

Research Article

Risk Factors and Duration of Smear-Positive and Culture-Negative Status in Patients with Sputum Smear-Positive Pulmonary Tuberculosis

Masanori Tanaka, Yoshiaki Minakata*, Mai Kato, Seigo Sasaki, Yuichiro Azuma, Kazumi Kawabe, and Hideya Ono

Department of Respiratory Medicine, National Hospital Organization Wakayama Hospital, Japan

***Corresponding author**

Yoshiaki Minakata, Department of Respiratory Medicine, National Hospital Organization Wakayama Hospital, 1138 Wada, Mihama-cho, Hidaka-gun, Wakayama, 641-0011, Japan. Tel: 81-738-22-3256

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- Cavity
- Culture
- Discharge
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- Smear

Abstract

Objective: The detection of smear-positive and culture-negative (SPCN) status during pulmonary tuberculosis treatment can be seen at a constant rate. However, its risk factors and duration of detection have not been well evaluated with short-interval follow-up of sputum examinations.

Methods and Materials: We retrospectively accumulated patients with sputum smear-positive pulmonary tuberculosis who were admitted to National Hospital Organization Wakayama Hospital in Japan from April 2018 to March 2021. The factors associated with the incidence and detection period of SPCN in sputum were analyzed with weekly sputum examinations by a multiple regression analysis.

Results: Among the 122 patients with pulmonary tuberculosis who were hospitalized and treated for sputum smear-positive disease, 103 (84.4%) had SPCN status. Risk factors for SPCN detection included female gender and high initial smear grade. The median duration of SPCN status was 35.5 days (range 7-76 days). Risk factors for prolonged SPCN duration included a high initial smear grade, the presence of cavities, and extensive lesions.

Conclusion: In a short-interval follow-up of sputum, a very large number of patients were found to have SPCN status. The severity of infection was related to the duration of SPCN detection.

INTRODUCTION

Tuberculosis remains a major global health problem. According to the 2021 Tuberculosis Registrant Information Survey, the incidence of TB in Japan was 9.2 per 100,000 population. [1]. The causative organism, *Mycobacterium tuberculosis*, is transmitted via respiratory droplet nuclei derived from patients with pulmonary tuberculosis. To prevent transmission, patients must be isolated until they recover to a non-infectious condition. How long it takes for patients to become non-infectious after receiving appropriate treatment is unclear [2], and the criteria for release isolation vary among countries. Some countries set the criteria for release from isolation at two weeks of treatment [3-5], but the rate of patients with a sputum smear-negative status after 2 weeks of treatment was reportedly 9%, and that with sputum culture-negative results was 5% [6], making it questionable whether or not these patients were truly non-infectious. The discharge criterion in Japan is the confirmation of either three consecutive negative sputum smears or three consecutive negative sputum cultures [7].

Sputum smear-positive and culture-negative (SPCN) status is a factor that prolongs the length of hospital stay under the Japanese discharge criteria. The recent increase in reported cases of SPCN is thought to be due to the strong bactericidal action of rifampicin on ribosomal components, leaving the cell wall stainable [8,9]. The incidence of SPCN in pulmonary tuberculosis patients being treated has been reported in the past to range from 5.4% to 20.4% [9-12], but its risk factors and the duration of detection have not been fully investigated.

The present study investigated the incidence and risk factors of SPCN status, the duration of SPCN detection, and the factors associated with the duration in patients with initial sputum smear-positive pulmonary tuberculosis.

METHODS AND MATERIALS**Subjects**

The patients with pulmonary tuberculosis admitted and

treated at National Hospital Organization Wakayama Hospital from April 2018 to March 2021 were retrospectively recruited. Entry criteria included patients who (1) had a positive sputum smear test at the start of treatment and (2) met the discharge criteria (three consecutive negative sputum smears or three consecutive negative sputum cultures) during hospitalization. Patients with (1) sputum smear-negative tuberculosis and (2) death, discharge, or transfer without meeting discharge criteria were excluded.

Evaluations

We evaluated the SPCN detection rate and compared the differences in patients' characteristics between those with and without SPCN status. Specifically, we collected information on age, gender, X-ray and CT scan on admission, amount of smear on admission, blood tests, and therapeutic drugs. We then measured the SPCN duration and evaluated the factors associated with the duration.

Sputum collection method

In our hospital, as a rule, sputum samples were collected regularly every week during hospitalization. If the smear test was negative on the collected sputum examination, sputum was also collected the next day. Culture tests were performed on solid medium (Ogawa medium) for eight weeks.

Definitions

The SPCN duration was defined as the period from the first SPCN detection date to the last SPCN detection date, plus seven days. The period during which SPCN was detected but after which culture-positive status was detected again before meeting the discharge criteria was excluded from the SPCN duration. According to the Japanese Society for Tuberculosis Classification of Diseases, the extent of the lesion on chest X-ray was defined as follows: 1, not exceeding the area of the lung field above the horizontal line passing through the anterior superior border of the second rib; 2, intermediate between 1 and 3; 3, exceeding the area of the unilateral lung field. In addition, a CT scan was performed to evaluate for cavities.

Parameters

We collected data on anthropometry, sputum test results, course of treatment, applied bacteriological discharge criteria (three consecutive negative smears or three consecutive negative cultures), and presence and duration of SPCN status.

Statistical analyses

The unpaired t-test, Mann-Whitney U-test, and χ^2 test were used to compare characteristics by SPCN detection, and simple and multiple regression analyses were used to examine factors related to SPCN duration. Calculations were performed using the GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics (IBM Japan, Tokyo, Japan) software programs. Statistical significance was defined by $p < 0.05$.

RESULTS

During the study period, 179 patients were recruited, and 57 were excluded, leaving 122 smear-positive pulmonary tuberculosis patients ultimately included in the analysis. Of these, 103 patients (84.4%) had SPCN detection. Among SPCN-detected cases, 50 (48.5%) met the criteria for discharge with 3 negative sputum smear tests, and 53 (51.5%) met the criteria for discharge with 3 negative sputum culture tests (Figure 1). There were 17 cases (16.5%) in which sputum and culture were positive again after 1 confirmation of SPCN.

The SPCN group had a higher proportion of women ($p=0.01$) and a higher initial smear grading ($p=0.01$) than the non-SPCN group. However, there were no significant differences between these patient groups with regard to the age, presence of cavities, extent of lesions, C-reactive protein level, albumin level, or usage of pyrazinamide. The median SPCN duration was 35.5 (quartile range 7-76) days (Table 1).

The presence of a cavity, an extensive lesion, and a higher initial smear grading were identified as factors related to the SPCN duration in a simple regression analysis (Table 2) as well as in a multiple regression analysis (Table 3). The median SPCN duration for patients with a cavity was 53.0 days, whereas that for those without a cavity was 28.0 days. The median SPCN durations for patients with a lesion spread of 1, 2, and 3 were 14.0, 43.0, and 56.0 days, respectively and those for patients with an initial smear grading of +/-, +, 2+, and 3+ were 7.0, 14.0, 39.0, and 50.5 days, respectively (Table 4 and Figure 2).

DISCUSSION

SPCN status was found in 84.4% of cases, and the risk factors for SPCN status included being female and having a high initial smear grade. The risk factors for prolonged SPCN duration included a cavity, an extensive lesion, and having a high initial smear grade.

In previous studies, Chao et al., reported that the SPCN detection rate was 13.8% among patients with sputum culture-positive pulmonary tuberculosis in 6 hospitals in Taiwan [10], Pefura-Yone et al., reported that the rate was 4.2% among patients with sputum smear-positive pulmonary tuberculosis in a single institution in Cameroon [11], Su et al., reported that the rate was 13.5% among patients with sputum culture-positive pulmonary tuberculosis in six hospitals in Taiwan [12], and Kim et al., reported that the rate was 20.4% among sputum culture-positive pulmonary tuberculosis patients [9].

The SPCN detection rate in the current study was 84.4%, which was very high compared to previous reports. One possible reason for the high detection rate of SPCN status is that most of the previous studies examined the status of SPCN in the second month of treatment. However, in our study, sputum examinations were performed at a short interval of one week of follow-up, which may have resulted in a higher detection sensitivity. Indeed, there were 40 cases in which SPCN was detected earlier than the second month, even though both smears and cultures were

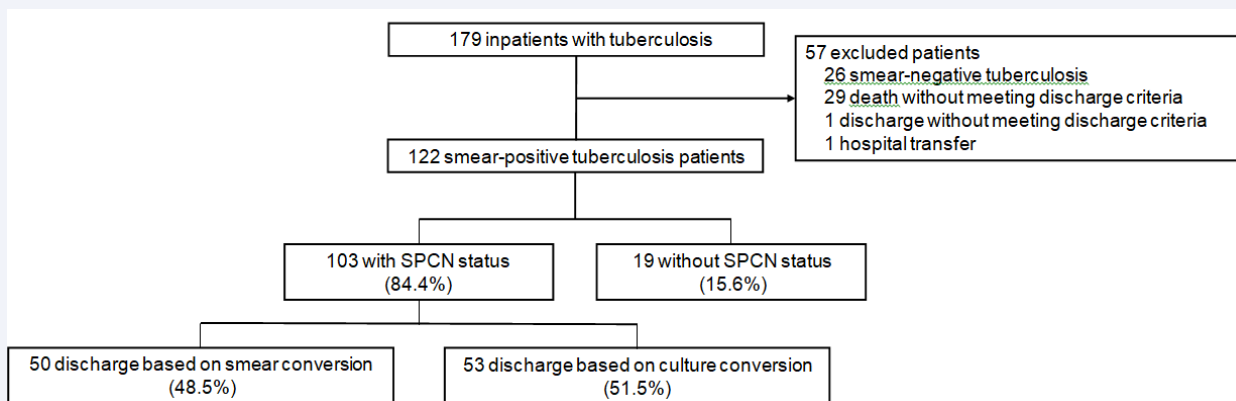


Figure 1 Flow diagram. SPCN; smear positive and culture negative.

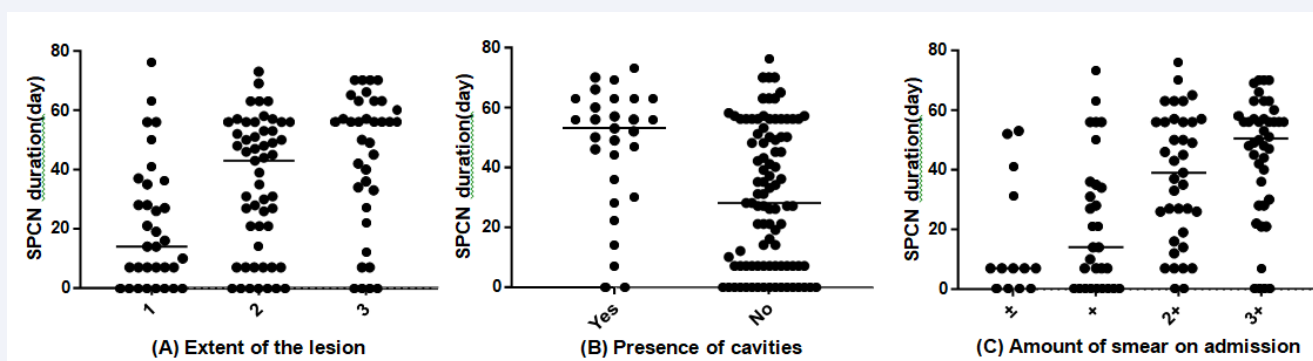


Figure 2 SPCN duration according to (A) extent of the lesion, (B) presence of cavities, and (C) amount of smear on admission. SPCN; smear positive and culture negative.

Table 1: Characteristics of patients

	With SPCN status	Without SPCN status	P value
n	103	19	
Age (median, IQR)	83 (70-89)	83 (79-89)	0.66
Gender (M/F)	48/55	15-Apr	0.01
Presence of cavities (Yes/No)	27/76	Feb-17	0.24
Extent of the lesion (1/2/3)	25/46/32	08-07-2004	0.26
Amount of smear on admission (±/+/2+/3+)	9/21/35/38	4/9/2/4	0.01
CRP (median, IQR)	3.77(0.85-8.07)	5.22(0.84-8.74)	0.44
Alb (median, IQR)	3.1(2.50-3.90)	2.9(2.28-3.55)	0.23
Pyrazinamide usage (Yes/No)	63/40	08-Nov	0.14
SPCN duration (median day)	35.5(range 7-76)	-	

Alb: Albumin; CRP: C-reactive protein, IQR: Interquartile range; SPCN: Smear Positive and Culture Negative.

negative at the second month, confirming that many patients developed SPCN earlier than the second month. Another possible reason is the differences in patients' background characteristics. In most previous studies, a large number of patients with initial smear-negative were recruited. The percentage of patients with an initially smear-negative status was 47% in Chao's report [10],

Table 2: Risk factors of longer SPCN duration in the simple regression analysis

	R	P value
Age	<0.001	0.996
Gender	0.059	0.516
Presence of cavities	0.091	<0.001
Extent of the lesion	0.128	<0.001
Amount of smear on admission	0.192	<0.001
CRP	0.15	0.103
Alb	0.075	0.414
Pyrazinamide usage	0.033	0.133

Alb: Albumin; CRP: C-reactive protein; IQR: Interquartile range; SPCN: Smear Positive and Culture Negative.

Table 3: Risk factors of longer SPCN duration in the multiple regression analysis

	β	Standardized β	P value
Constant	11.739		0.121
Amount of smear on admission	7.193	0.302	0.001
Presence of cavities	7.891	0.251	0.003
Nature of the lesion	-9.862	-0.179	0.033

R²=0.274, Durbin-Watson ratio 2.345

Table 4: SPCN duration

	n	SPCN duration(day(IQR))	P value
Age (median age 83 years)			0.52
≥83	65	36.0(7.0-56.0)	
<83	57	34.0(7.0-54.5)	
Gender			0.58
Male	63	31.0(7.0-56.0)	
Female	59	37.0(19.0-56.0)	
Presence of cavities			0.0007
Yes	29	53.0(33.0-63.0)	
No	93	28.0(7.0-52.0)	
Extent of the lesion			0.0004
1	33	14.0(3.5-35.5)	
2	53	43.0(10.5-54.5)	
3	36	56.0(28.5-62.3)	
Amount of smear on admission			<0.0001
+/-	13	7.0(0.0-36.0)	
+	30	14.0(0.0-35.25)	
2+	37	39.0(17.5-56.0)	
3+	42	50.5(29.5-57.25)	
CRP			0.30
<1mg/dl	31	27.0(7.0-52.25)	
≥1mg/dl	91	38.5(7.0-56.0)	
Alb			0.24
<3.0g/dl	58	46.5(7.0-56.0)	
≥3.0g/dl	64	28.0(7.0-56.25)	
Pyrazinamide usage			0.86
Yes	65	35(9.5-56.0)	
No	57	39.0(7.0-56.0)	

SPCN: Smear Positive and Culture Negative, Alb: Albumin, CRP: C-Reactive Protein, IQR: Interquartile Range.

43.4% in Su's [12], and 29.6% in Kim's [9], although those patients were not included in the current study. While the study of Pefura-Yone et al. included patients with pulmonary tuberculosis who were sputum smear-positive at the start of treatment, they did not confirm the presence of *M. tuberculosis* by polymerase chain reaction or culture, and their diagnosis was based only on sputum smear positivity and a medical history suggestive of pulmonary tuberculosis [11]. Therefore, the SPCN rate did not truly reflect only sputum smear-positive pulmonary tuberculosis, as it might have included patients with non-tuberculous mycobacteria and those whose smears were positive due to contamination with non-tuberculous mycobacteria during the smear examination.

SPCN status was observed significantly more often in patients with a high initial smear grading than in those with lower grades. Chao et al., and Kang et al., also reported that the amount of initial shedding was a factor involved in SPCN detection, which is consistent with the current results [8,10]. It has also been reported that sputum smear test negativity is delayed in cases with a high initial smear grade [12,14]. Rifampicin induction regimens increase SPCN [9,13], presumably because rifampicin exerts its potent bactericidal action by targeting ribosomal components while retaining cell wall staining [8]. As rifampicin is a key drug for tuberculosis treatment, there may be more opportunities to experience SPCN in cases with a high initial smear grade than in those with lower grades.

In previous studies, SPCN status was more often observed in patients with cavities [8,10], and extensive lesions [8], than in others, but this finding was not seen in the current study. Female gender was also a significant associated factor for SPCN detection in the current study. Gender was not extracted as an associated factor with delayed culture negative conversion in most previous reports [15-18]. Indeed, Guler et al. and do Socorro Nantua Evangelista et al., actually reported that male gender was associated with delayed sputum smear negativity [19,20]. These reports are inconsistent with the current results. Since the number of cases without SPCN was very small, it is possible that significant differences could not be detected for the presence or absence of cavities and extent of lesions, with significant differences for gender found only by chance.

The initial smear grade, presence of cavities, and lesion extent were extracted as factors associated with the SPCN duration in both simple and multiple regression analyses. There have been no studies to date that have followed sputum examinations at short intervals and examined SPCN, as in the current study, and there are no reports on SPCN duration and its associated factors. However, as noted above, the initial smear grade, presence of cavities, and lesion extent were shown to be related to the SPCN detection rates at the 2-month [10], and 5-month [8], time points, suggesting that these factors may also influence the duration of SPCN detection. Kang et al. speculated that when the bacterial load is high, the number of dead bacteria is increased, and it takes time to relieve this heavy load [8].

In the current study, 43.4% of the patients were discharged after the confirmation of 3 culture-negative results due to prolonged SPCN. In a Japanese report of 301 cases with smear-positive pulmonary tuberculosis, 25.6% of the patients were discharged after confirmation of 3 culture-negative results without 3 consecutive smear-negative results [21], which was lower than the rate in the present study. These authors performed two sputum tests every two weeks, which means that their testing protocol was less frequent than that utilized in the current study. Less frequent testing might therefore reduce the number of patients discharged with 3 culture-negative results, as some patients might be discharged with 3 smear-negative results before 3 culture-negative results.

Since SPCN cases require prolonged hospitalization in Japan, we investigated the possibility of discharge by confirming the SPCN result once. Seventeen patients (16.5%) had a culture-positive result again after the SPCN status was confirmed. Therefore, it is difficult to predict the negative results of culture after confirming an SPCN result once.

Several limitations associated with the present study warrant mention. First, the number of cases was small. The total number of cases analyzed was 122, and 15% of these cases did not have SPCN. Further large-scale studies are required in the future. Second, as the data were collected only until discharge, the duration of SPCN after discharge was unknown. In fact, 43.4% of all patients were discharged with persistent SPCN, suggesting that SPCN persists even after discharge. A study of SPCN at the

5th month of treatment also reported SPCN in 1.5%-5.4% of patients [8,22,23]. It is possible that the period of SPCN detection in some patients was evaluated as short. Third, this was a retrospective study. A prospective study might be required to confirm the features of SPCN. Fourth, co-morbid conditions were not evaluated, even though smoking and diabetes have been reported as risk factors for SPCN detection and delayed smear negativity [10]. As this study was a retrospective study, data on these factors were missing and could not be fully accumulated. Fifth, there were no Human Immunodeficiency Virus patients or rifampicin-resistant/multidrug-resistant tuberculosis patients in the current study; therefore, the effects of an immunosuppressed state or drug-resistant organisms were not evaluated.

CONCLUSIONS

The rate of SPCN was high, and the amount of initial smear grading, the presence of cavities, and the extent of lesions were found to be associated with SPCN duration. A method of determining SPCN status in sputum smear-positive patients undergoing treatment is needed.

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Authorship Statement

All authors contributed to the study conception. The literature search was performed and the first draft of the manuscript written by Masanori Tanaka. The manuscript was corrected by Yoshiaki Minakata. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Conflict of interest disclosure: The authors declare that they have no competing interests.

Ethics approval statement: The study was carried out in accordance with the principles of the Declaration of Helsinki and approved by the Ethics Committee of the National Hospital Organization Wakayama Hospital (approved number: 03-8; approval date March 23, 2022).

Patient consent statement: The contents of this study and the opportunity to reject the agreement were explained on the website of National Hospital Organization Wakayama Hospital. URL: <http://wakayama.hosp.go.jp>

Clinical trial registration: The trial was registered in the University Hospital Medical Information Network (UMIN000048368, July 15, 2022).

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