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Malaria Diagnostics, Where are we Now

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Introduction

Malaria, a worldwide disease of high morbidity and mortality, causes over 1 million deaths per year, mostlyin children less than 5 years of age [1,2]. Delayed treatment of malaria increases the chances of death and neurological disease [3]. In addition, to bring about malaria eradication and prolong accumulation of drug resistant parasites, treatment must follow an accurate diagnosis. To that end, a major goal in malaria diagnostics is to develop assays that are both sensitive and cost-effective for use in developing countries, which are economically and infrastructurally challenged. This review analyzes the current state of malarial diagnostics, from the gold standard of Giemsa-stained blood smears for microscopic examination to highly sensitive and novel technologies. Here, we suggest a future direction for malarial diagnostics.

Optical Imaging Techniques

Developed in the 1900s, microscopic examination of both thick and thin Giemsa-stained blood smears have long been the gold standard for malaria diagnosis [4]. Well trained microscopist can identify the species of the infecting Plasmodium parasite and quantify the density of parasite burden. Identification of the Plasmodium species and parasite density is necessary to determine the best treatment and subsequent monitoring of treatment success.Optimally, microscopic detection of parasitemia can measure as low as 5 parasites/μl or 0.0001% on thick smear; which is not sensitive enough to detect parasitemias in low transmission areas. Although this is the standard procedure, good quality staining reagents and microscopes, as well as, well trained microscopists are needed for this diagnostic technique, all of which developing countries may have a difficultymaintaining [3]. Microscopic diagnostic methods are also limited by the lack of a universal standard for quantifying parasites[4].There are other techniques that can enhance microscopic techniques by concentrating the parasites in heparinized blood smears or the quantitative buffy-coat method [3].

Diagnostic techniques utilizing alternative stains, such as Field's stain, acridine orange, or benzothiocarboxypurine have also been developed. Field's stain is a water-based Romanovsky stain that produces results that are comparable to that of Giemsa, however, the stain fades and is not available for review. Acridine orange and benzothiocarboxypurine users claim that these stains are easier to read and do not require an experienced microscopist. Nonetheless, these stains require a fluorescent microscope which may not be feasible in an isolated area lacking reliable electricity [4,5].

Diagnoses of malaria have also been conducted using dark-field and polarization microscopy. Jamjoom [6] showed that dark-field microscopy could be used for rapid detection of malaria or hemozoin pigment [6-8]. Polarization microscopy also detects malaria pigment in infected red blood cells compared to healthy red blood cells. It appears that these other light microscopy techniques may have a higher detection rate than Giemsa-stained blood smears with a simple compound microscope, although there is some problem with formalin pigment producing artifacts which may be mistaken as malaria pigment [9].

Molecular Techniques

While microscopy remains the most widely used method for diagnosing malaria, methods based on the polymerase chain reaction (PCR) of the small subunit ribosomal RNA gene (18SSU rRNA) were more recently developed. PCR based techniques are capable of detectingparasites at submicroscopic levels, quantifying the parasitic load, and identifying the species of Plasmodium responsible for the disease [10-12]. Moreover, in many cases, the sensitivity of PCR allows detection of asymptomatic or sub-clinical cases of malaria [13].

The gold standard among the PCR-driven malaria diagnostics, nested PCR is certainly the best established andinstrumental in detecting co-infections, which are prevalent in malaria-endemic areas [14-16]. Despite its success, well-trained laboratory personnel, high risk of contamination, and a lengthy process are drawbacks to its use. To circumvent these problems, a semi-nested PCR protocol was recently developed by Ongagna-Yhombi and colleagues using primers targeting the P. falciparumdihydrofolatereductase gene amplified from saliva of malaria infected patients [17].

Moreover, multiplex PCR and quantitative real-time PCR (qPCR) have also been proposed as potential tools for the diagnosis of clinical malaria [18,19]. Both multiplex and qPCR have the capacity to detect multiple Plasmodium species in a single reaction and qPCR can determine parasite loads at submicroscopic levels of parasites [18,19].

The loop-attenuated isothermal amplification (LAMP), a single cycle method that avoids the pitfalls of nested PCR, targets mitochondrial DNA [20-22]. Although LAMP has been proposed as a viable technique for malaria surveillance and has the advantage of being able to obtain more rapid results, it has not yet been established as a viable diagnostic tool that could be used on large number of samples in surveillance studies.Despite the recent advances in molecular techniques, the use of PCR-based methods for routine diagnosis of malaria remains elusive in endemic areas because of cost and the need for highly trained laboratory technicians.

Rapid Diagnostic Test

Rapid diagnostic tests (RDTs) utilize antibodies to detect Plasmodium-specific antigens from the peripheral blood circulation in

as little as 10 to 15 min. The majority of commercially available RDTs target histidine-rich protein-2 (HRP-2), a protein specific to the P. falciparum species, Plasmodium-lactate dehydrogenase (pLDH) or Plasmodiumadolase, enzymes found in the glycolytic pathway of all five Plasmodium spp. known to infect humans [23].

Recent studies of the diagnostic efficacy of microscopy, real-time qPCR, and PfHRP-2 / pLDH ELISA for identifying Plasmodium parasites found that PfHRP-2 ELISA detected malarial prevalence comparable to that of qPCR [24].While PfHRP-2 RDTs have displayed a high degree of sensitivity and specificity, they are not without their limitations. An antigen specific to P. falciparum, HRP-2 is not suitable for the detection of other Plasmodium spp. known to infect humans [25]. Genetic deletions of pfhrp2 and pfhrp3 genes, has been reported in Peru [26,27], Brazil [28], Mali [29], and India [30]. Although HRP-2 has been detected weeks after the cessation of symptoms [25], pLDH is only produced by viable Plasmodium spp. [31], and therefore, pLDH RDTs have the capacity to not only assess current parasitemias but also the efficacy of antimalarial therapies [32,33]. pLDH-based tests have also displayed similar specificity to HRP-2 assays (98%), albeit lower sensitivity (67%), in detecting P. falciparum infections in endemic areas [34]. However, pLDH RDTs have recently shown an inability to accurately identify mixed infections of parasites (P. falciparum, P. vivax, and P. knowlesi) [35-37].

Conclusion

Considering the state of malaria worldwide our efforts toward an accurate diagnosis malaria and treatment are lacking. Some antimalarial treatment is delivered based onclinical diagnosis (febrile patients) in which the patient may or may not have malaria, but some other disease with similar symptoms. Diagnostic methods are used that are not sensitive enough to detect low parasitemias. Infrastructure, finance, and trained personnel needed for accurate diagnosis is lacking in endemic areas. A major goal in malaria diagnostics is development of assays that are both sensitive and cost-effective for use in developing countries, which are economically and infrastructurally challenged. The gold standard of Giemsa-stained blood smears for microscopic examination and RDTs aresufficient to detect case of malaria in high transmission areas in patients with no immunity [3,34]. However, persons with immunity to malaria and are asymptomatic have low level parasitemiaswhich are not diagnosed and remain untreated, contributing to malaria transmission. Without a sensitive inexpensive diagnostic test for persons immune to malaria and those living in a low transmission areas, eradication of malaria will be less feasible [13]. Molecular tools are too expensive, complex and not rapid.Ideally, to eradicate malaria, curtail the overuse of expensive anti-malarial and the build-up of resistance to our depleted antimalarial arsenal; we recommend antimalarial treatment, only for patients in casesthat have a positive laboratory diagnosis for malaria. Patient samples found to be negative by microscopy or RDT should be screened by molecular techniques to discover low levels of parasitemias. If parasite burden is needed, microscopy should be used for detectable counts and qPCR should be used for low level burden. Nevertheless, in the real world, a highly sensitive economical test would yield a giant step toward malaria eradication.

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