Letter

Incidence of Active Tuberculosis over a Two-year Follow-up Period among Individuals with different Chest Radiographic Findings who were Excluded from Active Tuberculosis at Initial Baseline Screening

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Tuberculosis (TB) is a chronic infectious disease caused by *Mycobacterium tuberculosis* (MTB) that severely endangers human health. Despite significant progress in global TB control, TB remains one of the top ten causes of death worldwide. In its 2025 report, the World Health Organization (WHO) estimated 10.7 million new TB cases globally, with 1.23 million deaths. Early detection and treatment of TB are crucial for TB control. Community-based active case finding (ACF) is an active case detection strategy that is incorporated as a core component of Pillar 1 of the End TB Strategy and has demonstrated effectiveness in high-burden settings. Chest radiography (CXR) is highly sensitive and relatively easy to perform, making it a priority tool for systematic TB screening. The World Health Organization (WHO) and Chinese guidelines recommend ACF among the elderly and the general population in high-prevalence areas[1]. Systematic CXR-based TB screening involves identifying suspected TB cases (based on symptoms and/or CXR findings suggestive of TB) and referring them for diagnostic assessments aimed at early detection, prompt treatment initiation, effective reduction of TB incidence and mortality, and alleviation of the disease burden[2].

During ACF implementation, individuals identified as being at risk undergo bacteriological testing and are categorized as having clinically diagnosed TB, bacteriologically confirmed TB, or being bacteriologically negative. Previous studies have suggested that individuals who remain untreated despite having bacteriologically negative results (smear or culture) but CXR findings suggestive of TB have a risk of progression to smear- or culture-positive TB as high as 10%[3]. These studies also emphasize the importance of considering key host factors (symptoms, TB history, significant comorbidities, and immune risk profiles) in this population and conducting prospective follow-up studies to observe progression outcomes across all these dimensions. However, data on the impact of bacteriologically negative individuals with other non-suspicious lesions or normal CXR findings on the development of active TB are limited. It is necessary to investigate the risk factors for developing active TB among individuals with different CXR characteristics who are bacteriologically negative and are excluded as active TB cases, considering host characteristics. Implementing effective interventions for such individuals could prevent future TB and enhance the cost-effectiveness of systematic screening, particularly in aging populations and high-prevalence areas. Therefore, in 2021, we conducted CXR screening among the rural elderly in a high TB prevalence area in Zhejiang Province, China. We collected chest radiography (CXR) and bacteriological testing data to analyze active TB case detection at the time of screening based on different CXR findings. Furthermore, we established a cohort of bacteriologically negative individuals excluded as active TB cases, stratified them by their baseline CXR characteristics, and followed them for two years to observe the occurrence of bacteriologically positive TB cases. This provides data support for predicting TB risk and defining target populations for interventions.

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Zhejiang Province is located in eastern China. Quzhou City is a prefecture-level city in Zhejiang comprising six counties (districts/cities) and 100 townships. According to the Seventh National Population Census (2020), the resident population was 2.276 million, with 420,083 individuals (18.5%) aged 65 years or older. The reported TB incidence is high; the average annual reported pulmonary tuberculosis (PTB) incidence during 2017–2020 was 67.9 per 100,000 for the whole population, 175.7 per 100,000 for those aged ≥ 65 years, and the proportion of TB patients aged ≥ 65 years was 62.6% (1,752 of 2,799 cases), significantly higher than the 28.9% reported nationally in China in 2023[4].

Population data were obtained from the Quzhou City Population Census Yearbook (2020). Screening data were obtained from 105 primary healthcare centers in Quzhou, including information from resident health checkups and CXR results. Bacteriological test results, diagnoses, treatments, and treatment outcomes were obtained from the designated hospital's medical record system. TB patient data were extracted from the TB Information Management System of the Chinese Center for Disease Control and Prevention (China CDC). Mortality data were obtained from the Death Surveillance Management Information System, another subsystem of the Chinese center for disease control and prevention (China CDC).

Screening Method Permanent residents aged 65 years or older were screened from March to October 2021. Screening was organized by county-level health administrative departments, mobilized by village-level organizations, and implemented by the county-township integrated healthcare service system. The participants underwent resident health checkups and digital radiography chest examinations at township health centers. The CXR images were uploaded to county-level hospitals for radiological interpretation. Interpretation was performed manually, and the results were categorized as follows: no abnormality (A), any abnormality subdivided into (B) Suspected TB lesion(s), or (C) Non-suspected TB lesion(s). The results were fed back to the township health centers. Individuals with suspected TB (symptomatic and/or with CXR findings suggestive of TB) were referred for diagnostic evaluation (including bacteriological testing and clinical assessment) to confirm or exclude active PTB. Individuals not classified as having suspected TB were advised to seek diagnostic evaluation if they developed TB-related symptoms or during visits for other illnesses.

Follow-up Method The baseline cohort consisted of individuals categorized by their CXR characteristics who were bacteriologically negative and excluded as clinically diagnosed TB cases, follow-up primarily involved diagnostic evaluations focusing on bacteriological testing, individuals originally classified as suspected TB cases were contacted quarterly by phone. Those with no CXR abnormalities or non-suspected lesions were opportunistically informed during routine public health service visits. Additionally, quarterly clinical assessment information was collected from designated hospitals. Information was collected from county-level Centers for Disease Control and Prevention doctors visiting the diagnosing healthcare facilities for very few bacteriologically confirmed cases diagnosed outside Quzhou City. All participants were scheduled for a final visit at the end of the two-year follow-up period unless they were diagnosed with a case or died. Follow-up for 2022 spanned from January 1, 2022, to December 31, 2022. Follow-up for 2023 spanned from January 1, 2023, to December 31, 2023. The entire follow-up period was from January 1, 2022, to December 31, 2023. The follow-up period ended on December 31, 2023. Treatment outcome observations for bacteriologically positive cases were extended to December 30, 2024. To calculate person-time, follow-up for individuals who died, were diagnosed with clinical TB cases, or were bacteriologically confirmed TB cases during follow-up were censored at the date of the corresponding event.

Diagnostic evaluation was conducted at designated hospitals according to the People's Republic of China Health Industry Standard "Diagnostic Criteria for Tuberculosis (WS 288-2017)." The evaluation included clinical symptom inquiry, CXR, and laboratory tests. Laboratory tests included etiological diagnosis (sputum smear and MGIT-320 culture), immunological diagnosis, molecular biology, and drug susceptibility testing. Bacteriological testing included mycobacterial smears, liquid culture (MGIT), and GeneXpert MTB/RIF (Xpert). Induced sputum was used if the sputum volume or quality was inadequate. Bronchoscopic bronchoalveolar lavage (BAL) or lung tissue sampling was performed when necessary. Diagnosed cases included clinically diagnosed or bacteriologically confirmed TB cases. In this study, bacteriological testing refers to smears, cultures, and/or Xpert assays. The specimens were predominantly sputum; BAL fluid or lung tissue specimens were rarely used.

According to the People's Republic of China Health Industry Standard "Treatment of Tuberculosis (WS 288-2017)", individuals diagnosed as clinically diagnosed TB cases or bacteriologically confirmed TB cases received standardized, individualized treatment at designated hospitals after providing informed consent. The undiagnosed individuals did not receive any treatment or preventive therapy. Treatment outcomes included treatment success (cured, treatment completed) and treatment failure (death or other unsuccessful outcomes).

Quality Control CXR: Posteroanterior CXR were performed by radiological technicians or physicians at township health centers. CXR images were uploaded to the radiology department of county-level hospitals for manual interpretation. Interpretation was performed by a panel of three reviewers (2 infectious disease physicians and 1 radiologist). A consensus diagnosis by at least two reviewers was accepted as the radiological result. Panel physicians were required to hold practicing physician licenses, with at least one holding the title of attending physician or higher. All underwent training and passed qualification assessments by the Quzhou City TB Diagnosis and Treatment Quality Control Center. Additionally, during on-site quality control, 1% of X-ray films were randomly selected for re-evaluation. Sites achieving ≥ 90% concordance in re-evaluation were deemed qualified.

Laboratory Testing All laboratories met quality control requirements per guidelines and were capable of performing etiological diagnosis (sputum smear microscopy, MGIT-320 culture), immunological diagnosis, molecular biology, and drug susceptibility testing. Contamination rates for MTB cultures were maintained below 10%. Since 2015, all participating laboratories successfully completed 10 rounds of proficiency testing for TB molecular diagnosis and drug susceptibility testing organized by the National TB Reference Laboratory of the China CDC, with all verification results being satisfactory.

Statistical Methods Statistical analyses were conducted using IBM SPSS Statistics for Windows, Version 19.0 (IBM Corporation, Armonk, NY, USA). Categorical data are described as counts, proportions (%), and rates (%). Multivariate logistic regression was used to calculate the risk of PTB development among different CXR-finding groups at the screening stage. Cox regression models were used to assess the risk of progression from bacteriologically negative to bacteriologically positive TB. The incidence density was expressed as the number of new cases per 100 person-years. Risk is presented as adjusted hazard ratios (*HR*) with 95% confidence intervals (*CI*). A *P*-value < 0.05 was considered statistically significant.

Definitions Elderly permanent residents refer to those aged 65 years or older at the time of screening. Participation in Active Screening refers to participation in resident health checkups and CXR examinations. Any CXR abnormalities included suspected and non-suspected TB lesions. Suspected TB lesions are defined as CXR findings encompassing multiple nodular opacities; patchy, fluffy, or lobar consolidation; mass-like shadows; or enlargement of the hilar or mediastinal lymph nodes. These lesions are characterized by uneven density (higher centrally, lower peripherally), uneven distribution, and infiltrative changes; may be accompanied by thick-walled, thin-walled, tension cavities, or multiple moth-eaten cavities; and may also show adjacent "satellite lesions", bronchogenic spread, draining bronchi, lymphangitis, pleural effusion, etc. Nonsuspected TB lesions refer to other chest lesions on CXR — such as fibrotic scars, nodules, calcifications, and emphysema — that are not suspicious for TB. Bacteriological testing refers to smears, cultures, and/or molecular biology testing. Bacteriologically Negative refers to negative results on smear, culture, and/or molecular biology testing and is excluded as a clinically diagnosed TB case. Bacteriologically positive refers to positive results on smear, culture, and/or molecular biology testing. Bacteriologically confirmed TB cases are Xpert positive and/or culture-positive for MTB, and are also termed bacteriologically confirmed TB cases. Managed Hypertension refers to hypertension managed under the Essential Public Health Services program. Managed Diabetes refers to diabetes managed under the Essential Public Health Services program. Any TB symptoms refer to the self-reported presence of any TB-related symptoms (cough, fever, hemoptysis, night sweats, or weight loss) at screening. Incidence Density was defined as the number of bacteriologically positive cases during follow-up divided by the total person-years of follow-up. A history of Prior TB refers to a self-reported history of previous TB.

Background Screening Process and Baseline Characteristics In 2021, there were 420,083 permanent residents aged ≥ 65 years in the study area. Of these, 205,986 participated in resident health checkups, and 196,463 (46.8%) underwent CXR examinations and were included in the study. The CXR results showed no abnormalities in 140,293

(71.4%) and 56,170 (suspected TB lesions, 4,362; non-suspected lesions, 51,808) patients. Bacteriological testing was performed on 4,106 individuals (2.1%), of whom 210 were bacteriologically positive (204 bacteriologically confirmed TB cases, the other 6 were non-tuberculous mycobacteria) and 3,896 were bacteriologically negative (Supplementary Figure S1 and Supplementary Tables S1-1 to S1-5). Of the 3,896 bacteriologically negative individuals, excluding 55 deaths and 58 clinically diagnosed TB cases, the baseline cohort comprised 3,783 bacteriologically negative individuals who were excluded as active TB cases. Based on CXR findings, 1,419 patients had suspected TB lesions, 1,616 had non-suspected TB lesions, and 748 had no abnormalities. The population characteristics of each CXR stratum are shown in Table 1.

Follow-up and Incidence Among 3,783 baseline bacteriologically negative individuals, 113 developed

Table 1. Characteristics of study participants in whom active tuberculosis was excluded at baseline screening

Characteristics	Chest radiography at baseline *n* (%)			X^2	*P*
	Without abnormality	With abnormality suggestive of active TB	With abnormality suggestive of non-TB		
Total	748 (100)	1,419 (100)	1,616 (100)		NA
Sex				67.150	< 0.001
Male	424 (56.7)	1,049 (73.9)	1,074 (66.5)		
Female	324 (43.3)	370 (26.1)	542 (33.5)		
Age group (years)				17.446	< 0.001
65–	482 (64.4)	866 (61.0)	904 (55.9)		
≥ 75	266 (35.6)	553 (39.0)	712 (44.1)		
Body mass index (kg/m^2)				157.065	< 0.001
Underweight (< 18.5)	68 (9.1)	317 (22.3)	320 (19.8)		
Normal (18.5–)	482 (64.4)	975 (68.7)	1,082 (67.0)		
Overweight (≥ 25.0)	198 (26.5)	127 (8.9)	214 (13.2)		
Smoking				57.460	< 0.001
Current	181 (24.2)	545 (38.4)	458 (28.3)		
Former/never	567(75.8)	874 (61.6)	1,158 (71.7)		
Alcohol use				47.064	< 0.001
Current	26 (3.5)	175 (12.3)	141 (8.7)		
Former/never	722 (96.5)	1,244 (87.7)	1,475 (91.3)		
Any suspected TB symptoms				30.773	< 0.001
Yes	52 (7.0)	215 (15.2)	195 (12.1)		
No	696 (93.0)	1,204 (84.8)	1,421 (87.9)		
With any suspected symptoms of TB				58.954	< 0.001
Yes	71 (9.5)	323 (22.8)	332 (20.5)		
No	677 (90.5)	1,096 (77.2)	1,284 (79.5)		
Hypertension				45.861	< 0.001
Yes	170 (22.7)	387 (27.3)	571 (35.3)		
No	578 (77.3)	1,032 (72.7)	1,045 (64.7)		
Diabetes mellitus				6.990	0.030
Yes	59 (7.9)	99 (7.0)	155 (9.6)		
No	689 (92.1)	1,320 (93.0)	1,461 (90.4)		

Note. TB, tuberculosis.

bacteriologically positive active TB between 2022 and 2023 (90 in the suspected lesion group, 23 in the non-suspected lesion group, and 0 in the no abnormality group). The overall incidence rate was 1.62 per 100 person-years (95% *CI*: 1.32–1.91). Rates were 3.42 (95% *CI*: 2.73–4.12) for the suspected lesion group, 0.78 (95% *CI*: 0.46–1.10) for the non-suspected lesion group, and 0.00 for the no abnormality group. Compared with individuals with non-suspected TB lesions, those with suspected lesions had an *HR* of 4.215 (95% *CI*: 2.655–6.691) (Table 2 and Supplementary Table S2-1 to S2-6).

Comparison of characteristics within CXR strata: In the suspected lesion group, males had an *HR* of 1.814 (95% *CI*: 1.042–3.158) compared to females; other comparisons were not statistically significant (Supplementary Tables S2−S7). In the non-suspected lesion group, current alcohol consumption had an *HR* of 2.785 (95% *CI*: 1.034–7.500) compared to non-drinkers or former drinkers; other comparisons were not statistically significant (Supplementary Tables S2−S8). In the non-abnormality group, with no cases, statistical analysis was not feasible (Supplementary Tables S2−S9, Supplementary Figure S2). Comparison of characteristics between the suspected and non-suspected lesion groups adjusted for sex, age, any symptoms of TB, median body index, hypertension, diabetes mellitus, smoking, alcohol use, and history of previous TB. Across all the strata analyzed, age, sex, presence of any TB symptoms, diabetes status, smoking status, and history of prior TB as risk factors for progression were consistently higher in the baseline suspected TB lesion group than in the non-suspected lesion group. Notably, among individuals with a history of prior TB, the risk of progression in the baseline suspected lesion group was 12.47 times higher than in the baseline non-suspected lesion group (*HR* = 12.47; 95*% CI*: 1.58–98.06). Significant differences between the two groups were observed for all stratification factors, except for high body mass index and current alcohol consumption, where no statistically significant differences were found (Supplementary Figure S3 and Supplementary Tables S2−S10). As shown in Table 3, multiple logistic regression analysis indicated that male had increased risk of developing active disease (*HR* = 2.05; 95% *CI*: 1.11–3.81) compared with female.

Our study showed varying county-level screening coverage (27%–63%), prevalence of any CXR abnormality (28.6%), and detection rate of suspected TB lesions (2.2%), consistent with previous studies[5]. The proportion of individuals with suspected lesions who underwent bacteriological testing (38%, 1,658 people) aligned with the World Health Organization-reported rate of approximately 40%[6]. The risk of progression from baseline bacteriologically negative to positive was significantly higher in individuals with suspected or non-suspected lesions than in those with normal CXR. The incidence of progression in the suspected lesion group was 3.4% over two years, which was slightly lower than the 4% reported by Van't Hoog[7], with a higher risk observed in males. This may be explained by several factors: 1) ACF often detects subclinical cases in the early stages of TB with lower bacterial loads, and the reliance on smear microscopy in this study may have led to higher false-negative rates. 2) Sputum quality may have been an issue, as 77% (1,096/1,419) of the bacteriologically negative individuals with suspected lesions were asymptomatic, potentially making it more difficult to produce adequate sputum. 3) Early

Table 2. Incidence of confirmed active tuberculosis among study participants with respect to chest radiography at baseline

Characteristic	Abnormality, suspected	Abnormality, unsuspected	No abnormality
Subjects, *n*	1,419	1,616	748
Person-years[‡]	2,631.3	2,938.9	1,420.9
Incident confirmed tuberculosis, *n*	90	23	0
Cumulative incidence (95*% CI*) *%*	6.34 (5.07–7.61)	1.42 (0.85–2.00)	0.00 (0.00–0.00)
Incidence rate per 100 person-years (95*% CI*)	3.42 (2.73–4.12)	0.78 (0.46–1.10)	0.00 (0.00–0.00)
Adjusted hazard ratio (95*% CI*)[*]	4.191 (2.648–6.633)	Reference	–
Adjusted hazard ratio (95*% CI*)[&]	4.215 (2.655–6.691)	Reference	–

Note. [*]Adjusted for age and sex. [&]Adjusted for age, sex, body mass index, smoking, alcohol use, history of previous tuberculosis, any symptoms of tuberculosis, hypertension, diabetes. [‡]The total follow-up time period was defined as from January 1, 2022, to December 31, 2023. *CI*, confidence interval.

TB may involve intermittent bacillary excretion, which may be missed during testing. In this study, the median number of smear samples was three, and the median number of cultures was one (Supplementary Tables S1−S3). Estimates of the sensitivity and specificity of TB screening and diagnostic tests are influenced by the extent to which sequential testing procedures and patient selection pathways correspond to one or more dimensions of the underlying disease spectrum. This is particularly relevant, given that screening aims to detect a broad spectrum of diseases and typically employs multi-step algorithms[8].

We recommend prioritizing the follow-up of bacteriologically negative individuals with suspected or non-suspected lesions. Guidance on proper sputum collection and specimen quality is crucial. For those able to provide good-quality specimens, bacteriological diagnosis should favor highly sensitive, cost-effective, high-throughput molecular methods or repeated sputum examinations to increase detection. For those struggling to produce sputum, accelerating the deployment of non-sputum-based TB tests (e.g., urine and tongue swabs)[9] for community ACF is vital for reducing diagnostic delays and missed

Table 3. Risk factors related to the incidence of active in individuals with suspected abnormalities on CXR at baseline

Characteristic	At risk, *N*	Confirmed TB case, *N*	*HR* (95% *CI*)	*P*
All Participants	1,419	90		
Sex				0.023
Male	1,049	75	2.052 (1.106–3.806)	
Female	370	15	Reference	
Age group (years)				0.820
65–	866	55	Reference	
≥ 75	553	35	0.949 (0.607–1.486)	
Body index, kg/m^2				0.511
Underweight (< 18.5)	317	22	0.349 (0.038–3.190)	0.351
Normal (18.5–)	975	62	Reference	
Overweight (≥ 25.0)	127	6	0.719(0.301–1.716)	0.457
Smoking				0.529
Current	545	36	0.857 (0.531–1.385)	
Former/never	874	54	Reference	
Alcohol use				0.787
Current	175	11	0.911 (0.463–1.791)	
Former/never	1,244	79	Reference	
History of previous tuberculosis				0.629
Yes	215	13	0.866 (0.466–1.586)	
No	1,204	77	Reference	
Any symptoms of tuberculosis				0.286
Yes	323	23	3.281 (0.370–29.073)	
No	1,096	67	Reference	
Hypertension				0.956
Yes	387	24	1.014 (0.617–1.667)	
No	1,032	66	Reference	
Diabetes mellitus				0.501
Yes	99	7	1.328 (0.581–3.033)	
No	1,320	83	Reference	

Note. *HR,* hazard ratio; *CI*, confidence interval**.**

cases. The 1% progression rate in the non-suspected lesion group highlights that atypical CXR presentations in elderly patients with secondary PTB[10] can lead to missed diagnoses. We recommend strengthening the management of individuals with non-suspected lesions during ACF to ensure bacteriological testing for those with high-risk factors and implementing focused follow-up for those who test negative.

Based on this observational study, individuals who are bacteriologically negative and excluded as having active TB, but have either suspected or non-suspected lesions, are at a high risk for future TB development and should be prioritized for follow-up after screening. To enhance the cost-effectiveness of screening, particularly in large-scale community programs, it is essential to incorporate the management and outcomes of this group into the core monitoring and evaluation framework of ACF programs.

Limitations During the intensive ACF period, the large number of participants concentrated in a short timeframe and the heavy workload involved may have introduced bias in the collection of resident health checkup data, such as managed hypertension/diabetes status and self-reported symptoms at screening. CXR image classification relies solely on manual reading by radiologists, without using artificial intelligence (AI) systems. This may introduce bias in the CXR classification. Interpretation expertise reflects the mid-level physicians in county hospitals. The study period coincided with the COVID-19 pandemic, which may have affected screening participation rates and the proportion of individuals undergoing bacteriological testing. Bacteriological testing for individuals suspected to be at risk of TB was not uniformly comprehensive (i.e., not all individuals underwent smear, culture, and molecular testing as per the ideal protocol). Performing only one type of test (e.g., smear only) is common in this large-scale screening setting. During follow-up, only approximately 44% of the baseline cohort underwent at least one bacteriological test. Approximately 55% of patients received no bacteriological assessment during follow-up, which likely impacted the completeness of case detection.

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Competing Interests All authors declare no competing interests.

Ethics The study protocol was approved by the Ethics Committee of the Quzhou City Center for Disease Control and Prevention (approval No. IRB-2021-R-NO.001). All participants provided written informed consent for both the ACF program and subsequent follow-up at the time of screening.

Authors' Contributions Study conception and design: Ping Zhu, Lei Gao, Ying Du. Data collection and analysis: Ping Zhu, Ying Du, Yu Gao, Wei Wang. Drafting of the manuscript: Ping Zhu, Ying Du, Yan Qian. Critical revision of the manuscript: Lei Gao. Data organization: Ying Du, Yuhan Wang, Jianguo Liang. All authors contributed to and approved the final manuscript.

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Data Sharing All data relevant to the study have been included in the article or uploaded as supplementary information. The supplementary materials will be available in www.besjournal.com.

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