Malaria in the 21st century – still a threatening problem

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SUMMARY
There are six parasite species (P. falciparum, P. vivax, P. ovale curtisi, P. ovale wallikeri, P. malariae, and P. knowlesi) that cause malaria in humans. P. falciparum is responsible for most malaria-related deaths globally. P. vivax is the dominant malaria parasite in most countries outside of the Sub-Saharan Africa. In 2016, 91 countries reported a total of 216 million cases of malaria. The global tally of malaria deaths reached 445,000. In 2016, 24 cases of imported malaria were registered in the Republic of Serbia, with an incidence of 0.33/100,000. According to the World Health Organization recommendations, every suspected malaria case should be confirmed by microscopy or a rapid diagnostic test before treatment. The main stone of antimalarial therapy should be artemisinin-based combinations. Since malaria occurs in Europe as an imported (though rarely also autochthonous and a hospital-borne infection), the objective of this paper is to point out current problems and attitudes in the diagnosis and treatment of malaria, without entering the data field significant for professionals (infectologists, epidemiologists, intensivists).

Keywords: malaria; antimalarials; chemoprophylaxis; laboratory diagnostics

INTRODUCTION
There are six parasite species (P. falciparum, P. vivax, P. ovale curtisi, P. ovale wallikeri, P. malariae, and P. knowlesi) that cause malaria in humans. P. falciparum and P. vivax—pose the greatest threat. P. falciparum is the most prevalent malaria parasite on the African continent. In 2016, 91 countries reported a total of 216 million cases of malaria. The global burden of malaria deaths reached 445,000, mostly children under five years of age [1]. The number of confirmed malaria cases reported in the European Union and the European Economic Area (EU/EEA) from 2008 to 2012 ranged 5,000–7,000 [2]. Since the late 1990s, autochthonous malaria cases occurred in some European countries (Spain, Germany, Netherlands, France, Italy, and Greece) while between January 2016 and April 2018, six sporadic hospital transmissions of malaria were identified in the EU [3].

The last autochthonous case of malaria in former Yugoslavia was registered in 1964. Since then, malaria has been recorded only as an imported, tropical disease. In the 1990–2001 period, 158 cases of imported malaria were registered in the Republic of Serbia, while in the 2001–2009 period, malaria was diagnosed in 102 patients, mainly from the Afro-Asian region [4]. In 2016, 24 cases of imported malaria were registered in the Republic of Serbia, with an incidence of 0.33/100,000 [5]. However, epidemic potential for malaria transmission is relatively small in our community [6, 7].

ACCEPTED DIAGNOSTIC PROCEDURES

According to the World Health Organization (WHO) recommendations, every suspected malaria case should be confirmed by microscopy or a rapid diagnostic test before treatment. Parasitological diagnostics, a classic overview of thin and thick blood smear colored according to Giemsa, remain the “gold standard” of diagnostics. Thin and thick blood smear consists of a thick layer of lysed red blood cells. The blood elements, including parasites, are more concentrated, so the thick blood smear allows a more efficient detection of parasites even in small numbers (increased sensitivity). Morphology and the ratio of parasites to erythrocytes are preserved, so the typical forms of individual parasites can be identified. In the thin blood smear, the degree of parasitemia, the appearance of pigments in leukocytes, the number of thrombocytes, and other possible hematological changes can be assessed as well. A well-educated parasitologist, standardized laboratory procedures, and enough time to review are preconditions for quality performance reviews [8].

Rapid diagnostic tests detect specific antigens (proteins, enzymes) of malaria parasites. Some of the tests can detect only one species (P. falciparum), while others detect multiple species (P. vivax, P. malariae, and P. ovale). Immunochromatographic tests can target the histidine-rich protein 2 of P. falciparum, a pan-malarial plasmodium aldolase, and the...
parasite-specific lactate dehydrogenase. Some studies have found that the sensitivity was 86.7–93.4%, while the specificity was estimated at 98.2–99.3% [8–11].

Quantitative buffy coat method uses a fluorescence technique to detect parasites stained with acridine dye. For precise diagnosis, a check with a classic scanning technique is always recommended [12].

Molecular diagnostics most commonly use polymerase chain reaction (PCR), providing superior specificity and sensitivity compared to other mentioned methods, which is of particular importance in epidemiological and resistance studies [8, 13]. Real-time PCR may be useful as a method complementary to microscopy, particularly in cases of low parasitemia, and for species determination, especially in non- P. falciparum cases, in which most instances of misdiagnosis occur [13].

**ACTUAL RECOMMENDATIONS FOR THERAPY AND PROTECTION**

Actual therapeutic approaches have undoubtedly been marked by new therapeutic protocols. Particularly important items of data are related to the resistance of parasites [14]. Antimalarials come from different chemical structures. The 4-aminoquinolines are chloroquine, quinine, mefloquine, and amodiaquine, while the 8-aminoquinolone is primaquine. The antifolates class of antimalabotile medications such as pyrimethamine, proguanil, and sulfadoxine. The artemisinin derivatives (artesiminin, artesunate, arteether) are sesquiterpene lactones, obtained from the plant Artemisia annua. They are sesquiterpene lactone containing an unusual peroxide bridge. Artemisinins are considered prodrugs activated to generate carbon-centered free radicals or reactive oxygen species, and are the most potent antimalarial agents, effective against nearly all asexual and sexual parasite stages [20].

Artemisinin component in ACT (artemether, artesunate, or dihydroartemisinin) drastically reduces the number of parasites during the first three days of treatment, but potential disadvantage may be a higher risk of recrudescence when these drugs are used in monotherapy regimens. Recrudescence signifies the emergence of a clinical picture from parasites that persist in erythrocytes after the initial treatment. This is why drugs from other antimalarial groups are added, which eliminate the remaining parasites and in that way prevent recrudescent malaria [20].

In Serbia, malaria is treated in infectious departments of tertiary medical institutions, adapted to the WHO’s advice. Unfortunately, due to low consumption, most antimalarial drugs are not registered, so procurement takes place according to special procedures. Artemisinin-based treatment is the cornerstone for therapeutic approach, while artesunate is the preferred therapy for treatment of severe falciparum malaria.

**Table 1.** Treating uncomplicated *P. falciparum* malaria [16] – reproduced with WHO permission

| Treatment of uncomplicated *P. falciparum* malaria | Strong recommendation, high-quality evidence |
|---------------------------------------------------|---------------------------------------------|
| Treat children and adults with uncomplicated *P. falciparum* malaria (except pregnant women in their first trimester) with one of the following recommended ACTs: | |
| · artemether + lumefantrine | |
| · artesunate + amodiaquine | |
| · artesunate + mefloquine | |
| · dihydroartemisinin + piperaquine | |
| · artesunate + sulfadoxine–pyrimethamine (SP) | |
| **Duration of ACT treatment** | |
| ACT regimens should provide a 3-day treatment with an artemisinin derivative | |
| **Revised dose recommendation for dihydroartemisinin + piperaquine in young children** | |
| Children weighing < 25 kg treated with dihydroartemisinin + piperaquine should receive a minimum of 2.5 mg/kg of body weight per day of dihydroartemisinin and 20 mg/kg of body weight per day of piperaquine for 3 days | |

**ACT** – artemisinin-based combination therapy

intravascular coagulation, acute kidney injury, seizures, and severe infections, even with sepsis.

ACT is the mainstay of modern therapeutic protocols. Artemisinin and its semisynthetic derivatives, such as artesunate, artemether, and arteether dihydroartemisinin, are obtained from the plant *Artemisia annua*. They are sesquiterpene lactone containing an unusual peroxide bridge. Artemisinins are considered prodrugs activated to generate carbon-centered free radicals or reactive oxygen species, and are the most potent antimalarial agents, effective against nearly all asexual and sexual parasite stages [20].

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Side effects of artesinin occur rarely (3.4%). However, the greater concern is related to hemolysis which occurs in approximately 10–15% patients, and even more following intravenous artesunate treatment [21]. Delayed-onset anemia or postartesunate late hemolysis has been observed to occur two to three weeks following the initiation of IV artesunate, after complete parasite clearance, but this phenomenon is also described after oral administration of artemisinin drugs. Although there is no complete explanation
for this phenomenon, it unconditionally requires additional differential diagnostic and therapeutic efforts. Artemisinin resistance is a rare phenomenon, but the releases in the literature are found more often [22]. According to Centers for Control and Disease Prevention recommendations, chloroquine (or hydroxychloroquine) remains an effective choice for \( P. \) vivax and \( P. \) ovale infections. After the treatment of \( P. \) vivax / \( P. \) ovale infection, primaquine should be used, or recently introduced tafenoquine, due to the effects on hypnozoites in the liver, left after treatment, thus preventing malaria relapses [23].

It is said that 13 drugs are in advanced research development, two of which are in the advanced, final phase – artemefomel–ferroquine and lumefantrine-KAF156 [24].

Arterolane is a newer synthetic peroxide resembling the artemisinin derivative. Arterolane maleate and piperaquine effectively cures \( P. \) falciparum malaria by day 28 in pediatric patients, which justifies the clinical application of this combination [24, 25].

The US Food and Drug Administration approved tafenoquine for the prevention of relapse of \( P. \) vivax malaria on July 20, 2018. Tafenoquine, an 8-aminoquinoline, is used as a single-dose treatment for \( Plasmodium \) vivax relapse prevention. Administration of this drug, as well as primaquine, follow the same restriction and adverse events (glucose-6-phosphate dehydrogenase deficiency) [26].

**CHEMOPROPHYLAXIS**

Experiences of European authors show that only 10% of patients with severe malaria had taken antimalarial chemoprophylaxis and very few of them had been fully compliant [17].

The most commonly recommended regimen of chemoprophylaxis are as follows: doxycycline 100 mg once daily (started one day before traveling, and continued for four weeks after returning); mefloquine 250 mg once weekly (started 2.5 weeks before traveling, and continued for four weeks after returning); atovaquone/proguanil one tablet daily (started one day before traveling, and continued for one week after returning) [27].

Among the recommended drugs, the atovaquone–proguanil combination is the most justified one, especially in regions where there is a multi-resistant malaria. The impact of substituting atovaquone–proguanil for all mefloquine use resulted in a 2.3% decrease in estimated infections [28].

Advice on the protection from mosquito bites (repellents, insecticide impregnated net beds, etc.) are certainly an important part of the protection.

The vaccine remains an unfulfilled dream, although work on it is still being carried out with great enthusiasm today. In July 2015, the Committee for Medicinal Products for Human Use of the European Medicines Agency gave a positive opinion for the “candidate vaccine” Mosquirix. The vaccine is awaiting the final response from the WHO and African health authorities, with whose approval Phase III of its examination has been conducted [29]. The latest information favors the vaccine which consists of the central repeat the C-terminal domain of \( Plasmodium \) falciparum circumsporozoite protein, fused to hepatitis B virus surface antigen (HBsAg) in a 1:4 ratio. This vaccine demonstrated protective efficacy against clinical malaria in Phase III clinical trial [30].

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**REFERENCES**

1. World Health Organization. Guidelines for the treatment of malaria, third edition [internet]. WHO 2017 (cited 2018 Sept 8); Available from: http://www.who.int/malaria/publications/atoz/9789241549127/en/

2. Papareki T, Daikos G. Malaria in Europe: emerging threat or minor nuisance. Clin Infect Dis. 2016; 26(6):487–93.

3. Hospital-acquired malaria infections in the European Union – 30 April 2018, Stockholm, 2018 [internet]. RAPID RISK ASSESSMENT [cited 2018 Sept 28]; Available from: https://ecdc.europa.eu/en/publications-datarapid-risk-assessment-hospital-acquired-malaria-infections-european-union#

4. Dakić Z, Pelemis M, Djurković-Djakovic O, Lavadinovj L, Nikolić A, Stevanović G, et al. Imported malaria in Belgrade, Serbia, between 2001 and 2009. Wien Klin Wochenschr. 2011; 123 Suppl 1:15–9.

5. Institut za javno zdravlje Srbije, Dr Milan Jovanovic Batur. "Medical examination of zoonotic bolesti um Republici Srbiji za 2016. godinu [internet]. Urednik prim dr sc. med. Verica Jovanovic, Beograd 2017, str. 48–9. [cited 2018 Sept 26]; Available from: http://www.batut.org.rs/download/izvestaji/zaranezbolestiGodisnjizvestaj2016.pdf

6. Dakić Z, Kulisic Z, Stajkovic N, Pelemis M, Cobeljic M, Stanimirovic Z, et al. Ecology of Anopheles mosquitoes in Belgrade area: estimating vector potential for malaria retransmission. Acta Vet. 2008; 58(5–6):603–14.

7. Kavran M, Zgomba M, Weitze D, Petric D, Manz C, Becke N. Distribution of Anopheles daciae and other Anopheles maculipennis complex species in Serbia. Parasitol Res. 2018; 117(10):3277–87.

8. Mukry NS, Saud M, Sufaida GM, Shamsi TS. Laboratory diagnosis of malaria: comparison of manual and automated diagnostic tests. Can J Infect Dis Med Microbiol. 2017; 57.

9. Manjunath P, Salmani, Peerapur BV. Comparative study of peripheral blood smear, QBC and antigen detection in malaria diagnosis. J Clin Diagn Res. 2011; 5(5):967–9.

10. Wilosn LM. Malaria rapid diagnostic tests. Clin Infect Dis. 2012; 154(11):1637–41.

11. Centers for Control and Disease Prevention (CDC). Rapid diagnostic tests: how they work [internet]. CDC, 2015 (cited 2018 Sept 8); Available from: https://www.cdc.gov/malaria/malaria_worldwide/reduction/dx_rdt.html

12. Kuladeepa VA, Sukesh A. Quantitative buffy coat (QBC) test and other diagnostic techniques for diagnosing malaria: Review of literature. Natl J of Med Res. 2012; 2(3):386–8.

13. Dakić Z, Ivočić V, Pavlović M, Lavadinovj L, Marković M, Djurković-Djakovic O. Clinical significance of molecular methods in the diagnosis of imported malaria in returning travelers in Serbia. Int J Infect Dis. 2014; 29:24–30.

14. Eyasu M. Antimalarial drug resistance: In the past, current status and future perspectives. Br J Pharmacol Toxicol. 2015; 6(1):1–15.

15. Shreekant D, Bhimanna K. 4-aminoquinolines: An overview of antimalarial chemotherapy. Med chem. 2016; 6:001–011.

16. WHO. Guidelines for the treatment of malaria, third edition. Geneva: World Health Organization (WHO); 2015. (http://www.who.int/malaria/publications/atoz/9789241549127/en/)

17. Bouchaud O, Mühlberger N, Parola P, Calleri G, Matteelli A, Peyerl-Čanović P et al. Therapy of uncomplicated falciparum malaria in Europe: MALTHER – a prospective observational multicentre study. Malar J. 2012; 11(1):1–15.

18. Kurth F, Develoux M, Mechain M. Severe malaria in Europe: an 8-year multi centre observational study. Malar J. 2017; 16(1):57.

19. Centers for Control and Disease Prevention (CDC). Rapid diagnostic tests: how they work [internet]. CDC, 2015 (cited 2018 Sept 8); Available from: https://www.cdc.gov/malaria/malaria_worldwide/reduction/dx_rdt.html

20. Kuladeepa VA, Sukesh A. Quantitative buffy coat (QBC) test and other diagnostic techniques for diagnosing malaria: Review of literature. Natl J of Med Res. 2012; 2(3):386–8.

21. Dakić Z, Ivočić V, Pavlović M, Lavadinovj L, Marković M, Djurković-Djakovic O. Clinical significance of molecular methods in the diagnosis of imported malaria in returning travelers in Serbia. Int J Infect Dis. 2014; 29:24–30.

22. Eyasu M. Antimalarial drug resistance: In the past, current status and future perspectives. Br J Pharmacol Toxicol. 2015; 6(1):1–15.

23. Shreekant D, Bhimanna K. 4-aminoquinolines: An overview of antimalarial chemotherapy. Med chem. 2016; 6:001–011.

24. WHO. Guidelines for the treatment of malaria, third edition. Geneva: World Health Organization (WHO); 2015. (http://www.who.int/malaria/publications/atoz/9789241549127/en/)

25. Bouchaud O, Mühlberger N, Parola P, Calleri G, Matteelli A, Peyerl-Hoffmann G, et al. Therapy of uncomplicated falciparum malaria in Europe: MALTHER – a prospective observational multicentre study. Malar J. 2012; 11:212.
19. God PG, Frey A, Eisenhut M. Artemisinin derivatives versus quinine in treating severe malaria in children: a systematic review. Malar J. 2008; 7:210.

20. Cu L, Su Xi Z. Discovery, mechanisms of action and combination therapy of artemisinin. Expert Rev Anti Infect Ther. 2009; 7(8):999–1013.

21. Laloo DG, Shingadia D, Bell DJ, Beeching NJ, Whitty CJM, Chiodini PL; PHE Advisory Committee on Malaria Prevention in UK Travellers. UK malaria treatment guidelines 2016. J Infect. 2016; 72(6):635–49.

22. Baird KJ. Malaria caused by Plasmodium vivax: recurrent, difficult to treat, disabling, and threatening to life – averting the infectious bite preempts these hazards. Pathog Glob Health. 2013; 107(8):475–9.

23. Ashley EA, Dhorda M, Fairhurst RM, Amaratunga C, Lim P, Suon S, et al. Spread of artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med. 2014; 371(5):411–23.

24. Ashle AE, Pyae AP. Drugs in development for malaria. Drugs. 2018; 78(9):861–79.

25. Toure OA, Rulisa S, Anupkumar R, Anvikar AR. Efficacy and safety of fixed dose combination of arterolane maleate and piperaquine phosphate dispersible tablets in paediatric patients with acute uncomplicated Plasmodium falciparum malaria: a phase II, multicentric, open label study. Malar J. 2015; 14:469.

26. Watson J, Taylor WR, Bancone G, Bancone G, Chu CS, Jittamala P, et al. White implications of current therapeutic restrictions for primaquine and tafenoquine in the radical cure of vivax malaria. Open access, Research Article Published: April 20, 2018.

27. Schwartz E. Prophylaxis of malaria. Mediterr J Hematol Infect Dis. 2012; 4(1):e2012045.

28. Toovey S, Nieforth K, Smith P, Schlagenhauf P, Adamcova M, Tatt I. Comparative benefit of malaria chemoprophylaxis modelled in United Kingdom. Travel Med Infect Dis. 2014; 12(6):726–32.

29. Wilby KJ, Lau TT, Gilchrist SE. Mosquirix (RTS, S): A novel vaccine for the prevention of Plasmodium falciparum Malaria. Ann Pharmacother. 2012; 46(3):384–93.

30. Draper SJ, Sack BK, King CR, Nielsen CM, Rayner JC, Higgins MK, et al. Malaria vaccines: recent advances and new horizons. Cell Host Microbe. 2018; 24(1):43–56.

САЖЕТАК
Постоји шест врста паразита рода Plasmodium (P. falciparum, P. vivax, P. ovale curtisi, P. ovale vallikeri, P. malariae и P. knowlesi) који узрокују маларију код људи. P. falciparum је одговоран за већину смртних случајева везаних за маларију. P. vivax је доминантни паразит маларије у већини земаља изван подсахранске Африке. У 2016. години 91 земља је пријавила укупно 216 милиона оболелих од маларије. Број смртних случајева у 2016. години је 445.000. У 2016. години у Србији су регистрована 24 оболела од маларије (учесталост 0,33/100.000). У складу са препорукама WHO, свака сумња на маларију треба да се потврди микроскопијом или брзим дигностичким тестом пре лечења. Главни ослонац антималаричне терапије треба да буду комбинације са артемисинином. Будући да се маларија у великом броју европских земаља јавља као унесена (мада ретко и као аутохтона и болнички стечена инфекција), циљ овог рада је упознавање са актуелним проблемима и ставовима у дијагностици и лечењу маларије, без упуштања у детаљне значајне за професионале који се овим проблемима посебно баве (инфеколози, епидемиолози, интензивисти).

Кључне речи: маларија; антималарици; хемопрофилакса; лабораторијска дијагностика

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Маларија у 21. веку – и даље претећи проблем

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