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COMPARATIVE EVALUATION OF MICROBIOLOGICAL AND MOLECULAR METHODS IN THE DIAGNOSIS OF ABDOMINAL AND PULMONARY TUBERCULOSIS

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Abstract

There are certain diagnostic challenges in the detection and differentiation of abdominal and pulmonary tuberculosis using microbiological and molecular genetic tests of sputum and stools.

Aim — to evaluate the role of microbiological and molecular genetic tests of sputum and stool in the diagnosis of abdominal and pulmonary tuberculosis in adults.

Materials and Methods. A prospective case-control study included 203 adult patients: Group 1 comprised 48 patients with abdominal tuberculosis confirmed by Ziehl-Neelsen microscopy and/or nucleic acid amplification testing for *Mycobacterium tuberculosis* in stool samples, with histological confirmation from biopsy specimens; Group 2 included 35 cases of pulmonary tuberculosis confirmed by Ziehl-Neelsen microscopy, culture, and/or nucleic acid amplification testing of sputum and stool; Group 3 consisted of 120 cases of pulmonary tuberculosis diagnosed by Ziehl-Neelsen microscopy, culture, and/or nucleic acid amplification testing of sputum.

Results. Ziehl-Neelsen microscopy and nucleic acid amplification testing of stool samples did not allow differentiation between pulmonary and abdominal tuberculosis, highlighting the importance of clinical, histological, and radiological investigations. Patients with pulmonary tuberculosis presented predominantly with respiratory symptoms, whereas patients with abdominal tuberculosis reported abdominal symptoms; the diagnosis was confirmed by positive microscopic and molecular genetic testing of stool samples, histological findings, and major risk factors, including comorbid conditions (odds ratio = 4.5, confidence interval: 1.9–10.8) and pre-existing gastrointestinal diseases (odds ratio = 13.2, confidence interval: 5.2–33.9).

Conclusions. Microbiological examination and nucleic acid amplification testing of stool samples did not differentiate pulmonary from abdominal tuberculosis, indicating the need for comprehensive interpretation of results in conjunction with clinical, histological, and radiological findings.

Keywords: pulmonary tuberculosis, abdominal tuberculosis, nucleic acid amplification test.

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ПОРІВНЯЛЬНА ОЦІНКА МІКРОБІОЛОГІЧНИХ ТА МОЛЕКУЛЯРНИХ МЕТОДІВ У ДІАГНОСТИЦІ ТУБЕРКУЛЬОЗУ ОРГАНІВ ЧЕРЕВНОЇ ПОРОЖНИНИ ТА ЛЕГЕНЬ

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Резюме

Актуальність. Існують діагностичні труднощі у діагностиці та диференціюванні туберкульозу абдомінальної та легеневої локалізації за допомогою мікробіологічних та молекулярно-генетичних тестів мокротиння та калу.

Мета роботи — оцінити роль мікробіологічних та молекулярно-генетичних тестів мокротиння та калу у діагностиці туберкульозу органів черевної порожнини та легень у дорослих.

Матеріали та методи. Проспективне дослідження «випадок-контроль» включало 203 дорослих пацієнта: 1-ша група — 48 пацієнтів з абдомінальним туберкульозом, підтвердженим мікроскопією за Цілем-Нільсеном та/або тестом ампліфікації нуклеїнових кислот на наявність *M. tuberculosis* у зразках калу та біопсії з гістологічним підтвердженням; 2-га група — 35 випадків легеневого туберкульозу, підтверджених мікроскопією Ціля-Нільсена, посівом та/або тестом ампліфікації нуклеїнових кислот мокротиння та калу; 3-тя група — 120 випадків туберкульозу легень, діагностованих за мікроскопією Ціля-Нільсена, посівом та/або тестом ампліфікації нуклеїнових кислот мокротиння.

Результати. Мікроскопія за Цілем-Нільсеном та тест ампліфікації нуклеїнових кислот зразків калу не дозволяють відрізнити туберкульоз легень від абдомінального туберкульозу, що підкреслює важливість клінічних, гістологічних та радіологічних досліджень. Так, пацієнти з легеневою туберкульозом мали респіраторні симптоми, тоді як пацієнти з абдомінальним туберкульозом скаржилися на абдомінальні симптоми, діагноз яких підтверджувався позитивним результатом мікроскопічного та молекулярно-генетичним дослідженням зразків калу, гістологічно та основними факторами ризику: супутніми захворюваннями (OR = 4,5, CI: 1,9–10,8) та існуючими захворюваннями шлунково-кишкового тракту (OR = 13,2, CI 5,2–33,9).

Висновки. Мікробіологічне дослідження та тест ампліфікації нуклеїнових кислот зразків калу не дозволили диференціювати легеневої туберкульоз від абдомінального, що свідчить про необхідність комплексно оцінювати результати з клінічними, гістологічними та радіологічними результатами дослідження.

Ключові слова: туберкульоз легень, абдомінальний туберкульоз, тест ампліфікації нуклеїнових кислот.

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Introduction

Despite the availability of highly effective anti-TB drugs, tuberculosis (TB) continues to be a major cause of death worldwide [1]. Early detection is critical to initiate treatment and improve outcomes [2]. Conventional diagnostic methods face limitations in extrapulmonary

TB cases, in children, elderly individuals, and patients with severe mental illness—where obtaining respiratory samples is difficult [2]. Sputum is the standard sample for *M. tuberculosis* (MTB) detection, but children and patients with severe mental illness often cannot comply with sputum collection due to swallowing rather than coughing it up [2]. Invasive methods such as sputum induction or bronchoscopy are difficult to perform, as they require specialized equipment, trained personnel,

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and may pose risks to patients [3]. Diagnosis relies on chest X-rays, clinical signs and conventional microbiological methods, which demonstrated low sensibility in extrapulmonary forms and paucibacillary pulmonary TB [4]. Developing non-invasive, easy-to-collect testing methods, such as stool-based assays is critical to improve accuracy and reduce diagnostic delays in this vulnerable population [2]. While conventional culture on Lowenstein-Jensen media remains the gold standard to detect MTB due to its sensitivity and ability to test the drug susceptibility, results take 4–6 weeks, and sensitivity is low [3]. In contrast, nucleic acid amplification tests (NAATs) deliver results within hours, enabling genotypic study of drug susceptibility with high specificity and sensitivity [2]. While adults can provide sputum via coughing, children, elderly and patients with mental disorders often require gastric aspiration to obtain swallowed sputum from the stomach [2]. The stool-based methods (microscopy at Ziehl-Neelsen and NAATs) enable detection of MTB directly from stool samples, eliminating invasive procedures, such as fibroscopy and gastric aspiration [5]. Multiple studies in children and patients unable to produce sputum have demonstrated that stool Xpert testing can detect MTB with acceptable sensitivity and specificity. Consequently, in 2020, WHO endorsed stool as an alternative specimen for GeneXpert testing in children, particularly for diagnosing pulmonary TB when sputum is not available [5, 6]. Abdominal TB is an extrapulmonary type, which affects the gastrointestinal tract, peritoneum, abdominal lymph nodes, and sometimes other organs [7]. It accounts for 1–3 % of all TB cases and 6–13% of extrapulmonary TB cases [8]. Abdominal TB can develop from ingestion of infected sputum in pulmonary TB, hematogenous or lymphatic dissemination, direct extension from adjacent foci, or consumption of unpasteurized milk [9]. It commonly involves the ileocecal region, peritoneum, lymph nodes but can affect any gastrointestinal site and parenchymal organs (liver, spleen, pancreas) [10]. Delayed diagnosis and advanced disease can result in complications, including intestinal obstruction; intestinal perforation with peritonitis; fistula; mesenteric, psoas, or intra-abdominal abscesses; peritoneal complications; gastrointestinal hemorrhage; malabsorption syndrome [10]. Symptoms, such as abdominal pain, fever, weight loss, are often vague, nonspecific and lead to diagnostic delays [9]. Accurate diagnosis of abdominal TB requires distinguishing it from pulmonary TB, particularly when microbiological tests of stool samples are positive for MTB [12]. Early diagnosis and appropriate anti-TB therapy lead to better outcomes, whereas delayed detection often requires surgical intervention to manage complications, obtain tissue for histological confirmation, and increase the risk of developing drug resistance [12].

Objective — to evaluate the diagnostic challenges of abdominal and pulmonary TB in adults through sputum and stool analysis, with a particular focus on the role of nucleic acid amplification tests (NAATs). The hypothesis of the research was that NAATs, when applied

to sputum and stool samples, can detect abdominal and pulmonary TB in adults with acceptable sensitivity in association with clinical, laboratory and radio-imagistic data.

Materials and methods

The retrospective observational study involved patients hospitalized with tuberculosis and associated conditions from 2018 to 2024, comprising consecutive cases with comprehensive clinical, laboratory, and radiological records. The patients were examined through conventional microbiological methods (smear Ziehl-Neelsen staining for acid-fast-bacilli (AFB), conventional cultures on Lowenstein-Jensen and BACTEC media with antibiotic susceptibility testing of *Mycobacterium tuberculosis* (MTB) and NAAT - GeneXpert MTB/Rif for detection of MTB DNA and mutations in the *rpoB* gene responsible for multidrug-resistant TB (MDR-TB). The study included patients aged 18 years or older with a confirmed diagnosis of TB who provided informed consent. Patients were distributed in three groups: the 1st - diagnosed with 48 abdominal TB through microscopy and/or NAAT of the stool samples and biopsy with histological confirmation; the 2nd - included 35 pulmonary TB cases, confirmed through conventional microbiological tests of the sputum and having microscopy and/or NAAT positive in stool samples and radio-imaging (chest X-ray and HRCT) with TB-specific patterns and the 3rd group included 120 pulmonary TB cases, diagnosed through conventional microbiological methods and/or NAAT of sputum sample. Patients case management followed WHO recommendations. Statistical analysis was performed using SPSS 26.0. The Fisher's exact test was applied for non-parametric variables, while the Student's t-test was used for normally distributed variables, with statistical significance set at $p < 0.05$. To identify potential risk factors, odds ratios (OR) were calculated using logistic regression, with risk levels classified as low (OR 1.1–1.5), moderate (OR 1.6–2.9), and high (OR ≥ 3.0 , strong association). The likelihood ratio (LR) was applied to evaluate post-test probabilities of the disease, with a high LR value (>10) indicating a strong increase in the likelihood of the disease when the test result was positive.

Ethical considerations. The study was conducted in accordance with the declaration of Helsinki.

Results

The 3rd group demonstrated a significantly higher rate of men compared to 1st and 2nd groups ($\chi^2=11$;; $p<0.001$), while younger age (<45 years) and urban residence were more prevalent in 2nd and 3rd groups (age: $\chi^2=6.9$;; $p<0.05$; residence: $\chi^2=10.6$;; $p<0.001$). The average age of patients was 48 ± 3 years in the 1st group and 45 ± 6 in the 2nd group, both statistically higher than in the 3rd G (38 ± 7 ; $t=3.2$, $p<0.05$). Statistical analysis revealed significant associations between demographic factors and pulmonary TB, with male gender (OR = 3.3; 95% CI 1.6–6.8) and urban residence (OR = 3.7; 95% CI 1.9–8.6) identified as high-risk factors, while young age showed a moderate risk (OR = 2.2; 95% CI 1.1–4.6) (Figure 1).

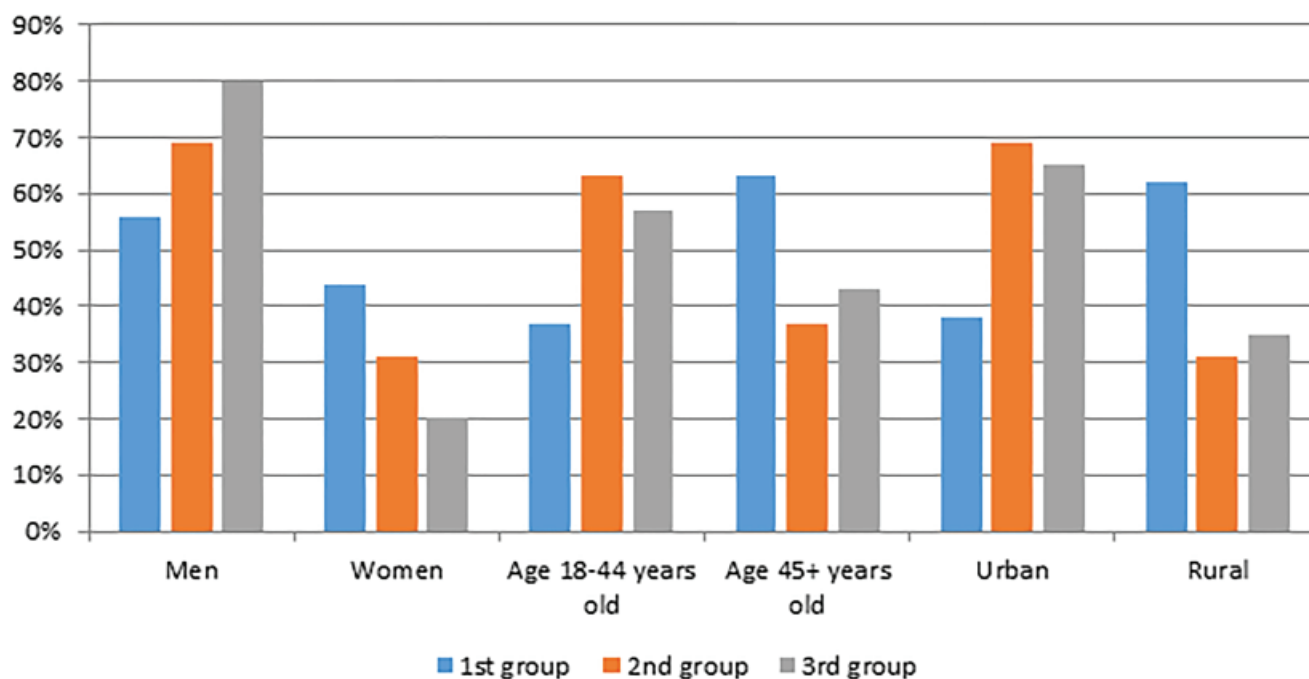


Fig. 1. Demographic characteristics

Most of the cases from the 2nd — 27 (88%) and 3rd groups — 89 (74%) were diagnosed by the primary healthcare workers compared with only 2 (5%) in the 1st group ($\chi^2=10.9$; $p<0.001$), while almost all cases from the 1st group were diagnosed by specialized healthcare services, and a lower proportion of patients in the 3rd group 31 (26%) and 2nd group - 8 (12%) cases ($\chi^2=14$; $p<0.001$). Patients were assessed based on prior exposure to anti-TB drugs. The 1st group showed a slightly higher proportion of patients who never received anti-TB treatment - 41 patients (85%), compared with 51 patients (78%) in the 2nd group and 91 patients (76%) in the 3rd group ($\chi^2=3.2$; $p>0.001$). All pulmonary TB patients from the 2nd and 3rd groups presented respiratory symptoms such as chronic cough, purulent sputum, while all patients with abdominal TB reported symptoms as chronic abdominal pain, distension and diarrhea. All patients presented general symptoms including fatigue, loss of weight, night sweats, and low-grade fever. Etiological diagnosis using microscopy, culture and/or NAAT-GeneXpert MTB/RIF test was applied in a statistical higher proportion of patients from the 2nd group 34 (96%), compared with the 3rd group 82 (68%) and 1st group 20 (42%) patients ($\chi^2=7.1$; $p<0.01$). While histological biopsy revealed tuberculous granuloma in a higher proportion of the 1st group 36 (75%) then 6 (17%) in the 2nd group and 11 (9%) in the 3rd group ($\chi^2=78$; $p<0.001$). AFB-positive sputum samples were established more frequently in the 2nd group, 22 patients (78%), compared with the 3rd group, 54 patients (45%), while none was detected in the 1st group ($\chi^2=7.6$; $p<0.001$). In contrast, AFB-positive stool samples were observed at similar rates in the 1st group, 20 patients (42%), and the 2nd group, 14 patients (40%), but were absent in the 3rd group ($\chi^2=6.1$; $p<0.001$). GeneXpert MTB/RIF testing of sputum samples was positive in a significantly higher proportion of patients in the 2nd group, 24 cases (68%),

compared with 61 cases (51%) in the 3rd group, while none were detected in the 1st group. GeneXpert MTB/RIF positivity in stool samples was recorded at similar rates in the 1st group, 19 cases (38%), and the 2nd group, 21 cases (31%), and was not performed in the 3rd group. Rifampicin resistance was identified in 6 cases (12%) from the 1st group, 5 cases (15%) from the 2nd group, and 19 cases (16%) from the 3rd group ($\chi^2=11.3$; $p<0.001$). The diagnostic performance of stool-based tests for abdominal TB showed an LR of 9.3 ($p = 0.002$) for microscopy, 11.9 ($p = 0.001$) for GeneXpert, and 76 ($p = 0.000$) for histological examination. Conventional cultures were performed only on sputum specimens, providing positive results in a slightly higher proportion in the 2nd group, 23 cases (66%), compared with 62 cases (52%) in the 3rd group. Drug-resistant TB was microbiologically confirmed in a similar proportion in all groups, 9 (19%) in the 1st group, 6 (17%) in the 2nd group and 17 (14%) in the 3rd G (figure 2.).

Radiological evaluation with chest X-ray and HRCT revealed that a slightly higher proportion of pulmonary TB patients demonstrated multi-segment lung involvement in the 2nd group, 27 cases (77%), compared with the 3rd group, 86 cases (71%). Lung destruction was observed in 18 patients (51%) in the 2nd group and in 52 patients (43%) in the 3rd group. Disseminated opacities were also more frequent in the 2nd group, 22 cases (78%), compared with 61 cases (51%) in the 3rd group. Lesions characteristic of post-TB lung disease (PTLD), including bronchiectasis, extensive fibrosis, calcifications, emphysema, fibrothorax, and recurrent respiratory infections, were identified at similar rates across all groups: 33 cases (27%) in the 3rd group, 12 cases (25%) in the 1st group, and 8 cases (22%) in the 2nd group.

Among risk factors analyzed, were assessed social, epidemiological, and biological determinants of TB. Contact with a person diagnosed with TB, considered as

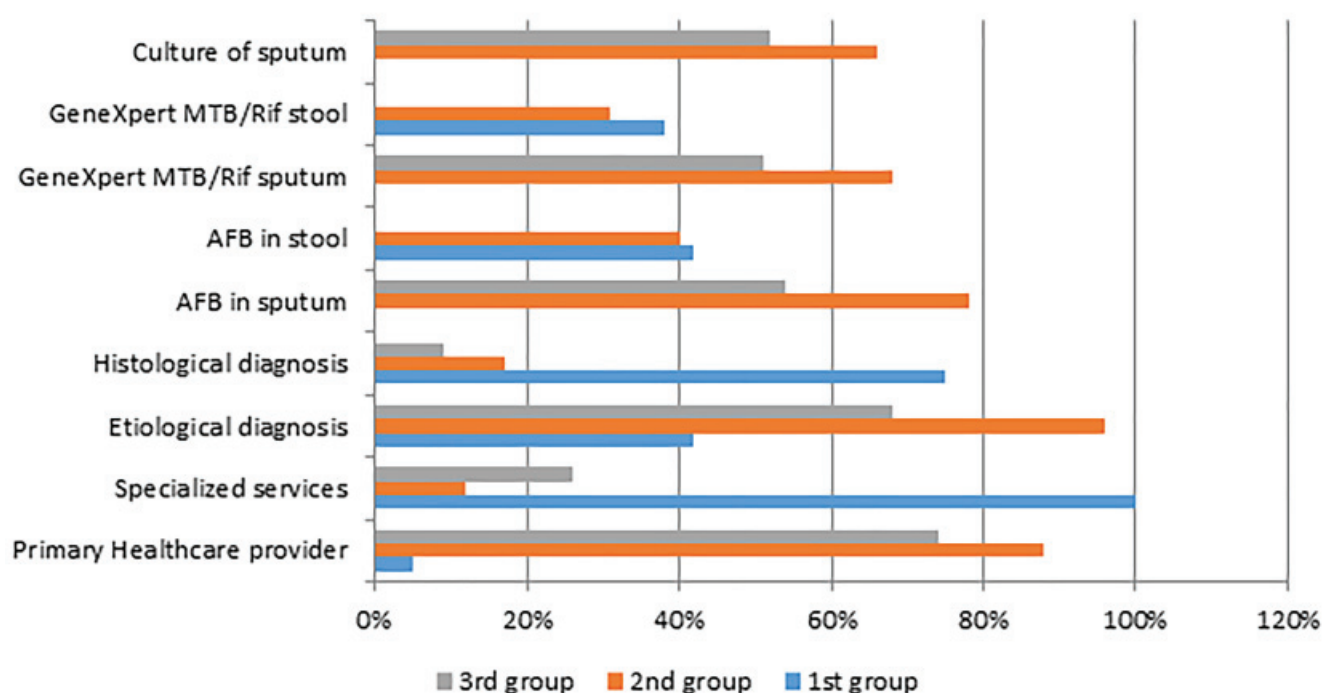


Fig. 2. Diagnostic and microbiological peculiarities

the major risk factor, was more frequently reported in the 3rd group, 43 cases (33%), and the 1st group, 11 cases (23%), compared with only 5 cases (14%) in the 2nd group. Patients who traveled abroad to high TB-burden regions within the previous 90 days were more frequently found in the 3rd group, 16 cases (20%), compared with similar rates in the 1st group, 4 cases (8%), and the 2nd group, 3 cases (8%). Co-morbidities statistically predominated in the 1st 41 (85%) and 2nd groups 31 (88%) then 3rd group 68 (56%), ($\chi^2=20$, $p<0.001$). The most commonly diagnosed conditions were gastrointestinal diseases (GID), including gastric or duodenal ulcer, chronic hepatitis, chronic pancreatitis, ulcerative colitis, all associated with undernutrition. The proportion of patients diagnosed with GID was

significantly higher in the 1st group, 42 cases (87%), compared with 42 cases (35%) in the 3rd and 12 cases (34%) in the 2nd groups ($\chi^2=41$, $p<0.001$). This was associated with undernutrition, defined by a body mass index below 19 kg/m², which was also more prevalent in the 1st group, 35 cases (73%), than in the 2nd group, 22 cases (63%), and the 3rd group, 56 (47%) cases ($\chi^2=24$, $p<0.001$). Malabsorption syndrome was identified in 28 patients (58%) of the 1st group, based on clinical indicators, chronic diarrhea, lack weight gain or ongoing weight loss, hematochezia, fecal fat excretion ≥ 7 g/day—and colonoscopic features such as ulceronodular lesions, pseudopolyps, strictures, deformed ileocecal valve, and/or mass lesions. Chronic alcoholism was diagnosed in a statistical higher proportion in the 2nd

Table 1.

The risk factors and treatment outcomes

Indicators	1 st G N=48	2 nd G N=35	3 rd G N=120	χ^2
TB contact	11 (23)	5 (14)	43 (33)	$\chi^2=1,1$, $p>0.05$
Returned from abroad	4 (8)	3 (8)	16 (20)	$\chi^2=0,7$, >0.05
Co-morbidities	41 (85)	31 (88)	68 (56)	$\chi^2=20$, $p<0.001$
HIV-infection	2 (5)	5 (14)	22 (18)	$\chi^2=7,1$, $p<0.05$
Gastro-intestinal diseases	42 (87)	12 (34)	42 (35)	$\chi^2=41$, $p<0.001$
Undernutrition	35 (73)	22 (63)	56 (47)	$\chi^2=24$, $p<0.001$
DM	3 (6)	6 (17)	21 (17)	$\chi^2=0,7$, $p>0.05$
Alcohol abuse	11 (23)	11 (31)	28 (23)	$\chi^2=1,1$, $p>0.05$
Tobacco smoking	26 (54)	31 (88)	98 (82)	$\chi^2=17$, $p<0.001$
Drug use	1 (2)	2 (6)	10 (8)	$\chi^2=1,2$, >0.05
Social vulnerable	46 (96)	26 (74)	107 (89)	$\chi^2=9,2$, $p<0.01$
Successfully treated	36 (75)	28 (80)	99 (83)	$\chi^2=8,1$, $p<0.01$
Died	12 (25)	5 (14)	11 (9)	
Lost to follow-up/failure	0	2 (6)	10 (8)	

group 11 (31%) compared with similar rates in the 1st group 11 (23%) and the 3rd group, 28 (23%) cases. All patients with alcoholism were also active tobacco smokers. HIV/AIDS as an immunosuppressive condition was diagnosed more frequently in the 3rd group, 22 cases (18%), compared with 5 cases (14%) in the 2nd group and 2 cases (5%) in the 1st group. Diabetes mellitus (DM) was diagnosed in similar rates in the 2nd - 6 (17%) cases and 3rd group 21 (17%) compared with 3 (6%) in the 1st group. Active tobacco smoking itself was recorded at a significantly higher rates in the 3rd group 98 (82%) and 2nd group 31 (88%) compared with 26 cases (54%) in the 1st group ($\chi^2=17$, $p<0.001$). The multivariable regression model including all peculiarities showed that the comorbid state was a high risk factor for abdominal TB (OR = 4.5, 95% CI 1.9–10.8), gastrointestinal diseases represented the strongest risk factor (OR = 13.2, 95% CI 5.2–33.9), and undernutrition also contributed as a high risk factor (OR = 4.2, 95% CI 2.1–8.7), while tobacco smoking emerged as a moderate risk factor for pulmonary TB (OR = 2.9, 95% CI 1.4–5.9).

The new cases with drug-susceptible TB received the standard 6-month regimen (2HRZE/4HR), consisting of 2 months of isoniazid (H), rifampicin (R), pyrazinamide (Z), and ethambutol (E), followed by 4 months of H and R, while patients with a prior anti-TB treatment were treated with a 6-month HRZE regimen. Patients with MDR-TB without fluoroquinolone resistance, received the standard all-oral regimen (comprising bedaquiline with levofloxacin or moxifloxacin, clofazimine, pyrazinamide, ethambutol, and ethionamide) during 6 months and followed by levofloxacin/moxifloxacin, clofazimine, ethambutol, and pyrazinamide for an additional five months or till one year if remained microbiological positive at the follow-up investigations. Treatment outcomes differed across groups, with the success rates which were highest in the pulmonary TB groups, with 99 (83%) in the 3rd group and 28 (80%) in the 2nd group, while the 1st group had the lowest rate at 36 (75%). The highest mortality rate was recorded in abdominal TB at 12 (25%) patients, followed by 5 (14%) in pulmonary TB patients from the 2nd group and 11 (9%) in the 3rd group. Other unfavorable outcomes were rare, with 10 (8%) lost to follow-up or treatment failure in the 3rd group and 2 (6%) in the 2nd group (Tab. 1).

Discussion

We performed the study to assess the role of microbiological and rapid molecular tests in diagnosis of abdominal TB and pulmonary TB in adults, utilizing sputum and stool samples. The main limitation of this study was that the use of stool samples was formally endorsed in the WHO's Consolidated Guidelines on Tuberculosis, Module 3: Diagnosis — Rapid diagnostics for tuberculosis detection in 2020 [2]. WHO strongly recommends using Xpert MTB/RIF, and later Xpert Ultra, on stool samples as an alternative method for diagnosing

pulmonary TB in symptomatic children when sputum cannot be obtained, but not for any form of extrapulmonary TB [2]. However, the certainty of evidence regarding stool test sensitivity is low due to lack of standardized protocol for sample preparation [5]. Additionally, the mycobacterial load in stool is heterogeneously distributed, and the presence of PCR inhibitors reduce the NAAT sensitivity leading to false-negative results [2]. The research demonstrated that all patients with abdominal TB complained chronic abdominal symptoms and pulmonary TB presented respiratory signs. Pulmonary TB was confirmed through microbiological testing of sputum samples in majority of pulmonary TB cases, whereas positivity for GeneXpert MTB/RIF in stool samples, together with histological confirmation, identified majority of abdominal TB cases. Finally, microbiological and NAAT of stool samples holds diagnostic value in certain high-risk groups—such as males, urban residents, and individuals with comorbidities, particularly gastrointestinal diseases—when supported by abdominal manifestations, histological confirmation and imaging findings, confirmed by other studies [5–10]. This risk stratification is also consistent with geospatial multi-agent simulations of TB spread, which reproduce clustered transmission patterns and heterogeneous exposure across urban micro-environments, supporting targeted screening approaches [12]. Treatment outcomes demonstrated higher success rates in patients with pulmonary TB compared to abdominal TB group, while mortality and loss to follow-up were reported in all groups, highlighting the impact of clinical and social determinants on TB evolution outcomes [13].

Conclusions

1. The microbiological and molecular genetic tests performed in stool samples cannot differentiate pulmonary from abdominal TB, demonstrating the importance of comprehensive clinical, histological, and radio-imagistic evaluation.
2. The major risk factors for abdominal TB were the comorbid state, especially gastro-intestinal diseases.
3. The diagnosis of abdominal TB was established based on clinical presentation dominated by abdominal symptoms and confirmed through histological biopsy, microscopy and/or GeneXpert MTB/RIF, being supported by high-risk factors — comorbidities, mainly gastrointestinal diseases and malnutrition. The absence of pulmonary lesions on chest imaging further distinguished it from pulmonary TB.
4. The diagnosis of pulmonary TB was established based on the respiratory symptomatology, associated to positive results of the conventional microbiological assays, associated to high risk factors — male gender, young age, urban residence, HIV-infection and tobacco-smoking.

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