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Mixed malaria: the paradox of visual diagnosis in the tropics.

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EDITORIAL

Malaria is the parasitic disease with the greatest impact on humans. It is an infectious disease caused by protozoa of the genus *Plasmodium spp*. and transmitted to humans by the bite of the mosquito *Anopheles spp*. Its incidence has increased in the tropical regions of Africa and Southeast Asia, areas already defined by the World Health Organization (WHO) as malarial zones **[1]**. In these places, malaria generally disappears at altitudes above 2.000 meters above sea level. The most frequently circulating parasites are *P. vivax* and *P. falciparum*; *P. malarie* is also widely distributed, but less frequent. In West Africa, *P. ovale* replaces *P. vivax*. The most widespread species in the world is *P. vivax*, which has been found from sea level to 2.770 meters above sea level. The endemic areas, however, are generally in the tropics.

By definition, the number of parasites found in the peripheral blood depends on the species: the highest number corresponds to *P. falciparum* which infects 10 to 40% of all red blood cells; it is worth mentioning that when parasitaemia of this species reaches more than 25%, it is usually fatal *[2]*. Multiple invasion of red blood cells is frequent with *P. falciparum*, rare with *P. vivax* (which has a predominance of reticulocytes) and very rare with *P. malariae* **[1- 3]**. Without treatment, maximum parasite multiplication is reached in 2 weeks in *P. vivax*, and in 10 days in the case of *P. falciparum*.

The simultaneous presence of erythrocytic parasitic forms (asexual and/or sexual) of two or more species of *Plasmodium spp*. is called mixed plasmodial infection. If, in addition, there are symptoms such as fever, chills, headache, sweating, among others, it corresponds to the disease called mixed malaria. In the world, the prevalence of this last entity is uncertain, depending on the diagnostic method used: 2% by light microscopy (e.g., thick blood smear [TBS] or peripheral blood smear) and up to 65% by polymerase chain reaction (PCR) **[4]**. In Latin America, prevalence is estimated at 0.46% by TBS and 12.8% by PCR.

In terms of performance, the thick smear is 20-30 times more sensitive than the thin smear (peripheral blood smear), although less specific for the identification of the erythrocytic asexual (young rings or trophozoites, mature trophozoites, schizonts) and sexual (gametocytes) forms of the five species of *Plasmodium spp*. that parasitize humans (*P. falciparum, P. vivax, P. ovale, P. malariae and P. knowlesi*), which explains an underdiagnosis of both clinical scenarios (both plasmodial infection and mixed malaria) by light microscopy, with serious implications for diagnostic and therapeutic guidance. Nevertheless, some methods have been developed to improve the diagnostic capability of TBS **[2,3]**:

- Fluorescent stains (acridine orange): used to increase sensitivity of point-of-care diagnosis, without improving specificity of species identification; additionally, some stains may be toxic.
- Fluorescence staining + microcentrifuge: increases diagnostic speed and sensitivity for *P. falciparum*; reduces sensitivity for other species.
- Magnetic deposition: takes advantage of the magnetic characteristics of hemozoin to precipitate the parasites to the plate with a magnet. It is an inexpensive method. It increases sensitivity; however, it is not species specific, without good representation of ring stage parasites.

Among the tests mentioned in a conventional manner, PCR has the best diagnostic performance, but its availability and cost limit its widespread use in developing countries which, paradoxically, are the ones with the most cases of malaria, according to sociodemographic and climatic conditions. It has some technical variants: conventional nested PCR, multiplex real-time PCR and reverse transcriptase PCR. Most of these

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methods target genes in the 18S rRNA of the malaria parasite **[4,7]**. PCR-based tests can be used as initial testing of suspected malaria cases; however, microscopy is often used to quantify parasitemia. PCR-based tests are especially useful for identifying asymptomatic patients and submicroscopic parasitemia that are missed by microscopy and rapid diagnostic tests (RDTs). Sensitivity and specificity for the different types of PCR range from 98% to 100% and 88% to 94%, respectively, when light microscopy is used as the gold standard **[4]**.

Rapid diagnostic dipstick tests identify *Plasmodium spp*. proteins (i.e., lactate dehydrogenase) and have a sensitivity of 75% and specificity of 89% for mixed malaria, but are also poorly available in some endemic regions. Occult *P. falciparum* infections can be detected 75% of the time with rapid diagnostic tests based on the detection of histidine-rich protein 2 (HRP2), but such tests are of little use in detecting cryptic *P. vivax* infections because of their poor sensitivity. It is common for mixed plasmodial infection to go unrecognized or underestimated, being 4-5 times higher with polymerase chain reaction (PCR) than with light microscopy (LM): 20.22% vs. 3.48%, respectively.

In particular, infection by *P. vivax* can represent a diagnostic challenge in patients with suspected reinfection by the same species vs. mixed malaria, especially in probable cases of hyperreactive malarial splenomegaly, due to the presence of fragmented erythrocytes in the thick blood smear, product of chronic antigenic stimulation, with hypergammaglobulinemia at the expense of IgM, which in turn could be confused with *P. falciparum* gametocytes **(Figure 1)**.

Figure 1

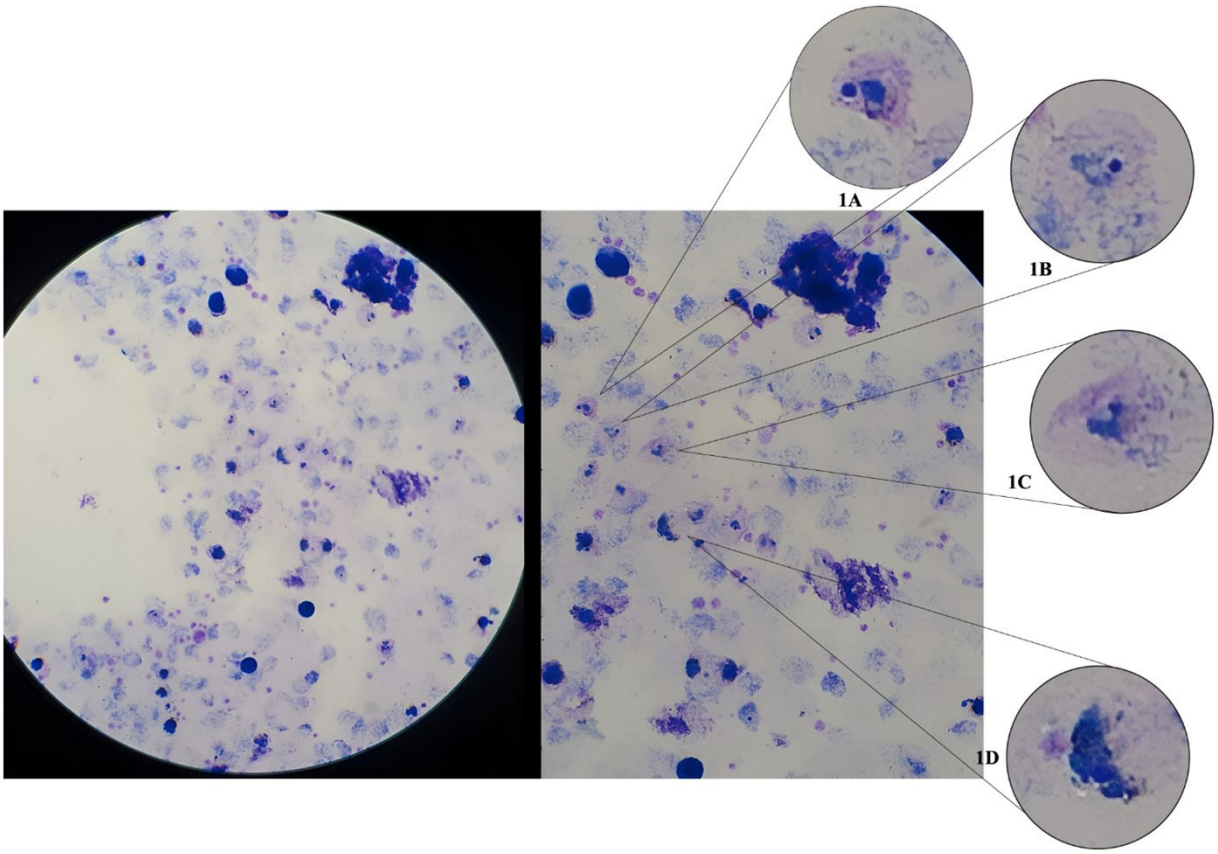


Figure 1. Thick Blood Smear (TBS). Trophozoites with irregular cytoplasm and malarial pigment are observed, in addition to Schüffner granulations characteristic of *Plasmodium vivax*. For the *P. vivax* count, no differentiation is made between the forms or stages observed, counting everything, without differentiation in the report (16.240 fp/mL). **1A.** Schüffner granulations. **1B.** Immature trophozoite with irregular cytoplasm. **1C.** Mature trophozoite. **1D**. Infected erythrocytes may appear fragmented by the hyperfunction of the spleen (reticuloendothelial system), simulating *P. falciparum* gametocytes, in which case the presence of Schüffner's granulations deters the diagnosis by image in favor of *P. vivax* (as in this case), ruling out mixed malaria (*P. vivax* - *P. falciparum*) prima fascie.

Cases of mixed malaria are usually detected after reactivation of an unidentified *Plasmodium spp*. at the start of treatment of a malaria patient. **P. vivax** and *P. ovale* can relapse because of their ability to generate hypnozoites, which can reactivate in numerous scenarios. The reactivation time depends on the drug used: less than 4 weeks with short half-life antimalarials (i.e.,

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artemisinin or quinine derivatives), and between 4 and 6 weeks with long half-life compounds (i.e., mefloquine or chloroquine). Likewise, **P. falciparum** can undergo a similar "reactivation" process in the course of **P. vivax** malaria, the occurrence of which can be explained by two reasons: **1.** erythrocytic asexual forms that do not respond to treatment for **P. vivax** (such as chloroquine) or **2**. errors at the time of diagnosis or a possible prolongation of the intrahepatic phase of *P. falciparum* during mixed infections **[3,4]**.

Mixed plasmodial infection is usually double, but triple and quadruple infections exist. The emergence of one plasmodial species after successful treatment of infection by another species is common. Plasmodial coinfections are mutually suppressive, with *P. falciparum* dominating *P. vivax* and *P. vivax* attenuating the severity of **P. falciparum** infection. The fact that one in five successfully treated *P. falciparum* patients develop a *P. vivax* episode shortly thereafter, and one in ten successfully treated *P. vivax* patients develop a *P. falciparum* episode shortly thereafter, forces one to think about current treatment strategies, especially in areas with a high frequency of mixed malaria **[4]**.

Late identification of coinfection with P. falciparum predisposes to more severe cases due to inappropriate treatment (it may be resistant to chloroquine) and/or more rapid progression to target organ damage due to its avidity for capillary microcirculation in the central nervous system, splanchnic and pulmonary beds, with mortality ranging from 10-40% in non-immune adults. **Table 1** shows the clinical and laboratory criteria for severe malaria **[5]**, according to current clinical practice guidelines. For Colombia, the 2022 epidemiological bulletin reported 71,573 accumulated cases of malaria, of which 98% were classified as uncomplicated malaria, with the following distribution by causal species: *P. vivax*: 60.7% (43,427); *P. falciparum*: 38.3% (27,437); mixed infection (*P. vivax - P. falciparum*): 1.0% (709) **[6]**.

Table 1. Diagnostic criteria for severe malaria. Adapted from: **[5]**

Clinical criteria	Laboratory criteria
Altered state of consciousness	Acidosis (BE >8 mEq/L; HCO3 <15 mmol/L)
Prostration	Hypoglycaemia <49 mg/dL
Seizures >2 in 24h	Severe anaemia (<7 g/dL and <20% + Parasitaemia >10.000/uL)
Jaundice (BT >3 mg/dL + >100 mil/uL)	Kidney injury: Cr >3 mg/dL or BUN >20
Pulmonary oedema	Hyperparasitaemia >10% of parasitised red blood cells (Colombia: >5% or >50.000/uL)
Significant bleeding	
Shock	

It is therefore suggested that the use of the thick blood smear as a diagnostic tool for malaria may be insufficient, leading to underreporting (mainly in cases of mixed malaria) and to limitations in the provision of timely treatment. Therefore, the suspicion of *Plasmodium spp*. in the samples analyzed by this method require, if possible, confirmation with more sensitive techniques such as molecular techniques, which allow an adequate diagnosis of malaria mainly in symptomatic population **[7]** or, failing that, a consensual analysis with laboratory personnel with greater experience in the mounting and reading of this type of samples, which allows elucidating determining details in favor of a species, as in the case of Schüffner's granulations in cases of *P. vivax.*

REFERENCES

1. Varo R, Chaccour C, Bassat Q. Update on malaria. Med Clin (Barc). 2020 Nov 13;155(9):395-402. English, Spanish. doi: 10.1016/j.medcli.2020.05.010. Epub 2020 Jun 30. PMID: 32620355.

2. Savi MK. An Overview of Malaria Transmission Mechanisms, Control, and Modeling. Med Sci (Basel). 2022 Dec 23;11(1):3. doi: 10.3390/medsci11010003. PMID: 36649040; PMCID: PMC9844307.

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3. Mayxay M, Pukrittayakamee S, Newton PN, White NJ. Mixed-species malaria infections in humans. Trends Parasitol. 2004 May;20(5):233-40. doi: 10.1016/j.pt.2004.03.006. PMID: 15105024.

4. Cardona-Arias JA, Salas-Zapata W, Carmona-Fonseca J. A systematic review of mixed studies on malaria in Colombia 1980-2022: what the "bifocal vision" discovers. BMC Public Health. 2023 Jun 17;23(1):1169. doi: 10.1186/s12889-023- 16098-5. PMID: 37330477; PMCID: PMC10276438.

5. White NJ. Severe malaria. Malar J. 2022 Oct 6;21(1):284. doi: 10.1186/s12936-022- 04301-8. PMID: 36203155; PMCID: PMC9536054.

6. Serna-Trejos, Juan Santiago, & Bermúdez-Moyano, Stefanya Geraldine. (2022). Contexto epidemiológico de malaria en Colombia, 2022. Revista Cubana de Medicina, 61(4), . Epub 01 de diciembre de 2022. Recuperado en 17 de mayo de 2024, de http://scielo.sld.cu/scielo.php?script=sci_arttext&pid=S0034-75232022000400018&lng=es&tlng=es.

7. Siwal N, Singh US, Dash M, Kar S, Rani S, Rawal C, Singh R, Anvikar AR, Pande V, Das A. Malaria diagnosis by PCR revealed differential distribution of mono and mixed species infections by Plasmodium falciparum and P. vivax in India. PLoS One. 2018 Mar 22;13(3):e0193046. doi: 10.1371/journal.pone.0193046. PMID: 29565981; PMCID: PMC5863947.