Risk factors for anaemia in patients on prolonged linezolid therapy for chronic osteomyelitis: a case–control study

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Objectives: The intrinsic properties of the new antibiotic linezolid make it an attractive candidate for the treatment of chronic osteomyelitis. However, data regarding the tolerance of long-term linezolid administration are still lacking.

Methods: The medical charts of patients given linezolid for >4 weeks were retrospectively analysed, especially their haematology. In a case–control study, we compared the respective characteristics of patients who developed anaemia during linezolid therapy and those who did not.

Results: Forty-five adults with chronic osteomyelitis received 600 mg linezolid intravenously twice daily for 7 days, and then orally, for a mean total duration of 15.9 weeks (range, 6–36). Anaemia episodes requiring blood transfusion occurred in 13/45 patients (28.9%). Median time from treatment initiation to anaemia onset was 7.4 weeks (range, 4–16). Anaemia was significantly associated with premature linezolid therapy cessation ($P = 0.0012$). No linezolid-related thrombocytopenia was observed. By univariate analysis, four variables were associated with the occurrence of anaemia: age >58 years, alcohol abuse, diabetes mellitus and low haemoglobin before linezolid treatment. Logistic regression analysis revealed two independent risk factors for anaemia: age >58 years (OR = 20.5, 95% CI 0.69–599; $P = 0.0001$) and pre-treatment haemoglobin <10.5 g/dL (OR = 16.49, 95% CI 1.06–255; $P = 0.04$).

Conclusions: Profound anaemia may occur in adult patients with chronic osteomyelitis on prolonged linezolid therapy, and often necessitates linezolid cessation. These patients are likely to be aged >58 years and to have low pre-treatment haemoglobin. The results for the present series might help physicians to identify patients who should not be given long-term linezolid treatment for chronic osteomyelitis.

Keywords: oxazolidinones, toxicity, myelosuppression

Introduction

Chronic osteomyelitis is an uncommon but serious infection that results in pain, functional incapacity, long-term therapy and hospitalization.1 The most common actiological agents involved in this infection are Staphylococcus aureus and Staphylococcus epidermidis, which exhibit resistance to methicillin in most cases in Europe.2 Prolonged therapy with a combination of antimicrobial agents that achieve high bone concentrations is recommended for the treatment of chronic osteomyelitis, especially in patients with an infected orthopaedic device.3 Rifampicin is a very useful agent for the treatment of such infection, but must be combined with another active antimicrobial agent to prevent failure due to the selection of rifampicin-resistant mutants.4 Fluoroquinolones have been widely used for patients with chronic osteomyelitis as a rifampicin partner.5,6 However, most of the staphylococcal strains currently resistant to methicillin are also resistant to fluoroquinolones.2 The combination of rifampicin and fusidic acid may result in liver toxicity, and failures due to the selection of rifampicin-resistant mutants have been reported.5 As a result, long-term therapy combining rifampicin with vancomycin or teicoplanin may be required but its use is often limited by
Anaemia risk factors in osteomyelitis patients on long-term linezolid

Patients and methods

Patients

The medical records of patients treated with linezolid for 4 weeks or more for chronic osteomyelitis were reviewed. The diagnosis of chronic osteomyelitis was assessed on the basis of the following data: fever >38°C, inflammation or purulent discharge in the area of the osteosynthesis devices or protheses, or biological inflammatory syndrome (erythrocyte sedimentation rate >50 mm/h and C-reactive protein >10 mg/L), radiological evidence of loose osteosynthesis devices or protheses (luxation or pseudarthrosis), evidence of chronic osteomyelitis on plain radiography, presence of leucocytes on direct examination of intraoperative samples and Gram-positive pathogens (methicillin-resistant *S. aureus* or coagulase-negative staphylococci, and *Enterococcus* spp.). Infection was considered chronic when it had started >4 weeks before the diagnosis of osteomyelitis. All microbiological cultures were obtained from intraoperative bone biopsies or joint aspirations. Bacteria were assessed on direct examination of intraoperative samples and Gram-positive cocci (*S. aureus* 29 cases including 21 methicillin-resistant strains), coagulase-negative staphylococci (14 cases including 14 methicillin-resistant strains), others (eight cases including two anaerobes). Mixed flora, including Gram-negative bacteria, were found in four patients. Forty-one patients (91.1%) received linezolid combined with another agent: rifampicin for 26 patients (57.8%), clindamycin for three, fluoroquinolones for eight including levofloxacin in four cases, glycopeptides for two and cefepime for two.

Anaemia occurred in 13/45 patients (28.9%) and was significantly associated with the premature cessation of linezolid therapy (*P* = 0.0012, Figure 1). Three patients without anaemia had to stop linezolid therapy because they developed peripheral sensitive neuropathy. Among the patients with anaemia and those without, 10 (76.9%) and 14 (43.7%), respectively, had an abnormal pre-treatment haemoglobin as defined above. Mean pre-treatment haemoglobin was 10.1 g/dL in patients who developed anaemia and 11.8 in those who did not (*P* = 0.01). The mean ratios of the expected/observed duration of linezolid treatment were 17.2/11.6 and 14.7/18.6 weeks in patients with and without anaemia, respectively. All patients with anaemia required blood transfusions because their haemoglobin levels were <8.0 g/dL. A low absolute reticulocyte count was associated with anaemia. The median time from linezolid therapy the potential toxicity of these agents and the need for long-term parenteral administration.

Linezolid is a synthetic antibiotic which belongs to the new class of oxazolidinones. Certain recent results support the notion that linezolid might be useful for the management of multidrug-resistant Gram-positive chronic osteomyelitis.5–12 At present, however, available clinical data are not sufficient to determine whether prolonged linezolid therapy is safe for patients with chronic osteomyelitis.

Since most clinical studies of linezolid have only covered periods of <2 weeks, very little is known about its long-term toxicity, especially as regards myelosuppression. A few cases of reversible myelosuppression, especially thrombocytopenia and occasionally anaemia, have recently been reported.13–16 Most patients who experienced anaemia were receiving concomitant medications known to cause bone marrow suppression, and many had complex illnesses.17

For the last 3 years, we have been using linezolid combined with other antibacterial agents, especially rifampicin, as long-term therapy for patients with Gram-positive chronic osteomyelitis. None of our patients experienced linezolid-related thrombocytopenia, but several of them developed profound linezolid-related anaemia and required blood transfusions. The present case–control study was undertaken to explore the possible existence of factors associated with the development of anaemia in osteomyelitis patients on long-term linezolid therapy.

### Treatment protocol

Linezolid was administered intravenously at a dosage of 600 mg twice daily for 7 days, and then orally at the same dosage until the completion of therapy. In agreement with previous reports, the expected durations of linezolid treatment in the present patients were at least 3, 4 and 6 months for chronic osteomyelitis or osteosynthesis devices, total hip prosthesis and total knee prosthesis, respectively. Antibiotic treatment was combined with surgical debridement or removal of the orthopaedic material in some situations (unstable device and severe sepsis) and in delayed re-implantation. In other cases, patients retained the infected material during treatment. When there was mixed infection that included Gram-negative bacteria, appropriate antibiotics were added to linezolid.

### Follow-up

Haematology was assessed weekly and included haemoglobin, haematocrit, counts of red and white blood cells (WBC), absolute neutrophil counts and platelet counts (PLTC). For patients with normal or abnormal baseline values, anaemia, thrombocytopenia and leucopenia were defined, respectively, as haemoglobin and PLTC <75% and WBC <50% of the lower limit of normal (i.e. 12 g/dL, in men and 11 g/dL in women for haemoglobin, 150 × 10³ PTLC/mm³ for PTLC and 4 × 10³ cells/mm³ for WBC) or baseline values. In cases of anaemia, levels of vitamin B12, folates, iron and haptoglobin were measured, reticulocyte counts and bone marrow analysis was performed if the patient accepted the procedure.

### Statistical analysis

Variables were compared using the Wilcoxon rank sum test for quantitative variables and Fisher’s exact test for qualitative variables adapted to small numbers if necessary. Logistic regression analysis was used for the multivariate study.

### Results

From 1 October 1999–31 December 2002, 45 adults with chronic osteomyelitis comprising 28 males and 17 females [mean age: 54.2 (range, 25–82) years], were given long-term linezolid treatment. The subjects included 23 with orthopaedic device infection. Of these, 20 had a total joint prosthesis (12 of the hip and eight of the knee) and 22, various forms of osteomyelitis. The duration of linezolid therapy was in the range 6–36 weeks (mean: 15.9). All patients were infected with at least one of the following. Gram-positive cocci: *S. aureus* (29 cases including 21 methicillin-resistant strains), coagulase-negative staphylococci (14 cases including 14 methicillin-resistant strains), others (eight cases including two anaerobes). Mixed flora, including Gram-negative bacteria, were found in four patients. Forty-one patients (91.1%) received linezolid combined with another agent: rifampicin for 26 patients (57.8%), clindamycin for three, fluoroquinolones for eight including levofloxacin in four cases, glycopeptides for two and cefepime for two.

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initiation to onset of anaemia was 7.4 weeks (range, 4–16). All patients with anaemia had normal baseline serum iron, vitamin B12 and folic acid levels, and none had either underlying haematological disease or severe renal impairment. No events involving leucopenia were reported, and only one case of thrombocytopenia occurred under linezolid treatment but that was due to alcohol abuse. Bone marrow analysis in seven of the 13 patients with anaemia showed moderate eosinophilia, decreased cellular marrow with red blood cell aplasia but no myelopoietic dysplasia and normal platelet development.

Four variables associated with the occurrence of anaemia were identified by univariate analysis: age, alcohol abuse (>80 g daily), diabetes mellitus and low pre-treatment haemoglobin (Table 1). By logistic regression analysis, two independent risk factors for anaemia were identified (Table 2): age >58 years [odds ratio (OR) = 20.5, 95% confidence interval (CI) 0.69–599; \( P = 0.0001 \)] and pre-treatment haemoglobin <10.5 g/dL [OR = 16.49, 95% CI 1.06–255; \( P = 0.04 \)]. The critical age and pre-treatment haemoglobin value were determined by receptor operating curve (ROC) analysis.

### Discussion

This case–control study was designed to identify risk factors for anaemia in patients with chronic osteomyelitis receiving long-term treatment with linezolid. The main goal of the study was to give physicians useful data enabling them to identify patients for whom long-term linezolid treatment might safely be considered without an undue risk of anaemia. We found that anaemia requiring blood transfusion was responsible for the premature cessation of linezolid therapy in about one-third of patients with chronic osteomyelitis who were given >4 weeks of treatment. Patients who experienced anaemia and those who did not were treated, respectively, for 67.4% and 126% of the expected minimal duration of linezolid treatment. The second cause of premature cessation of linezolid therapy in the present study was peripheral neuropathy.

The oxazolidinones, initially developed as monoamine oxidase inhibitors for the treatment of depression, were not used in humans for some time despite their antimicrobial activity, because they were found to be lethal in animals. The development of their use for clinical purposes started with the discovery of linezolid, which was markedly less toxic than the original agents. The precise mechanism of action of linezolid is not known, but it is thought to interfere with protein synthesis at a

### Table 1. Comparison by univariate analysis of the clinical and demographic characteristics of chronic osteomyelitis patients with and without anaemia

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>anaemia</th>
<th>no anaemia (controls), ( n = 32 )</th>
<th>OR</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (± S.D.) age (years)(^a)</td>
<td>49.8 ± 17.1</td>
<td>65.6 ± 13.3</td>
<td>NA</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Surgery with bleeding(^b)</td>
<td>6 (46)</td>
<td>4 (12)</td>
<td>3.69</td>
<td>0.06</td>
</tr>
<tr>
<td>Alcohol abuse(^c)</td>
<td>4 (30)</td>
<td>1 (3)</td>
<td>9.84</td>
<td>0.04</td>
</tr>
<tr>
<td>Diabetes mellitus(^d)</td>
<td>7 (53)</td>
<td>4 (12)</td>
<td>4.30</td>
<td>0.03</td>
</tr>
<tr>
<td>Orthopaedic device infection(^e)</td>
<td>8 (61)</td>
<td>13 (40)</td>
<td>1.51</td>
<td>0.31</td>
</tr>
<tr>
<td>Methicillin-susceptible staphylococci(^f)</td>
<td>4 (30)</td>
<td>2 (6)</td>
<td>4.92</td>
<td>0.08</td>
</tr>
<tr>
<td>Linezolid–rifampicin combination(^g)</td>
<td>5 (38)</td>
<td>20 (62)</td>
<td>0.61</td>
<td>0.30</td>
</tr>
<tr>
<td>Chronic heart disease(^h)</td>
<td>4 (30)</td>
<td>4 (12)</td>
<td>2.46</td>
<td>0.21</td>
</tr>
<tr>
<td>Hypertension(^i)</td>
<td>6 (46)</td>
<td>7 (21)</td>
<td>2.10</td>
<td>0.20</td>
</tr>
<tr>
<td>Mean baseline haemoglobin (g/dL)(^j)</td>
<td>10.1</td>
<td>11.8</td>
<td>NA</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^a\)Quantitative variables compared by the Wilcoxon rank sum test.

\(^b\)Qualitative variables compared using Fisher’s exact test (adapted to small numbers when necessary).

NA, not applicable.
with chloramphenicol toxicity. This might be due to certain abnormalities not entirely different from those seen in patients. Bone marrow examinations performed in our patients showed erythropoiesis occurred after the cessation of linezolid therapy. and those included in previous studies, rapid recovery of normal linezolid and in other recent studies: thus, Monson and co-
addition, in vitro studies have shown that combining linezolid with rifampicin prevented the occurrence of rifampicin- or linezolid-resistant isolates. These data support the notion that linezolid could be used together with rifampicin to treat chronic bone and joint infections due to resistant Gram-positive cocci as a replacement for glycopeptides, which are not easy to administer in a long course of parenteral therapy.

However, the prolonged use of linezolid can lead to myelosuppression, including neutropenia, thrombocytopenia and anaemia. As a result, weekly monitoring of haematological values is recommended for patients on linezolid therapy. Reversible myelosuppression with red-cell hypoplasia after therapy with linezolid has already been reported in Phase 3 clinical trials of linezolid and in other recent studies: thus, Monson and co-workers reported a case of reversible pure red blood cell aplasia that developed in a patient who had been on linezolid therapy for 8 weeks, and Green and co-workers reported reversible linezolid-associated hypoproliferative anaemia in three patients who were receiving 600 mg linezolid twice daily for 6–12 weeks. Decreased haemoglobin/haematocrit levels were reported in 4.1% of patients treated for miscellaneous multidrug-resistant Gram-positive infections, and subsequently in 5% of diabetic patients with foot infections.

The present results for the overall haematological tolerance of linezolid therapy differ slightly from those of previous authors, who reported thrombocytopenia episodes in up to 10% of the patients, but rarely anaemia. The inclusion in previous studies of patients with more severely compromised immune status, haematological disorders and septic conditions than those of our patients may explain these differences. In particular, most of our patients had no sepsis or concomitant medications potentially toxic for the bone marrow. Our data confirm previous reports that myelosuppression rarely occurs before day 14 of linezolid therapy and appears to be due to the prolonged duration of therapy. In our patients and those included in previous studies, rapid recovery of normal erythropoiesis occurred after the cessation of linezolid therapy. Bone marrow examinations performed in our patients showed abnormalities not entirely different from those seen in patients with chloramphenicol toxicity. This might be due to certain similarities between the mechanisms of action of linezolid and phenicols. In animal studies, hypersegmentation of megakaryocytes and ringed sideroblasts were reported to be present in bone marrow samples. Here, the respective roles of deficient B-vitamins, abnormal folates and renal impairment could not be evaluated, because they were not present in any of our patients. Nevertheless, these abnormalities should probably be taken into account before considering long-term linezolid therapy, because they may impair haematopoiesis. After discontinuation of linezolid therapy, we observed no cases of anaemia in patients given glycopeptides instead of linezolid. The high frequency of anaemia episodes in our patients was probably due to the very long duration of their therapy, because most of them had chronic infection of an orthopaedic device. In univariate analysis, age, haemoglobin baseline values, diabetes mellitus and alcohol abuse were significantly associated with anaemia. By multivariate analysis, we found that age >58 years and a pre-treatment haemoglobin <10.5 g/dl were independent risk factors for the occurrence of anaemia in patients treated for chronic osteomyelitis. Note that the haemoglobin level of some of the present patients who had a value of <10.5 g/dl at the start of linezolid therapy rose during treatment. These patients had normal haemoglobin before surgery and no other risk factor for anaemia (data not shown). Therefore, patients with low pre-treatment haemoglobin but no other risk factors could probably be given long-term linezolid therapy, provided their haemoglobin level before surgery is normal. We did not find any correlation between anaemia and the antimicrobial agent associated with linezolid. In particular, patients treated with the rifampicin–linezolid combination did not differ from those treated with other combinations as regards myelosuppression. All the patients with an infected orthopaedic device received linezolid combined with another antistaphylococcal agent, because the emergence of resistance during therapy has been reported in patients with indwelling prosthetic devices who were given linezolid.

Conclusions

Linezolid therapy constitutes a significant advance in the treatment of patients with resistant Gram-positive infections, who until now were treated with parenteral glycopeptides. In this study, age >58 years and low pre-treatment haemoglobin were found to be risk factors for the occurrence of anaemia in patients on prolonged linezolid therapy. However, because the study population was small, the present findings must be confirmed in larger studies. Nevertheless, they may already help physicians to improve the safety of prolonged linezolid therapy in patients with chronic osteomyelitis.

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