3AA; however, this is not consistent with the effectiveness of the therapy on *P. falciparum*. Another hypothesis is the natural or induced drug resistance of the parasite, which has not previously been reported in quartan malaria, except for one case of chloroquine resistance in Indonesia; while artemisinin resistance has been reported only in *P. falciparum* on the Thai–Cambodian border, and in unconfirmed reports from South America and some African countries. A positive molecular biology test for *P. malariae* and a normal lumefantrine blood level would confirm this hypothesis: both tests could not be done in our case as this suspicion arose 38 days later, when samples were no longer available. A third possibility is the ineffectiveness of artesunate/lumefantrine against the particularly long-lasting schizogonic pre-erythrocytic phase of *P. malariae*. Our patient had a minimum incubation period of 8 days for his *P. falciparum* malaria episode and of 54 days for *P. malariae*, so that *P. falciparum* may have been treated before *P. malariae* had appeared in his blood. In this case, the use of a longer-acting compound, such as mefloquine or piperaquine, or one with liver-stage activity, such as atovaquone/proguanil, might be preferable.

Several hypotheses on possible biological interactions between *P. malariae* and other malaria parasites in mixed infections can be made, regarding respective disease suppression: the resolution of *P. falciparum* infection may have allowed the clinical manifestation of *P. malariae*, but the possible intervention of antimalarial drugs in these mechanisms has yet to be tested. Moreover, the presence of HIV infection in this patient must be taken into account. Although it is debated whether HIV coinfection can lead to the worsening of clinical malaria, the production of antibodies to *P. falciparum* is reduced in HIV-infected patients and other aspects of the antimalarial immune response, such as antigen-stimulated cell proliferation and the production of certain cytokines, are impaired. We can not exclude HIV infection-related immune deficiency as a cofactor in *P. malariae* reappearance, particularly in an individual who has probably lost his semi-immunity to the parasite. In such travellers, the importance of malaria prophylaxis should not be underestimated, since their risk of infection as well as of severe disease may be increased.

However, our case raises the problem of treating mixed infections also with the latest antimalarials and highlights the fact that *P. malariae*, in spite of its lower incidence and lower pathogenicity, deserves more attention.

The patient gave written informed consent regarding the publication of these data.

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

### Transparency declarations
None to declare.

### References


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**Reversion of naturally occurring high-level resistance mutations to NS3 protease inhibitors in two treatment-naive individuals infected with hepatitis C virus**

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Sir,

HIV-infected patients could profoundly benefit from the use of the new anti-hepatitis C virus (HCV) direct-acting antivirals, the NS3 protease inhibitors (PIs), because their chance of reaching a sustained virological response is dramatically less than in HCV-monoinfected persons. The prevalence of natural resistance within the NS3 protease domain of HCV in HIV-infected individuals is estimated to be 7.9%–16%. Currently only a few studies have evaluated the stability of HCV resistance mutations in vivo in the absence of drugs.
In the present study we investigated the dynamics of naturally occurring strains of HCV resistant to NS3 PIs in two HIV/HCV-coinfected patients.

The two patients were recruited for virological and immunological studies of HIV/HCV infection in March 2000. The study conformed to the ethical guidelines of the Declaration of Helsinki and the subjects gave written informed consent. Some of the data were generated as part of the routine laboratory and clinical work performed for standard care of outpatients.

Case 1

An HIV-infected individual infected with HCV genotype 1b, naive to anti-HCV treatment, was recruited for virological and immunological studies of HIV/HCV infection. The anti-HIV treatment schedule, HCV-RNA levels and NS3 protease dynamics are summarized in Figure 1.

At baseline, the HIV-RNA load was 30000 copies/mL, the absolute CD4+ cell count was 319 cells/mm³ and the alanine aminotransferase (ALT) level was 135 IU/L. A double resistance mutant harbouring Ala-156-Thr and Asp-168-Val amino acid changes was detected. Twenty-seven weeks later, the Val-55-Ile mutant was detected; the CD4+ cell count was 249 cells/mm³, the HIV-RNA level was 17000 copies/mL and the ALT value was 158 IU/L.

At week 63, reversion to the wild-type strain was observed; the CD4+ cell count was 306 cells/mm³, the HIV-RNA load was 79 copies/mL and the ALT level was 38 IU/L. In this case, phylogenetic analysis showed that the mutated sequence at baseline was related to that detected at week 63, which was wild-type, and both clustered with HCV genotype 1b, while the mutated strain found at week 27 clustered with HCV genotype 1a (data not shown).

Case 2

An HIV-infected individual infected with HCV genotype 1a was recruited in the same study as patient 1.

At baseline evaluation, the Arg-155-Lys mutation was identified as the dominant strain; the CD4+ cell count was 331 cells/mm³, the HIV-RNA load was 120000 copies/mL and the ALT value was 160 IU/L. The anti-HIV treatment schedule, HCV-RNA levels and NS3 protease dynamics are summarized in Figure 1. Antiretroviral treatment (ART) was stopped at week 40 due to the patient’s decision. At week 58, when ART was restarted, the CD4+ cell count was 167 cells/mm³, the ALT level was 193 IU/L and the HIV-RNA load was 150000 copies/mL. At week 76, a reversion to wild-type sequence was found. The CD4+ cell count was 136 cells/mm³, the HIV load was 29000 copies/mL and the ALT value was 227 IU/L. Twenty-four weeks later, when the wild-type was still dominant, the CD4+ T cell count was 218 cells/mm³, the HIV-RNA level was 79 copies/mL and the ALT value was 119 IU/L. Phylogenetic analysis showed that the patient’s sequences were closely related (data not shown).

We report here two cases found to be infected with naturally occurring strains of HCV resistant to NS3 PIs that reverted to wild-type during 63 or 100 weeks of follow-up.

Patient 1 harboured two concomitant mutations not previously described in natural strains. To our knowledge, this is the first report describing a case infected with HCV genotype 1b showing a combination of two mutations conferring resistance to anti-HCV NS3 PIs that spontaneously reverted to wild-type. The phylogenetic tree showed that the HCV infecting strain, evaluated at different timepoints, clustered with different subtypes. Remarkably, at week 27 of follow-up the Val-55-Ile variant was detected, which has been found to occur naturally in HCV genotype 1a but not in genotype 1b.6 This interesting finding could be the consequence of a mixed HCV infection, characterized by alternate dominance of one subtype over the other, or a new infection with a different subtype. In this case, the reversion to the HCV wild-type strain was detected when the HIV load was dramatically decreased and ALT levels were within normal ranges. We can argue that ART-mediated control of HIV replication, probably ameliorating the immune response, may modify the viral dynamics of HCV.

In the second case, the Arg-155-Lys amino acid mutation within the NS3 protease domain was revealed at baseline. It has been reported that the Arg-155-Lys mutant exhibits lower fitness in vitro with respect to the wild-type.7 In two recent

Figure 1. Longitudinal evaluation of plasma HCV-RNA levels and NS3 protease mutational pattern in (a) patient 1 and (b) patient 2. NFV, nelfinavir; SQV, saquinavir; d4T, stavudine; LPV/r, lopinavir/ritonavir; ABC, abacavir; ddl, didanosine; TDF, tenofovir. Week 0 = baseline evaluation.
case reports it was shown that the persistence of this variant did not display clinically a reduced viral fitness compared with the wild-type.4,5

In our patient exhibiting an Arg-155-Lys mutant at baseline, the HCV load was decreased when wild-type appeared and increased again when the wild-type sequence was possibly stabilized. This finding is in accordance with data obtained in vitro, suggesting different fitness of the natural Arg-155-Lys mutant and wild-type in vivo.7

In conclusion, our findings indicate that it is important to continue to collect data on the presence and dynamics of natural resistance to anti-HCV PIs. Whether this information will have an additional predictive value on the response to anti-HCV PI-based therapy in HIV-infected individuals remains to be established.

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**Transparency declarations**

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