Transdermal Diagnosis of Malaria Using Vapor Nanobubbles

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To the Editor: Establishing reliable noninvasive methods for diagnosis of malaria has been a challenge. Lukianova-Helb et al. should be applauded for developing such a method on the basis of hemozoin (Hz) detection (*1*). The authors reported a proof of principle and are preparing for "large-scale studies in humans" (*2*). Such large endeavors should be based on firm evidence, so it is surprising that the results presented were from a single patient, remarkable for the unusual quadruple drug treatment (*2*). In such a scenario, to compensate for the limited data, the results should be of convincing scientific quality.

However, the case described raises several doubts that could have been addressed, such as the reliability of the diagnosis if only a thin film and a rapid test were used (co-infection excluded) and why parasitemia was not determined at the time of the device test (instead of 4 hours before and 9 hours after). What developmental stages were the parasites in at the time of the evaluation (for example, already early trophozoites containing Hz or Hz-rich gametocytes)? Why was the patient not re-evaluated to find out if repeated measurements would become appropriately negative (test-of-cure)?

The methods and results used in the study contrast with the extraordinary numbers for the limit of detection (LOD): 0.0001% in human blood and 0.00034% in a rodent model (*1*,*2*). However, the LOD is a virtual, inferred parasitemia rate based on the detection of free Hz added to uninfected blood (*1*). An LOD can be obtained from serially diluted cultures or samples (*3*). In rodent models, detection of Hz tends to be much easier (*4*). Moreover, in *Plasmodium falciparum* infections, only immature forms have been observed, with little or no detectable Hz (*5*).

The prospects of a noninvasive test for malaria are exciting. However, in times of cost restraints, any diagnostic test or intervention should provide sufficiently convincing results before consideration of resource-intensive large-scale trials.

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etymologia

Hemozoin [he"mo-zo'in]

From the Greek *haima* ("blood") + *zoon* ("animal"), hemozoin is a pigment produced by malaria parasites from hemoglobin in the host's red blood cells. This pigment was first observed by Johann Heinrich Meckel in 1847 in the blood and spleen of a mentally impaired person. In 1849, Rudolf Virchow made the connection to malaria, but it was initially believed that it was produced in the patient's spleen as a part of the immune response to malaria. In 1880, Charles Louis Alphonse Laveran observed pigmented parasites in the blood of an Algerian soldier and realized that the parasites, not the patient, produce "malaria pigment." The term "hemozoin" was coined by Louis Westenra Sambon.

Isolated *Plasmodium falciparum* hemozoin, Ernst Hempelmann, via Wikipedia

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In Response:

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In Response: The letter by Rebelo et al. (*1*) that questions our previously described noninvasive malaria diagnostics (*2*,*3*) misinterprets both articles. The main objection comes to our alleged call for "large-scale studies in humans"; no such statement appeared in our 2014 article (*2*), and in the 2015 article (*3*), we clearly stated that large-scale studies will be considered after the optimization of a new prototype and improving its sensitivity. The authors' final questioning of our eligibility for resources is a nonscientific opinion.

Concerning the quality of the standard clinical diagnosis, both thin blood film analysis and rapid diagnostic test results were obtained in a certified US clinical laboratory and returned consistent data. The lack of re-evaluation of the patient and the diagnostic timing are indeed limitations but were caused by the clinical restrictions. Our goal in the 2015 article (*3*) was to demonstrate the first noninvasive diagnosis of malaria in a human, which was achieved. The additional parameters discussed in the letter were not the subject of this study. Their letter further misinterprets our 2014 study, stating that parasitemia was virtual in that article; in fact, we studied actual infections among mice (*2*).

The criticism of Rebelo et al. might have been fueled by their own limited detection of hemozoin with flow cytometry and microscopy (*4*), in which they used parasite cultures and an unspecified number of malaria patients. That the methods they used might not have performed well does not mean that the novel technology we described, based upon a different mechanism, would have the same limitations in detecting hemozoin.

In conclusion, we agree with the need for optimization of the technology and additional testing. We are currently developing and testing our technology in a malaria-endemic country. Nevertheless, the letter by Rebelo et al. does not alter the fact that our novel noninvasive malaria diagnostic technology worked in a human.

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Malaria in French Guiana Linked to Illegal Gold Mining

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