Plasmodium falciparum Chloroquine-Resistance Transporter Gene Detection in Imported Plasmodium falciparum Malaria Cases

To the Editor—We share the cogent sentiments expressed by Farcas et al. [1] about the great interest of PCR-based methods for the diagnosis of imported malaria, given that, in most westernized countries, morbidity and death due to this protozoan infection among returned travelers is on the rise [2]. Because we have tested >900 patients per year since October 1999, we have accumulated a large amount of routine experience in the molecular diagnosis of imported malaria. First using conventional PCR [3], and now using real-time PCR [4], we have found that the detection of mixed-species infections was sharply improved by both techniques. Overall, however, real-time PCR demonstrates a decisive feature for our clinician colleagues: the capacity to ensure, in a turnaround time of <4 hours, 6 days a week, that a given febrile traveler does not have malaria.

In addition to its utility in the diagnosis of malarial infection, PCR appeared to be a must for epidemiological surveys focused on Plasmodium falciparum with resistance to aminoquinolines, as assessed by the detection of point mutations in the P. falciparum chloroquine-resistance transporter (pfCRT) and P. falciparum multidrug-resistance (pfMDR1) genes. Performed in cohorts of returned travelers or immigrants, such molecular surveillance studies showed that a large proportion of patients (1%–3%) who presented with falciparum malaria harbored the K76 (wild-type) P. falciparum strain [5, 6]. In other words, the malarial infections of these subjects probably would have been cured by chloroquine (CQ), which remains one of the cheapest and safest drugs ever used for malaria treatment. So, we are in full agreement with Farcas et al. [1], who claimed that the molecular assessment of CQ sensitivity, based on the detection of T76 mutation by real-time PCR, appeared to offer an interesting perspective, as previously suggested in a review article [7].

In this view, the sensitivity of a molecular test for the detection of CQ resistance would be of crucial importance. That is, such an assay should display good absolute sensitivity, but it should also be able to detect a minority mutant fraction in a sensitive wild-type population. The test performed by Vessière et al. [8] in the Department of Parasitology (Rangueil University Hospital, Toulouse, France) exhibited 2% sensitivity for the detection of the K76T mutation, which appeared to be insufficient to avoid the occurrence of RI/RII resistance in the event of therapeutic use of CQ. We therefore developed a more sophisticated assay, in which locked nucleic acid probes blocked the amplification of wild genotypes, so that the detection of mutated parasites was boosted. As a result, the sensitivity peaked at 0.063%, translating to 1 mutated P. falciparum parasite of 1600 wild-type P. falciparum asexual forms [9]. Given the design of both primers and probes, this PCR assay could detect all K76T pfCRT haplotypes, and it provided 2 different melting temperature points (Tm), 1 for mutated and 1 for wild-type haplotypes. Thus, the result was not affected by the geographical origin of the parasite isolate [1].

We were, therefore, surprised that Farcas et al. [1] did not assess the sensitivity of their method using, for example, artificial admixtures of mutated and wild P. falciparum strains. Moreover, these authors did not find any mixed infection (infection with wild-type and mutated strains) among 200 isolates studied [1]. In comparison, Vessière et al. [8] reported 11 of 131 imported malaria cases were mixed infections, most of African origin. By locked nucleic acid PCR, 21 mixed infections were detected in the same batch of patients (A. Berry, personal communication). A specificity problem could be excluded, because none of these 11 or 21 patients had positive results for malaria by both conventional and real-time PCR techniques for a species other than P. falciparum. Because Farcas et al. [1] did not give any description of the primers they used, any analytical comparison with our studies was impossible. The sensitivity level of their assay remains, therefore, questionable, which is a pending problem for a kit that could have a commercial destiny.

Farcas et al. [1] criticized the assay used by Vessière et al. [8] because of “the lack of head-to-head comparison with an accepted reference standard” [1, p. 626]. As far as we know, there is no available “reference standard” for the K76T mutated P. falciparum strain; moreover, we did not find any use for such a standard in Farcas and colleagues’ article. Concerning “the lack of negative controls” [1, p. 626], we will refer back to figure 1 in Vessière and colleagues’ article, in which an admixture containing 0% of mutated parasites was used as a negative control.

Finally, we fully agree with Farcas et al. [1] about the need for high-quality requirements in laboratories performing malaria diagnosis. In France and in most countries in the European Union, any private or public laboratory must implement Good Laboratory Practices, a minimal legal obligation. However, far more rigorous quality constraints should be expected from specialized laboratories handling molecular techniques for routine diagnosis of infectious diseases. Our department is in the process of completing accreditation by the French Committee for Accreditation, the French branch of the International Accreditation Forum [10]. Under review are the molecular diagnostic procedures for malaria (including the de-
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References


A Simple Algorithm for the Diagnosis of AIDS-Associated Genitourinary Tuberculosis

To the Editor—As has been shown in surveys in Canada, the United Kingdom, and the United States, genitourinary tuberculosis (TB) is a common form of nonpulmonary TB, accounting for 27% (range, 14%–41%) of the extrapulmonary TB cases [1]. Among patients with AIDS, the incidence of genitourinary TB may be even higher. In an autopsy study in India, 24 of 35 kidneys from patients who died of AIDS showed evidence of infection, including 17 cases of TB [2]. In a similar study in Mexico City, renal disease was demonstrable in 87 (63%) of 138 autopsies performed on patients with AIDS; infection was the cause of the renal disease in 36 cases, with 19 being due to Mycobacterium tuberculosis [3]. However, no data of the prevalence of genitourinary TB in living patients with AIDS can be found in the literature. TB of the urinary tract is very common among our patients with AIDS in our hospital, we applied a simple algorithm based on the absence of positive results of culture on routine media for patients with AIDS and pyuria, albuminuria, or hematuria in the urine examination. Patients with a diagnosis of pulmonary or extrapulmonary TB were excluded from this study. Of 88 patients classified as having AIDS category C [5] and being hospitalized between September 2003 and December 2004, 22 patients with sterile pyuria, albuminuria, or hematuria had a urine examination and received a diagnosis of TB of the urinary tract. Three overnight urine samples that were neutralized with bicarbonate were obtained from each patient. The samples were centrifuged, and a slide was prepared for Ziehl-Neelsen staining, and, posterior to decontamination with 2% NaOH, the samples were inoculated on 2 L-J slants. Urine samples for 6 patients (27%) were positive for acid-fast bacteria (2 by smear examination and culture and 4 by culture only). All isolates were identified as M. tuberculosis using standard techniques. The patients—5 men and 1 woman—had a mean age of 39.1 years (SD, ± 6.2 years). Albuminuria was the most common laboratory abnormality (5 of 6 patients), followed by pyuria (4 of 6 patients) and hematuria (3 of 6 patients). None of the patients had positive skin test results. One patient had an abnormal chest radiograph, but no pulmonary TB was diagnosed on processing of 3 sputum samples. In addition, another patient received a diagnosis of lymph node TB when his urine examination became positive for M. tuberculosis 4 weeks later. We conclude that genitourinary TB is very common among our patients with AIDS and that an algorithm based on a simple urine examination has a very high predictive value for the diagnosis of genitourinary TB and should be included in the differential diagnosis of patients with AIDS and sterile pyuria, albuminuria, or hematuria.