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ISONIAZID-RESISTANT TUBERCULOSIS - A SERIES OF CLINICAL CASES

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Abstract

The emergence of M. tuberculosis strains resistant to Isoniazid (INH) and Rifampicin (RIF), the two most potent drugs of first-line anti-TB treatment is termed multidrug drug-resistant TB (MDR-TB). Multidrug-resistant tuberculosis has been a severe medical and epidemic problem worldwide.

We present a series of clinical cases consisting of two patients diagnosed with isoniazid-resistant tuberculosis. Histopathological examination supports the diagnosis of tuberculous granulomas. The pathology molecular examination revealed the presence of Isoniazid-resistant Mycobacterium tuberculosis 1, 2 via the following mutation c.947G>A; p.Gly316Asp. Isoniazid-resistance is associated with mutations in the furA-katG and fabG1-inhA operons and in the ahpC gene. 64% of the isoniazid-resistance phenotypes were associated with the kat G 315 mutation worldwide. The second most common mutation is inhA-15, reported in 19% of the Isoniazid-resistant isolates. The significant association between the two mutations, inhA c-15 and kat G 315 respectively, and the high-level resistance is of interest in the interpretation of current and future molecular diagnostic testing, as an early prediction of the level of Isoniazid-resistance is essential to decide the benefit of high-dose Isoniazid use.

Keywords: *multidrug-resistant tuberculosis, genetic mutations, clinical cases.*

Rezumat

Apariția tulpinilor de M. tuberculosis rezistente la izoniazidă (INH) și rifampicină (RIF), cele mai puternice două medicamente din prima linie de tratament anti-TB este denumită TB multidrog rezistentă (MDR-TB). Tuberculoza multirezistentă reprezintă o problemă medicală și epidemică gravă la nivel mondial.

Prezentăm o serie de cazuri clinice constând din doi pacienți diagnosticați cu tuberculoză rezistentă la izoniazidă. Examenul histopatologic susține diagnosticul de granuloame

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tuberculoase. Examenul molecular a evidențiat prezența Mycobacterium tuberculosis rezistent la izoniazidă prin următoarea mutație: c.947G>A; p.Gly316Asp. Rezistența la izoniazidă este asociată cu mutații ale operonilor furA-katG și fabG1-inhA și ale genei ahpC. La nivel mondial, 64% dintre fenotipurile care conferă de rezistență la izoniazidă au fost asociate cu mutația kat G 315. A doua cea mai frecventă mutație este inhA-15, raportată la 19% dintre tulpinile rezistente la izoniazidă izolate. Asocierea semnificativă dintre cele două mutații, respectiv inhA c-15, respectiv kat G 315 și rezistența la nivel înalt este de interes în interpretarea testelor de diagnostic molecular actuale și viitoare, deoarece o predicție timpurie a nivelului de rezistență la izoniazidă este esențială pentru a decide beneficiul utilizării de izoniazidă în doze mari.

Cuvinte cheie: *tuberculoză multirezistentă, mutații genetice, cazuri clinice.*

Introduction

Tuberculosis continues to be a public health issue nowadays, being endemic in many parts of the world and among the top 10 causes of death worldwide, despite significant progress in the last decade[1].

Drug-resistant tuberculosis (DR TB) constitutes a major threat, despite the End TB Strategy which calls for early diagnosis and prompt treatment of all people of all ages with any form of TB, sensitive or resistant to anti-TB drugs[2,3]. Multidrug-resistant tuberculosis (MDR TB) is a type of tuberculosis caused by Isoniazid- and Rifampicin-resistant Mycobacterium tuberculosis[3]. MDR TB continues to be a significant problem, both domestically and globally, and the lack of a safe and effective MDR regimen is a significant impediment to providing adequate treatment for patients with active MDR TB[3].

Major challenges to eradicating DR TB include the difficulty of providing universal access to rapid and accurate diagnosis via the development of genetic testing for Rifampicin-resistance, comprehensive treatment regimens, appropriate to identified drug-resistance, and social and psychological support[1,3].

In 2017, 558,000 new DR TB patients developed resistance to Rifampicin (RR TB) worldwide, Rifampicin being the most effective first-line drug, and 82% of them were MDR TB[1]. Out of these, only 160,684 cases (25%) were diagnosed and notified in

2017[3]. Only 87% of diagnosed and notified cases were included in a therapeutic regimen with second-line drugs[3]. The consequences are not favourable, i.e., 80% of the patients assessed as MDR TB treatment failure died within the next three years[3].

Antituberculosis drug-resistance patterns are divided into categories based on the results of genotypic or phenotypic bacteriological testing, as follows[3,4,5,6]:

- Mono-resistance: resistance to only one first-line antituberculosis drug.
- Poly-resistance: resistance to several first-line antituberculosis drugs other than Isoniazid and Rifampicin.
- Multidrug-resistance (MDR): resistance to Isoniazid and Rifampicin, with or without resistance to other drugs.
- Extensive drug-resistance (XDR): resistance to Isoniazid and Rifampicin associated with resistance to any Fluoroquinolone and at least to one of the second-line injectable drugs (Kanamycin, Amikacin or Capreomycin).
- Rifampicin-resistance (RR): resistance detected by genotypic or phenotypic methods, with or without resistance to other anti-TB drugs. It includes any resistance to Rifampicin, in the form of mono-resistance, poly-resistance, MDR or XDR.

Even if there is a clinical suspicion of DR TB, there must be a positive diagnosis based on antibiogram results[7]. A final diagnosis of DR TB requires identification of the Mycobacterium tuberculosis complex (bacterial cultures and specific immunochromatographic identification methods, Atg MPT64) for which rapid molecular (to detect MTB DNA and resistance-associated mutations) and conventional (using solid or liquid media) tests will be performed, i.e. antibiogram in Lowenstein Jensen solid medium or in liquid medium[3].

If genetic testing and antibiograms for Isoniazid and Rifampicin are suggestive for resistant TB, customized treatment will be initiated, taking into account the patient's treatment history and any previous contact with a known case of DR TB. Subsequently, after receiving the result of the extended antibiogram, the treatment regimen will be adjusted in order to achieve maximum therapeutic efficacy[6].

Treatment of non-MDR mono- and poly-drug resistant conditions

Identification of rifampicin resistance or rifampicin-associated poly-drug resistance (RE, RS, RZ, RZE, RZS, RZSE) is treated according to the same principles and with the same therapeutic regimens as MDR TB, since Rifampicin resistance is a surrogate marker of MDR TB (it can be associated with INH resistance in up to 85% of the cases)[3].

According to the latest recommendations, WHO states that Isoniazid mono-resistance/ HE, HS, HZ poly-resistance are treated as follows [8]: 6 R Z E Q (MFX/LFX) 7/7 from the moment the Fluoroquinolone is added.

In DR TB, adjuvant treatment consists in a variety of non-pharmacological measures that help cure the disease by complementing the pharmacological therapy or by increasing the patient's acceptability and tolerance to medication. The main adjuvant measures are surgical treatment, nutritional, psychological, and social support[3].

Surgical treatment is an adjuvant therapy option decreasingly recommended after the introduction of effective chemotherapy. The aim of the surgical treatment is to resolve major, potentially life-threatening complications (i.e., haemoptysis, pneumothorax, pleural empyema) or to complement anti-



tuberculosis treatment when this is not effective enough. Surgery can remove or limit active cavity lesions, prevent late complications (bronchopleural fistula, empyema, remaining cavities) or relapse[3].

Higher therapeutic success rates have been described regarding the combination of pharmacological treatment with partial lung resection versus pharmacological treatment without surgery or combined with pneumonectomy in selected cases[9,10,11]. The effectiveness of the surgical treatment has been shown to be better when performed in bacteriologically negative cases[9,10,11].

Case study

Case no. 1

A 20-year-old non-smoking female patient with no significant personal pathological history and no need for chronic treatment at home comes to the „Marius Nasta" NPI for a dry cough in the last 6 months and an episode of low-grade hemoptysis. There where no clinically significant findings at the physical examination and preserved cardio-respiratory status. In the clinical and paraclinical context, the following preliminary investigations were requested: laboratory tests (complete blood count, electrolytes levels, coagulation tests, inflammatory markers), sputum microscopy for non-specific bacteria, and Koch's bacillus, GeneXpert, chest radiography, electrocardiogram. The chest X-ray revealed a heterogeneous left pulmonary basal consolidation process, and the following imaging investigations, such as a chest CT scan, found a left pulmonary lower lobe consolidation process with a tendency to cavitation, raising the suspicion of bacillary foci (Figure 1).

Sputum examination for Koch's bacillus showed the presence of Mycobacterium tuberculosis (9 AFB per 100 HPF), with positive GeneXpert genetic testing (low detected) and no resistance detected. Specific investigations were carried out by performing bronchoscopy with bronchial biopsy, which revealed periorificial congestive infiltrative lesions at the apical level of the left lower lobe, with the histopathological examination supporting the diagnosis of endobronchial tuberculosis. Anti-tuberculosis treatment regimen I was initiated as follows INH 300 mg per day, RMP 600 mg per day, EMB 800 mg per day, PZA1500 mg per day.

At T2 (negative AFB at the microscopic examination and cultures), the patient has a good radiological outcome but with symptoms persistence, so the decision is to continue the intensive phase until T3. At T3, bronchoscopic and imaging reassessment is performed. The bronchoscopy showed mutilating bronchial statics changes in the LIL segments, with punctiform stenosis of one of

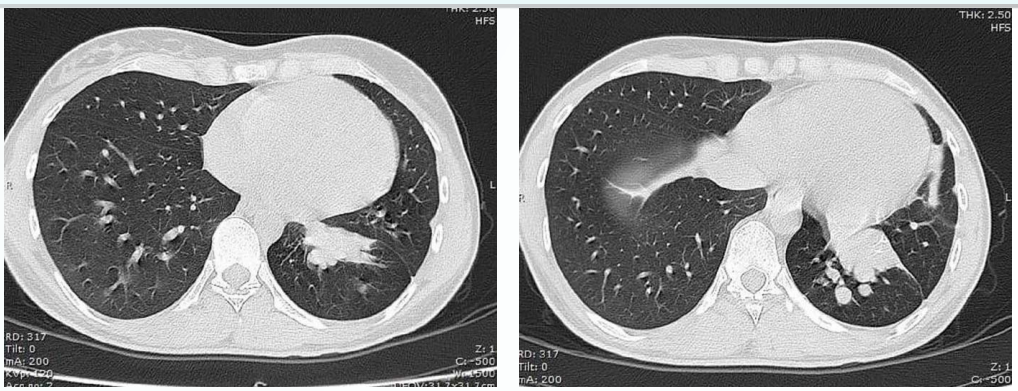


Figure 1. *Initial CT exam of the thorax with contrast substance reveals the following: heterogeneous consolidation process with a tendency to cavitation, moderate iodophillia and peripheral microcalcifications, associating tubular bronchiectasis occupied by mucoid impacts, designed LIL*

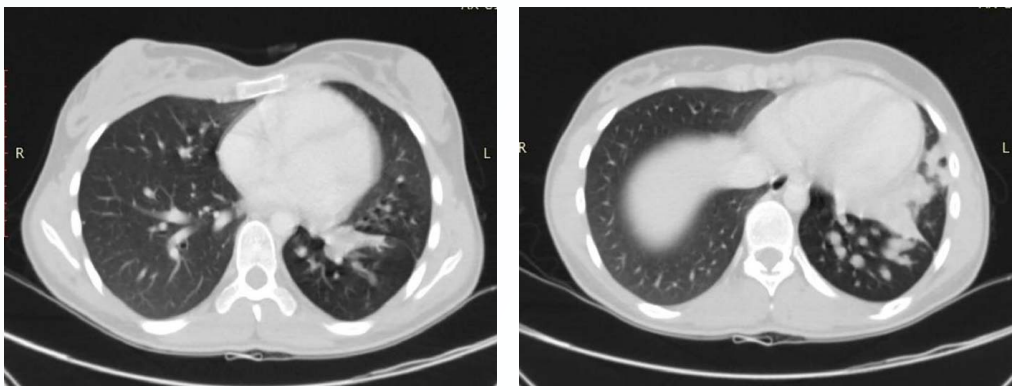


Figure 2 - *CT exam of the thorax with contrast substance in contact with the previous CT exam revealsthe following: Heterogeneous consolidation process with moderate iodophillia and peripheral microcalcifications, associating tubular bronchiectasis occupied by mucoid impacts, designed LIL. Multiple images of microcalcifications with centropulmonary and subpleural topography with dimensions between 3 and 6 mm bilaterallydispersed. No pleural or pericardial fluid accumulations. Without mediastinal adenopathy or hiatus with a pathological visa. Medium-sized media with opacified homogeneous size. Conclusions: dimensional progression of the retro-stenotic triangular condensation with sacral bronchiectasis and endo-luminal secretion retention.*

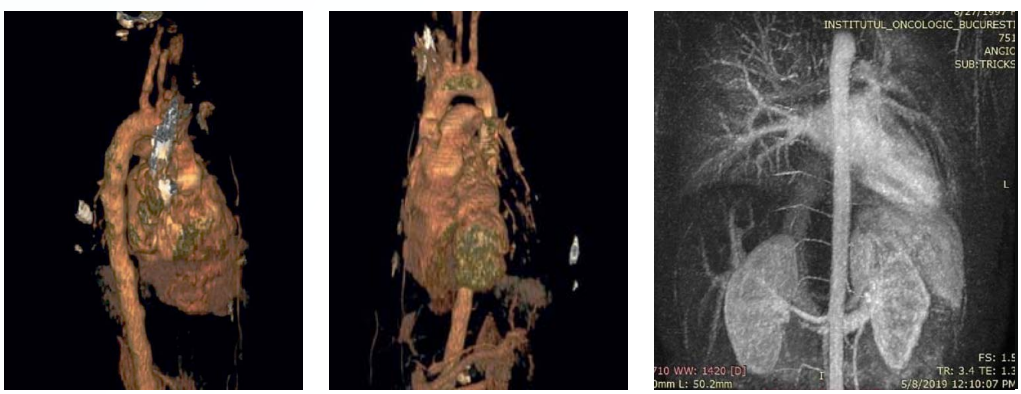


Figure 3. *The angio IRM exam with iv contrast enhancement, the toraco-abdominal aorta, highlights : Angiography of ECM 3D CE, of the dorsal-lumbar aorta, from infrarenal training to angiography TRICKS (12 acquisitions in the coronal plane) does not reveal aberrant tracts of thoracic aorta, right or right atrial renal artery with specificity for pulmonary seizure syndrome tributary to the condensation process in the basal medial segment of the lower left pulmonary lobe. Segmental pulmonary changes will be characterized as cystic bronsiectatic ystic cavities, panopacified by serum mucinous fluid.*

the segments. And the imaging exam revealed the persistence of the left pulmonary lower lobe consolidations processes with air bronchogram and bilateral non-calcified pulmonary micronodular lesions. It is decided to move on to the continuation phase of the treatment with a customised regimen as follows: INH 600 mg per day, RMP 600 mg per day and EMB 1200 mg per day.

At T8, the patient is clinically asymptomatic, endoscopically unchanged due to the LIL segment's punctiform stenosis; the retrostenotic changes are persistent at the imaging exam. It is not deemed necessary to continue the tuberculostatic treatment, and no surgical therapy is recommended for the remaining lesions. Over the next 12 months, multiple episodes of retrostenotic pneumonia occur occasionally. Imaging shows a clear lesional dynamics (Figure 2).

Therefore, there is a suspicion of pulmonary malignancy, including pulmonary sequestration syndrome, and further investigation by MR angiography of the thoracic aorta shows no aberrant arterial tracts from the thoracic aorta, left renal artery or the right atrium, specific to the pulmonary sequestration syndrome due to LIL consolidation (Figure 3). Subsequently, a surgical left lower lobectomy was performed, with a good outcome and complete recovery (Figure 4). Histopathological examination supports the diagnosis of tuberculous granulomas. The molecular pathological examination revealed the presence of Isoniazid-resistant *Mycobacterium tuberculosis* 1,2 (kaC gene) via the following mutation *c.947G>A; p.Gly316Asp*.

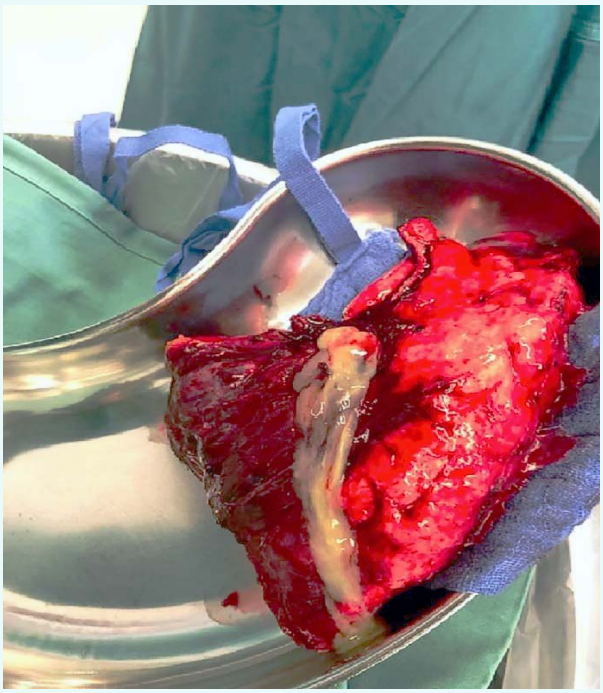


Figure 4. *Left lower lobe lobectomy. Macroscopy - Lobectomy specimen from the lower lobe. Centrally, there is a 4.5 cm long staple line, directly adjacent to it is a polypoid of grey-green gel-like material protruding from the parenchyma. The pleural surface is partly coarse dark red with scarred connective tissue. The parenchyma is prominently solidified on the lamellar cut surface at all levels. There are multiple, smaller foci with a crumby, consistent cut surface. In the area of the dark red colored pleura with the palpably solidified parenchyma, numerous dilated alveoli, these filled with similar gel-like material surrounding parenchyma homogeneously hemorrhagically imbibed. - Histopathological examination revealed partial lung resection with chronic bronchitis and bronchiolitis as well as bronchiectasis and centrally necrotizing epithelioid cell granulomas encapsulated in connective tissue, some with calcifications, consistent with tuberculous granulomas.*

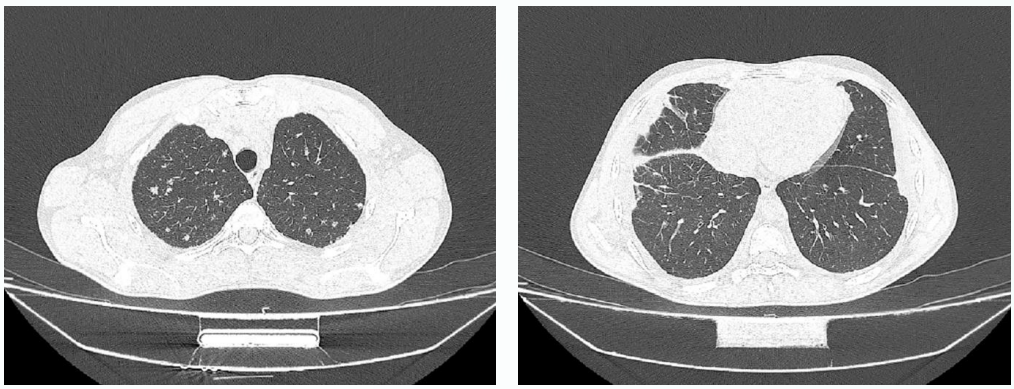


Figure 5. *CT exam of the thorax with contrast substance highlights multiple micronodules and small juxacentimeter pulmonary nodules distributed bilaterally, predominantly in the upper ½, apparently in slight numerical progression. Bilateral pleural effusion with a lateral-thoracic constricted appearance, with a thickness of 19mm on the left side and 30mm on the right side, which associates underlying pulmonary compression with a lamellar appearance in the lower lobes and medium lobe. They layered mediastinal adenopathies with slightly inhomogeneous iodophilia and sizes up to 17/12mm lower right paratracheal in progression. Multiple adenopathies in the abdominal floor were included in the examination, most found in the root of the mesentery, with sizes up to 19/15mm. Permeable pulmonary vascular axes.*

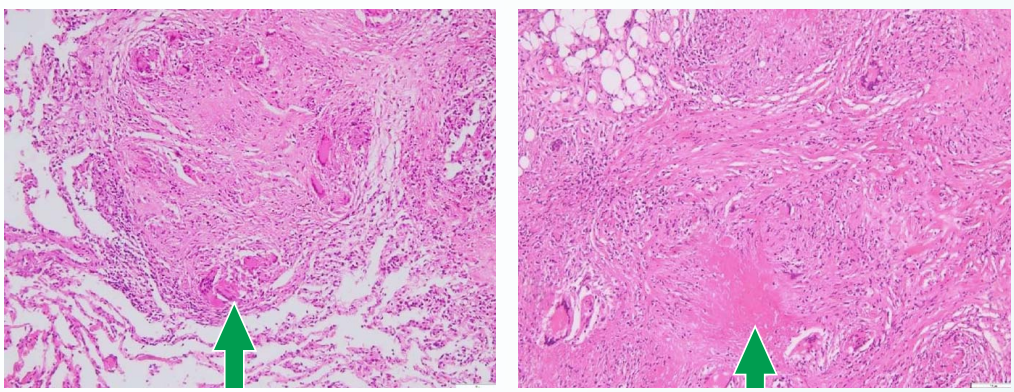


Figure 6. *Conglomerate gigantocellular epithelioid granulomas (green arrow); multinucleated giant Langhans cells are observed towards the periphery of the granulomas. Confluent, necrotizing gigantocellular epithelioid granulomas. The arrow indicates caseous necrosis, bordered by epithelioid histiocytes and giant Langhans cells.*



No mutation was detected for Rifampicin (rpoB gene) and Fluoroquinolones (gyrA gene). The final overall assessment has proven the presence of Mycobacterium tuberculosis, and there is evidence of isoniazid resistance. There is no evidence of rifampicin or fluoroquinolone resistance. The rifampicin (525) and capreomycin (tlyA gene) resistance testing do not produce usable results. The mutation c.947G>A; p.Gly316Asp in the katC gene has not yet been reported in the medical literature. This method covers regions 507 and 526-531 for rifampicin in the rpoB gene. Further resistance mechanisms cannot be ruled out. The patient's outcome is good, and it is not considered necessary to resume treatment.

Case no. 2

A 21-year-old male patient, smoker, with no occupational exposure to respiratory toxic substances and no significant personal pathological history, came to „Marius Nasta" NPI with fever, chills, fatigability, posterior thoracic pain in the right hemithorax, poor appetite and slight weight loss, recurrent symptoms with onset 1 year ago. From the recent history, we note that the patient has been investigated multiple times in other medical services in the last 12 months and has had many chest CT scans showing bilateral upper lobe micronodular lesions, mediastinal micro-lymphadenopathy, and recurrent left pleural effusion. The previous imaging examination also revealed minimal pelvic ascites.

Patient with serial negative sputum examinations for Koch's bacillus, at microscopy and cultures, diagnostic suspicion of Langerhans cell histiocytosis was raised. At that time, the investigations continued with a skull X-ray, which revealed multiple osteolytic lesions and, following a haematological evaluation, a bone marrow biopsy was recommended to rule out a diagnosis of multiple myeloma. This hypothesis was later refuted by histopathological and immunohistochemical testing.

In the clinical and paraclinical context, the following investigations were requested and performed: laboratory tests (complete blood count, electrolytes levels, coagulation tests, inflammatory markers), sputum microscopy for non-specific bacteria and Koch's bacillus, GeneXpert, contrast-enhanced CT scans of the chest and brain, complex respiratory functional explorations, i.e. plethysmography + DLco-measurements, cardiology consultation and echocardiography, bronchoscopy with bronchoalveolar lavage and thoracentesis for diagnostic and therapeutic purposes.

Laboratory tests revealed mild microcytic hypochromic anaemia and a significant inflammatory syndrome. Imaging examinations showed multiple bilateral diffuse

pulmonary nodules and micronodules, in numerical progression from the previous assessments, as well as layered mediastinal adenopathy with slightly non-homogeneous iodophilia, progressive in size, and bilateral lateralthoracic loculated pleural effusion. Brain CT refuted the previously detected osteolytic lesions (Figure 5).

Pleural fluid analysis revealed an exudate (total proteins = 5.5 g/dL) with elevated ADA (adenosine deaminase) levels (77.7 U/L), and the cytological smear showed moderate lymphocytosis, with no tumour cells on the examined smear. Functionally, the patient has moderate restrictive ventilatory dysfunction, normal central flow resistance, and severely decreased alveolar-capillary membrane gas transfer factor. At endoscopy, autofluorescence bronchoscopy did not detect actual or suspected proliferating lesions. Bronchial aspirate and bronchoalveolar lavage were non-specific.

Diagnostic investigations continued with a right thoracotomy with lung biopsy and parietal pleura biopsy. Histopathological examination revealed pulmonary and pleural granulomatosis suggestive of a TB etiology (Figure 6). Tuberculostatic treatment regimen I HRZE adjusted to the patient's weight was initiated as follows INH 300 mg per day, RMP 450 mg per day, EMB 800 mg per day, PZA 1500 mg per day.

Since the beginning of the tuberculostatic treatment, the clinical outcomes have been less favourable, with the occurrence and persistence of gastrointestinal symptoms (postprandial abdominal pain, nausea, vomiting, and loss of appetite), with intolerance to Isoniazid.

At T2, the results of the liquid medium cultures for Koch's bacillus from the lung fragment are positive, and the antibiogram showed resistance to Isoniazid, so it was decided to replace it with Levofloxacin 500 mg per day in the treatment regimen, with monthly ECG monitoring. The patient had a good drug tolerance, but mild gastrointestinal symptoms persisted.

At T5, the patient required emergency admission to the General Surgery Department for diffuse abdominal pain, feculent vomiting, and altered general condition. Surgical treatment was performed intraoperatively, and findings were inflammatory bowel disease, subocclusive syndrome and prevesical properitoneal abscess bilaterally fused in the paracolic gutters, for which viscerolysis, evacuation, lavage, and drainage were performed. Following the investigations, the diagnosis of prevesical properitoneal TB bilaterally fused in the paracolic gutters was established. The patient's postoperative outcome was good, with complete remission of the gastrointestinal symptoms.

At T9, the patient is clinically asymptomatic, with a favourable outcome of the imaging changes described above. Therefore there was no indication for continuing the tuberculostatic treatment.

Discussions

Drug resistance occurs when mutations or chromosomal replication errors occur in the genes encoding drug targeting or biotransformation mechanisms. It significantly impacts the efficacy of tuberculostatic therapy[12,13,14,15].

Isoniazid has potent bactericidal activity against Mycobacterium tuberculosis, primarily by inhibiting mycolic acid synthesis[16,17]. Isoniazid resistance is associated with mutations in the furA-katG and fabG1-inhA operons, and in the ahpC gene[16,18,19,20]. INH is a prodrug activated by the catalase-peroxidase enzyme encoded by the



katG gene[18]. Mutations in the katG gene, reducing the gene activity related to INH, are among the main mechanisms of the INH resistance[16,18]. Alternatively, INH-resistance may arise due to point mutations within the promoter region of the inhA operon or the inhA gene. Mutation within the promoter region of inhA can lead to an overexpression of inhA, resulting in a relative resistance to INH. InhA mutations can be the cause of cross-resistance to ethionamide[16,19,20].

64% of the isoniazid-resistance phenotypes were associated with the katG315 mutation worldwide[21,22,23]. Although more than 300 katG mutations have been identified, mutations within codon 315 are the most prevalent[22, 23]. Moreover, a specific amino acid substitution (from serine to threonine) is responsible for 95% of the katG 315 mutations[23]. Mutations within the katG are associated with moderate to high-level Isoniazid resistance[21].

The second most common mutation is inhA-15, reported in 19% of the Isoniazid-resistant isolates[23]. Mutations within the promoter region of inhA tend to result in a low-level phenotypic resistance. And they also confer resistance to second-line drugs, namely ethionamide and prothionamide[22, 24]. The most prevalent mutation within the inhA promoter region is the c-15t mutation, which is present in a median of 19% of the clinically Isoniazid-resistant isolates worldwide[21,23].

Mutations within the inhA promoter region were not associated with a poor outcome (treatment failure or death) of the first-line regimen I tuberculostatic treatment. However, such mutations were associated with an increased risk of relapse. On the other hand, katG 315 mutations have been associated with a poor treatment outcome[21,25].

These two mutations, katG315 and inhA-15, combined with ten of the most common mutations within the inhA promoter and ahpC-oxyR intergenic region explain 84% of the global Isoniazid-resistance phenotypes[21].

Conclusions

The significant association between the two mutations, inhA c-15 and katG 315, respectively, and the high-level resistance is of interest in the interpretation of current and future molecular diagnostic testing, as an early prediction of the level of Isoniazid resistance is essential to decide the benefit of high-dose Isoniazid use[21]. LPA testing, such as MTBDRplus (Hain test), can reliably identify isolates containing these mutations and thus can predict the level of Isoniazid resistance[26, 27]. However, an isolated katG or inhA mutation may suggest highly variable levels of minimum inhibitory concentration. Thus, inhA mutations do not always predict low resistance levels and katG mutations high resistance levels, so LPA testing may not be

used to decide whether or not Isoniazid will be useful in a combination therapy regimen when only one of these mutations is detected[21]. Moreover, further research is necessary to investigate the real benefits of high-dose Isoniazid use in patients with low or moderate resistance caused by only one of the two mutations[21]. Local variability of the individual mutation frequency may limit the sensitivity of molecular diagnostic testing[23]. Therefore, well-designed systematic surveys and whole genome sequencing are needed to identify mutation frequencies across geographic regions where rapid molecular testing is currently implemented, providing a context for interpreting of the results and the opportunity to improve the next generation of diagnostic tests[23].

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References

1. Global Tuberculosis Report 2022. World Health Organization, 2022.

2. The End TB Strategy. World Health Organization; 2014. http://www.who.int/tb/strategy/end-tb/en/. Accessed March 14, 2019.

3. National Guideline for the Management of Drug-resistant Tuberculosis Cases 2020. Romanian Society of Pneumology 2020. Official Gazette no. 917 bis 2020.10.8

4. Paul Ioan Stoicescu et al. Management of Multidrug Resistant Tuberculosis Cases. Ministry of Health, "Marius Nasta" Institute of Pneumology, National Tuberculosis Control Program; 2005

5. Companion Handbook to the WHO Guidelines for the Programmatic Management of Drug-Resistant Tuberculosis. World Health Organization; 2014

6. Arghir OC, Chiotan DI, Cioran NV, et al. Methodological Guideline for the implementation of the National Program for the Prevention, Surveillance and Control of Tuberculosis. Ministry of Health, "Marius Nasta" Institute of Pneumology, National Tuberculosis Control Program; 2015

7. Daniela Homorodean, Adriana Moisoiu. National Guideline for the TB Laboratory Network. Ministry of Health, "Marius Nasta" Institute of Pneumology, National Tuberculosis Prevention, Surveillance and Control Program; 2017

8. WHO Treatment Guidelines for Isoniazid-Resistant Tuberculosis: Supplement to the WHO Treatment Guidelines for Drug-Resistant Tuberculosis. World Health Organization; 2018

9. Ahuja SD, Ashkin D, Avendano M, et al. 'Aultidrug Resistant Pulmonary Tuberculosis Treatment Regimens and Patient Outcomes: An Ir/hvidual Patient Data Meta-analysis of 9,153 Patients. PLOS Med. 2012; 9(8):e 1001300

10. Fox GJ, Mitnick CD, Benedetti A et al. Surgery as an adjunctive treatment for multidrug-resistant tuberculosis: an individual patient data metaanalysis. Clin Infect Dis. 20l6; 62(7):887-895

11. Harris RC, Khan MS, Martin LJ, et al. The effect of surgety on the outcome of treatment for multidrug-resistant tuberculosis: a systematic review and meta-analysis. BMC Infect Dis. 2016; 16:262

12. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. PLoS One. 2015 Mar 23;10(3):e0119628. doi: 10.1371/journal.pone.0119628. PMID: 25799046; PMCID: PMC4370653.

13. World Health Organization. Global Tuberculosis Report. Geneva, Switzerland; WHO press; 2013.

14. World Health Organization; Multidrug and Extensively Drug-Resistant TB 2010 Global Report of



Surveillance and Response. Geneva, Switzerland; WHO press; 2010.

15. Sougakoff W. Molecular epidemiology of multidrug-resistant strains of Mycobacterium tuberculosis. Clin Microbiol Infect. 2011; 17: 800–805. 10.1111/j.1469-0691.2011.03577.x

16. Erdenegerel Narmandakh, Oyuntuya Tumenbayar, Tsetsegtuya Borolzoi, Baasansuren Erkhembayar, Tsolmon Boldoo, Naranzul Dambaa, Buyankhishig Burneebaatar, Naranbat Nymadawa, Satoshi Mitarai, Sarantuya Jav, Chen-Yuan Chiang. Genetic Mutations Associated with Isoniazid Resistance in Mycobacterium tuberculosis in Mongolia. ASM Journals, Antimicrobial Agents and Chemotherapy Vol. 64, No. 7. https://doi.org/10.1128/aac.00537-20

17. Jindani A, Aber VR, Edwards EA, Mitchison DA. 1980. The early bactericidal activity of drugs in patients with pulmonary tuberculosis. Am Rev Respir Dis 121:939–949.

18. Zhang Y, Heym B, Allen B, Young D, Cole S. 1992. The catalase-peroxidase gene and isoniazid resistance of Mycobacterium tuberculosis. Nature 358:591–593.

19. Banerjee A, Dubnau E, Quemard A, Balasubramanian V, Um KS, Wilson T, Collins D, de Lisle G, Jacobs WR, Jr. 1994. inhA, a gene encoding a target for isoniazid and ethionamide in Mycobacterium tuberculosis. Science 263:227–230.

20. Vilchèze C, Jacobs WR. 2014. Resistance to isoniazid and ethionamide in Mycobacterium tuberculosis: genes, mutations, and causalities. Microbiol Spectr 2:MGM2.

21. Lempens P, Meehan CJ, Vandelannoote K, Fissette K, de Rijk P, Van Deun A, Rigouts L, de Jong BC. Isoniazid resistance levels of Mycobacterium tuberculosis can largely be predicted by high-confidence resistance-conferring mutations. Sci Rep. 2018 Feb 19; 8(1):3246. doi: 10.1038/s41598-018-21378-x. PMID: 29459669; PMCID: PMC5818527.

22. Vilcheze C, Jacobs WR., Jr. Resistance to isoniazid and ethionamide in mycobacterium tuberculosis: genes, mutations, and causalities. Microbiology Spectrum. 2014;2:MGM2-0014-2013. doi: 10.1128/microbiolspec. MGM2-0014-2013.

23. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. PloS one. 2015;10:e0119628. doi: 10.1371/ journal. pone. 0119628.

24. Böttger, E. C. In Antituberculosis Chemotherapy Vol. 40 (eds P. R. Donald & P. D. van Helden) Ch. 14, 128–144 (Karger, 2011).

25. Huyen MN, et al. Epidemiology of isoniazid resistance mutations and their effect on tuberculosis treatment outcomes. Antimicrobial agents and chemotherapy. 2013;57:3620–3627. doi: 10.1128/ AAC.00077-13.

26. Ramaswamy SV, et al. Single nucleotide polymorphisms in genes associated with isoniazid resistance in Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2003;47:1241–1250. doi: 10.1128/AAC.47.4.1241-1250.2003.

27. Jagielski T, et al. Identification and analysis of mutations in the katG gene in multidrug-resistant Mycobacterium tuberculosis clinical isolates. Pneumonologia i alergologia polska. 2013;81:298–307.