EBioMedicine 56 (2020) 102781

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.elsevier.com/locate/ebiom

Commentary

Uncovering hidden pfhrp2/3 deletion Plasmodium falciparum

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ARTICLE INFO

Article History:
Received 20 April 2020
Accepted 21 April 2020
Available online xxx

Deaths and transmission from malaria have declined significantly in the past decade, although the progress has somewhat stalled in recent years [1]. Accurate and timely diagnosis is essential to providing adequate treatment to malaria cases. *Plasmodium falciparum* histidine-rich protein 2 (PfHRP2) based rapid diagnostic test (RDT) is widely used especially in Africa, where *P. falciparum* accounts for 98% of estimated malaria cases [1]. Despite its importance, reports of parasites lacking *pfhrp2* gene emerging across the globe are threatening the importance of this malaria diagnostic tool. Peru is the first country that reported a high prevalence of *pfhrp2* and *pfhrp3* deleted strains in the field and warned that PfHRP2-based RDT should be replaced with a non-PfHRP2-based RDT or quality microscopic diagnosis [2]. The threat of false-negative PfHRP2-based RDT alarmed the World Health Organization (WHO) and recommendation for accurate reporting was updated in 2014 [3], followed by multiple reports by WHO Global malaria program [4]. In the African continent, where the burden of malaria mortality and morbidity are the highest, a recent review showed that the level of pfhrp2/3 gene deletion varied among 12 countries where *pfhrp2/3* deletions were tested in Africa – the highest being Eritrea (62%), and the lowest being Angola (0.4%), with a caveat that sample collection and assay varied across studies [5]. Standardized surveillance protocol is now developed by WHO to study *pfhrp2*/3 deletion in malaria-endemic countries [6]. Based on the protocol, suspected PfHRP2-RDT false negative individuals, who are febrile, should be screened by microscopy or a pf-pLDH RDT [6]. Being negative with PfHRP2-based RDT, but positive with other screening methods indicate potential deletion, and if suspected deletion exceeds 5%, WHO urges to switch to non-PfHRP2-based RDTs or microscopy [6]. Although molecular confirmation of *pfhrp2/3* deletion is currently not required, detecting the emergence of *pfhrp2/3* deletion parasite and monitoring it can assist malaria-endemic countries to make a plan accordingly. When screening for deletion, infection with multiple clones of parasites can cause difficulty to analyze the results, since the presence of *pfhrp2/3* deletion parasite can be masked by the presence of *pfhrp2/3* un-deleted parasites.

In an article in *EBioMedicine*, Grignard and colleagues [7] propose a novel multiplex qPCR assay that addresses the issue of polyclonal infection. The authors went through meticulous optimization and validation process. In the final multiplex qPCR assay, host DNA (human beta tubulin (*HumTuBB*)), a single-copy parasite house-keeping gene (*P. falciparum* lactate dehydrogenase (*pfldh*)), as well as *pfhrp2* and *pfhrp3* are simultaneously detected using specific primers and probes. They used *pfldh* to confirm the presence as well as quantification of parasite DNA, and used *HumTuBB* to normalize relative parasite densities in each sample. Using the developed and validated assay, the authors tested various field samples including; dried blood spots (DBS) from Eritrea ($n = 50$), whole blood collected from symptomatic individuals in Kenya ($n = 150$) and Tanzania ($n = 149$), and confirmed *P. falciparum* in whole blood samples collected from UK travelers ($n = 113$) at Public Health England Malaria Reference Library. Of those tested, 31 (62%), 0 (0%), 1 (0.7%), and 1 (3.5%) were *pfhrp2/3* double deletions among samples from Eritrea, Kenya, Tanzania and UK travelers, respectively. Furthermore, when polyclonal infections were taken into account, the authors reported additional *pfhrp2, pfhrp3* or *pfhrp2/3* deletions among samples from Kenya, and UK travelers. The current UK recommendation for malaria diagnosis is a thick and thin smear, but when a microscopist is inexperienced or unavailable, RDT plays a back-up diagnostic role. Given the caveats reported in this paper, the authors recommend that PfHRP2-based RDT results should be interpreted cautiously if that is used when microscopy analysis is not available.

The use of *pfldh* quantification as normalizer without the human gene was previously reported [8]. Grignard and colleagues report that they have tried both methods (delta Ct vs delta delta Ct) and are comparable while adding a complex layer of internal control measure [7]. As it is commonly known that field samples can be collected and prepared in various ways. DBS is one of the sources of DNA the authors used in this manuscript and this could potentially contribute to a sample quality bias – partially filled or oversaturated. Incorporating an additional layer of normalization to the analysis is one of the ways to ensure the results are comparable across time and location. Detecting *pfhrp2/3* deletion parasites masked by polyclonal infection may not change the result of the diagnosis, however, detecting the emergence and monitoring its spread over the course of time

DOI of original article: http://dx.doi.org/10.1016/j.ebiom.2020.102757.
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https://doi.org/10.1016/j.ebiom.2020.102781

will enable countries and communities affected by malaria plan for the change in malaria diagnostic tools before the surge of parasite strains that are undetectable by current PfHRP2-based RDTs.

Declaration of Competing Interest

Dr. Kobayashi has nothing to disclose.

References

[1] World Health Organization. World MALARIA Report 2019. Geneva.
[2] Gamboa D, Ho MF, Bendezu J, Torres K, Chiodini PL, Barnwell JW, et al. A large proportion of P. falciparum isolates in the Amazon region of Peru lack pfhrp2 and pfhrp3: implications for malaria rapid diagnostic tests. PLoS One 2010:5.
[3] Cheng Q, Gatton ML, Barnwell J, Chiodini P, McCarthy J, Bell D, et al. Plasmodium falciparum parasites lacking histidine-rich protein 2 and 3: a review and recommendations for accurate reporting. Malar J 2014:13.
[4] WHO Global malaria programme. False-negative RDT results and P . falciparum histidine-rich protein 2 / 3 gene deletions2015;2:1–8.
[5] Agaba BB, Yeka A, Nsobya S, Arinaitwe E, Nankabirwa J, Opigo J, et al. Systematic review of the status of pfhrp2 and pfhrp3 gene deletion, approaches and methods used for its estimation and reporting in Plasmodium falciparum populations in Africa: review of published studies 2010-2019. Malar J 2019;18:1–10.
[6] World Health Organization. Surveillance template protocol for pfhrp2/pfhrp3 gene deletions. 2020.
[7] Grignard L, Nolder D, Sepúlveda N, Berhane A, Mihreteab S, Kaaya R, et al. A novel multiplex qPCR assay for detection of plasmodiumfalciparum with histidinerich protein 2 and 3 (pfhrp2 and pfhrp3) deletions in polyclonal infections. EBioMedicine 2020 Available from DOI: http://10.1016/j.ebiom.2020.102757.
[8] Schindler T, Deal AC, Fink M, Guirou E, Moser KA, Mwakasungula SM, et al. A multiplex qPCR approach for detection of pfhrp2 and pfhrp3 gene deletions in multiple strain infections of Plasmodium falciparum. Sci Rep 2019;9:1–10.