Malaria diagnostic resistance: implications for endemic regions and global treatment programmes

Essay Summary

Malaria accounts for over 435,000 deaths per year. A key method of malaria diagnosis is the pfHRP2 rapid diagnostic test (RDT), which has been proven reliable and accurate. Recent reports have however questioned the reliability and accuracy of the test. The cause of this is unclear, however deletions in the pfHRP2 gene are the most likely suggestion. This has major implications for the investigation and diagnosis of malaria around the world, as false negatives could delay treatment and lead to increased mortality.

The prevalence of pfHRP2 deletion is also increasing, suggesting the deletion provides an evolutionary benefit for P. falciparum. This draws parallels with HIV's evolved resistance to treatment, and necessitates a similar combinatorial approach is taken to solving the problem by combining RDT's together.

It could be argued the goal to develop cheap, universal tests for infectious diseases such as malaria has provided a narrow selection pressure that the organism can overcome, and so perhaps a 'one size fits all' approach is not best. Evolution of the organism must be met by evolution in management and prevention, with adaptability and ingenuity key.

Essay

Why Malaria Matters

Despite decades of concerted international efforts, malaria continues to account for approximately 435,000 people each year. (WHO, 2018). Plasmodium falciparum (P.falciparum) is the most common and lethal malaria species. In resource limited settings diagnosis and subsequent treatment of malaria has been transformed by the rapid diagnostic tests (RDTs). These are cheaper and require almost none of the infrastructure and expertise required for microscopy diagnosis. RDTs test for a variety of proteins produced by plasmodium species, but the most common and widely used RDT detects P.falciparum histidine rich protein 2 (pfHRP2). In original implementation studies this test demonstrated high levels of consistency and performance (Beadle et al., 1994). It is no understatement to pronounce that pfHRP2 RDTs are the backbone of clinical malaria management in areas of high endemicity. In Sub-Saharan Africa, 75% of tests for malaria are performed with RDT's (WHO, 2018)

The Rapid Diagnostic Test

RDT's utilize lateral flow immunochromatography. The pfHRP2 RDT contains antibodies labelled with a dye, which can bind to an antigen e.g. pfHRP2. Antibodies fixed in position can also bind the antigen. When a sample if added to the RDT, antigens first bind to dye labelled antibodies, and then move down the RDT and are trapped by a line of fixed antibodies. This leads to the formation of a line of antigens bound to the fixed antibodies and to the dye labelled antibodies. The dye will mean this line is coloured. The presence of this coloured line, along with a control line to indicate the test is valid, shows that a person has malaria. It is an excellent example of a robust, quick and easy-to-use point of care test.

The specificity of the antigen antibody complex means that RDTs are highly specific for their protein of choice. The ability of pfHRP2 RDT's to function therefore relies on pfHRP2 being present in P.falciparum's genome and being translated into a protein.

The global malaria research community has been shocked by recent studies that describe radically impaired sensitivity of the pfHRP2 RDT compared with studies only one decade before(Gamboa et al., 2010). Given the widespread usage of pfHRP2 RDTs in malaria diagnosis, it is essential to ascertain the cause of reductions in sensitivity and false negative results. Misdiagnosis of malaria due pfHRP2 deletions has led to delays in treatment and could cause increased mortality should only RDT testing be performed. (Houzé et al., 2011) Several suggestions have been made as to why pfHRP2 RDT sensitivity has decreased.

Alterations in pfHRP2 Genome

One hypothesis put forward for the cause of false negative RDTs is the loss of the pfHRP2 gene from the plasmodium genome. During research for a WHO RDT evaluation program, (Gamboa et al., 2010) it was found that some patients who were positive for P.falciparum based on microscopy tested negative on a pfHRP2 RDT. PCR was then used to confirm loss of pfHRP2 and pfHRP3 genes, while ELISA showed a lack of pfHRP2/pfHRP3 protein expression. pfHRP3 is another malaria antigen used in malaria RDTs. 41% of samples were shown to lack pfHRP2, and 70% lacked pfHRP3. Subsequent studies in Brazil, clearly separated in time and place from sub-Saharan Africa, have reproduced this finding that some patients testing positive for microscopy but negative on pfHRP2 RDT had deletions of the pfHRP2 gene (Maltha et al., 2012). Interestingly, Maltha and colleagues also found that 2 false negative strains of P. falciparum did not have pfHRP2 deleted but still tested negative on RDT. The authors hypothesize that a mutation or deletion in strains with pfHRP2 present might have altered the binding of RDT antibody to pfHRP2 antigen, and so have led to false negatives.

This hypothesis was explored previously (Baker et al., 2005), with analysis finding certain pfHRP2 sequences could be detected at low parasitaemia (<250a (parasites/ µl, while other could not, but no variation at higher levels of parasitaemia. Many of the sequences used came from laboratory lines and not from field isolates. In a subsequent study (Baker et al., 2010), no relationship was found between the pfHRP2 gene sequence and RDT detection rate for P. falciparum even at low parasitaemia. Subsequent studies (Kumar Bharti et al., 2017) have also analysed pfHRP2 sequences and found no link between pfHRP2 RDT positivity and pfHRP2 sequence.

The most likely explanation for false negative RDT results therefore appears to be gene deletions, due to the wide variety of reports in the literature that show microscopy positive, RDT negative test subjects with deletions of pfHRP2. As well as the aforementioned studies in Peru, studies in Brazil (Houzé et al., 2011), Mali (Koita et al., 2012), Eritrea (Berhane et al., 2018), Rwanda (Kozycki et al., 2017) and India (Bharti et al., 2016) have corroborated that pfHRP2 deletions occur and lead to false negative results.

The Prozone Effect

Another explanation of false negative pfHRP2 RDTs is a prozone-like effect. In malaria RDTs, both the dye labeled antibody and capture antibody must bind to an antigen to cause a coloured line indicating malaria infection. Patients with high levels of antigen in

their blood might oversaturate dye labeled antibodies and cause interference in the ability of capture antibodies to also bind. It has been found that increasing quantities of pfHRP2 eventually lead to a reduction in the intensity of the line indicating a person is malaria positive, although the line does not vanish. (Luchavez et al., 2011) It is possible therefore, that an RDT may be interpreted incorrectly due to a reduction in the intensity of the line. Other studies have found a prozone effect in 16 of 17 pfHRP2 RDTs tested, but no such effect for other RDT's such as LDH and aldolase.(Gillet et al., 2009) The prozone effect can therefore account for reductions in line intensity, but this would rely on this effect only causing issues since pfHRP2 deletions began to occur, as it has not seemed to cause issues for two decades prior. Furthermore, an increasing prozone effect would mean that parasite levels in the bloodstream have been increasing worldwide, to levels that do not seem conceivable. (Luchavez et al., 2011) As mentioned in the above studies, line visibility would not be an issue except in extremely high parasitaemias, combined with user error. These are both relatively rare occurrences and so for them to account for the majority of false negative pfHRP2 RDT's seems unlikely.

Why is pfHRP2 Being Deleted?

The cause for mutations occurring is unclear, but it could be an evolutionary mechanism to overcome the selection pressure of RDT's. If a patient does not test positive, they will not receive treatment, and the parasite can continue to reproduce. It would therefore be expected that P. falciparum strains with a pfHRP2 deletion would have a survival advantage and therefore be more likely to reproduce and increase in number. The deletions likely existed before RDT's were developed and so have only proved useful since their widespread usage.

The origins and causes of increasing pfHRP2 RDT negative malaria strains in Peru has been investigated. (Akinyi et al., 2013) It was found that the prevalence of pfHRP2 deletions has increased from 20% in samples from 1998-2001, to 40% in 2003-2005. This seems to suggest there is a selection pressure for pfHRP2 to be deleted, but this could not be confirmed from the study. Furthermore, the deletion of pfHRP2 was not a one-off event, but occurred multiple times in Peru. The authors point out however that RDT's were rarely used in Peru at the time due to the wide availability of microscopy. One study however is not definitive, and it would be of great interest to see further studies analysing the prevalence and origins of the pfHRP2 in different areas.

Key to understanding why pfHRP2 is being deleted will be finding the function of the protein, which is currently unknown. There appears to be no survival advantage by modifying the protein, however due to its subteloromic location it mutates frequently. (Baker et al., 2010) More work is therefore needed to understand the function of pfHRP2 and why it can mutate so much without any loss of survival fitness.

Solutions to Decreased pfHRP2

Sensitivity Several fields of medicine suffer problems of resistance, but typically this is to a treatment rather than an investigation. Organisms will always adapt to the selection pressures exerted on them, and so it is unsurprising to see the prevalence of pfHRP2 deletions increasing in the P.falciparum genome due to the proteins used in an RDT.

Solutions used to treatment resistance in HIV medicine rely on combining multiple treatments together, known as highly active antiretroviral therapy (HAART). Perhaps

combining multiple RDT's would allow increased sensitivity in detecting P. falciparum than with one RDT alone. A candidate to combine with pfHRP2 would be the lactate dehydrogenase protein.

Lactate dehydrogenase (LDH) is produced by all four malaria species which infect humans (Mouatcho & Goldring, 2018) and so can detect species other than P. falciparum. A systematic review comparing LDH and pfHRP2 RDT's for diagnosis of P. falciparum found that LDH RDT's were more specific, and pfHRP2's are more sensitive. (Li et al., 2017) Sensitivity and specificity has been shown to be higher when pfHRP2 and LDH RDT's are combined. In addition, the LDH RDT picked up 53% of pfHRP2 negative samples which tested negative on pfHRP2 RDT's. This shows that combination RDT's can be more effective than either alone, and perhaps provide a solution to the problem of pfHRP2 deletions.

Another candidate to replace the single RDT is whole genome PCR testing for malaria. A key feature of the malaria genome would be used as a PCR primer, ensuring the parasite could not mutate to avoid detection. PCR is more sensitive than microscopy or RDT (Das et al., 2017) and can detect ultra-low levels of parasitaemia. Currently PCR is too expensive, labour intensive and equipment heavy to be used in place of other testing methods, but it is almost certain these barriers will reduce over time, and allow PCR to be used as widely and frequently as RDT's are currently.

Future Questions and Conclusion

Several unanswered questions emerge from the deletions in pfHRP2. The cause, although speculated to be due to pfHRP2 RDT introduction, is unclear. The function of pfHRP2 also remains unclear, and so it is difficult to hypothesize the causes of deletion prevalence increasing. Additionally, some patients testing false negative on pfHRP2 RDT had no deletion in the gene. This would seem to indicate factors apart from deletions of the pfHRP2 gene are at play. These questions need to be answered quickly, as over 150 million RDT's are carried out annually in Sub Saharan Africa alone. (WHO, 2018) Aside from causing an increased malaria burden, false negative malaria testing would cause economic consequences as patients require hospital admissions following more severe infections.

Traditionally, a goal for infectious diseases such as malaria has been simple, fast and cheap rapid diagnostic testing. It is perhaps pertinent to ask whether the focus on a universal test has provided an ideal situation for the parasite, which can use evolution to overcome the selection pressure of an RDT, and whether having a wider range of investigations is more useful for organisms capable of evolving. This is a question not limited to malaria, but to other infectious diseases that rely heavily on a single diagnostic tool, such as GeneExpert for diagnosis of TB in Sub Saharan Africa. In conclusion, the decreasing sensitivity of pfHRP2 RDT's is likely to be due to deletions of the gene from P. falciparum's genome. New approaches may include combined RDT's and PCR, however there will never be a perfect investigation as the parasite will always evolve to combat a selection pressure. Ultimately, it is not possible to prevent malaria and other infectious organisms from evolving, as this is their nature. What is necessary however is the ability for healthcare professionals and researchers to adapt to the changes that occur, and come up with novel solutions to the problems they face.

Bibliography

Akinyi, S. et al. (2013) ‘Multiple genetic origins of histidine-rich protein 2 gene deletion in Plasmodium falciparum parasites from Peru’, Scientific Report, 3. doi: 10.1038/srep02797

Baker, J. et al. (2005) Genetic Diversity of Plasmodium falciparum Histidine-Rich Protein 2 (PfHRP2) and Its Effect on the Performance of PfHRP2-Based Rapid Diagnostic Tests. Available at: https://academic.oup.com/jid/article-abstract/192/5/870/803166 (Accessed: 16 November 2018)

Baker, J. et al. (2010) ‘Global sequence variation in the histidine-rich proteins 2 and 3 of Plasmodium falciparum: Implications for the performance of malaria rapid diagnostic tests’, Malaria Journal, 9(1), p. 129. doi: 10.1186/1475-2875-9-129

Beadle, C. et al.(no date) Diagnosis of malaria by detection of Plasmodium falciparum HRP-2 antigen with a rapid dipstick antigen-capture assay. Available at: https://ac.els-cdn.com/S0140673694915202/1-s2.0-S0140673694915202-main.pdf?_tid=7c7b094c-2b80-41a0-9d23-29ae2d78f717&acdnat=1542798030_d311d65f6818e978ac7721d654cdf305 (Accessed: 21 November 2018)

Berhane, A. et al.(2018) ‘Major Threat to Malaria Control Programs by Plasmodium falciparum Lacking Histidine-Rich Protein 2, Eritrea’, Emerging Infectious Diseases, 24(3), pp. 462–470. doi: 10.3201/eid2403.171723

Bharti, P. K. et al. (2016) ‘Prevalence of pfhrp2 and/or pfhrp3 Gene Deletion in Plasmodium falciparum Population in Eight Highly Endemic States in India’, PLOS ONE. Edited by G. Snounou. Public Library of Science, 11(8), p. e0157949. doi: 10.1371/journal.pone.0157949

Das, S. et al. (2017) ‘Performance of a High-Sensitivity Rapid Diagnostic Test for Plasmodium falciparum Malaria in Asymptomatic Individuals from Uganda and Myanmar and Naive Human Challenge Infections’, Am. J. Trop. Med. Hyg , 97(5), pp. 1540–1550. doi: 10.4269/ajtmh.17-0245.

Gamboa, D. et al.(2010) ‘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. Edited by A. Bjorkman. Public Library of Science, 5(1), p. e8091. doi: 10.1371/journal.pone.0008091

Gillet, P. et al.(2009) ‘Assessment of the prozone effect in malaria rapid diagnostic tests’, Malaria Journal, 8(1). doi: 10.1186/1475-2875-8-271.Houzé, S. et al. (2011) ‘Combined Deletions of pfhrp2 and pfhrp3 Genes Result in Plasmodium Falciparum Malaria Flase-Negative Rapid Diagnostic Test’, Journal of Clinical Microbiology. American Society for Microbiology Journals, 49(7), pp. 2694–2696. doi: 10.1128/JCM.00281-11.

Koita, O. A. et al.(2012) ‘False-negative rapid diagnostic tests for malaria and deletion of the histidine-rich repeat region of the hrp2 gene.’, The American

journal of tropical medicine and hygiene. The American Society of Tropical Medicine and Hygiene, 86(2), pp. 194–8. doi: 10.4269/ajtmh.2012.10-0665.

Kozycki, C. T. et al. (2017) 'False-negative malaria rapid diagnostic tests in Rwanda: impact of Plasmodium falciparum isolates lacking hrp2 and declining malaria transmission', Malar J, 16, p. 123. doi: 10.1186/s12936-017-1768-1.

Kumar Bharti, P. et al. (2017) 'Sequence variation in Plasmodium falciparum Histidine Rich Proteins 2 and 3 in Indian isolates: Implications for Malaria Rapid Diagnostic Test Performance', Scientific Reports. Nature Publishing Group, 7(1), p. 1308. doi: 10.1038/s41598-017-01506-9.

Li, B. et al. (2017) 'Performance of pfHRP2 versus pLDH antigen rapid diagnostic tests for the detection of Plasmodium falciparum :a systematic review and meta-analysis', Archives of Medical Science. Termedia, 3(3), pp. 541–549. doi: 10.5114/aoms.2017.67279.

Luchavez, J. et al.(2011) Laboratory demonstration of a prozone-like effect in HRP2-detecting malaria rapid diagnostic tests: implications for clinical management. doi: 10.1186/1475-2875-10-286

Maltha, J. et al. (2012) 'Rapid Diagnostic Tests for Malaria Diagnosis in the Peruvian Amazon: Impact of pfhrp2 Gene Deletions and Cross-Reactions', PLoS ONE. Edited by G. Snounou. Public Library of Science, 7(8), p. e43094. doi: 10.1371/journal.pone.0043094

Mouatcho, J. C. and Dean Goldring Correspondence J P Dean Goldring Goldringd, J. P. (2018) 'Malaria rapid diagnostic tests: challenges and prospects'. doi: 10.1099/jmm.0.052506-0

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