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Decoding microbiological discordance in tuberculosis: clinical phenotypes of molecular-only, culture-only, and dual confirmation in an Italian South TB Network (ISTB-Net)

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Decoding microbiological discordance in tuberculosis: clinical phenotypes of molecular-only, culture-only, and dual confirmation in an Italian South TB Network (ISTB-Net)

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Abstract

Purpose: Rapid molecular assays have increased microbiological confirmation of tuberculosis (TB), but the clinical meaning of discordant molecular and culture results remains incompletely defined. We compared the clinical phenotypes of patients with molecular-only, culture-only, and dual microbiological confirmation in pulmonary and extrapulmonary TB.

Methods: A multicenter observational cohort study was conducted in 22 Italian centres from 1 January 2021 to 30 May 2026. Patients with pulmonary TB (pTB) or pure extrapulmonary TB (epTB) and with both molecular and culture results were eligible. Patients were categorized accordingly to testing results as molecular-only (molecular positive/culture negative), culture-only (molecular negative/culture positive), or dual-confirmed (both positive). Logistic regression models estimated odds ratios (ORs) and adjusted odds ratios (aORs) for molecular-only and culture-only confirmation compared with dual-confirmed disease.

Results: 861 patients were included, of whom 685 with pTB (79.6%) and 176 with pure epTB (20.4%). In pTB, 83/685 (12.1%) were molecular-only, 76/685 (11.1%) culture-only, and 526/685 (76.8%) dual-confirmed, while in pure epTB, cases were respectively 29/176 (16.5%), 28/176 (15.9%), and 119/176 (67.6%). In pTB, molecular-only confirmation was independently associated with migrant status (aOR 24.24, 95% CI 2.97-197.66; p=0.003) reflecting the marked predominance of migrants among molecular-only cases (82/83, 98.8%), diabetes (aOR 6.87, 95% CI 3.00-15.74; p<0.001), and chronic pulmonary disease (aOR 3.62, 95% CI 1.30-10.12; p=0.014). Culture-only pTB was associated with older age (aOR per 10 years 1.37, 95% CI 1.11-1.69; p=0.003), female sex (aOR 2.28, 95% CI 1.08-4.82; p=0.031), and respiratory failure (aOR 2.66, 95% CI 1.25-5.65; p=0.011). In pure epTB, molecular-only confirmation was inversely associated with smear positivity (aOR 0.14, 95% CI 0.04-0.46; p=0.001). Culture-only epTB was associated with lower odds of systemic symptoms (aOR 0.08, 95% CI 0.02-0.35; p<0.001), longer total diagnostic delay (aOR per 30 days 1.06, 95% CI 1.02-1.11; p=0.008), and lower odds of multiple extrapulmonary sites (aOR 0.31, 95% CI 0.10-0.99; p=0.048).

Conclusions: Molecular and culture results identify overlapping but non-identical microbiological phenotypes. Molecular-only TB should not be interpreted as weak evidence of disease, while culture-only TB underlines the persistent role of culture in diagnostic pathways and resistance profiling.

Introduction

The complex diagnostic work-up of tuberculosis (TB) has long represented a major challenge to TB elimination. TB diagnosis relies on the integration of distinct criteria including epidemiological risk factors, clinical presentation, radiological findings, and immunological-microbiological confirmation whenever possible. The microbiological diagnosis is based on acid-fast bacilli (AFB) smear microscopy, nucleic acid amplification tests (NAATs), and mycobacterial culture, often integrated with genotypic and/or phenotypic drug susceptibility testing [1]. Multiple factors including the limited sensitivity of smear microscopy in detecting and estimating AFB, the prolonged turnaround time of mycobacterial culture and the need for trained laboratory personnel and specifically equipped facilities contribute to making TB control programs more expensive and challenging. Since molecular methods were introduced, improved sensitivity and shorter diagnostic turnaround times have facilitated both early diagnosis and active case finding [1].
Although the benefits of molecular diagnostics were initially evident in high-burden settings, a positive impact has also been observed in low-burden countries. In these settings, improved differentiation of TB from more common conditions with similar clinical presentations helps reduce the diagnostic delay of an old but often overlooked endemic disease. Over the last two decades, molecular testing has improved the diagnosis of extrapulmonary TB, overcoming some of the sensitivity limitations of conventional methods [2], and has enabled earlier treatment initiation, particularly in settings where limited TB-specific expertise might otherwise have delayed or prevented clinicians from initiating treatment for presumptive TB [3].
Furthermore, since molecular testing has been incorporated into the microbiological criteria for bacteriologically confirmed TB, its widespread implementation has contributed to redefining the clinical spectrum of the disease. TB is now recognized as a continuum of clinical presentations, and terms such as *asymptomatic TB*, *subclinical TB*, and *incipient TB* have become central to the current debate among TB specialists [4, 5]. Consequently, the clinical significance of molecular testing positivity has become an emerging area of research, as the detection of *Mycobacterium tuberculosis* (*M. tb*) DNA in the absence or presence of culture positivity may reflect different stages or phenotypes of TB disease [3].
Therefore, the aim of this study was to determine whether different microbiological confirmation patterns are associated with distinct demographic characteristics, comorbidities, clinical profiles, diagnostic delay, disease extent, and drug-resistance profiles in patients with pulmonary TB (pTB) and pure extrapulmonary TB (epTB).

Methods

Study design and setting

A multicentre observational cross-sectional cohort study was conducted using the Italian South TB Network (ISTB-Net) database, including consecutive TB patients managed in 22 participating centres across 7 Italian regions (Puglia, Calabria, Basilicata, Campania, Sicilia, Molise, Lazio) from 1 January 2021 to 30 May 2026. The present analysis was restricted to those patients for whom both molecular and culture results were available, because the primary objective was to compare microbiological confirmation patterns defined by the combination of these two tests. List of participant centers is in *Supplementary file 1*.

Study population and Disease localization

Patients were considered eligible if they had records of both a molecular and a culture test result, each coded as positive or negative. Patients with negative results of both molecular and culture tests (M-/C-) were excluded from the primary microbiologically-confirmed pattern analysis. Patients with at least one positive test (M+/C-; M-/C+; M+/C+) were considered microbiologically-confirmed cases for the purpose of this study. Records that could not be classified as pulmonary TB (pTB) or pure extrapulmonary TB (epTB) were excluded from the main analytical cohort.
Specifically, pTB was defined as any pulmonary involvement recorded in the database (either with or without evidence for concomitant extrapulmonary involvement), while pure epTB was defined as mere extrapulmonary disease without

recorded pulmonary involvement. In sensitivity analysis, pTB was further restricted to isolated pulmonary disease by excluding patients with concomitant extrapulmonary localization.

Microbiological confirmation patterns

Within pTB and pure epTB, patients were classified into three mutually exclusive confirmation groups: molecular-only confirmation, defined as a positive molecular result and negative culture (M+/C-); culture-only confirmation, defined as a negative molecular result and positive culture (M-/C+); and dual confirmation, defined as positivity of both molecular testing and culture (M+/C+). The dual-confirmed group was used as the reference category in regression analyses because it represents concordant microbiological confirmation by both methods. In the text the expression "molecular-only" and "culture-only" imply the term "confirmed", which will not be repeated each time. Molecular testing was performed using Xpert MTB/RIF, Xpert MTB/RIF Ultra, or TaqMan MTB PCR, according to the diagnostic platform used at the participating centres. Mycobacterial culture was performed according to standard laboratory procedures according international guidelines. Molecular testing and culture were performed on the same clinical specimen, using separate aliquots of the specimen for the two assays.

Variables and definitions

Age was calculated as year of TB diagnosis minus year of birth and analysed per 10-year increase in regression models. Migrant status was defined as country of origin being other than Italy. Geographical origin was classified according to WHO regions of origin. Italy was retained as a separate category, while the European Region (EURO) included countries of the WHO European Region other than Italy. The other areas corresponded to the following WHO official regions: African Region (AFRO), Eastern Mediterranean Region (EMRO), South-East Asian Region (SEARO), Region of the Americas (AMRO), and Western Pacific Region (WPRO).

Comorbidities included diabetes mellitus (DM), current smoking, chronic obstructive pulmonary disease (COPD), human immunodeficiency virus (HIV) infection, and previous TB.

Clinical variables included cough, fever, undesired weight loss, night sweats, dyspnoea, haemoptysis and chest pain. For pure epTB, systemic symptoms were defined as the presence of fever and/or weight loss and/or night sweats.

Patient-dependent diagnostic delay was defined as the interval between symptom onset and first access to the National Health Service. Health system-dependent diagnostic delay was defined as the interval between first healthcare facility access and start of anti-TB treatment. Total diagnostic delay was defined as the sum of patient-dependent and health system-dependent diagnostic delay. When the total diagnostic delay information was missing but both components of delay were available, total delay was calculated as a sum of the two components.

For pTB, disease burden was described using smear positivity, TIMIKA score (20 points for each affected lung lobe plus 40 points if any cavitation is present), high radiological burden, respiratory failure and concomitant extrapulmonary involvement. A TIMIKA score ≥80 was regarded as a high radiological burden. The extensive disease proxy in pTB was defined as the presence of at least one of the following: smear positivity, TIMIKA score ≥80, respiratory failure, or concomitant extrapulmonary involvement.

For pure epTB, disease extent was described using the number and type of extrapulmonary sites recorded in the database. Multiple extrapulmonary sites were used to define the disease-extension variable and as the extensive disease proxy. Recorded extrapulmonary localizations were grouped into the following categories: lymph node, pleural, abdominal, meningeal/central nervous system (CNS), genitourinary/renal, pericardial, bone/joint isolated, multiple extrapulmonary sites, pleuro-pulmonary or mixed site recorded, and not recorded. The "pleuro-pulmonary or mixed site recorded" category was retained descriptively for records classified as pure epTB according to the main pulmonary involvement variable but with a mixed site description in the free-text localization field.

Accordingly to the WHO definition [6], drug-resistance variables included rifampicin-resistant TB (RR-TB), rifampicin-susceptible isoniazid-resistant TB (Hr-TB), multidrug-resistant TB (MDR-TB), any drug resistance, pre-extensively drug-resistant TB (pre-XDR-TB), and extensively drug-resistant TB (XDR-TB). RR-TB was defined as resistance to rifampicin, irrespective of resistance to other drugs. Hr-TB was defined as resistance to isoniazid with susceptibility to rifampicin.

MDR-TB was defined as resistance to at least both rifampicin and isoniazid. Pre-XDR-TB was defined as MDR/RR-TB with additional resistance to any fluoroquinolone. XDR-TB was defined as MDR/RR-TB with additional resistance to any fluoroquinolone and to at least one additional Group A drug, namely bedaquiline or linezolid.

Treatment outcome was classified as treatment completion or unfavourable outcome. Unfavourable outcome was defined as death or loss to follow-up. Patients still on treatment at the time of data extraction were excluded from treatment-outcome denominators.

Statistical analysis

Categorical variables are presented as number and percentage. Continuous variables are presented as median and interquartile range (IQR). Analyses were performed separately for pTB and pure epTB. Comparisons among molecular-only (M+/C-), culture-only (M-/C+) and dual-confirmed (M+/C+) groups used the chi-square test or Fisher exact test for categorical variables and the Kruskal-Wallis test for continuous variables. Agreement between molecular testing and culture was summarized using observed agreement, Cohen's kappa and Gwet's AC1, and visualized using agreement plots.

Unadjusted odds ratios (ORs) were estimated for molecular-only and culture-only confirmation, each compared with dual-confirmed disease. Multivariable logistic regression models were then fitted separately for each comparison. For pTB, Model A included age, sex, migrant status, diabetes, current smoking, chronic pulmonary disease and total diagnostic delay. Model B additionally included smear positivity, high pulmonary disease burden (TIMIKA score ≥80), respiratory failure and previous TB. Model B was specified as a secondary exptloratory model to assess whether the observed associations persisted after additional adjustment for markers of disease burden. Model B was interpreted as an exploratory adjustment for disease burden rather than as a causal model. For pure epTB, parsimonious exploratory models included age, sex, systemic symptoms, total diagnostic delay, smear positivity and multiple extrapulmonary sites. Migrant status was included in the molecular-only (M+/C-) epTB model but excluded from the culture-only (M-/C+) epTB model because all culture-only epTB cases with available data were migrants, producing quasi-complete separation.

Sensitivity analyses were performed by restricting pTB to isolated pulmonary disease and by estimating centre-clustered standard errors for the main pTB models. All regression analyses were complete-case. No imputation was performed. Because the analysis was exploratory and designed to identify clinically interpretable patterns, p-values were not adjusted for multiple comparisons. WHO region of origin and extrapulmonary disease sites were described overall and according to disease localization. Extrapulmonary disease sites were also summarized by microbiological confirmation pattern as a descriptive analysis, without formal modelling because of the limited number of events in several site categories.

Results

Cohort selection and confirmation patterns

Among 1,242 records, molecular test results were available for 1,096 patients (88.2%) and culture results for 1,007 patients (81.1%). A total of 964 patients (77.6% of the overall database) had both molecular and culture results available. Of these, 101/964 (10.5%) had both tests negative and were excluded from the confirmed-pattern analysis. Among the remaining 863 patients with at least one positive test (89.5% of those with both tests available), 2/863 (0.2%) were excluded because disease localization could not be classified as pTB or pure epTB. The final analytical cohort therefore included 861 patients (69.3% of the overall database and 89.3% of patients with both tests available): 685 with pTB (79.6%) and 176 with pure epTB (20.4%) (*Supplementary file 2*).

The distribution of microbiological confirmation patterns differed by disease localization (p=0.043). In pTB, 83/685 patients (12.1%) were molecular-only, 76/685 (11.1%) were culture-only, and 526/685 (76.8%) were dual-confirmed. In pure epTB, 29/176 patients (16.5%) were molecular-only, 28/176 (15.9%) were culture-only, and 119/176 (67.6%) were dual-confirmed. Single-method positivity confirmation was therefore more frequent in pure epTB than in pTB.

Geographical origin

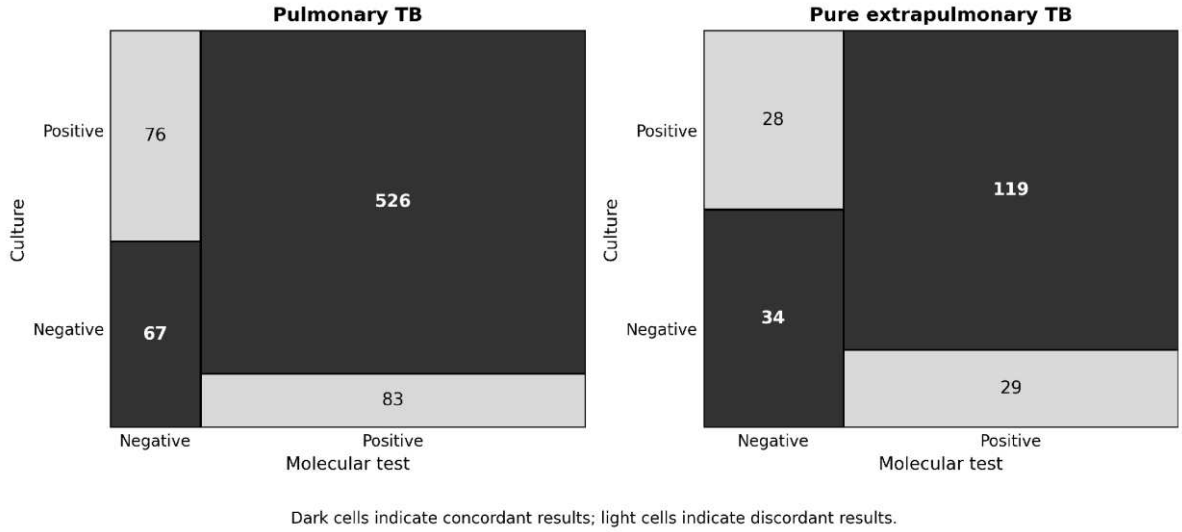
Overall, the most represented WHO regions of origin were the African Region (AFRO; 278/861, 32.3%), Italy (165/861, 19.2%), the European Region excluding Italy (EURO; 154/861, 17.9%), the South-East Asian Region (SEARO; 135/861, 15.7%), and the Eastern Mediterranean Region (EMRO; 99/861, 11.5%). The Region of the Americas (AMRO) and Western Pacific Region (WPRO) accounted for 17/861 (2.0%) and 13/861 (1.5%) patients, respectively.

The distribution of WHO region of origin differed between pTB and pure epTB. Compared with pTB, pure epTB included a higher proportion of patients from AFRO (65/176, 36.9% vs 213/685, 31.1%) and SEARO (45/176, 25.6% vs 90/685, 13.1%), while patients from Italy represented 23/176 (13.1%) of pure epTB and 142/685 (20.7%) of pTB. The full distribution of WHO regions of origin by disease localization and microbiological confirmation pattern is reported in *Supplementary Table S3 - S4*.

Agreement between molecular testing and culture

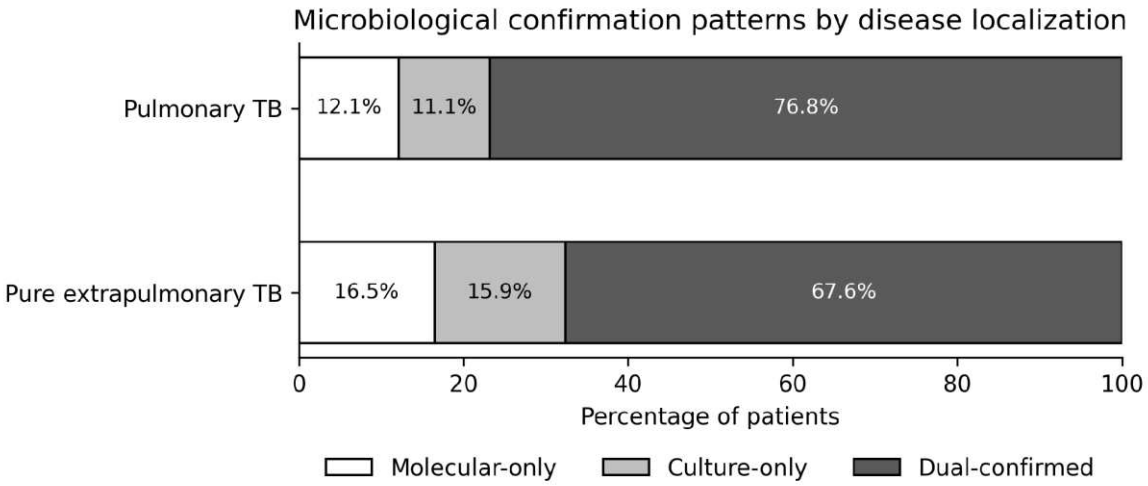
Observed agreement was 0.775, Cohen's kappa was 0.338, and Gwet's AC1 was 0.659. In pTB, observed agreement was 0.789, Cohen's kappa 0.326, and Gwet's AC1 0.692. In pure epTB, observed agreement was 0.729, Cohen's kappa 0.351, and Gwet's AC1 0.534. These results indicate that molecular testing and culture were related but not interchangeable diagnostic signals, with lower agreement in pure epTB (Figure 1-2). Among the 964 patients with both molecular and culture results available, including 101 patients with concordant negative results, 646 were positive by both methods, 112 were molecular-positive/culture-negative, 105 were molecular-negative/culture-positive, and 101 were negative by both methods. Accordingly, agreement analyses were based on the full cohort of 964 patients with paired test results, whereas confirmation-pattern analyses were restricted to the 861 patients with at least one positive test.

Figure 1. Agreement between molecular testing and culture by disease localization.



Abbreviations: TB: tuberculosis.

Figure 2. Microbiological confirmation patterns by disease localization.



Legend: Bars show the proportion of molecular-only, culture-only and dual-confirmed cases in pTB and pure epTB.
Abbreviations: TB: tuberculosis; pTB: pulmonary tuberculosis; epTB: extrapulmonary tuberculosis.

Pulmonary tuberculosis

Among 685 patients with pTB, the three confirmation groups differed in demographic profile, comorbidity burden, diagnostic delay and disease-burden markers (Table 1). Median age was 39 years (IQR 30.5-48) in molecular-only cases, 44 years (IQR 36.8-59.5) in culture-only cases and 40 years (IQR 29-53) in dual-confirmed cases (p=0.048). Female sex did not differ significantly across groups (p=0.351). Migrant status was markedly more frequent in molecular-only pTB: 82/83 patients (98.8%), compared with 62/76 (81.6%) culture-only and 399/526 (75.9%) dual-confirmed cases (p<0.001). Diabetes was also more frequent in molecular-only pTB (20/83, 24.1%) than in culture-only (6/76, 7.9%) or dual-confirmed disease (49/526, 9.3%; p<0.001).

Symptom patterns suggested that culture-only pTB was less systemically symptomatic. Fever was present in 19/83 molecular-only cases (22.9%), 8/76 culture-only cases (10.5%) and 132/526 dual-confirmed cases (25.1%; p=0.019). Weight loss was recorded in 17/83 (20.5%), 5/76 (6.6%) and 109/526 (20.7%), respectively (p=0.013). Cough, night sweats, dyspnoea and haemoptysis did not differ significantly across the three groups.

Diagnostic delay was longest in molecular-only pTB. Median patient delay was 120 days (IQR 60-225) in molecular-only cases, 70 days (IQR 20-200) in culture-only cases and 55 days (IQR 15-150) in dual-confirmed cases (p<0.001). Median total diagnostic delay was 123 days (IQR 71-300), 95 days (IQR 45-210) and 90 days (IQR 37-205), respectively (p=0.005). Health-system delay did not differ significantly (p=0.951).

Disease-burden variables also differed across confirmation patterns. Smear positivity was documented in 63/83 molecular-only cases (75.9%), 39/72 culture-only cases (54.2%) and 411/509 dual-confirmed cases (80.7%; p<0.001). Median TIMIKA score was highest in molecular-only pTB: 80 (IQR 40-100), compared with 60 (IQR 40-80) in culture-only and 70 (IQR 60-80) in dual-confirmed disease (p=0.002). High radiological burden (TIMIKA score ≥ 80) was present in 52.3%, 27.3% and 38.8% of the three groups, respectively (p=0.018). The extensive disease proxy was present in 95.2% of molecular-only pTB, 71.1% of culture-only pTB and 86.9% of dual-confirmed pTB (p<0.001). Respiratory failure was most frequent in culture-only pTB (36.8%) compared with molecular-only (16.9%) and dual-confirmed disease (26.0%; p=0.016).

Drug-resistance variables were not markedly different in pTB, although isoniazid resistance showed a borderline difference across groups: 20.5% in molecular-only, 27.6% in culture-only and 16.5% in dual-confirmed pTB (p=0.055). Rifampicin resistance was recorded in 7.2%, 2.6% and 7.4%, respectively (p=0.302). Length of stay differed significantly, with a median of 50 days (IQR 25-70) in molecular-only pTB, compared with 30 days in both culture-only and dual-confirmed pTB (p=0.012). Treatment outcome data were available for 77, 68 and 510 patients with pTB, respectively, because treatment was still ongoing in the remaining cases. Treatment completion was lower in molecular-only and culture-only pTB than in dual-confirmed pTB: 60/77 (77.9%), 51/68 (75.0%) and 447/510 (87.6%), respectively (p=0.004).

Pure extrapulmonary tuberculosis

Among 176 patients with pure epTB, confirmation patterns were also associated with distinct clinical profiles (Table 2). Median age was numerically higher in molecular-only cases, although the difference was not significant: 54 years (IQR 26-58.5) in molecular-only, 39 years (IQR 27.5-43.5) in culture-only and 36.5 years (IQR 25-55) in dual-confirmed disease (p=0.397). Migrant status differed significantly across groups: 22/29 molecular-only cases (75.9%), 28/28 culture-only cases (100.0%) and 103/119 dual-confirmed cases (86.6%; p=0.025). Among the 176 patients classified as pure epTB, the most frequent recorded localizations were lymph node TB (36/176, 20.5%), pleural TB (31/176, 17.6%), multiple extrapulmonary sites (28/176, 15.9%), abdominal TB (14/176, 8.0%), meningeal/CNS TB (12/176, 6.8%), genitourinary/renal TB (11/176, 6.2%) disseminated or multisite disease (7/176, 4.0%), pericardial TB (7/176, 4.0%), and isolated bone/joint TB (5/176, 2.8%). Extrapulmonary site was not recorded in 11/176 patients (6.2%). A pleuro-pulmonary or mixed site was recorded in 14/176 patients (8.0%) despite the absence of pulmonary involvement according to the main pulmonary TB variable; these cases were retained in the pure epTB cohort according to the prespecified classification rule and described separately. The distribution of extrapulmonary sites differed across confirmation patterns. Molecular-only epTB included a higher proportion of pericardial disease (7/29, 24.1%), genitourinary/renal disease (5/29, 17.2%), and isolated bone/joint disease (3/29, 10.3%). Culture-only epTB was enriched for meningeal/CNS disease (10/28, 35.7%) and abdominal TB (4/28, 14.3%). Dual-confirmed epTB was most represented by lymph node TB (30/119, 25.2%) and pleural TB (27/119, 22.7%). The site distribution is reported in *Supplementary Table S5*.

Systemic symptoms were much less frequent in culture-only epTB. They were present in 20/29 molecular-only cases (69.0%), 6/28 culture-only cases (21.4%) and 69/119 dual-confirmed cases (58.0%; p<0.001). Fever showed the same pattern, occurring in 51.7%, 14.3% and 42.9% of the three groups, respectively (p=0.007). Weight loss and night sweats did not differ significantly.

Diagnostic delay in pure epTB had a different pattern from pTB. Median patient delay was 30 days (IQR 7-150) in molecular-only cases, 190 days (IQR 45-271.2) in culture-only cases and 60 days (IQR 21-155) in dual-confirmed disease (p=0.005). In contrast, median health-system delay was longest in molecular-only epTB: 30 days (IQR 7-60), compared with 4 days (IQR 2-15.2) in culture-only and 8 days (IQR 3-17) in dual-confirmed disease (p=0.001). Total diagnostic delay

was numerically longest in culture-only epTB but did not reach conventional statistical significance in the three-group comparison (p=0.092).

Smear positivity was least frequent in molecular-only epTB: 7/29 (24.1%), compared with 18/28 (64.3%) culture-only and 68/119 (57.1%) dual-confirmed cases (p=0.003). Multiple extrapulmonary sites were more common in molecular-only and dual-confirmed disease than in culture-only epTB: 51.7%, 46.2% and 17.9%, respectively (p=0.013). Isoniazid resistance was absent in molecular-only epTB and present in 21.4% of culture-only and 21.0% of dual-confirmed cases (p=0.024). Treatment outcome data were available for 24, 26 and 113 patients with pure epTB, respectively, because treatment was still ongoing in the remaining cases. Treatment completion did not differ across confirmation patterns: 21/24 (87.5%), 22/26 (84.6%) and 95/113 (84.1%), respectively (p=0.914).

Adjusted analyses

In adjusted pTB Model B, molecular-only confirmation remained independently associated with migrant status (aOR 24.24, 95% CI 2.97-197.66; p=0.003), diabetes (aOR 6.87, 95% CI 3.00-15.74; p<0.001) and chronic pulmonary disease (aOR 3.62, 95% CI 1.30-10.12; p=0.014. In Model B, respiratory failure showed an inverse association with molecular-only confirmation (aOR 0.37, 95% CI 0.15–0.90; p=0.029). Total diagnostic delay showed a borderline association with molecular-only pTB (aOR per 30 days 1.04, 95% CI 1.00-1.08; p=0.065).

Culture-only pTB was independently associated with older age (aOR per 10 years 1.37, 95% CI 1.11-1.69; p=0.003), female sex (aOR 2.28, 95% CI 1.08-4.82; p=0.031) and respiratory failure (aOR 2.66, 95% CI 1.25-5.65; p=0.011). High TIMIKA score showed a borderline inverse association with culture-only confirmation (aOR 0.51, 95% CI 0.24-1.07; p=0.075).

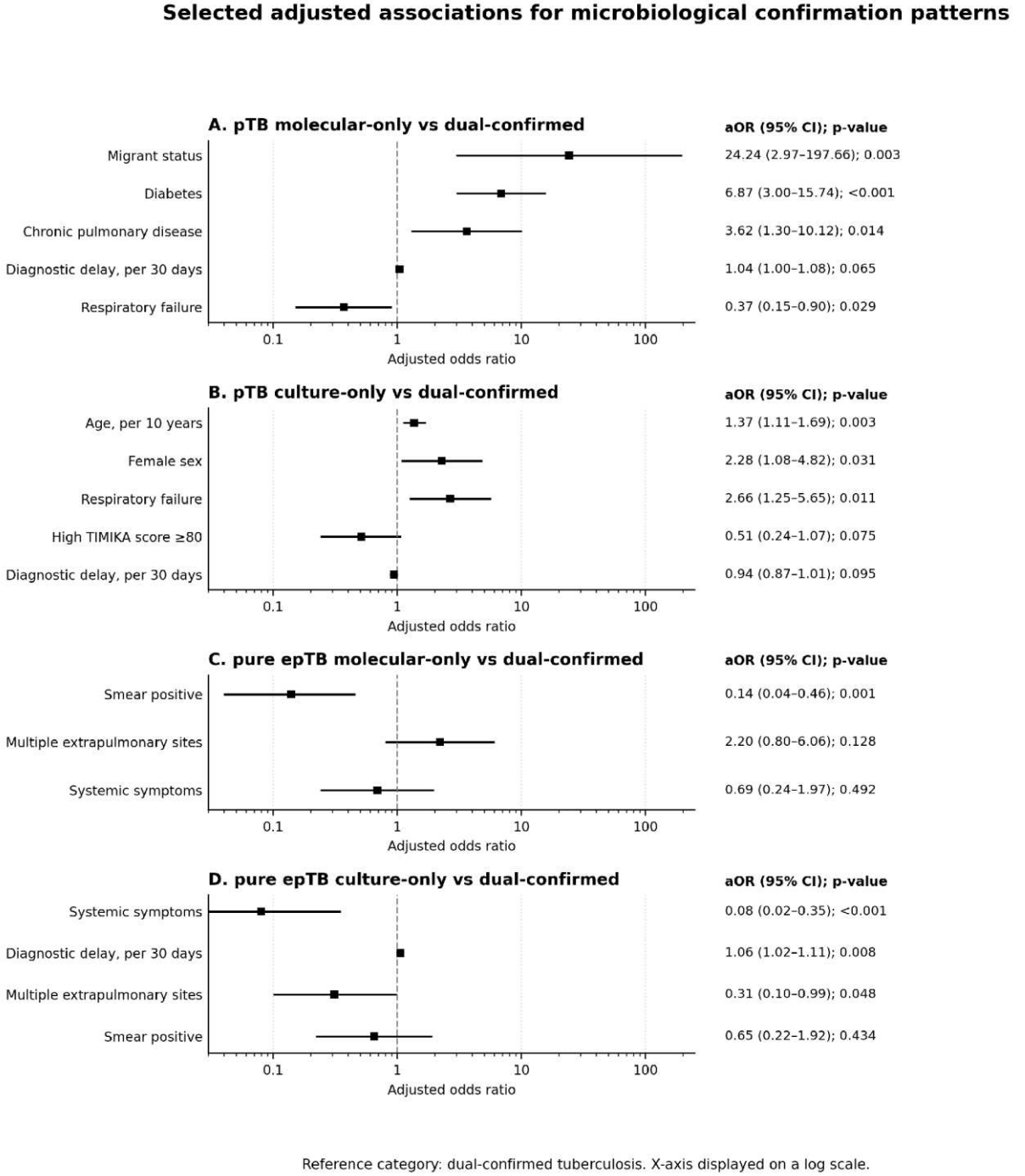
In pure epTB, molecular-only confirmation was independently associated with lower odds of smear positivity (aOR 0.14, 95% CI 0.04-0.46; p=0.001). In the exploratory adjusted analysis of pure epTB, culture-only confirmation showed associations with systemic symptoms(aOR 0.08, 95% CI 0.02-0.35; p<0.001), total diagnostic delay (aOR per 30 days 1.06, 95% CI 1.02-1.11; p=0.008) and multiple extrapulmonary sites (aOR 0.31, 95% CI 0.10-0.99; p=0.048). Given the limited number of culture-only cases, these estimates were considered exploratory and interpreted cautiously. The selected adjusted associations are summarized in Table 3 and Figure 3.

Sensitivity analyses

When the pTB cohort was restricted to isolated pulmonary disease, 661 patients remained: 80 molecular-only (12.1%), 75 culture-only (11.3%) and 506 dual-confirmed (76.6%). The main findings were unchanged. Molecular-only isolated pTB remained associated with migrant status (aOR 24.30, 95% CI 3.02-195.74; p=0.003) and diabetes (aOR 7.43, 95% CI 3.20-17.25; p<0.001), while respiratory failure remained inversely associated (aOR 0.38, 95% CI 0.15-0.96; p=0.041). Culture-only isolated pTB remained associated with older age (aOR per 10 years 1.36, 95% CI 1.10-1.67; p=0.004), female sex (aOR 2.55, 95% CI 1.20-5.42; p=0.015) and respiratory failure (aOR 2.54, 95% CI 1.19-5.41; p=0.016).

Using centre-clustered standard errors for the main pTB models did not materially change the strongest associations. Molecular-only pTB remained associated with migrant status (p=0.004), diabetes (p=0.001) and chronic pulmonary disease (p<0.001). Culture-only pTB remained associated with older age (p<0.001), female sex (p=0.015) and respiratory failure (p=0.028). The sensitivity analysis are summarized in Table 4.

Figure 3. Selected adjusted associations for microbiological confirmation patterns.



Abbreviations: pTB: pulmonary tuberculosis; epTB: extrapulmonary tuberculosis; aOR: adjusted odds ratio; CI: confidence interval.

Table 1. Pulmonary tuberculosis characteristics by confirmation pattern.

Variable	M+/C-	M-/C+	M+/C+	p-value
N	83	76	526	
Age, years, median (IQR)	39 (30.5–48)	44 (36.8–59.5)	40 (29–53)	0.048
Female sex, n/N (%)	20/83 (24.1)	24/76 (31.6)	126/526 (24.0)	0.351
Migrant status, n/N (%)	82/83 (98.8)	62/76 (81.6)	399/526 (75.9)	<0.001
Diabetes, n/N (%)	20/83 (24.1)	6/76 (7.9)	49/526 (9.3)	<0.001
Current smoker, n/N (%)	32/83 (38.6)	33/76 (43.4)	179/526 (34.0)	0.234
COPD, n/N (%)	10/63 (15.9)	4/65 (6.2)	50/487 (10.3)	0.193
HIV-positive, n/N (%)	0/83 (0)	2/76 (2.6)	16/526 (3.0)	0.274
Previous TB, n/N (%)	8/83 (9.6)	2/76 (2.6)	21/526 (4.0)	0.050
Any symptom, n/N (%)	36/83 (43.4)	39/76 (51.3)	285/526 (54.2)	0.182
Cough, n/N (%)	24/83 (28.9)	26/76 (34.2)	197/526 (37.5)	0.302
Fever, n/N (%)	19/83 (22.9)	8/76 (10.5)	132/526 (25.1)	0.019
Weight loss, n/N (%)	17/83 (20.5)	5/76 (6.6)	109/526 (20.7)	0.013
Night sweats, n/N (%)	10/83 (12.0)	13/76 (17.1)	49/526 (9.3)	0.104
Dyspnoea, n/N (%)	7/83 (8.4)	5/76 (6.6)	32/526 (6.1)	0.718
Haemoptysis, n/N (%)	2/83 (2.4)	3/76 (3.9)	44/526 (8.4)	0.076
Patient delay, days, median (IQR)	120 (60–225)	70 (20–200)	55 (15–150)	<0.001
Health-system delay, days, median (IQR)	10 (3–21)	7.5 (4.2–15.8)	7 (3–23)	0.951
Total diagnostic delay, days, median (IQR)	123 (71–300)	95 (45–210)	90 (37–205)	0.005
Smear positive, n/N (%)	63/83 (75.9)	39/72 (54.2)	411/509 (80.7)	<0.001
TIMIKA score, median (IQR)	80 (40–100)	60 (40–80)	70 (60–80)	0.002
High radiological burden (TIMIKA ≥80), n/N (%)	34/65 (52.3)	15/55 (27.3)	176/454 (38.8)	0.018
Respiratory failure, n/N (%)	14/83 (16.9)	28/76 (36.8)	137/526 (26.0)	0.016
Concomitant extrapulmonary involvement, n/N (%)	3/83 (3.6)	1/76 (1.3)	20/526 (3.8)	0.544
Extensive disease proxy, n/N (%)	79/83 (95.2)	54/76 (71.1)	457/526 (86.9)	<0.001
Rifampicin resistance, n/N (%)	6/83 (7.2)	2/76 (2.6)	39/526 (7.4)	0.302
Isoniazid resistance, n/N (%)	17/83 (20.5)	21/76 (27.6)	87/526 (16.5)	0.055
RR/MDR-TB, n/N (%)	2/83 (2.4)	2/76 (2.6)	21/526 (4.0)	0.683
Length of stay, days, median (IQR)	50 (25–70)	30 (16.8–52)	30 (20–60)	0.012
Complete treatment*	60/77 (77.9)	51/68 (75.0)	447/510 (87.6)	0.04
Negative outcome	17/77 (22.1)	17/68(25)	63/510 (12.4)	0.04

Legend: p-values compare molecular-only, culture-only and dual-confirmed pTB. pTB includes pulmonary disease with or without concomitant extrapulmonary localization. Extensive disease was defined as smear positivity and/or TIMIKA score ≥ 80 and/or respiratory failure and/or concomitant extrapulmonary involvement. Negative outcome: lost to follow-up+death

* "Outcome data were available for 77, 68 and 510 patients, respectively, as treatment was still ongoing in the remaining cases

Abbreviations: TB, tuberculosis; pTB, pulmonary tuberculosis; HIV, human immunodeficiency virus; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; TIMIKA, radiographic severity score; RR/MDR-TB, rifampicin-resistant or multidrug-resistant tuberculosis.

Table 2. Pure extrapulmonary tuberculosis characteristics by confirmation pattern.

Variable	M+/C-	M-/C+	M+/C+	p-value
N	29	28	119	
Age, years, median (IQR)	54 (26–58.5)	39 (27.5–43.5)	36.5 (25–55)	0.397
Female sex, n/N (%)	5/29 (17.2%)	8/28 (28.6%)	29/119 (24.4%)	0.589
Migrant status, n/N (%)	22/29 (75.9%)	28/28 (100.0%)	103/119 (86.6%)	0.025
Diabetes, n/N (%)	3/29 (10.3%)	5/28 (17.9%)	23/119 (19.3%)	0.523
Current smoker, n/N (%)	7/29 (24.1%)	13/28 (46.4%)	57/119 (47.9%)	0.066
COPD, n/N (%)	2/29 (6.9%)	2/22 (9.1%)	13/108 (12.0%)	0.704
HIV-positive, n/N (%)	0/29 (0.0%)	1/28 (3.6%)	7/119 (5.9%)	0.381
Previous TB, n/N (%)	3/29 (10.3%)	2/28 (7.1%)	7/119 (5.9%)	0.692
Systemic symptoms, n/N (%)	20/29 (69.0%)	6/28 (21.4%)	69/119 (58.0%)	<0.001
Fever, n/N (%)	15/29 (51.7%)	4/28 (14.3%)	51/119 (42.9%)	0.007
Weight loss, n/N (%)	8/29 (27.6%)	5/28 (17.9%)	36/119 (30.3%)	0.420
Night sweats, n/N (%)	6/29 (20.7%)	2/28 (7.1%)	11/119 (9.2%)	0.163
Patient delay, days, median (IQR)	30 (7–150)	190 (45–271.2)	60 (21–155)	0.005
Health-system delay, days, median (IQR)	30 (7–60)	4 (2–15.2)	8 (3–17)	0.001
Total diagnostic delay, days, median (IQR)	67 (55–155)	192 (46–274.5)	84 (32.5–204)	0.092
Smear positive, n/N (%)	7/29 (24.1%)	18/28 (64.3%)	68/119 (57.1%)	0.003
Extensive disease proxy, n/N (%)	15/29 (51.7%)	5/28 (17.9%)	55/119 (46.2%)	0.013
Rifampicin resistance, n/N (%)	0/29 (0.0%)	1/28 (3.6%)	12/119 (10.1%)	0.124
Isoniazid resistance, n/N (%)	0/29 (0.0%)	6/28 (21.4%)	25/119 (21.0%)	0.024
RR/MDR-TB, n/N (%)	0/29 (0.0%)	0/28 (0.0%)	1/119 (0.8%)	0.786
Length of stay, days, median (IQR)	25 (24–62)	42.5 (22–64)	33 (15–60)	0.131
Complete treatment*	21/24 (87.5)	22/26 (84.6)	95/113 (84.1)	0.914
Unfavourable outcome	3/24 (12.5)	4/26 (15.4)	18/113 (15.9)	

Legend: p-values compare molecular-only, culture-only and dual-confirmed pure epTB. Extensive disease corresponds to multiple extrapulmonary localizations. Unfavourable outcome: lost to follow-up+death

*Outcome data were available for 24, 26 and 113 patients, respectively, as treatment was still ongoing in the remaining cases

Abbreviations: TB, tuberculosis; epTB, extrapulmonary tuberculosis; HIV, human immunodeficiency virus; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; RR/MDR-TB, rifampicin-resistant or multidrug-resistant tuberculosis.

Table 3. Selected adjusted logistic regression models for microbiological confirmation patterns.

Population	Outcome vs dual-confirmed	Predictor	N	Events	aOR (95% CI)	p-value
Pulmonary TB	Molecular-only	Migrant status	450	48	24.24 (2.97–197.66)	0.003
Pulmonary TB	Molecular-only	Diabetes	450	48	6.87 (3.00–15.74)	<0.001
Pulmonary TB	Molecular-only	Chronic pulmonary disease	450	48	3.62 (1.30–10.12)	0.014
Pulmonary TB	Molecular-only	Total diagnostic delay, per 30 days	450	48	1.04 (1.00–1.08)	0.065
Pulmonary TB	Molecular-only	Respiratory failure	450	48	0.37 (0.15–0.90)	0.029
Pulmonary TB	Culture-only	Age, per 10 years	444	42	1.37 (1.11–1.69)	0.003
Pulmonary TB	Culture-only	Female sex	444	42	2.28 (1.08–4.82)	0.031
Pulmonary TB	Culture-only	Respiratory failure	444	42	2.66 (1.25–5.65)	0.011

Population	Outcome vs dual-confirmed	Predictor	N	Events	aOR (95% CI)	p-value
Pulmonary TB	Culture-only	High TIMIKA score ≥80	444	42	0.51 (0.24–1.07)	0.075
Pulmonary TB	Culture-only	Total diagnostic delay, per 30 days	444	42	0.94 (0.87–1.01)	0.095
Pure epTB	Molecular-only	Smear positive	141	27	0.14 (0.04–0.46)	0.001
Pure epTB	Molecular-only	Multiple extrapulmonary sites	141	27	2.20 (0.80–6.06)	0.128
Pure epTB	Molecular-only	Systemic symptoms	141	27	0.69 (0.24–1.97)	0.492
Pure epTB	Culture-only	Systemic symptoms	141	27	0.08 (0.02–0.35)	<0.001
Pure epTB	Culture-only	Total diagnostic delay, per 30 days	141	27	1.06 (1.02–1.11)	0.008
Pure epTB	Culture-only	Multiple extrapulmonary sites	141	27	0.31 (0.10–0.99)	0.048
Pure epTB	Culture-only	Smear positive	141	27	0.65 (0.22–1.92)	0.434

Legend: All models use dual-confirmed disease as the reference category. pTB estimates are from Model B. Pure epTB estimates are from parsimonious exploratory models. Complete-case analyses were used.

Abbreviations: TB, tuberculosis; pTB, pulmonary tuberculosis; epTB, extrapulmonary tuberculosis; aOR, adjusted odds ratio; CI, confidence interval; TIMIKA, radiographic severity score.

Table 4. Sensitivity analyses.

Sensitivity analysis	Outcome vs dual-confirmed	Predictor	aOR (95% CI)	p-value
Isolated pTB only	Molecular-only	Migrant status	24.30 (3.02-195.74)	0.003
Isolated pTB only	Molecular-only	Diabetes	7.43 (3.20-17.25)	<0.001
Isolated pTB only	Molecular-only	Chronic pulmonary disease	2.88 (1.00-8.31)	0.051
Isolated pTB only	Molecular-only	Respiratory failure	0.38 (0.15-0.96)	0.041
Isolated pTB only	Culture-only	Age, per 10 years	1.36 (1.10-1.67)	0.004
Isolated pTB only	Culture-only	Female sex	2.55 (1.20-5.42)	0.015
Isolated pTB only	Culture-only	High TIMIKA score ≥80	0.45 (0.21-0.96)	0.039
Isolated pTB only	Culture-only	Respiratory failure	2.54 (1.19-5.41)	0.016
Centre-clustered SE, pTB	Molecular-only	Migrant status	24.24 (2.81-209.13)	0.004
Centre-clustered SE, pTB	Molecular-only	Diabetes	6.87 (2.17-21.77)	0.001
Centre-clustered SE, pTB	Culture-only	Age, per 10 years	1.37 (1.16-1.61)	<0.001
Centre-clustered SE, pTB	Culture-only	Respiratory failure	2.66 (1.11-6.36)	0.028

Legend: Isolated pTB analysis excluded patients with concomitant extrapulmonary localization. Centre-clustered analyses used robust standard errors clustered by participating centre for the main pTB models.

Abbreviations: pTB, pulmonary tuberculosis; aOR, adjusted odds ratio; CI, confidence interval; TIMIKA, radiographic severity score; SE, standard error.

Discussion

Our study aims to highlight potential diagnostic pitfalls or management gaps and to reflect on the different presentations of TB disease based on the results of different diagnostic tests. As previously reported by other authors, molecular and culture tests are complementary rather than interchangeable, and both are useful in establishing the final diagnosis because culture alone misses a substantial number of cases [7, 8]. In addition, our data show encouraging concordance between the two methods, supporting the use of molecular testing in the TB diagnostic work-up. In more than 10% of cases, molecular testing confirmed the diagnosis despite a negative culture result. Furthermore, different disease presentations and demographic characteristics were observed in our study population.
Among pulmonary TB (pTB) cases, respiratory failure was less common in molecular-only cases. This may be due to the stronger inflammatory response triggered to control replicating bacilli [9], which may contribute to worse respiratory performance in culture-positive disease. Nevertheless, more severe radiological findings were observed in molecular-only cases. This suggests that replicating bacilli and their related effects are not the only determinants of TB disease, and that the overall impact of infection on the lungs should also be considered, as suggested by studies on post-TB lung disease and TB-associated lung disease [10]. Furthermore, TB infection follows a multifaceted and dynamic course. The clinical spectrum may evolve bidirectionally from symptomatic to asymptomatic disease, and consequently culture positivity may vary within the same individual during different stages of infection [3]. This may reflect the interaction between the host immune system and *Mycobacterium tuberculosis*, which can drive the transition between dormant and actively replicating bacilli [11]. DM is known to alter immune function and has important effects on TB infection [12]. This may explain the association between DM and molecular-only confirmation. Likewise, the well-established association between COPD and TB [13] suggests that a chronic and more indolent disease course may be associated with changes in the replicative status of bacilli. This may explain the mild association between molecular-only diagnosis and COPD observed in our study. In addition, COPD patients may present polymicrobial flora, and the decontamination procedures used before culture may reduce culture sensitivity [14]. Nevertheless, these considerations regarding DM and COPD remain speculative, given the lack of studies specifically addressing this issue.
Interestingly, smear positivity was less frequent in culture-only cases than in molecular-only cases, confirming that molecular diagnosis cannot distinguish between dead and viable bacilli [15]. A strong association between migrant status and molecular-only diagnosis was also observed. Although sparse data may have affected this finding, it may reflect different stages of infection among individuals from endemic countries, resulting either from long-standing latent infection or from recently acquired infection during the migratory journey. Likewise, this finding, together with the similar observations in patients with DM and COPD, further emphasizes the importance of molecular diagnostics in screening high-risk populations. The high proportion of migrants among molecular-only cases may also explain why diagnostic delay and length of hospitalization were longer in molecular-only pTB [16].
Some considerations regarding epTB should also be made. The sensitivity of molecular and culture methods varies according to the type of clinical specimen analyzed [17]. This heterogeneity, together with the limited number of epTB cases, precludes robust statistical interpretation of the findings, which are reported for completeness only. Nevertheless, single-method confirmation appeared to be more frequent in pure epTB than in pTB. Moreover, systemic symptoms were less common in culture-only cases, and, consistently, patient delay was longer in this subgroup. These findings further highlight the importance of combining molecular and culture methods to achieve an accurate diagnosis. The low frequency of multisite disease and its weak inverse association with culture-only diagnosis among epTB cases also support the need for analyses stratified by specimen type. However, because of the limited sample size, subgroup statistical analyses were not performed.
Finally, differences in treatment outcomes were limited, and the slightly better outcome observed in the dual-confirmed pTB group was of marginal statistical significance. Therefore, no considerations regarding the treatment regimen can currently be made based on these findings.
This study has several limitations. The data were observational, routinely collected, and retrospectively analyzed; therefore, residual confounding and missing data cannot be excluded. Molecular testing was analyzed as recorded in the database and may reflect differences in diagnostic platforms, specimen types, and laboratory workflows across

participating centers. The high analytical sensitivity of Xpert Ultra enables the detection of trace amounts of *M. tuberculosis* DNA even in specimens that remain culture-negative [18]. The limited availability of data on the diagnostic methods employed precludes a robust pooled analysis of the performance of individual diagnostic tools. Additionally, data about possible confounders which may have invalidated culture test, such as specimen storage, transportation, decontamination, non-tuberculous mycobacteria co-infection or start of anti-TB treatment before performing test, were unavailable. Some radiological variables, including cavitation and bilateral lung involvement, were not consistently available. Therefore, disease severity was described using the TIMIKA score, smear positivity, respiratory failure, and concomitant extrapulmonary involvement. Multivariable models should be considered exploratory, particularly for pure epTB, where the limited sample size and model separation restricted the number of variables that could be included. Finally, the analysis included only patients with both molecular and culture results available, which may limit the generalizability of the findings to the overall TB population represented in the database.

In conclusion, molecular-only TB should not simply be considered culture-negative TB. In this multicenter cohort, discordant molecular and culture results may identify distinct clinical phenotypes among patients with pulmonary TB. Further studies are needed to better define the clinical significance of molecular-only confirmation in the diagnostic work-up of extrapulmonary TB. Molecular testing and culture should therefore be regarded as complementary components of TB diagnosis, each providing distinct and valuable information for patient management, drug resistance assessment, and public health surveillance.

Ethics approval and consent to participate

All procedures in studies involving human participants were performed in accordance with the ethical standards of the Declaration of Helsinki and were approved by the Ethics Committee of Istituto Tumori Giovanni Paolo II (approval no. 9128, November 21, 2023). A subsequent amendment (approval no. 2231, November 10, 2025) was approved to include additional participating study centers. The Ethics Committee granted an exemption from requiring specific additional informed consent for the study, beyond the standard consent obtained from patients for the collection and use of data as part of their routine clinical care.

Consent for publication

Not applicable.

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