Review

Title: Hematologic Toxicity of Linezolid in Multidrug Resistant and Extensively Drug Resistant Tuberculosis (MDR/XDR-TB): the role of mitochondria

Amaylia Oehadian¹, Prayudi Santoso¹, Dick Menzies², Rovina Ruslami³

¹Amaylia Oehadian, MD
Division of Hematology and Oncology Medic, Department of Internal Medicine Faculty of Medicine, Padjadjaran University/ Dr. Hasan Sadikin General Hospital, Bandung, Indonesia;
amaylia.oehadian@unpad.ac.id, ORCID : https://orcid.org/0000-0002-7853-2221

¹Prayudi Santoso, PhD
Division of Hematology and Oncology Medic, Department of Internal Medicine Faculty of Medicine, Padjadjaran University/ Dr. Hasan Sadikin General Hospital, Bandung, Indonesia;
prayudi@unpad.ac.id, ORCID: https://orcid.org/0000-0002-7182-386X

²Prof. Dick Menzies, MD, MSc
McGill International TB Centre Respiratory Epidemiology and Clinical Research Unit, Montreal, Canada; dick.menzies@mcgill.ca
https://orcid.org/0000-0003-1601-4514

³Prof Rovina Ruslami, MD, PhD
Department of Biomedical Science, Division of Pharmacology, Padjadjaran University/ Dr. Hasan Sadikin General Hospital, Bandung, Indonesia;
Jl. Prof. Eickman No.38, Pasteur, Bandung 40161, West Java, Indonesia
Phone +62 22 2038114
rovina.ruslami@unpad.ac.id, ORCID : https://orcid.org/0000-0003-4995-5054

Corresponding author
Prof Rovina Ruslami, MD, PhD
Department of Biomedical Science, Division of Pharmacology,
Padjadjaran University/ Dr. Hasan Sadikin General Hospital, Bandung, Indonesia;
Jl. Prof. Eyckman No.38, Pasteur, Bandung 40161, West Java, Indonesia
Phone +62 22 2038114
rovina.ruslami@unpad.ac.id, ORCID: https://orcid.org/0000-0003-4995-5054

Running Title: Hematologic Toxicity of Linezolid in MDR/XDR-TB

Acknowledgements: The authors thank Kevin Yonatan, MD for final editing for submitting the manuscript

Declaration of personal interests: we have nothing to declare.

Declaration of funding interests: This research and APC was funded by Doctoral Dissertation Research Program, an internal Universitas Padjadjaran research funding number 1595/UN6.3.1/PT.00/2021

Author Contributors

Conceptualization: Oehadian A, Santoso P, Menzies D, Ruslami R
Writing-original draft preparation: Oehadian A, Santoso P
Writing-review and editing: Menzies D, Ruslami R
Approval of final manuscript: All authors

Competing interests None.
Concise Clinical Review: Hematologic Toxicity of Linezolid in Multidrug-Resistant and Extensively Drug-Resistant Tuberculosis: Role of Mitochondria

Amaylia Oehadian¹, Prayudi Santoso¹, Dick Menzies², Rovina Ruslami³

¹Department of Internal Medicine, Hasan Sadikin Hospital, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia,
²McGill International TB Centre Respiratory Epidemiology and Clinical Research Unit, Montreal Canada, Director of the WHO McGill Collaborative Centre for TB Research,
³Department of Biomedical Science, Division of Pharmacology, Faculty of Medicine Universitas Padjadjaran, Bandung, Indonesia

Corresponding author: Prof Rovina Ruslami, MD, PhD
Department of Biomedical Science, Division of Pharmacology, Universitas Padjadjaran, Bandung, Indonesia;
Jl. Prof. Eyckman, No. 38, Pasteur, Bandung 40161, West Java, Indonesia. Phone: +62 22 2038114;
email: rovina.ruslami@unpad.ac.id; ORCID: https://orcid.org/0000-0003-4995-5054

Running Title: Hematologic Toxicity of Linezolid in MDR/XDR-TB

Abstract

Multidrug-resistant tuberculosis (MDR-TB) is resistant to both rifampicin and isoniazid. Extensively drug-resistant TB (XDR-TB) is a rare type of MDR-TB which is resistant to quinolone and one of the group A TB drugs, i.e., linezolid or bedaquiline. In 2018, the World Health Organization revised the groupings of TB medicines and reclassified linezolid as a group A drug for the treatment of MDR-TB. Linezolid is a synthetic antimicrobial agent in the oxazolidinone class. Although linezolid has good efficacy, it can cause substantial adverse events, especially hematologic toxicity. In both TB infection and linezolid mechanism of action, mitochondrial dysfunction plays an important role. In this concise review, linezolid characteristics as an antiTB drug are summarized, including its efficacy; pathogenesis of
hematologic toxicity, highlighting mitochondrial dysfunction; and the monitoring and management of hematologic toxicity.

Keywords: MDR-TB, XDR-TB; Linezolid; Hematologic Toxicity; Mitochondria
Introduction

Multidrug-resistant tuberculosis (MDR-TB) is resistant to both rifampicin and isoniazid, the two most effective anti-TB drugs, and as a result, it requires treatment with a second-line regimen\(^1\). Extensively drug-resistant TB (XDR-TB) is MDR-TB with resistance to quinolone and one of group A TB drugs, i.e., linezolid or bedaquiline\(^2\). Currently, drug-resistant TB is a major public health burden. In 2020, the World Health Organization (WHO) reported approximately 500,000 people worldwide have rifampicin-resistant tuberculosis (RR-TB), 78% of which whom develop MDR-TB. The global treatment success rate for MDR/RR-TB is 57%\(^3\).

In 2018, the WHO revised the groupings of TB medicines and reclassified linezolid as a group A drug for the treatment of MDR-TB. Unless contraindicated, linezolid should be the first concern in the initial regimen for MDR/XDR-TB\(^4\). In 2020, the WHO Global TB Programme announced updated guidelines on the treatment of drug resistant TB. Most MDR/XDR TB patients can be treated with solely oral drug regimens without the use of injectable drugs\(^5\). In cooperation with the American Thoracic Society, Centers for Disease Control and Prevention, the European Respiratory Society, and the Infectious Diseases Society of America released guidelines for the treatment of drug-resistant TB in November 2019. These guidelines prioritize the use of an all-oral regimen, and linezolid is recommended in the regimen for MDR-TB\(^6\).

Linezolid use has a substantial risk of toxicity, with the optimal dose and duration remaining undefined. However, linezolid is safe and well tolerated when used in short courses (<28 d) with infections other than TB. With cases of longer treatment, such as when used for MDR/XDR-TB treatment (typically 6 months or longer), linezolid is associated with frequent serious, dose- and duration-dependent adverse effects, including anemia, neutropenia, thrombocytopenia, peripheral neuropathy, and more rarely, optic neuropathy, lactic acidosis, pancreatitis, and rhabdomyolysis\(^7\). A detrimental event (particularly hematological,
neurological, and gastrointestinal) was experienced in 58.9% subjects, mostly in a cohort of individuals treated with an oral daily dosage >600 mg\textsuperscript{8,9}. These adverse events can result in early cessation or reduction in the dose, which may compromise the efficacy of the drug.

Risks and predictor factors for hematological toxicities with linezolid remain unclear. The current focus is primarily on the efficacy and toxicity of a linezolid regimen in MDR-TB and XDR-TB. Comprehensive review of linezolid itself is limited, including pathogenesis, risk factors of hematologic toxicities, and on how to monitor and manage any problems that might occur arise. Linezolid inhibits mitochondrial ribosomes, which may be the cause of hematological alterations. However, little is known about the mechanisms involved in linezolid-associated mitochondrial toxicity in humans\textsuperscript{10,11}.

Because of the importance of linezolid in the treatment of MDR/XDR-TB and the high number of reports on hematologic toxicities, the goal of this review is to increase awareness and understanding of hematologic toxicities with a focus on the role of mitochondria.

1. Data Sources

The databases searched were Medline via PubMed, Cochrane Database of Systematic Reviews via Ovid, and EmBase (January 2000 to July 2020). Searches were performed in July 2020. Data for this review were identified by searches of PubMed combining “multidrug resistant tuberculosis,” “extensively resistant tuberculosis,” and “linezolid” as free text and MeSH terms, combined with other terms including “hematologic toxicity,” “bone marrow suppression,” or myelotoxicity (see Figure 1).

2. Linezolid: Drug invention, Chemistry, Mode of Action, and Pharmacology

Drug Invention

Linezolid was discovered in the mid 1990s and was approved for commercial use in 2000 as an antibiotic for the treatment of all major gram-positive bacteria that are pathogenic
to humans. Linezolid is a synthetic antimicrobial agent in the oxazolidinone class and since its approval in 2000, has remained the primary drug for that class. It is used in therapy because of its distinctive mode of action, which involves inhibition of protein synthesis. As a synthetic antibiotic, linezolid blocks the biosynthesis of bacterial proteins via binding to rRNA on both the 30S and 50S ribosomal subunits.

**Chemistry**

The empirical formula of linezolid is \( \text{C}_{16}\text{H}_{20}\text{FN}_{3}\text{O}_{4} \) (molecular weight: 337.35 g/mol) (see Figure 2). Studies on structure–activity relations of oxazolidinones indicate that the N-aryl group and 5-S configuration are crucial for activity. The 5-acylaminomethyl group is responsible for the activity. Activity is high because of the electron-withdrawing group in the aryl ring. Extra replacement on the proximal aromatic ring does not affect antibacterial activity but can change solubility and pharmacokinetics.

**Pharmacokinetics**

Oral absorbance of linezolid is remarkable, with 100% bioavailability, not influenced by food. An intravenous route can be changed to an oral one in clinically stable patients. The plasma protein-binding level of the molecule is approximately 31%, and plasma half-life of linezolid is from 3.4 to 7.4 h. Linezolid has volume distribution that approximates whole-body water content of 40–50 L. The compound is metabolized to inactive metabolites, including hydroxyethyl glycine and aminoethoxy-acetic acid. Linezolid has a clearance rate of \( 80 \pm 29 \) mL/min through both nonrenal and renal mechanisms, and renal tubular reabsorption may occur. An Unaltered form of some fraction of the dose is excreted in urine. After a fivefold increase in dose, a low level of nonlinearity was found, with a 30% decrease in clearance. Elderly patients, those with mild to moderate hepatic damage or
mild to moderate chronic renal failure, and healthy or young volunteers show no difference in plasma concentration. A seven to eightfold increase in exposure to drug metabolites is reported in patients with severe renal impairment on dialysis, compared with patients with normal renal function. Clearance of linezolid is higher in children than in adults, and therefore, higher daily doses of drug per kilogram of body weight are required in children than in adults.\textsuperscript{14}

Pharmacodynamics

Linezolid is an antimicrobial agent with a long duration and time-dependent activity. The value of the area under the curve/minimum inhibitory concentration (AUC/MIC) and the time that the plasma concentration is above the MIC (%T > MIC) are used as parameters to evaluate pharmacodynamics (PD). Linezolid has a post antibiotic effect. The best pharmacokinetic/pharmacodynamics (PK/PD) parameters to determine its activity are time with serum concentrations higher than the minimum inhibitory concentration (T > MIC) and AUC/MIC ratio. Linezolid is primarily a bacteriostatic antimicrobial agent, and T > MIC of at least 40% is predictive of its efficacy. In healthy volunteers, this objective can be achieved for pathogens with MICs of 2–4 mg/L by administration of 600 mg intravenously twice a day.\textsuperscript{15}

3. Efficacy of Linezolid Regimen for Multidrug-Resistant Tuberculosis and Extensively Drug-Resistant Tuberculosis

Most trials of linezolid effects on TB included subjects with MDR-TB and XDR-TB. Efficacy of linezolid in treatment of drug-resistant TB is evaluated in several reviews. Zhang et al.\textsuperscript{16} conducted systematic review and meta-analysis of 15 studies (367 patients), and 83% (95% confidence interval (CI), 75%–90%) of outcomes were favorable (either cure or treatment completion). The pooled rate of culture conversion was 89% (95% CI, 83%–95%), 46.3% of the subjects had XDR-TB. Cox et al.\textsuperscript{17} reviewed 11 studies (148 patients), and the pooled
proportion for treatment success was 67.9% (95% CI, 58.0%–78.9%). Of the subjects, 28% were infected with XDR-TB strains. Agyeman et al.\(^8\) conducted systematic review and meta-analysis of 23 studies in 14 countries involving 507 patients. The pooled proportion for treatment success was 77.4% (95% CI, 71.4%–82.8%), with a culture conversion rate of 88.4% (95% CI, 83.8%–92.4%). Lifan et al.\(^9\) conducted systematic review and meta-analysis of 72 original studies that included 302 patients with XDR-TB treated with linezolid. Pooled estimates for sputum culture conversion and treatment success rates were 93.2% and 67.4%, respectively.

Individual patient analysis was reported in three meta-analyses. Sotgiu et al.\(^8\) reported individual data from 121 patients treated with linezolid. The pooled estimates of anti-TB treatment success and culture conversion were 82% and 93%, respectively. Median times to sputum smear and culture conversion were 43.5 d and 61 d, respectively. Chang et al.\(^20\) evaluated 194 patients from 20 articles and found that linezolid increased the probability of favorable outcome by 55% (10%–121%). Ahmad et al.\(^21\) evaluated 12,030 patients from 25 countries in 50 studies. Overall treatment success was 7,346 (61%), whereas 1,017 (8%) suffered failure or relapse and 1,729 (14%) died. Treatment success was positively associated with the use of linezolid (adjusted risk difference 0.15; 95% CI, 0.11–0.18), compared with failure or relapse.

Two randomized studies found improved treatment outcomes with linezolid in XDR-TB. Lee et al.\(^22\) evaluated 41 patients with XDR-TB and reported higher culture conversion four months after randomization (relative risk (RR) 2.26; 95% CI, 1.19–4.28) when linezolid was given immediately than with a delay of two months. By four months, 15 of the 19 patients (79%) in the immediate-start group and 7 of the 20 (35%) in the delayed-start group had culture conversion (\(p = 0.001\)).
Tang et al.\textsuperscript{23} conducted a multicenter, prospective, randomized, controlled study of 65 subjects with XDR-TB. They found a significantly higher cure rate (RR 2.36; 95% CI, 1.13–4.90) and lower failure rate (RR 0.26; 95% CI, 0.10–0.70) in subjects treated with linezolid than in those who were not. The proportion of sputum culture conversion in the linezolid therapy group was 78.8% by 24 months, significantly higher than that in the control group (37.6%, \( P < 0.001 \)). The treatment success rate in the linezolid therapy group was 69.7%, significantly higher than that in the control group (34.4%, \( p = 0.004 \)).

4. Mitochondria in Tuberculosis and Linezolid Toxicity

\textit{Mitochondria in Tuberculosis}

Mitochondria have important roles in the cell death pathway and TB infection\textsuperscript{24-26}. \textit{Mycobacterium tuberculosis} (M.t.b) targets mitochondria to increase replication through disturbance of the cellular death pathway in alveolar macrophages\textsuperscript{27}. \textit{Mycobacterium tuberculosis} secretes proteins that cause changes in structure in the structure (size, form, number, distribution, fragmentation) and function of mitochondria. Inhibition of mitochondrial function leads to increased growth of M.t.b and continuing infection\textsuperscript{28,29}.

Two processes affect macrophages in TB infection, i.e., necrosis and apoptosis. Infection with M.t.b virulent strain (H37Rv) causes damage to mitochondrial membranes in macrophages, increasing cytochrome c release from intermembrane spaces and causing further necrosis\textsuperscript{29,30}. Necrosis of macrophages caused by M.t.b is the mechanism by which there is further dissemination of the pathogen and development of disease\textsuperscript{26}.

Infection with attenuated M.t.b strain (H37Ra) causes apoptosis of macrophages, which had retained M.t.b in apoptotic bodies to inhibit pathogen dissemination. Apoptosis of macrophages promotes antibacterial activity by increasing antigen presentation, allowing the innate immune system to reduce infection\textsuperscript{26}. 

10
Mitochondria in Linezolid Toxicity

Linezolid inhibits bacterial protein synthesis and further interferes with growth of bacteria by a mechanism that involves disturbance of bacterial mitochondria\textsuperscript{10,31}. Mitochondria are important organelles that initiate or amplify signals of apoptotic cell death\textsuperscript{11,32}. Mitochondria originated as ancient proteobacteria that were engulfed by eukaryotic cells in order to obtain energy from utilization of oxygen. Therefore, there are evolutionary resemblances between mitochondrial and bacterial ribosomes. Interactions between ribosomal-inhibitor antibiotics, such as linezolid, and the host can have severe clinical effects common to primary mitochondriopathies\textsuperscript{10,33-38}.

Linezolid binds to mitochondrial ribosomes, damages expression and biosynthesis of mitochondrial proteins encoded by the mitochondrial genome, and blocks the action of cytochrome c-oxidase and mitochondrial oxidative activity\textsuperscript{31,39}. The degree of exposure of a microorganism to the antibiotic as estimated by the AUC/MIC ratio is associated with the effectiveness of linezolid. Inhibition of mitochondrial ribosomes is also correlated with the level of linezolid exposure\textsuperscript{40}. Little is known about linezolid tissue specificity. Mitochondrial haplogroup U, mutations in 12S rRNA, and m.2706A \(\rightarrow\) G, m.3197T \(\rightarrow\) C, and m.3010G \(\rightarrow\) A polymorphisms in 16S rRNA tend to be associated with increased mitochondrial and clinical adverse effects\textsuperscript{32}.

5. Hematologic Toxicity

Pathogenesis of Hematologic Toxicity

Suppression of bone marrow by linezolid is usually related to dose and duration of treatment, although it occurs infrequently during days 10–14 of drug administration\textsuperscript{34}. Two possible mechanisms are considered to be responsible for thrombocytopenia side effects. One
is bone marrow suppression of platelet production\textsuperscript{41-43}. Linezolid increased myosin light chain 2 phosphorylation, followed by repression of platelet release from mature megakaryocytes, in an in vitro investigation using human megakaryoblast (MEG-01)\textsuperscript{42}. The second mechanism is increased platelet destruction due to an immune mechanism\textsuperscript{34,44}.

Linezolid or its metabolites bind to glycoprotein membrane IIb/IIIa to form immunoglobulin G (IgG) antibody complex by fragment antigen-binding (Fab). The fragment crystallizable (Fc) portion attaches to macrophages. The reticuloendothelial system destroys the platelet–linezolid–IgG complex\textsuperscript{44}.

An in vivo study with mice showed that linezolid affects precursor colony forming unit–erythroblasts, burst forming unit–erythroblasts, and erythroblasts in bone marrow. Linezolid represses proliferation and cellular metabolite activity and interferes with mitochondrial function. Linezolid blocks mitochondrial protein biosynthesis and decreases ATP production in bone marrow precursor cells\textsuperscript{45}. Mechanisms underlying linezolid-induced anemia are not clear, but vacuolated pronormoblasts suggest the mechanism of anemia is identical to that of chloramphenicol-induced myelosuppression. Suppression of mitochondrial respiration via inhibition of mitochondrial protein synthesis is the likely mechanism. Pure red cell aplasia has also been reported as one mechanism of linezolid-induced anemia\textsuperscript{46,47}.

Figure 3 shows a schematic of linezolid-induced hematologic toxicities.

Bacterial ribosomes are composed of two subunits: a large 50S subunit and a small 30S subunit. Linezolid binds to 23S rRNA in the large subunit of the prokaryotic ribosome, preventing bacterial protein synthesis and inhibiting bacterial growth. Mitochondrial ribosomes are similar to their bacterial counterparts. Linezolid interrelates with mitochondrial ribosomes, interferes with mitochondrial protein synthesis, and reduces ATP in bone marrow precursor cells in subjects at risk of drug accumulation and in those inherently more susceptible to
mitochondrial toxicity. Such mitochondrial dysfunction causes bone marrow suppression, leading to anemia, leukopenia, and thrombocytopenia.

**Incidence**

The incidence of myelosuppression (anemia or neutropenia), anemia, and thrombocytopenia is shown in Table 1.

<table>
<thead>
<tr>
<th>Myelosuppression</th>
<th>Anemia</th>
<th>Thrombocytopenia</th>
<th>n (subjects)</th>
<th>Starting dose (mg/d)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>NA*</td>
<td>32%</td>
<td>19</td>
<td>1200</td>
<td>Attassi et al.44</td>
</tr>
<tr>
<td>NA</td>
<td>4.1%</td>
<td>7.4%</td>
<td>796</td>
<td>1200</td>
<td>Birmingham et al.48</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>17%</td>
<td>42</td>
<td>1200</td>
<td>Niwa et al.49</td>
</tr>
<tr>
<td>NA</td>
<td>NA</td>
<td>37.8%</td>
<td>331</td>
<td>1200</td>
<td>Takahashi et al.50</td>
</tr>
<tr>
<td>NA</td>
<td>38.10%</td>
<td>11.8%</td>
<td>121</td>
<td>450-1200</td>
<td>Sotgiu et al.8</td>
</tr>
<tr>
<td>4%</td>
<td>NA</td>
<td>NA</td>
<td>51</td>
<td>300</td>
<td>Koh et al.51</td>
</tr>
<tr>
<td>85%</td>
<td>NA</td>
<td>NA</td>
<td>13</td>
<td>600</td>
<td>Tse-Chang et al.52</td>
</tr>
<tr>
<td>32.9% (95% CI, 23.1%-43.5%)</td>
<td>NA</td>
<td>NA</td>
<td>507</td>
<td>300-1200</td>
<td>Agyeman et al.18</td>
</tr>
<tr>
<td>48%</td>
<td>NA</td>
<td>NA</td>
<td>109</td>
<td>1200</td>
<td>Conradie et al.53</td>
</tr>
</tbody>
</table>

*NA: not available.

6. Risk Factors for Hematologic Toxicity

Risk factors for hematologic toxicity are shown in Table 2.

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>Study design</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin pre-treatment &lt;10.5 g/dL</td>
<td>Case control</td>
<td>Senneville et al.34</td>
</tr>
<tr>
<td></td>
<td>Case report and literature review</td>
<td>Luo et al.47</td>
</tr>
<tr>
<td>Platelet count</td>
<td>Clinical trial</td>
<td>Gerson et al.41</td>
</tr>
<tr>
<td>Baseline platelet count &lt;173,000/mm³</td>
<td>Prospective observational</td>
<td>Grau et al.55</td>
</tr>
<tr>
<td>Baseline platelet count &lt;240,700/mm³</td>
<td>Observational retrospective cohort</td>
<td>González-Del Castillo et al.56</td>
</tr>
<tr>
<td>Baseline platelet level of &lt;200,000/mm³</td>
<td>Retrospective</td>
<td>Kılıç et al.57</td>
</tr>
</tbody>
</table>
**Table 1.** Characteristics of included studies.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Daily dosage &gt;18.7 mg/kg/d</th>
<th>Retrospective</th>
<th>Chen et al.²⁹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Doses</td>
<td>&gt;600 mg/d</td>
<td>Systematic review and meta-analysis</td>
<td>Agyeman et al.¹⁸</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Duration</th>
<th>&gt;14 d</th>
<th>Clinical trial</th>
<th>Gerson et al.⁴¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&gt;10 d</td>
<td>Open-label, noncomparative, nonrandomized clinical trial</td>
<td>Birmingham et al.⁴⁸</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Renal function</th>
<th>Creatinine clearance &lt;88.3 mL/min/1.73 m²</th>
<th>Retrospective</th>
<th>Chen et al.⁵⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td>Creatinine clearance rates of &lt;60 mL/min</td>
<td>Retrospective</td>
<td>Hanai et al.⁵⁹</td>
<td></td>
</tr>
<tr>
<td>Hemodialysis</td>
<td>Retrospective</td>
<td>Hanai et al.⁵⁹</td>
<td></td>
</tr>
<tr>
<td>Renal failure: creatinine clearance &lt;50 mL/min</td>
<td>Observational retrospective cohort</td>
<td>González-Del Castillo et al.⁵⁶</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Serum albumin concentration &lt;33.5 g/L</th>
<th>Retrospective</th>
<th>Chen et al.⁵⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebrovascular disease</td>
<td>Observational retrospective cohort</td>
<td>González-Del Castillo et al.⁵⁶</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Moderate or severe liver disease</th>
<th>Observational retrospective cohort</th>
<th>González-Del Castillo et al.⁵⁶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malignancy</td>
<td>Observational retrospective cohort</td>
<td>González-Del Castillo et al.⁵⁶</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Combination therapy</th>
<th>Caspofungin and levofloxacin therapy</th>
<th>Retrospective</th>
<th>Chen et al.⁵⁸</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbapenem treatment combination therapy</td>
<td>Retrospective</td>
<td>Kılıç et al.³⁷</td>
</tr>
</tbody>
</table>

---

Figure 4 provides a schematic summary of the role of mitochondria in TB and hematologic toxicity of linezolid. *Mycobacterium tuberculosis* disrupts the mitochondrial permeability transition pore complex (mPTPC) and causes mitochondrial dysfunction. However, linezolid can also cause mitochondrial dysfunction. The two mechanisms inhibit mitochondrial protein biosynthesis, decrease ATP in bone marrow precursor cells, and cause myelosupression (anemia, neutropenia, and thrombocytopenia). Other mechanisms that contribute to hematologic toxicities include disturbance in phosphorylation of myosin light chain 2 in megakaryocytes, which suppresses platelet release, and the complex of linezolid–platelet...
membrane glikoprotein IIb/IIIa–Immunoglobulin G. Dose, duration, platelet baseline, renal failure, liver dysfunction, albumin, cardiovascular disease, malignancy, caspofungin, levofloxacin, and carbapenem combination also influence the development of hematologic toxicities.

7. Monitoring

With linezolid use, it is necessary to monitor blood counts (weekly during initial phase, then monthly) and for signs and symptoms of peripheral neuropathy and retinitis. Monitoring varies considerably among studies. Natsumoto measured platelet counts two to three times per week, and Hanai et al. assessed hematological and biochemical parameters after four days of linezolid treatment. Conradie et al. evaluated side effects of treatment weekly to week 16, at weeks 20 and 26, and then at one, two, and three months after the end of treatment and every three months thereafter to 24 months after the end of treatment.

In 2016, the Curry International Tuberculosis Center recommended complete blood counts monthly for patients on linezolid. The United States Agency for International Development Tuberculosis (USAID TB) CARE recommends a complete blood count check before starting linezolid, weekly during first month, and then monthly, with additional checks if there are any symptoms or signs of myelosuppression.

The WHO recommends monitoring for linezolid treatment, with hemoglobin and white blood cell count monitored weekly at first and then monthly or as needed based on symptoms. Any adverse events throughout treatment should be managed immediately to relieve suffering, minimize the risk of treatment interruptions, and prevent morbidity and mortality.

8. Clinical Management of Myelosuppression According to Severity Grading
The clinician should make reasonable judgments whether to continue, reduce, or stop linezolid when myelosuppression occurs. The risks and benefits of continuing linezolid should be considered carefully in this situation. The EndTB Consortium recommends clinical management of myelosuppression according to severity grading (Table 3).

**Table 3. Clinical management of myelosuppression according to severity grading**

<table>
<thead>
<tr>
<th>Severity grade</th>
<th>Grade 1 Mild</th>
<th>Grade 2 Moderate</th>
<th>Grade 3 Severe</th>
<th>Grade 4 Life-threatening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>9.5–10.5 g/dL</td>
<td>8.0–9.4 g/dL</td>
<td>6.5–7.9 g/dL</td>
<td>&lt;6.5 g/dL</td>
</tr>
<tr>
<td>Platelets</td>
<td>75,000–99,999/mm³</td>
<td>50,000–74,999/mm³</td>
<td>20,000–49,000/mm³</td>
<td>&lt;20,000/mm³</td>
</tr>
<tr>
<td>White blood cells</td>
<td>&lt;LLN*/mm³</td>
<td>2,000–3,000/mm³</td>
<td>&lt;2,000/mm³</td>
<td>&lt;1,000/mm³</td>
</tr>
<tr>
<td>Absolute neutrophil count</td>
<td>1,000–1,500/mm³</td>
<td>500–999/mm³</td>
<td>500–749/mm³</td>
<td>&lt;500/mm³</td>
</tr>
</tbody>
</table>

**Action**

- Monitor carefully, and consider reduction of linezolid dose (300 mg daily or 600 mg thrice weekly).
- Monitor carefully, and consider reduction of linezolid dose (300 mg daily or 600 mg thrice weekly); in case of Grade 2 neutropenia, stop linezolid immediately. In case of Grade 2 anemia, consider eritropoietin. Restart at reduced dose once toxicity has decreased to Grade 1.
- Stop linezolid immediately. In case of Grade 3 anemia, consider eritropoietin. Restart at reduced dose once toxicity has decreased to Grade 1.
- Stop linezolid immediately. Consider transfusion or eritropoietin. Restart at reduced dose once toxicity has decreased to Grade 1.

*LLN: Lower limit of normal

There are no firm guidelines on how to re-administer linezolid after stopping the drug. However, there are several previous studies that recommend how to re-administer linezolid. A Nix-TB study used linezolid at 1,200 mg per day as the initial dose. According to the protocol, dose reduction to 600 mg daily and 300 mg daily or temporary cessation of linezolid is permitted. Cattaneo et al. recommend therapeutic drug monitoring (TDM) of linezolid to maintain the AUC/MIC ratio of 100 and adjust the linezolid dose. The linezolid dose has been...
reduced to less than 300 mg/d without compromising efficacy. A dose reduction to 200 or even 150 mg of linezolid once daily can be considered when the AUC/MIC ratio is sufficiently high. For patients who develop dose-dependent toxicity, TDM should be considered to reduce linezolid doses on re-administration\textsuperscript{65,66}.

9. **Conclusions**

Linezolid is a promising option for treating MDR-TB and XDR-TB, however hematologic toxicity is the major concern with a linezolid-containing regimen. Several clinical factors are considered as risk factors for hematologic toxicity. Linezolid blocks mitochondrial protein biosynthesis, reduces ATP production in bone marrow precursor cells, and causes myelosuppression. Increased sensitivity to linezolid in mitochondrial ribosomes may be associated with genetic variation in human mitochondria. Further studies need to address how to identify patients who will develop myelosuppression. Such studies should begin by identifying potential biomarkers of mitochondrial dysfunction and genetic susceptibility to predict myelosuppression.

In the future, it will be necessary to evaluate whether adjustment of the concentration in serum to obtain the pharmacodynamic target is a reasonable strategy to avoid adverse events in patients receiving prolonged courses of linezolid.

10. **Funding Details**

This research and article processing charge (APC) were funded by the Doctoral Dissertation Research Program, with an internal Universitas Padjadjaran research funding number of 1595/UN6.3.1/PT.00/2021, and ALG (Academic Leadership Program), with internal Universitas Padjadjaran research funding with contract number 3855/UN.C/LT/2019.
11. Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

12. Acknowledgments

The authors thank Kevin Y. Budiman, MD, for final editing and submitting the manuscript.
References


Table legends

N/A

Figure legends

Figure 1. Study selection
Figure 2. Chemical structure of linezolid\textsuperscript{14}
Figure 3.

Bacterial ribosomes are composed of two subunits: a large 50S subunit (LSU) and a small 30S subunit (SSU). Linezolid binds to 23S rRNA in the large subunit of the prokaryotic ribosome, preventing bacterial protein synthesis and inhibiting bacterial growth. Mitochondrial ribosomes are generally similar to their bacterial counterparts. Linezolid interrelates with mitochondrial ribosomes, interferes with mitochondrial protein synthesis, and reduces ATP in bone marrow precursor cells in subjects at risk of drug accumulation and in those inherently more susceptible to mitochondrial toxicity. The mitochondrial dysfunction causes bone marrow suppression leading to anemia, leukopenia, and thrombocytopenia.
Figure 4.
*Mycobacterium tuberculosis* disrupts the mitochondrial permeability transition pore complex (mPTPC) and causes mitochondrial dysfunction. Linezolid can also cause mitochondrial dysfunction. The two mechanisms inhibit mitochondrial protein biosynthesis, decrease ATP in bone marrow precursor cells, and cause myelosuppression (anemia, neutropenia, and thrombocytopenia). Other mechanisms that contribute to hematologic toxicities are disturbance in phosphorylation of myosin light chain 2 in megakaryocytes, which suppresses platelet release, and the complex of linezolid–platelet membrane glikoprotein IIb/IIIa–Immunoglobulin G. Dose, duration, platelet baseline, renal failure, liver dysfunction, albumin, cardiovascular disease, malignancy, caspofungin, levofloxacin, and carbapenem combination also influence the development of hematologic toxicities.