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Evaluation of the Hematological and Biochemical Markers of Iron Metabolism in Pulmonary Multidrug-Resistant Tuberculosis (MDR-TB)

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Abstract

Medical management of Multidrug-Resistant Tuberculosis (MDR-TB) has become a major public health issue. The outbreak of this form of tuberculosis is partly due to anemia observed in patients with (MDR-TB). In order to improve the medical management of these patients, it is therefore necessary to define the characteristics of this anemia. The main objective of this study was to investigate the biochemical and haematological parameters related to the anemia observed in patients with Multi-Drug Resistant Tuberculosis (MDR-TB). Thus, 100 (MDR-TB) patients and 100 non-tuberculous patients as many women than men and between 18 and 55 years of age were selected. The hematological markers were assayed with the Sysmex Kobe automate, Japan, while the biochemical parameters such as ferritin and transferrin were performed using Cobas C311 from Roche Diagnostic, France and as for the serum iron, an Atomic Absorption Spectrometer (AAS) of the Varian Spectr AA-20 Victoria® type, Australia was used.

Results showed that 32% of women and 18% of men had mixed (iron deficiency and inflammatory) anemia, while the remaining 68% of women and 82% of men had inflammatory anemia.

Keywords: Abidjan; Anemia; MDR-TB; Micronutrients

Abbreviations

MCV	:	Mean Cell Volume
MCHC	:	Mean Corpuscular Hemoglobin Concentration
RMCH	:	Rates of Mean Corpuscular Hemoglobin
TBC	:	Total Iron Binding Capacity
TSC	:	Transferrin Saturation Coefficient

Introduction

Multidrug-Resistant Tuberculosis (MDR-TB) is a disease caused by *Mycobacterium tuberculosis* which is at least resistant to two major TB drugs in first-line therapy, such as Isoniazid and Rifampicin [1]. The outbreak of this kind of tuberculosis is a real threat against all efforts made recently to control and eradicate tuberculosis [2].

According to the World Health Organization (WHO) 480,000 people have contracted Multidrug-Resistant Tuberculosis (MDR-TB) and 190,000 deaths was recorded worldwide [3]. The prevalence rate in Africa and Côte d'Ivoire are respectively 14% [4] and 2.5% [5]. The medical management of this form of tuberculosis is not easy on account of the high rates of therapeutic failures leading to the selection of mycobacterial bacilli with new resistance to second-generation anti-tuberculosis drugs. These therapeutic failures stand for a threat to the Sustainable Development Goals (MDGs) which aims at eradicating any kinds of TB in 2030 [6]. According to WHO report [3], the rate of treatment failures in the cohort of MDR-TB cases detected in 2010 was more than 50% and that about 9.6% of these MDR-TB evolved to a form of Ultra-Drug-Resistant Tuberculosis (UDRTB) in 2012. One of the main findings generally brought back by studies carried out on tuberculosis studies and particularly on MDR-TB is anemia

[7,8]. Anemia is multifactorial: it can be of infectious origin or due to a micro-nutritional deficiency. Thus, an effective management of this form of tuberculosis requires to specify the origins of anemia. The aim of this study was to investigate the biochemical and haematological parameters related to the observed anemia in people with Multidrug-Resistant Tuberculosis (MDR-TB).

This study consisted in:

- Evaluating the haematological and biochemical profile of multidrug-resistant tuberculosis (MDR-TB) before and during second line TB treatment.
- Determining the main forms of anemia recorded in MDR-TB patients according to those haematological and biochemical data.

Materials and Methods

This study was conducted at the Institute Pasteur of Côte d'Ivoire (IPCI). It involved blood samples of MDR-TB patients. These samples were collected from five Anti-Tuberculosis Centers (ATC) of Abidjan from January 2014 to December 2015. One hundred (100) Multidrug-Resistant Tuberculosis Patients (MDR-TB) as many men as women and one hundred (100) non-tuberculous volunteers used as control with 50 men and 50 women were selected for this study. The age of patients and control ranged from 18 to 55 years. Blood samples were performed at different stages of patients follow-up:

- The M_0 stage involved initial assessment after the GenXpert® MTB / RIF test to confirm multidrug resistance before starting any treatment [9].
- Stages M_3 and M_6 for the follow-up assessment at respectively 3 and 6 months for second-line anti-tuberculosis treatment.

Two samples of MDR-TB were collected at each follow up stage (M_0, M_3 and M_6) and two (2) samples from each non-tuberculosis control patients in a non-anticoagulant tube (red cap tube) and in an EDTA tube (Ethylene Diamine Tetra-acetic Acid), 5 mL of blood was collected in each tube. 600 MDR-TB samples (300 tubes without anticoagulant and 300 EDTA tubes) and 200 samples as control (100 tubes without anticoagulant and 100 EDTA tubes) were selected for this study. The samples of tubes without anticoagulant were then centrifuged at 3000 rpm for 5 minutes using a horizon 642 VES centrifuge, THE DRUCKER CO., USA. The serum was then collected for analysis of the biochemical markers. As for the EDTA tube, it was used for the determination of haematological markers of iron metabolism. Hematological markers such as hemoglobin, mean cell volume, rate of mean corpuscular hemoglobin was measured using Sysmex XN-1000i Kobe Japan [10]. Analysis of iron in serum was assayed by atomic absorption spectrometer (SAA) of Varian Spectr AA-Victoria® type, Australia [11]. As for ferritin and transferrin, different assays were performed with Cobas C311 from Roche Diagnostic, France [12,13].

Results

The results of this study showed a significant decrease in hematic markers of iron metabolism at the onset of the experiment compared to the non-tuberculosis controls groups $P < 0.05$. During anti-tuberculosis treatment, only the rate of hemoglobin (Hb) remained significantly lower compared to non-tuberculosis control groups $P < 0.05$.

On the other hand, this hemoglobin level increased significantly during anti-tuberculosis treatment compared to the initial analysis (M_0) $P < 0.05$. However, it should be noted that, apart from the hemoglobin level and total iron binding capacity (TBC) which showed a significant increase during treatment $P < 0.05$, the other haematological markers did not undergo any significant change (Figures 1-4).

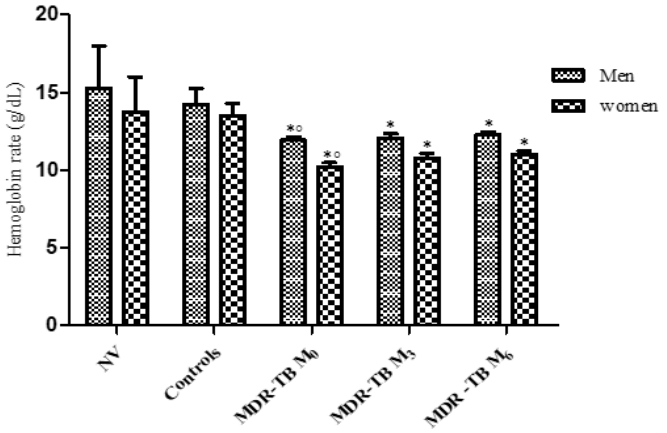

Figure 1: Hemoglobin rate of MDR-TB and controls.

Hemoglobin rate for MDR-TB patients and non-tuberculosis control groups. M_0: initial assessment. M_3, M_6: follow-up assessment at 3 and 6 months of treatment. NV: Normal values. *: significant Difference between MDR-TB and non-tuberculosis control groups $P < 0.05$. Significant difference between the various stages of the follow-up, $P < 0.05$.

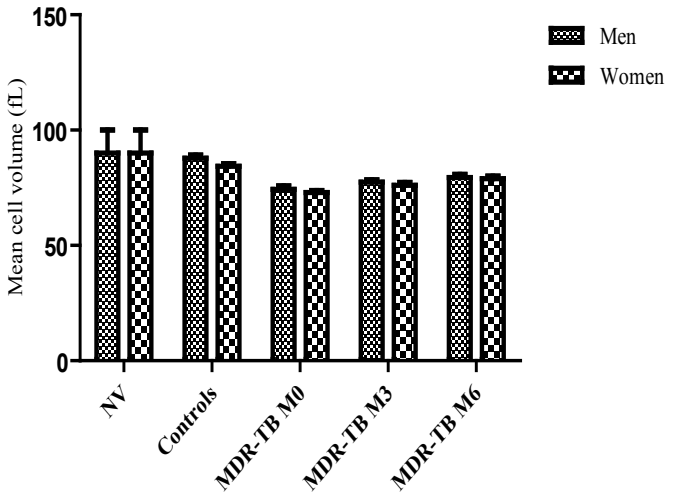

Figure 2: Mean cell volume of MDR-TB and controls.

Mean cell volume (MCV) for Multidrug-Resistant Tuberculosis (MDR-TB) and non-tuberculosis control groups. M_0: initial assessment. M_3, M_6: follow-up assessment at 3 and 6 months of treatment. NV: Normal values. *: significant Difference between MDR-TB and non-tuberculosis control groups $P < 0.05$.

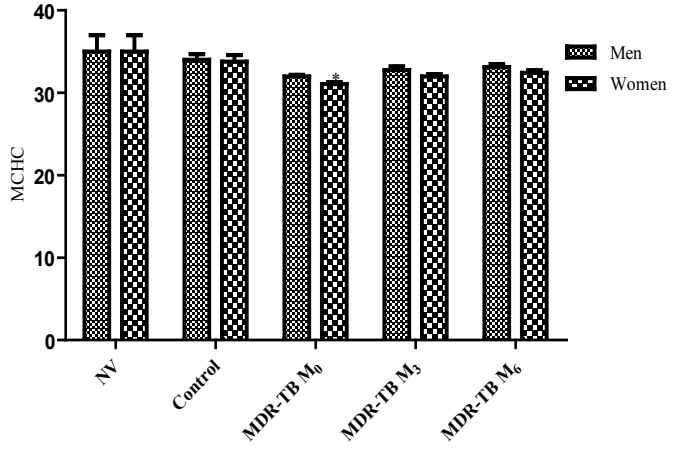

Figure 3: Mean corpuscular hemoglobin concentration.

Mean corpuscular hemoglobin concentration for Multidrug-Resistant Tuberculosis (MDR-TB) and non-tuberculosis control groups. M_0: initial assessment. M_3, M_6: follow-up assessment at 3 and 6 months of treatment. NV: Normal values. *: significant Difference between MDR-TB and non-tuberculosis control groups $P < 0.05$.

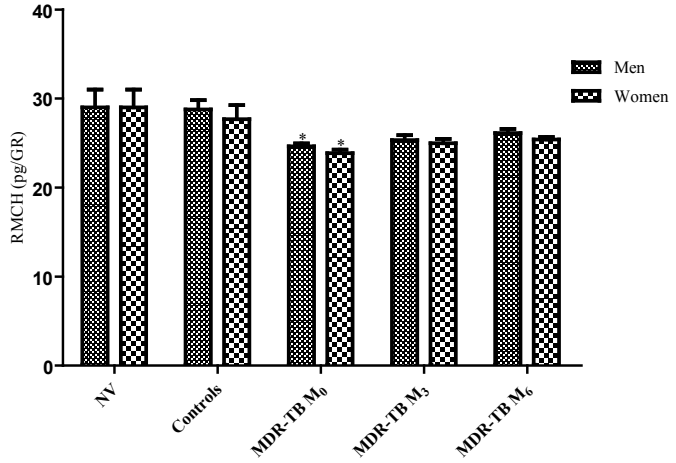

Figure 4: Rate of mean corpuscular hemoglobin in MDR-TB and controls.

Rates of mean corpuscular hemoglobin (RMCH) for multidrug-resistant tuberculosis (MDR-TB) and non-tuberculosis control groups. M_0: initial assessment. M_3, M_6: follow-up assessment at 3 and 6 months of treatment. NV: Normal values. *: significant Difference between MDR-TB and non-tuberculosis control groups $P < 0.05$.

This study also showed a significant decrease in serum iron concentrations and Total Iron Binding Capacity (TBC) in patients with multidrug-resistant tuberculosis, notwithstanding the follow up stage of patients compared to controls ($P < 0.0001$) (Figures 5,6). In contrast to serum iron that did not undergo any significant change, TBC increased significantly during anti-TB treatment compared to baseline ($P < 0.0001$) (Figure 6).

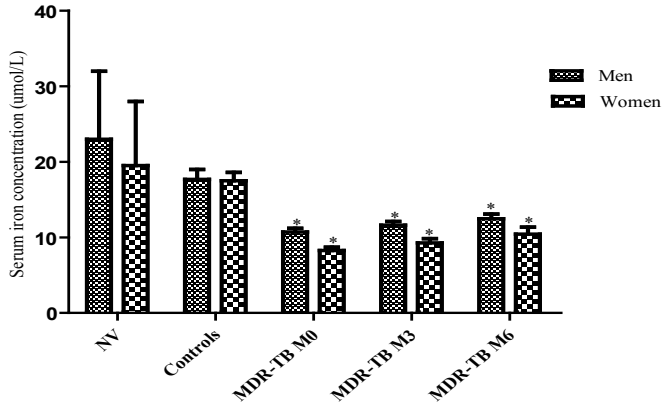

Figure 5: Serum iron concentration for MDR-TB and controls.

Serum iron concentration for Multidrug-Resistant Tuberculosis (MDR-TB) and non-tuberculosis control groups. M_0: initial assessment. M_3, M_6: follow-up assessment at 3 and 6 months of treatment. NV: Normal values. *: significant Difference between MDR-TB and non-tuberculosis control groups $P < 0.05$.

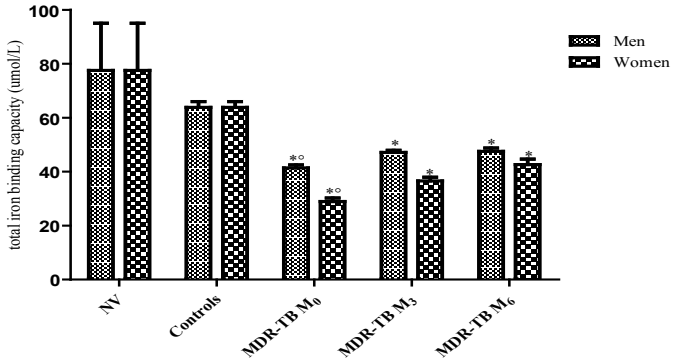

Figure 6: total iron binding capacity (TBC) for MDR-TB and controls.

Total Iron Binding Capacity (TBC): Rate of bound iron when transferrin is saturated at 100%. MDR-TB: multidrug resistant Tuberculosis. M_0: initial assessment. M_3, M_6: follow-up assessment at 3 and 6 months of treatment. NV: Normal values. *: significative difference between MDR-TB and non-tuberculosis control groups, $P < 0.0001$. º: significative difference between initial assessment and MDR-TB follow up assessment, $P < 0.0001$.

It should be noted that despite increases in iron concentrations and TBC values during treatment, reduction percentages of these markers remained high after six months of treatment (M6) (Figures 7,8).

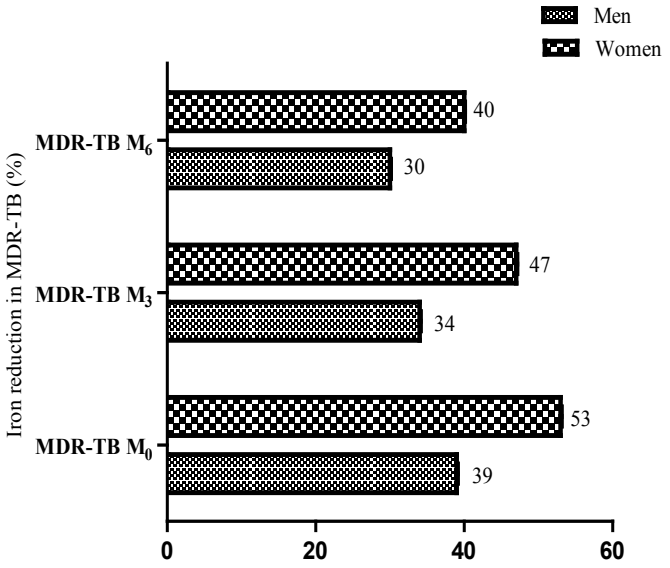

Figure 7: Iron reduction percentage in MDR-TB.

Iron reduction percentage in MDR-TB at the follow up different stages compared to non-tuberculosis control group

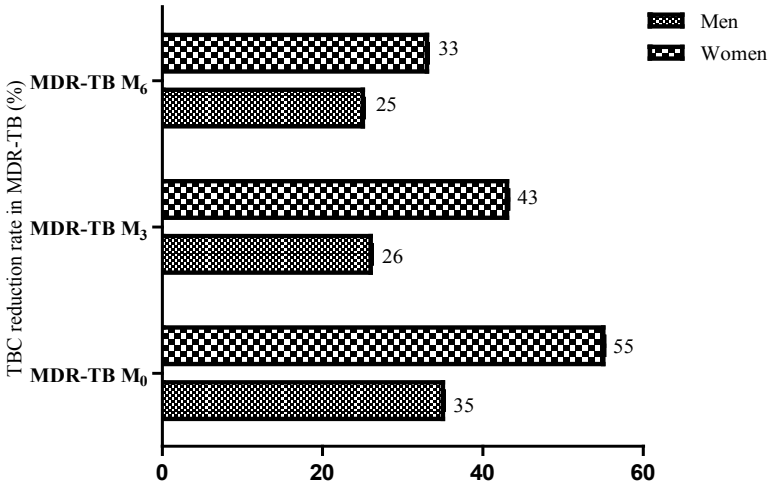

Figure 8: Reduction percentage of TBC.

Total Iron binding capacity (TBC) in MDR-TB at the follow up different stages compared to non-tuberculosis control group.

Concerning serum ferritin and Transferrin Saturation Coefficient (TSC), 16 women out of 50, or 32% of women and 9 men out of 50, or 18% of men, had a significantly lower serum ferritin concentration and TSC compared to controls and the usual values ($P < 0.05$) (Tables 1,2). The remaining 68% of women and 82% of men had ferritinemia and TSC in the normal range (Table 1,2).

Table 1: Values of serum ferritin concentrations in MRD-TB and controls.

	MDR-TB with low ferritin (µg/L)		MDR-TB with normal ferritin (µg/L)		Non tuberculosis control group (µg/L)	
	Men (9) or (18%)	Women (16) or (32%)	Men (41) or (82%)	Women (34) or (68%)	Men (50) or (100%)	Women (50) or (100%)
M_0	14,9 ± 7,3*	12,2 ± 5,7*	211,6 ± 21,5	166,5 ± 10,0	164,4 ± 18,2	96,6 ± 13,1
M_3	18,7 ± 5,5*	14,9 ± 4,3*	151,3 ± 11,9	163,4 ± 11,6		
M_6	21,6 ± 6,4*	17,5 ± 3,2*	154,6 ± 9,8	117,6 ± 11,6		

Ferritin concentration in multidrug resistant tuberculosis (MDR-TB) at initial assessments (M_0) and follow-up assessment at 3 and 6 months of treatment (M_3 and M_6) with those of non-tuberculosis volunteer control groups. *: Significative difference, $P < 0.05$.

Table 2: Values of Transferrin Saturation Coefficient (TSC) in MDR-TB and the controls.

	MDR-TB with low TSC (%)		MDR-TB with normal TSC (%)		Non tuberculosis control group (%)	
	Men (9) or (18%)	Women (16) or (32%)	Men (41) or (82%)	Women (34) or (68%)	Men (50) or (100%)	Women (50) or (100%)
M_0	10,9 ± 3,1*	09,4 ± 2,1*	21,9 ± 1,4	27,3 ± 1,7	28,6 ± 2,3	26,9 ± 1,8
M_3	11,7 ± 2,1*	11,1 ± 1,3*	22,7 ± 1,1	23,8 ± 1,4		
M_6	12,3 ± 2,3*	12,3 ± 1,2*	23,8 ± 1,3	22,6 ± 2,2		

Transferrin Saturation Coefficient (TSC): percentage of transferin iron binding. MDR-TB: Multidrug resistant tuberculosis. M_0: initial assessment. M_3, M_6: follow-up assessment at 3 and 6 months of treatment. *: Significative difference, $P < 0.05$. Serum ferritin concentrations and Transferrin Saturation Coefficient (TSC).

Discussion

This study showed a significant decrease in haematological markers such as Hemoglobin (Hb), Mean Cell Volume (MCV), Mean Cell and Hemoglobin Concentrations (MCH and MCHC) in Multidrug-Resistant TB Patients (MDR-TB) compared to non-tuberculosis control groups. These results define the microcytic and hypochromic character of anemia found in these MDR-TB. Several authors have reported anemia in tuberculosis treating patients, though these studies are not precise on the type of anemia [8]. These results could highlight a lack of iron supply to the erythropoietic process.

Furthermore, analysis of the biochemical parameters of this study showed a significant decrease in serum iron concentrations and Total Iron Binding Capacity (TBC) in Multidrug-Resistant Tuberculosis (MDR-TB) compared to non-tuberculosis control group and to normal values. Serum ferritin concentrations and Transferrin Saturation Coefficient (TSC) decreased significantly in 32% of women with MDR-TB and in 18% of men with MDR-TB, while they remained within the range of normal values in the rest of patients. These results are characteristics of inflammatory anemia or anemia of chronic diseases on the one hand (ferritin and normal TSC) and a mixed anemia (iron deficiency and inflammatory anemia) on the other hand (ferritin and low TSC) as reported by several studies [14,15] The inflammatory anemia is due not to an iron deficiency but rather to a functional deficiency of iron. Indeed, the normal ferritin values representing the reserve pool reflecting an absence of iron deficiency, while the low values of TBC, which is the functional iron pool, might characterize a functional deficit of available iron [16]. These results are in agreement with those of Lovey et al. [17] who showed that ferritin levels above 100 µg / L exclude the hypothesis of iron deficiency and that the transferrin saturation coefficient only decreases when iron reserves are completely depleted. On the other hand, low serum iron concentrations and decreased Total Iron Binding Capacity (TBC) could be explained by a high retention of iron in macrophages and a decrease in the iron supply to erythropoiesis during chronic inflammation induced by Mycobacterial infection [18].

The mechanisms leading to the introduction of this type of anemia involve the production of various cytokines including interferon-γ, TNF-α and interleukins 1 and 10 which could lead to the sequestration of iron resulting from the degradation of red blood cells by macrophages and repression of the synthesis of erythropoietin. This process could be amplified by an excessive synthesis of hepcidin. A pro-inflammatory protein mostly produced

by the hepatocyte and excreted in the bloodstream. This protein would interact with ferroportin which is the exporter of iron present in enterocytes and macrophages causing degradation of the latter. This leads to a decrease in intestinal iron absorption and iron retention in macrophages and hepatocytes, the two main routes of iron supply to the body [14]. This set of mechanisms would lead to decreased serum iron concentration and iron binding capacity to transferrin [16]. This difficulty of mobilizing iron from the reserves might lead to a decrease in the synthesis of hemoglobin, hence the hypochromic character of anemia (low hemoglobin concentration).

At the same time, there is a reactionary increase in the number of mitoses resulting in a production of small red blood cells, which is reflected by the Microcytic Character of the Anemia (low MCV). In addition, the decrease in Total Iron Binding Capacity (TBC) is due to a decrease in plasma of transferrin levels.

Indeed, during inflammation, the reduction level of this plasma iron transport protein is linked to its hyper catabolism in the inflammatory zone and on the other hand to the reduction of its synthesis due to the reserves in full iron, as shown by the normal serum ferritin concentrations [17]. Mix Anemia (iron deficiency and inflammation anemia) could be explained by an insufficient iron intake in addition to the inflammatory process. However, the slight increase in serum iron concentrations during second-line therapy in MDR-TB was not in agreement with the study of Edem et al. [19], which showed a progressive decrease in iron concentrations in tuberculosis patients during First-line treatment. This observation is due to the fact that second-line antituberculosis molecules have no haemolytic action in MDR-TB compared to first-generation anti-tuberculosis drugs such as Rifampicin [20]. Moreover, despite the significant increase in iron concentrations and TBC values during treatment, the percentages of reduction of these markers remained high after six months of treatment (M6). This may suggest inaction of the second generation anti-tuberculosis molecules on the inflammatory process caused by the *Mycobacterium tuberculosis* bacillus.

Conclusion

The management of multidrug-resistant tuberculosis has become a major public health issue. The outbreak of this form of tuberculosis is partly due to the anemia observed in these people with a MDR-TB. In order to improve the medical management of these patients, it is important to define the origin of this anemia observed in these patients. Thus, our study on the profile of hematological and biochemical markers of iron metabolism made it possible to specify the inflammatory and mixed characteristics of anemia observed in these multidrug resistant tuberculosis (MDR-TB). The persistence of these metabolic disorders after six months of the intensive phase of second-line anti-tuberculosis treatment advocates additive measures such as the effective management of inflammation in parallel with anti-tuberculosis treatment.

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