Plasma concentrations of atovaquone given to immunocompromised patients to prevent *Pneumocystis jirovecii*

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**Objectives:** Atovaquone is one of the alternatives to trimethoprim/sulfamethoxazole for prophylaxis of *Pneumocystis jirovecii* pneumonia (PCP) in immunocompromised patients. In volunteers, there was wide inter-individual variability in atovaquone bioavailability. The aim of this study was to assess the plasma concentrations of atovaquone in immunocompromised patients under PCP prophylaxis.

**Methods:** Adult haematology or HIV-positive patients receiving atovaquone (750 mg oral suspension twice a day) for PCP prophylaxis were included. Plasma concentrations were assessed using UV-HPLC, around 12 h after the evening dose (Cmin) and 1–5 h after the morning dose (Cmax).

**Results:** A total of 82 measurements were performed in 33 patients. This included 19 HSCT recipients, 7 haematology non-transplant patients and 7 HIV-positive patients. The median Cmin (IQR) was 11.3 μg/mL (6.2–27.8) and the median Cmax was 13.4 μg/mL (6.0–28.3). The Cmin and Cmax of atovaquone were not different between HIV-negative and HIV-positive patients, or between HSCT and non-HSCT patients. Atovaquone concentrations were not influenced by the co-administration of valaciclovir (n = 20) or ciclosporin (n = 11), by gut graft-versus-host disease (n = 7) or by the intake of atovaquone with food. Nineteen of the 33 (58%) patients had Cmin, 15 μg/mL, a threshold associated with a low rate of clinical response in PCP treatment.

**Conclusions:** Atovaquone is poorly absorbed in more than half of immunocompromised patients and its bioavailability varies between individuals. These unpredictable variations raise the question of therapeutic drug monitoring, in order to identify patients with low concentrations and those who could benefit from regimen adaptation or from alternatives.

**Introduction**

*Pneumocystis jirovecii* pneumonia (PCP) is a life-threatening infection in immunocompromised patients. Trimethoprim/sulfamethoxazole is the gold standard for prophylaxis.1-3 In case of allergy or intolerance to trimethoprim/sulfamethoxazole, atovaquone is one of the alternative regimens.2,4 Seventeen percent of allogeneic HSCT recipients5 and roughly half of HIV-positive patients6 may need alternative regimens, which are considered to have lower efficacy compared with trimethoprim/sulfamethoxazole.2,4 However, the reasons for this low efficacy are not well understood.

Atovaquone is a hydroxynaphthoquinone, a highly lipophilic compound, active in vitro against *Plasmodium, Pneumocystis* and *Toxoplasma* species by inhibiting mitochondrial pyrimidine biosynthesis.7 Its absorption is variable, increased with a meal8,9 and was fat dose-dependent.9 Also, atovaquone is subject to drug-drug interactions with aciclovir, anti-diarrhoeals, opiates, efavirenz and PIs.10 In HIV-positive patients, atovaquone was shown to have prophylactic efficacy comparable to that of dapsone, but to be better tolerated.11 Atovaquone was found to be equally effective as pentamidine aerosols at the dose of 1500 mg once daily.12 However, although a daily dose of 750 mg was not as efficient as a dose of 1500 mg once daily,12 none of these studies assessed the atovaquone plasma concentrations as a cause of prophylactic failure. So far, there is no recommendation for therapeutic drug monitoring (TDM) of atovaquone, despite known variable bioavailability of the drug and prophylactic failures.
The objective of this observational study was to assess the plasma concentrations of atovaquone in immunocompromised patients under PCP prophylaxis with atovaquone and evaluate whether they were in the expected ranges.

**Methods**

All adult patients receiving atovaquone for PCP prophylaxis in our haematology and clinical immunology wards between May and September 2016 were proposed to be assessed for atovaquone plasma concentrations. All patients were given atovaquone (750 mg twice a day, oral suspension) for a minimum of 2 weeks, and their plasma atovaquone concentrations were therefore considered to be in a steady state. Other medication data within the last 14 days were recorded. Biological material was obtained only for routine purposes. According to the French Health Public Law (CSP Art L1121-1.1), such investigation does not require informed consent or ethics committee approval. The refusal to give additional recommendations for the drug intake was deliberate, in order to collect data in routine conditions. The first blood sample was collected ~12 h after the last drug intake, to assess $C_{\text{min}}$. Afterwards, the patients took their morning dose and a second blood sample was collected 1–5 h thereafter, in order to measure $C_{\text{max}}$. Plasma was immediately separated and frozen at −20°C until proceeding to the assay.

Atovaquone plasma levels were measured using UV-HPLC as well as UV detection as previously described. In the absence of an established minimal atovaquone concentration threshold value for PCP prophylaxis, a randomized study was referred to. That study showed that the response rate to atovaquone was significantly better with a $C_{\text{min}}$ > 15 µg/mL (98%) than below this threshold (66%). Therefore, the concentrations in the patients were compared with this threshold.

**Statistical analysis**

$C_{\text{min}}$ and $C_{\text{max}}$, and all quantitative variables are presented as median (IQR). The Mann–Whitney test was used to compare the first assessment of concentrations. For patients who were assessed twice, the signed rank test was used to compare the first and second assessments of concentrations. Univariate analysis using logistic regression was performed to investigate the relationship between atovaquone concentrations and the potential associated factors. ORs and their 95% CIs were estimated separately for each potential risk factor. All tests were two-tailed. P values < 0.05 were considered to be significant. Statistical analyses were conducted with Stata statistical software 12.0 (Stata Corporation, College Station, TX, USA).

**Results**

**Patients**

Thirty-three patients were included in the study: 26 haematology patients (including 19 allogeneic HSCT recipients and 7 non-transplanted patients) and 7 HIV-infected patients (Table 1). All patients received atovaquone because of allergy or intolerance to trimethoprim/sulfamethoxazole.

A total of 82 measurements were performed. All patients were sampled at least once for $C_{\text{min}}$ and 30 for $C_{\text{max}}$. In addition, 11 patients were sampled a second time between 1 and 8 weeks (median 4 weeks) after the first assessment.

**Plasma concentrations of atovaquone and possible associated factors**

The median $C_{\text{min}}$ of atovaquone was 11.3 µg/mL (6.2–27.8; n = 33) and was not significantly different from the median $C_{\text{max}}$ [13.4 µg/mL (6.0–28.3; n = 30)] ($P = 0.073$). Nineteen of the

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<th>Table 1. Characteristics of the 33 patients receiving atovaquone as P. jirovecii prophylaxis</th>
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<tr>
<td>Age (years)</td>
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<td>Male/female, n/n (%)</td>
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<tr>
<td>Patients with haematological diseases, n (%)</td>
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<td>non-transplanted patients, n (%)</td>
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<tr>
<td>underlying disease</td>
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<tr>
<td>AML, n</td>
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<td>aplastic anaemia, n</td>
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<td>allogeneic HSCT recipients, n (%)</td>
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<td>underlying disease</td>
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<td>multiple myeloma, n</td>
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<tr>
<td>myeloproliferative disorder, n</td>
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<td>lymphoma, n</td>
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<tr>
<td>GVHD, n/n (%)</td>
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<tr>
<td>previous or active gut GVHD, n/n (%)</td>
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<td>HIV-positive patients, n (%)</td>
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<td>CD4 cells/mm$^3$</td>
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<td>neutropenia (PNN$^a \leq 500/\text{mm}^3$), n (%)</td>
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<tr>
<td>albumin (g/L)</td>
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<td>diarrhoea, n (%)</td>
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<tr>
<td>concomitant medications, n (%)</td>
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<tr>
<td>ciclosporin</td>
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<td>benzoazepine</td>
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<td>posaconazole</td>
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<td>anti-protease drug</td>
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<td>voriconazole</td>
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<td>paracetamol</td>
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Values shown are median (IQR) unless indicated otherwise.

$^a$PNN, polynuclear neutrophils.

33 (58%) patients presented $C_{\text{min}}$ below the threshold of 15 µg/mL (Figure 1a). The $C_{\text{min}}$ and $C_{\text{max}}$ were not influenced by the presence of HSCT, graft-versus-host disease (GVHD), oral intake of atovaquone with food, or concomitant administration of valaciclovir, ciclosporin or of any drug known to interfere with atovaquone metabolism (ciclosporin, paracetamol voriconazole, posaconazole, anti-protease drugs, benzoazepine) or with albumin, hepatic enzymes or creatinine values (Table S1, available as Supplementary data at JAC Online). HIV-positive patients showed a non-significant trend of higher concentrations than haematology patients. The time elapsed between the intake of atovaquone and the blood sample did not influence the $C_{\text{min}}$ [median 12.6 h (11.9–13.3)] or the $C_{\text{max}}$ [median 1.5 h (1.1–2.5)].

Among the 11 haematology patients who were sampled twice at an interval of several weeks, the second assessment of concentrations showed little variation when compared with the first assessment (Figure 1b).

**Discussion**

This study shows that, with a regimen of 750 mg twice a day of atovaquone for PCP prophylaxis, the atovaquone plasma
Figure 1. Concentrations of atovaquone in 33 patients with haematological diseases or HIV infection. The cross represents the mean, the horizontal line represents the median and the box represents the IQR. (a) Comparison of $C_{\text{min}}$ and $C_{\text{max}}$ of atovaquone. (b) Comparison of first and second assessment of atovaquone concentration.
concentrations (steady state and post-administration) in immuno-compromised patients are low. With a mean $C_{\text{min}}$ of 11.3 $\mu$g/mL, these findings are below the steady-state concentrations reported in HIV-positive patients taking 750 mg twice or three times a day, and below the expected $C_{\text{min}}$ mentioned in the summary of product characteristics (SmPC) (22 $\mu$g/mL) for PCP treatment with a daily dose of 750 mg twice a day. Finally, only 42% of the patients had $C_{\text{min}}$ levels $\geq 15 \mu$g/mL—a steady-state concentration threshold associated with a probability of 98% success in a prospective study of 133 patients treated for PCP—with only 36% of patients had $C_{\text{min}}$ levels $\geq 22 \mu$g/mL.

So far, atovaquone pharmacokinetics have been studied in HIV-positive patients in Phase I and II studies, or in treatment studies of PCP, but not in haematology patients. The absorption of atovaquone varies among individuals. The recommended dose of 1500 mg per day for PCP prophylaxis was supported by several studies in HIV-positive patients. Unfortunately, none of these studies measured the atovaquone plasma concentrations. Consequently, there is no clear indication of the optimal prophylactic concentrations of atovaquone in humans.

For malaria prophylaxis or treatment, the failures of atovaquone/proguanil have been associated with low atovaquone plasma concentrations. Two cases of PCP prophylaxis failure with low doses of atovaquone were reported after transplant, but these patients were not assessed for atovaquone plasma concentrations. Although atovaquone resistance conferred by mutations of the cytochrome b gene of *P. jirovecii* may be observed, sub-optimal serum concentrations could be another cause for drug failure in PCP prophylaxis. This hypothesis is supported by the documented relationship between atovaquone dose and PCP prophylactic effect in HIV-infected patients and in animal models.

Our study confirms the poor absorption of the drug and the large inter-individual variability of plasma concentrations of atovaquone. With more than half of the patients with atovaquone concentrations $<15\mu$g/mL, the main concern is the need for TDM. On the other hand, very little intra-individual variability of concentrations among the 11 patients assessed twice was found. In case of low concentrations in optimal conditions (no drug-drug interactions, intake with a fatty meal), considering that a higher dose may not significantly increase the plasma concentrations, and that no intravenous formulation is available, the final decision would be to switch to dapsone or aerosolized pentamidine, or to consider cautious re-introduction of trimethoprim/sulfamethoxazole. The use of atovaquone TDM could prevent the onset of PCP in high-risk patients.

There are some limitations to this study. First, the absence of any PCP cases prevented the detection of any relationship between atovaquone plasma concentrations and prophylaxis failure, and the establishment of a minimal efficient concentration. However, PCP is a very rare event even in high-risk patients. Using a threshold of 15 $\mu$g/mL established in a PCP treatment study, more than half of our patients had a $C_{\text{min}}$ below the threshold. It is unknown whether this threshold is clinically relevant for PCP prophylaxis. However, we can hypothesize that the cut-off for prophylaxis should be either comparable to or lower than the cut-off for therapeutic efficacy, so we may have overestimated it, but are unlikely to have underestimated it. Second, we did not record the amount of fat ingested during intake, although taking atovaquone with a fatty meal is recommended in the SmPC. However, this recommendation may not be routinely delivered to the patients. Based on these results, it was proposed to better educate patients on the intake of the drug with fatty food.

**Conclusions**

These findings illustrate the great inter-individual variability of atovaquone absorption and the low plasma concentrations in immunocompromised patients. This finding raises the question of TDM, in order to identify patients who could benefit from regimen adaptation or from alternatives. Whether these low concentrations explain some cases of prophylactic failure deserves large prospective studies using routine TDM. Only large studies will allow establishment of a minimal threshold of atovaquone plasma concentrations for an optimal prophylactic effect.

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This study was carried out as part of our routine work.

**Transparency declarations**

None to declare.

**Author contributions**


**Supplementary data**

Table S1 is available as Supplementary data at JAC Online.

**References**


