J Clin Tuberc Other Mycobact Dis 16 (2019) 100106

Contents lists available at ScienceDirect

J Clin Tuberc Other Mycobact Dis

journal homepage: www.elsevier.com/locate/jctube

The benefit of stool mycobacterial examination to diagnose pulmonary tuberculosis for adult and elderly patients

Aya Konno[a], Osamu Narumoto[a,*], Hirotoshi Matsui[a], Keita Takeda[a,b], Yuta Hirano[a], Kyota Shinfuku[a], Hiroyuki Tashimo[a], Masahiro Kawashima[a], Akira Yamane[a], Atsuhisa Tamura[a], Hideaki Nagai[a], Ken Ohta[a,c], Shigeto Tohma[a]

[a] Center for Pulmonary Diseases, National Hospital Organization Tokyo National Hospital, Takeoka 3-1-1, Kiyose, Tokyo, Japan
[b] Department of Basic Mycobacteriology, Graduate School of Biomedical Science, Nagasaki University, Japan
[c] JATA Fukujuji Hospital, Japan

ARTICLE INFO

Keywords:
Stool
Tuberculosis
Diagnosis

ABSTRACT

Background: In patients with suspected pulmonary tuberculosis, who have difficulty in expectorating sputum, alternative specimens by invasive procedures, gastric aspirate or sputum suction, are not always available in the feeble elderly. Several studies report the benefit of stool test for pediatric or HIV infected patients, but few in adult patients.

Objective: To evaluate the benefit of stool examination as non-invasive alternative test to detect *Mycobacterium tuberculosis* (MTB) infection.

Methods: Stool specimens were examined for mycobacteria in 187 cases of microbiologically-diagnosed pulmonary tuberculosis between September 2013 and August 2017. We retrospectively reviewed the medical records to determine the positive detection rate of MTB with stool specimens and investigated factors related to MTB detection.

Results: Among 187 patients included, positive rate of MTB in stool was 12.8% (24/187) by stool acid-fast bacilli smear, 68.1% (98/144) by TRC Rapid®, and 40.6% (76/187) by culture. Multivariate logistic regression analysis revealed two contributing factors to MTB detection in stool; cavitation and male. The adjusted odds ratio with 95% confidence interval (CI) for cavitation was 2.9 (95%CI 1.48–5.69) and 2.1 (95%CI 1.08–3.93) for male.

Conclusion: We recommend stool examination for those who are unable to give sputum and have risks for invasive procedures.

1. Introduction

The tuberculosis (TB) patient population in Japan is aging faster. While the number of newly diagnosed TB patients in Japan have decreased from 39,384 in 2000 to 16,789 in 2017, TB patients aged over 85 years increased from 3148 to 4318 [1]. Surprisingly, TB patients aged over 80 years accounted for 40% in 2017 [1]. Age related factors like immune suppression from comorbid diseases increases the reactivation of TB and increases the development of TB [2,3]. The Global tuberculosis report 2018 from WHO report "In the WHO Eastern Mediterranean, South-East Asia and Western Pacific regions, the TB epidemic is a markedly ageing one with a progressive increase in the notification rate with age, and a peak among those aged 65 years or over [4]".

Microscopic examination of sputum smear is often the first test to be used in suspicion of pulmonary TB. However, some elderly patients have difficulties in expectorating sputum. Compared to middle age, low grade positive smears are reported to be more frequently seen in the elderly and pediatric age [5,6]. In patients who have difficulty coughing up sputum, alternative respiratory tract specimens can be acquired only by invasive procedures, including gastric aspiration or bronchoscopic examinations, which is not always available in the feeble elderly.

Diagnosing tuberculosis in elderly patients is challenging, when they are unable to provide sputum, and alternative noninvasive method to diagnose tuberculosis is necessary. In pediatric or HIV infected patients(sputum is known to be scanty [7]) the benefit of mycobacterial examination of stool (smear, culture, and nucleic acid amplification test) as non-invasive alternative test to detect MT infection have been indicated in several studies [8–15], but few studies in adult patients

* Corresponding author.
E-mail address: narumoto-tky@umin.ac.jp (O. Narumoto).

https://doi.org/10.1016/j.jctube.2019.100106

Table 1
Characteristics of 187 patients.

Characteristics (n = 187)	Median(quartile) or percentage
Sex (male%)	60.4% (113/187)
Age	74 (54–85)
BMI	17.8 (15.9–20.2)
Alubumin (g/dl)	2.6 (2.1–3.3)
Diabetes mellitus	20.3% (38/187)
HIV	3.7% (7/187)
Miliary tuberculosis	23.5% (44/187)
Cavity formation	45.5% (85/187)
Area of consolidation (0/1/2/3)*	2/19/69/97

* Classification by total areas of abnormalities based on chest x-ray based on the Japanese society for tuberculosis.(0: no abnormalities on chest-x-ray, 1: Affected area less than one-third of a unilateral lung field, 2: between 1 and 3, 3: Affected area greater than the area of a unilateral lung).

Table 2
Characteristics of stool TRC or culture positive patients.

Characteristics	Positive[a] MT detection in stool (n = 124) median(quartile)or percentage	Negative MT detection in stool(n = 63)	*p-value*
Sex (male%)	67.7% (84/124)	46.0% (29/63)	0.0041*
Age	71 (54–84)	81 (55–87)	0.13†
BMI	17.7 (15.8–20.1)	18.0 (15.9–21.6)	0.50†
Albumin (g/dl)	2.5 (2–3.3)	2.7 (2.1–3.3)	0.54†
DM	19.4% (24/124)	22.2% (14/63)	0.65*
HIV	2.4% (3/124)	6.3% (4/63)	0.22**
Miliary tuberculosis	18.5% (23/124)	33.3% (21/63)	0.024*
Areas of consolidation (0/1/2/3)	0/9/48/67	2/10/21/30	

[a] Positive MT detection means combined result of positive TRC or culture.
* Chi-square test (pearson).
** Fisher's exact test.
† Wilcoxon/Kruskal–Wallis test.

Table 3
Results of multivariate logistic regression analysis.

	p-value	Adjusted odds ratio (95% confidence interval)
Cavity formation	0.015	2.9 (1.48–5.69)
Sex (Ref = Male)	0.028	2.1 (1.08–3.93)

Prior to multivariate logistic regression, STEPWISE selection was conducted using JMP13.0 (inclusion criteria $p < 0.25$, exclusion criteria $p < 0.05$). Whole model test : *p-value* 0.0001, R2 square test 0.07, AICc 227, BIC 236, LOF *p-value* 0.15.

[16–18]. We investigated the sensitivity of mycobacterial examination of stool for the diagnosis of pulmonary TB in Japan where TB patients are aging rapidly.

2. Methods

2.1. Patient selection and data collection

We retrospectively investigated adults aged 20 years or older who were admitted to Tokyo National Hospital, Kiyose city, Tokyo, Japan from September 2013 to August 2017 with the diagnosis or in suspicion of TB. Patients were ineligible if they were not TB, did not give both sputum and stool specimens. We also excluded if they did not have pulmonary TB. Patient factors and backgrounds were investigated from the chart. Chest X-ray (CXR) were assessed by two expert doctors for tuberculosis. We classified CXR into four categories by areas of consolidation (Table 1). Chest computed tomography (CT) images were also checked for cavity formation. The diagnosis of intestinal tuberculosis was made according to the medical records of the attending physician.

2.2. Specimen collection, and laboratory methods

We compared the positive isolation rate of *M. tuberculosis* organisms between sputum and stool specimens collected from the same patients. One stool and three respiratory samples were collected from all patients. Both sputum and stool specimens were collected in separate tubes. About 100 to 200 μl of stool specimens were used for further treatment. Both samples were pre-treated with a semi-alkaline protease proteinase (Sputazyme, Kyokuto Pharmaceutical, Co. Ltd., Japan) [19] according to the manufacturer's instructions. Stool specimens were spun down and the supernatants were used for further treatment. All specimens were further decontaminated using N-acetyl-L-cysteine-sodium hydroxide (NALC−NaOH), followed by neutralization with phosphate buffer (PB, pH 6.8) and centrifugation (3000 g, 15 min at 4 ˚C). Auramine O fluorescence microscopy was performed as per standard literature for both sputum and stool samples [20]. Transcription Reverse-transcription Concerted reaction (TRC Rapid®) system (TOSOH, Tokyo, JAPAN) was performed according to the manufacturer's instructions. And also, those samples were cultured using the Mycobacteria Growth Indicator Tube (MGIT®) system (Becton Dickinson, Sparks, MD, USA). Liquid MGIT cultures were incubated at 37 ˚C for up to 6 weeks. If there was no growth, cultures were declared negative. Culture with solid medium (2% Ogawa medium) was also used if specimens were suspected of contamination during culture with MGIT® and incubated at 37 ˚C for up to 8 weeks.

2.3. Statistical analyses

Chi-square test (Pearson) , Fisher's exact test and Wilcoxon/Kruskal-Wallis test were used to compare the characteristics between two groups. Logistic regression analysis was used to detect factors related to positive detection of MTB from stool specimens. All statistical analyses were performed using JMP 13.00 (SAS Institute Inc. Cary, NC, USA). Statistical significance was accepted at $p < 0.05$.

2.4. Ethics statement

The study protocol was approved by the Tokyo National Hospital Research Ethics Committee. Informed consent was waived because of

Table 4
Characteristics of the five patients diagnosed only by stool.

	Age	Sex	Tuberculosis	Stool acid smear	Stool culture	Stool TRC	HIV	Cavity formation	Area of consolidation
1	41	M	Pulmonary and intestinal	–	+	+	–	–	1
2	70	F	Pulmonary	–	–	+	–	–	1
3	79	M	Pulmonary	–	+	+	–	+	2
4	19	F	Intestinal, urinary tract, pulmonary and peritoneum	–	–	+	–	–	1
5	40	F	Pulmonary	–	–	+	+	+	2

Table 5
Comparison between stool and sputum examination.

Smear (n = 187)	Stool AFB smear positive	Stool AFB smear negative
Sputum AFB smear positive (at least one time)	24	90
Sputum AFB smear negative	0	73
Culture (n = 187)	**Stool AFB culture positive**	**Stool AFB culture negative**
Sputum AFB culture positive (at least one time)	72	91
Sputum AFB culture negative	4	20
TB-TRC (n = 138)	**Stool TRC positive**	**Stool TRC negative**
Sputum TRC positive	80	15
Sputum TRC negative	13	30

the retrospective nature of the study.

3. Results

From September 2013 to August 2017, 2063 patients were admitted to the Tokyo National Hospital with the diagnosis or in suspicion of TB. Stool specimens were examined for mycobacteria in 300 cases. Among them, 187 cases were microbiologically diagnosed with pulmonary tuberculosis and were retrospectively reviewed. Patient's characteristics are summarized in Table 1. Among 187 patients enrolled, 113 (60.4%) were men; with mean age of 74 years; and of whom 38 (20.3%) had diabetes mellitus (DM) and 7 (3.7%) had human immunosuppressive virus (HIV) infection; 44 had milliary tuberculosis and 7 (3.7%) had intestinal tuberculosis and 85 (45.5%) had cavity formation (Table 1).

For sputum specimen, we have done AFB smear and culture for three specimens from different days, and TRC for one specimen. The results demonstrated AFB smears were positive in 60.6% (114/187), TRC was positive in 67.3% (126/187), and cultures were positive in 87.2% (163/187) of patients.

Stool examination was conducted one time for each patient. Stool smear for acid fast bacteria (AFB) was positive in 12.8% (24/187), TRC was positive in 68.1% (98/144, 33 missing data), culture was positive in 40.6% (76/187) of patients. The clinical characteristics of stool TRC and/or culture positive patients and those of negative patients are shown in Table 2. Male sex, cavity formation and absence of miliary tuberculosis had a statistically significant ($p < 0.05$) difference, favoring positive results (Table 3).

We ran univariate logistic regression on characteristics and turned out 3 same variables, cavity formation, miliary tuberculosis and male sex were statistically significant ($p < 0.05$). Prior to multivariate logistic regression, stepwise selection was conducted using JMP13.0 (inclusion criteria $p < 0.25$, exclusion criteria $p < 0.05$) . The results indicate male sex and cavity formation are statistically significant. The adjusted odds ratio (OR) with 95% confidence interval (CI) for presence of cavity was 2.9 (95%CI 1.48–5.69), and 2.1 (95%CI 1.08–3.93) for male.

We could not find any common characteristics among 5 patients whose MTB was proved by only stool samples (Table 4). Detailed comparison between stool and sputum examination are shown in Table 5.

4. Discussion

Not all tuberculosis-suspected patients can produce adequate and appropriate sputum samples. In such cases, diagnosing TB is challenging and often requires invasive procedures like bronchoscopy. Stool samples have been reported to be useful for the diagnosis of TB in pediatric patients and in HIV patients (especially for patients with low CD4 counts) [9,10,12–15]. Although the usefulness of stool examination for adult patients has also been documented in a few articles [16,18], the usefulness is not commonly recognized.

In our study, stool TRC for MTB was positive in 68.1% (98/144, 43 missing data) of patients, while the sputum TRC for MTB was positive in 67.3% (126/187). This was surprising because the result shows stool TRC for MTB have almost same sensitivity as TRC from sputum samples. We can see there are reasonable number of patients with positive TRC in either stool specimens or sputum specimens, and they seems to complement each other (Table 5). Stool culture for MTB was positive in 40.6% (76/187) of patients, while the sputum culture was positive in 87.2% (163/187) of cases. Culture positive rate for stool examination was lower than that of sputum examination, and even lower than the positive rate for stool TRC. This result is consistent with other reports, and most of the MTB in the stool specimen could be considered to be dead and only DNAs are detected.

Multivariate logistic regression analysis revealed positive rate for stool examination was high in male patients and patients with cavity formation. The amount of MTB in sputum and the positive rate of MTB in stool is reported to have a correlation [16]. The rate of MT detection in stool specimen (positive TRC or culture) was higher in Sputum AFB positive patients (80%, 92/114) compared to sputum AFB negative patients (44% 32/73) in this study too. We suspect that the patients with cavity formation on the CXR tend to shed MTB more than those who do not have cavities. Therefore, patients who have cavity formation would have more chance to swallow MTB and cause positive rate of stool specimens become higher. The reasons for high positivity in males are unknown and require further study. However there are some papers that reports females tends to have lower acid-fast bacillary counts in the sputum [5,21].

The rate of patients who were microbiologically diagnosed on the basis of stool positivity alone was 2.7% (5/187). When considering the cost-effectiveness of stool specimen, stool examination may not be necessary if patients can give enough sputum.

However, we showed positive rate of TRC for MTB was almost identical between stool and sputum samples. Therefore, for those who can't give enough sputum, especially those with cavity formation in x-ray, we can expect good diagnostic sensitivity of stool examination.

In previous reports, the positive rate for stool examination ranged from 25% to 94% [12,14,18]. In the article which reported 94% of sensitivity, all patients were all smear positive. Our study participants were smear positive in 60.6% of patients, which may have reduced the sensitivity. Another paper reported that increasing the amount of stool used for the examination may improve the sensitivity [8]. Further optimization of laboratory processing methods may improve the sensitivity of stool examination.

There are some limitations to this study. Due to the retrospective study design, patients included in this study may be biased for sputum smear negative patients. However, the sensitivity of stool tests was reasonably good even for such patients.

In conclusion, *M. tuberculosis* positive rate of stool specimens, especially by TRC is comparable with sputum positivity rate. We

recommend stool examination for adults who are unable to expectorate sputum and have risks for invasive procedures.

Declaration of Competing Interest

None.

Acknowledgments

None.

Funding sources

None.

Reference

[1] JATA. Tuberculosis in Japan Annual Report2018. The Research Institute of Tuberculosis Japan Anti-tuberculosis Association. 2018; (http://jata.or.jp/english/decr.html#factsheet (Accessed Feb 2019)).
[2] Davies PD. Tuberculosis in the elderly. Epidemiology and optimal management. Drugs Aging 1996;8(6):436–44.
[3] Dutt AK, Stead WW. Tuberculosis in the elderly. Med Clin North Am 1993;77(6):1353–68.
[4] WHO. Global Tuberculosis Report2018. https://wwwwhoint/tb/publications/global_report/en/ (Accessed Feb 2019). 2018.
[5] Rieder HL, Lauritsen JM, Naranbat N, Katamba A, Laticevschi D, Mabaera B. Quantitative differences in sputum smear microscopy results for acid-fast bacilli by age and sex in four countries. Int J Tuberc Lung Dis 2009;13(11):1393–8.
[6] Palanivel C KS, Pruthu T, Gomathi R, Mahalakshmy T, Kavita V. Age and sex differences in sputum smear microscopy results for acid fast bacilli in a Tertiary Care Centre, South India. J Respir Med 2014;2014:1–5.
[7] Padmapriyadarsini C, Narendran G, Swaminathan S. Diagnosis & treatment of tuberculosis in HIV co-infected patients. Indian J Med Res 2011;134(6):850–65.
[8] Banada PP, Naidoo U, Deshpande S, Karim F, Flynn JL, O'Malley M, et al. A novel sample processing method for rapid detection of tuberculosis in the stool of pediatric patients using the Xpert MTB/RIF assay. PLoS One 2016;11(3):e0151980.
[9] Hasan Z, Shakoor S, Arif F, Mehnaz A, Akber A, Haider M, et al. Evaluation of Xpert MTB/RIF testing for rapid diagnosis of childhood pulmonary tuberculosis in children by Xpert MTB/RIF testing of stool samples in a low resource setting. BMC Res Notes 2017;10(1):473.
[10] LaCourse SM, Pavlinac PB, Cranmer LM, Njuguna IN, Mugo C, Gatimu J, et al. Stool Xpert MTB/RIF and urine lipoarabinomannan for the diagnosis of tuberculosis in hospitalized HIV-infected children. AIDS 2018;32(1):69–78.
[11] Moussa H, Bayoumi FS, Mohamed AM. Gene Xpert for direct detection of mycobacterium tuberculosis in stool specimens from children with presumptive pulmonary tuberculosis. Ann Clin Lab Sci 2016;46(2):198–203.
[12] Nicol MP, Spiers K, Workman L, Isaacs W, Munro J, Black F, et al. Xpert MTB/RIF testing of stool samples for the diagnosis of pulmonary tuberculosis in children. Clin Infect Dis 2013;57(3):e18–21.
[13] Oramasionwu GE, Heilig CM, Udomsantisuk N, Kimerling ME, Eng B, Nguyen HD, et al. The utility of stool cultures for diagnosing tuberculosis in people living with the human immunodeficiency virus. Int J Tuberc Lung Dis 2013;17(8):1023–8.
[14] Walters E, Demers AM, van der Zalm MM, Whitelaw A, Palmer M, Bosch C, et al. Stool culture for diagnosis of pulmonary tuberculosis in children. J Clin Microbiol 2017;55(12):3355–65.
[15] Wolf H, Mendez M, Gilman RH, Sheen P, Soto G, Velarde AK, et al. Diagnosis of pediatric pulmonary tuberculosis by stool PCR. Am J Trop Med Hyg 2008;79(6):893–8.
[16] Kokuto H, Sasaki Y, Yoshimatsu S, Mizuno K, Yi L, Mitarai S. Detection of mycobacterium tuberculosis (MTB) in fecal specimens from adults diagnosed with pulmonary tuberculosis using the Xpert MTB/rifampicin test. Open Forum Infect Dis 2015;2(2):ofv074.
[17] Ouedraogo AS, Kabore DO, Poda A, Sanogo B, Birba E, Sanou I, et al. [Evaluation of stool microscopy and culture to assist the diagnosis of pulmonary tuberculosis in a tuberculosis endemic country]. Med Sante Trop 2016;26(1):97–100.
[18] Rahman SMM, Maliha UT, Ahmed S, Kabir S, Khatun R, Shah JA, et al. Evaluation of Xpert MTB/RIF assay for detection of Mycobacterium tuberculosis in stool samples of adults with pulmonary tuberculosis. PLoS One 2018;13(9):e0203063.
[19] Burdz TV, Wolfe J, Kabani A. Evaluation of sputum decontamination methods for Mycobacterium tuberculosis using viable colony counts and flow cytometry. Diagn Microbiol Infect Dis 2003;47(3):503–9.
[20] Kent PKG. Public health mycobacteriology: a guide for the level III laboratory. US department of health and human services, public health service. Centers for Disease Control; 1985. US Department of Health and Human Services, Public Health Service, Centers for Disease Control.
[21] Lawson L, Lawson JO, Olajide I, Emenyonu N, Bello CS, Olatunji OO, et al. Sex differences in the clinical presentation of urban Nigerian patients with pulmonary tuberculosis. West Afr J Med 2008;27(2):82–6.