%) and of 3rd quartile 14 (45.2%) (Table 4).

Binary logistic regression was performed to assess the impact of several factors on RR among the Rifampicin sensitive and resistant cases of EPTB. The model contained five independent variables (Age, gender, residence, diabetes and wealth index). One of the two predictor of RR was younger age, reporting an odds ratio of 1.04. This indicated that age was significantly lower in RR cases, younger children were over 3.331 times more likely to have RR. Another predictor was wealth index where RR was found significantly higher among wealthy family.

Discussion

AEPTB needs special attention to ensure access of quality diagnosis, drug susceptibility testing and prompt initiation of appropriate therapy as drug resistant TB affecting extra-pulmonary site may continue to increase. This study was aimed at determining the rate of bacteriologically confirmed TB in extra-pulmonary and non-sputum respiratory samples; to explore the importance of demographics and life style variables; to determine the extra-pulmonary sites and Rifampicin resistance rate.

Total 3918 study samples of suspected TB patients with sign and symptoms were enrolled. Among them, 853 (21.77%) were MTB positive with 31(3.63%) cases of Rifampicin resistance by Xpert MTB/RIF. While, 868(22.15%) cases were culture positive with 33(3.80%) cases of Rifampicin resistance by DST method. Among the 853 (21.77%) Xpert detected cases, 713(28.8%) and 140(9.70%) were detected in

Table 4: Distribution of RR-TB cases by TB Population trait

Variables	New case (n=683)		Prev. treated (n=170)		Total (n=853)		P value
	Total	RR	Total	RR	Total	RR	
Age group (years)							
1 - 10	38 (5.6)	3 (16.7)	14 (8.2)	2 (15.4)	52 (6.1)	5 (16.1)	0.016
11 – 20	191 (28.0)	7 (38.9)	42 (24.7)	5 (38.5)	233 (27.3)	12 (38.7)	
21 - 30	261 (38.2)	5 (27.8)	64 (37.6)	4 (30.8)	325 (38.1)	9 (29.0)	
31 - 40	87 (12.7)	1 (5.6)	30 (17.6)	1 (7.7)	117 (13.7)	2 (6.5)	
41 - 50	50 (7.3)	1 (5.6)	9 (5.3)	0 (0.0)	59 (6.9)	1 (3.2)	
51 - 60	27 (4.0)	1 (5.6)	7 (4.1)	1 (7.7)	34 (4.0)	2 (6.5)	
61 - 70	20 (2.9)	0	2 (1.2)	0	22 (2.6)	0	
71 - 80	9 (1.3)	0	2 (1.2)	0	11 (1.3)	0	
Gender							
Male	312 (45.7)	8 (44.4)	74 (43.5)	5 (38.5)	386 (45.3)	13 (41.9)	0.705
Female	371 (54.3)	10 (55.6)	96 (56.5)	8 (61.5)	467 (54.7)	18 (58.1)	
Residence							
Urban	538 (78.8)	14 (77.8)	27 (15.9)	4 (30.8)	565 (66.2)	18 (58.1)	0.327
Rural	145 (21.2)	4 (22.2)	143 (84.1)	9 (69.2)	288 (33.8)	13 (41.9)	
DM							
Non-DM	495 (72.5)	14 (77.8)	129 (75.9)	9 (69.2)	624 (73.2)	23 (74.2)	0.966
Pre-DM	112 (16.4)	2 (11.1)	22 (12.9)	3 (23.1)	134 (15.7)	5 (16.1)	
Diabetic	76 (11.1)	2 (11.1)	19 (11.2)	1 (7.7)	95 (11.1)	3 (9.7)	
Wealth							
1st quartile	301 (44.1)	3 (16.7)	3 (1.8)	2 (15.4)	304 (35.6)	5 (16.1)	0.021
2nd quartile	321 (47.0)	9 (50.0)	3 (1.8)	3 (23.1)	324 (38.0)	12 (38.7)	
3rd quartile	61 (8.9)	6 (33.3)	164 (96.5)	8 (61.5)	225 (26.4)	14 (45.2)	
HIV status (positive)	0	0	0	0	0	0	

Table 5: Factors Associated with RR among the Rifampicin Sensitive and Resistant Cases of EPTB Using Binary Logistic Regression

Variables	RR	RS	Unadjusted		Adjusted	
			OR (95% CI)	P value	OR (95% CI)	P value
Age Gender	21.70±12.89	27.99±14.26	1.04 (1.01-1.08)	0.016	1.04 (1.00 – 1.07)	0.013
Male	13 (41.9)	373 (45.4)	Ref			
Female	18 (58.1)	449 (54.6)	1.15 (0.42–1.87)	0.705		
Residence						
Urban	18 (58.1)	547 (66.5)	Ref			
Rural	13 (41.9)	275 (33.5)	1.43 (0.70-2.16)	0.327		
Diabetic						
Non-DM	23 (74.2)	601 (73.1)	Ref			
Pre-DM	5 (16.1)	129 (15.7)	1.03 (0.06-2.00)	0.948		
Diabetic	3 (9.7)	92 (11.2)	0.85 (0 – 2.06)	0.792		
Wealth						
1st quartile	5 (16.1)	299 (36.4)	Ref			
2nd quartile	12 (38.7)	312 (38.0)	1.03 (0.29-1.76)	0.932	0.41 (0.14 – 1.18)	0.101
3rd quartile	14 (45.2)	211 (25.7)	2.38 (1.66-3.10)	0.016	0.24 (0.08 – 0.68)	0.007

extra-pulmonary and non-sputum respiratory samples respectively. These cases were a combination of both new and previously treated TB cases. This data is in concordance with the trends seen in 2014. The proportion of EPTB varies from 15-20% of all TB cases in HIV negative setting[14]. However, we did not find any HIV positive case in this study. The percentage of EPTB among new and relapse TB cases in South Asia ranged from 19% to 23% in 2014[15]. A study in Malaysia showed 13% prevalence of HIV in

EPTB patients[16]. The proportion and sites of EPTB varies according to geographic location, comorbidity, presenting symptoms, epidemic situation, time period and a wide variety of host factors[17, 18]. Lymph node tuberculosis was the most common presentation of EPTB in our study (43%) followed by pus and lymph node aspirates (42% and 39%). This finding is alike to other studies[16,19,20].

The major contribution in our study comes from adolescent and early adult age group, between 15-34 years of age (78%) also no particular age group was found to be free of detection of TB among the extra-pulmonary and non-sputum respiratory samples. Higher proportion of detection among non-sputum respiratory samples in lower age group indicates recent transmission from close contact. A study from India also shows the similar finding; 38% of the patients were in the age 15-24 years followed by 25% in age 25-34 years21. A finding of 55% EPTB patients within 16 to 45 years age group has also been observed in Bangladesh[13]. The likelihood of development of EPTB is higher among females than males. In this study, we also found EPTB more in females among extra-pulmonary samples (58.6%) whereas in non-sputum respiratory samples MTB detection rate was totally reverse (male 65%). Similar findings are also reported of 56.3% and 51.6% female EPTB patients[19,20]. Possible factors accounting for more females maybe due to overcrowding and a stigma associated with having TB and lack of access to health care, particularly in developing countries[21]. Recent Indian studies have also noted a higher prevalence of EPTB in children than adults (47% vs 16% respectively), with the greater affection for females (63% vs 33% respectively)[22].

We found 66.2% MTB positive cases from urban (including metropolitan) area compared to 33.8% in rural area. This population distribution pattern was also observed by[23]. We also found 38% of EPTB cases were from middle income families (2nd quartile of wealth index) but 45% of RR-TB cases were from high income group (3rd quartile). This finding may be due to having more junk food, less exposure to sunlight resulting in decreased Vitamin D concentration and less immunity of the wealthy people. A study in India found around 59% of EPTB cases were from poor families having monthly income of 5,000-10,000 rupees 24 which goes in contrast to our study.

Prevalence of diabetes and pre-diabetes among new cases were 11.1% and 16.4% respectively and among previously treated cases were 11.2% and 12.9% respectively. There was no significant difference among Rifampicin sensitive and resistant cases. But study conducted in Malaysia[16] and Georgia[25] found patients with DM at greater risk of EPTB. As Bangladesh is a low HIV prevalent country (0.1%)[26], prevalence of HIV among Rifampicin sensitive and resistant cases of EPTB were 0 % that reflects there is very low risk of HIV infection to cause RR EPTB.

We found that GeneXpert detected 31(3.63%) cases of Rifampicin resistance among 853 MTB cases in 2018 at NTRL. The number of RR TB cases was 16(2.8%) and 09(6.3%) in new and previously treated cases of extra-pulmonary samples. Whereas among the non-sputum respiratory samples, RR TB cases were 03(2.6%) and 03(11.1%) in new and previously treated cases respectively. The prevalence of Rifampicin resistance in the current study is discordant with other published studies[28]. However, in total EPTB samples RR was 1.01% which was alarming. As NSRS are non-sputum respiratory samples, it gives a reflection of PTB where RR was found 0.41% among total 1443 samples. As MTB detection rate is higher among 15 - 34 age group, so RR EPTB was also found higher in that age group which was statistically significant along with the 3rd quartile of wealth index. Age group and wealth index related data for Rifampicin resistant EPTB is scarce. Studies in Bangladesh conducted among the Rifampicin resistant/MDR PTB also found younger age group to be predominant as well[23,29]. Our study revealed female predominance among the Rifampicin resistant EPTB. This is similar to a study conducted in India 30. But previous studies of Bangladesh among the Rifampicin resistant PTB patients showed male predominance[23,29,31]. However, the very few numbers of patients with Rifampin resistance in this study may limit the comparison between males and females.

Conclusion

Drug resistance is one of the uprising challenges among the cases of EPTB. We believe and aspire that this very preliminary information will certainly bring positive change in the future systematic studies to incisively address the epidemiological scenario of drug resistance EPTB in our country.

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Conflict Of Interest

All authors declared no conflict of interests.

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Authors' contributions
Adeeba Khanduker, Sadia Sharmin, Afroza Begum, S.M. Abdur Razzaque, Pronab Kumar Modak, Md. Shirajul Islam conceived and designed the study and wrote up the draft manuscript. Shamim Hossain, Tanvir Huda, Md. Roknuzzaman Khan, Elma Mostofa contributed to the collection and analysis of the data, laboratory work, spelling and grammer checking and critically reviewing the manuscript. Farzana Mahzabin Monika analyzed the data, interpreted the results. S.M. Mostofa Kamal involved in the manuscript review and editing. All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a retrospective study the written informed consent was not obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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