Study protocol

Infections in migrants: the importance of malaria and other parasites

2021-08-01

Study protocol

Infections in migrants: the importance of malaria and other parasites

Overall aim

The purpose of this project is to improve the health in migrants and immigrants focusing on malaria and other parasitic infections.

Specific aims

1. To assess the parasite prevalence in newly arrived migrants and the need to introduce screening for malaria and other parasites in migrants from certain countries.
2. To assess the parasite prevalence and specific antibody responses to malaria and other parasitic infections in immigrants who live in Sweden.

Background

Malaria is a major threat to human health, especially in Sub-Saharan Africa [1]. Global migration has resulted in a surge of refugees and asylum applications in Europe, many from malaria endemic countries.

The symptoms of malaria range from mild febrile illness to life-threatening multi-organ failure [3]. Disease severity is influenced by several host-parasite interactions, where host immunity is believed to one of the most important [4].

Immunity to malaria is gradually acquired after repeated infections in populations living in high-endemic areas [4]. However, sterilizing immunity does not seem to evolve, and asymptomatic low-level infections are common. Individuals may carry a low grade parasitemia for extended periods of time [5]. Chronic parasitemia may lead to anaemia [6], cognitive dysfunction [7], adverse events during pregnancy [8] and may have hidden cost on telomere degradation and ageing [9].

Migrants from malaria endemic areas may present with febrile malaria but may also harbour asymptomatic malaria infection. Moreover, other parasitic infection can cause silent infections with adverse effect on health, such as schistosomiasis causing bladder calcification and strongyloides that may progress to a life-threatening disease called strongyloides hyperinfection, especially in patients receiving corticosteroids or other immunomodulatory treatment.

Thus, estimating the burden of malaria and other parasitic infections in newly arrived migrants as well as in immigrants with longer residency in Sweden is important in order to improve health in these populations.

Previous studies have reported 3-30% parasite prevalence in asymptomatic refugees from malaria endemic areas [11-14]. In Sweden, health screening is offered to newly arrived immigrants, including HIV, tuberculosis and viral hepatitis. Currently, malaria is not included in the screening program in

Sweden, although other countries recommend screening [15-17] and in the US, presumptive treatment is offered without testing [18]. Neither is there any regular testing for other parasites, not even preceding long term corticosteroid treatment.

Moreover, duration of asymptomatic parasite carriage in non-endemic countries is not fully established, however, case reports suggest that infections may persist for several years [20].

The immunologic response to malaria infection consists largely of antibodies towards a variety of parasite antigens, and develops over the course of several symptomatic malaria infections [22]. In highly endemic areas, immunity gradually protects against the severe forms of the disease and on further exposure, protection against clinical symptoms evolves [4, 22]. However, the protective immunity wanes over time with ceased exposure and thus previously protected individuals living in non-endemic countries are at risk for developing a more severe disease upon re-exposure for example when travelling to visit friends and relatives [23]. The longevity of circulating antibodies towards malaria is not fully elucidated, and therefore the risk cannot be fully appreciated.

This project focuses on the burden of malaria and other parasitic infections in newly arrived migrants and immigrants with longer residency in Sweden. We will evaluate the parasite prevalence and if screening for malaria and other parasites should be offered in migrants from Sub-Saharan Africa. In addition, we will assess longevity of malaria-specific immune responses as well as how migrants navigate through the Swedish health system.

Study design: Cross-sectional observational study.

Study population:

1. Newly arrived migrants born in countries where malaria is endemic (ie Sub-Saharan Africa) will be invited to participate in the study at migration health care units in Stockholm and Västerås. Blood samples will be collected at the time of routine sampling. According to sample size calculation based on expected 5% prevalence (see below) a total of 715 individuals will then be included in total. This number will be adjusted based on results in the pilot study.
2. Immigrants from malaria endemic countries living in Sweden will be invited to participate with voluntary blood sampling on one occasion. The study will be advertised in posters in waiting areas at health clinics in Stockholm.
3. Letter invitations to indivudials arriving in Sweden from Democratic Republic of the Congo and Uganda between 2015 and 2019 and with a postal address in the Stockholm County, will be sent an invitation letter offering to participate in the study.

Inclusion criteria: Born in a malaria endemic country, defined as a country with indigenous spread of malaria reported in the World Malaria Report (WHO) 2019.

Exclusion criteria: Inability to understand patient study information or inability sign the informed consent form, with exception to children under 15 where legal guardian may approve and sign consent.

Sampling and analyses: A venous blood samples (EDTA tube) will be collected on one occasion. To minimize the amount of venipunctures and time for the study participants, blood samples will be collected on the same occasion as other blood samples taken within the routine screening program for newly arrived migrants. A short questionnaire including questions about patient origin and previous malaria as well as other diseases and previous treatment will be completed at the time of sampling with the aid of a translator if needed.

Blood samples will be sent to the research laboratory for malaria diagnostics and haemoglobin concentration. A rapid diagnostic test (RDT) will be performed for malaria. The sample will thereafter be centrifuged, aliquoted, and stored frozen at the research laboratory. Presence of parasites will be analysed by realtime-PCR of the species specific 18SRNA gene in a multiplex assay [25]. In study population 4, malaria microscopy will be performed at the same time as RDT to increase sensitivity.

RDT is less sensitive than microscopy and PCR but offers a rapid result, the same day with this set up, and all positive individuals will be referred to the Department of Infectious Diseases at the regional hospital for further investigations and treatment. Likewise, all individuals found to be positive for malaria by microscopy and/or PCR at a later stage will be contacted and referred for complete assessment and treatment at the Department of Infectious Diseases. Results from complete blood count will be assessed and followed up according to clinical routine at each centre where samples were collected.

Contact information to the study participant will be included as part of the recruitment and consent procedures in order to be able to contact the study participants for referral.

Complementary to the proposed study, health economic aspects of malaria screening will be assessed for cost-effectiveness estimation.

Moreover, PBMC will be prepared from a subset of patients to assess the cellular immune response. Plasma samples will be analyzed for the presence and levels of antibodies against crude whole *P. falciparum* parasite extract using ELISA, and against a panel of specific pre-hepatic and blood stage antigens using a multiplex Luminex [26]. The magnitude and breadth of antibody responses will be related to duration of residency in a malaria free country. This will allow us to estimate the long-term decay kinetics using a sero-epidemiological mathematical model [27]. In addition, serological markers for schistosomiasis and strongyloides will be analysed at the Public Health Agancy of Sweden.

A subset of volunteers among immigrants living in Sweden (n=100) will be asked to contribute with a second blood sample after 6-12 months. This will allow us to estimate the long- term decay kinetics using a sero-epidemiological mathematical model [27].

Statistical analysis

The analyses will be performed using Stata version 14.2 (StataCorp, College Station, TX, USA). Categorical variables will be summarized by proportions, and the numerical variables by medians and interquartile ranges (IQR). In comparative analyses, categorical variables will be analyzed using the Pearson’s chi-squared test, or the Fisher's exact test when appropriate. The Mann-Whitney's U-test will be used for comparing the distribution of numeric variables between two groups and the Kruskal-Wallis's test among multiple groups. Backward stepwise univariable logistic regression will be used to potential risk factors for parasitic infection. The variables with a p-value less than 0.05 will be

kept in the final multivariable model. The Wald's test will be used to test the regression coefficients. P-values less than 0.05 were considered statistically significant.

Significance

Migration health and integration is of great concern and importance. The level of asymptomatic parasite carriage is unknown in migrants arriving to Sweden, however, chronic asymptomatic malaria may later on result in symptomatic disease or cause another burden on the host. Thus, the findings in this project have the potential to guide the prevention and management of malaria in migrants.

Establishing the longevity and breadth of immune responses to malaria will contribute to vaccine design and will also help identification of populations at risk in areas of declining transmission and optimisation of sero-epidemiological models.

Results from the questionnaire will be able to guide improved web-based information to immigrants about health care of febrile illness after tropical visits and in newly arrived migrants. The research and dissemination of results will improve the management of fever and malaria in high risk groups including children, adults and pregnant women originating from malaria endemic areas.

Ethical considerations

There are several ethical considerations in the proposed research project, related both to the study design and features of the study population. The study is performed on human volunteers after informed consent. However, migrants arriving in Sweden from malaria endemic countries come from diverse backgrounds and experiences of health care in their former home countries that could significantly differ from the organisation of the Swedish health care system. In this context, information about the project and how it is different from the offered health care (screening for other diseases) needs extra attention.

We believe that bridging the cultural and language barriers will be possible with the aid of translators that are present during the health check-ups in the clinic. We have previously experienced that malaria is a well-known disease among individuals originating in malaria endemic areas and that it is not associated with any significant stigma, which will facilitate bilateral understanding.

Furthermore, advantages of better malaria interventions justify blood sampling with only minor discomfort. The possible benefits of the study will favour future migrants from malaria endemic countries.

References

1. *World Malaria Report 2016*. 2016, WHO.
2. SCB. Available from: http://www.scb.se/contentassets/ae2169ea762240feadca8779dc3662a0/be0101-utrikes-fodda-fland-invar-2016.xlsx.
3. White, N.J., et al., *Malaria.* Lancet, 2014. **383**(9918): p. 723-35.
4. Doolan, D.L., C. Dobano, and J.K. Baird, *Acquired immunity to malaria.* Clin Microbiol Rev, 2009. **22**(1): p. 13-36, Table of Contents.

5. Babiker, H.A., et al., *Characteristics of Plasmodium falciparum parasites that survive the lengthy dry season in eastern Sudan where malaria transmission is markedly seasonal.* Am J Trop Med Hyg, 1998. **59**(4): p. 582-90.
6. Sifft, K.C., et al., *Asymptomatic only at first sight: malaria infection among schoolchildren in highland Rwanda.* Malar J, 2016. **15**(1): p. 553.
7. Fernando, S.D., C. Rodrigo, and S. Rajapakse, *The 'hidden' burden of malaria: cognitive impairment following infection.* Malar J, 2010. **9**: p. 366.
8. Okell, L.C., et al., *Factors determining the occurrence of submicroscopic malaria infections and their relevance for control.* Nat Commun, 2012. **3**: p. 1237.
9. Asghar, M., et al., *Chronic infection. Hidden costs of infection: chronic malaria accelerates telomere degradation and senescence in wild birds.* Science, 2015. **347**(6220): p. 436-8.
10. *World malaria report 2017*. 2017, World Health Organization: Geneva.
11. Marangi, M., et al., *Prevalence of Plasmodium spp. in malaria asymptomatic African migrants assessed by nucleic acid sequence based amplification.* Malar J, 2009. **8**: p. 12.
12. Matisz, C.E., et al., *Post-arrival screening for malaria in asymptomatic refugees using real-time PCR.* Am J Trop Med Hyg, 2011. **84**(1): p. 161-5.
13. Maroushek, S.R., et al., *Malaria among refugee children at arrival in the United States.* Pediatr Infect Dis J, 2005. **24**(5): p. 450-2.
14. Monge-Maillo, B. and R. López-Vélez, *Migration and Malaria in Europe.* Mediterranean Journal of Hematology and Infectious Diseases, 2012. **4**(1): p. e2012014.
15. Karki, T., et al., *Screening for infectious diseases among newly arrived migrants in EU/EEA countries--varying practices but consensus on the utility of screening.* Int J Environ Res Public Health, 2014. **11**(10): p. 11004-14.
16. Monge-Maillo, B. and R. Lopez-Velez, *Is screening for malaria necessary among asymptomatic refugees and immigrants coming from endemic countries?* Expert Rev Anti Infect Ther, 2011. **9**(5): p. 521-4.
17. Chaves, N.J., et al., *The Australasian Society for Infectious Diseases and Refugee Health Network of Australia recommendations for health assessment for people from refugee-like backgrounds: an abridged outline.* Med J Aust, 2017. **206**(7): p. 310-315.
18. Stauffer, W.M., et al., *Pre-departure and post-arrival management of P. falciparum malaria in refugees relocating from sub-Saharan Africa to the United States.* Am J Trop Med Hyg, 2008. **79**(2): p. 141-6.
19. Njama-Meya, D., M.R. Kamya, and G. Dorsey, *Asymptomatic parasitaemia as a risk factor for symptomatic malaria in a cohort of Ugandan children.* Trop Med Int Health, 2004. **9**(8): p. 862-8.
20. Szmitko, P.E., M.L. Kohn, and A.E. Simor, *Plasmodium falciparum malaria occurring 8 years after leaving an endemic area.* Diagn Microbiol Infect Dis, 2009. **63**(1): p. 105-7.
21. Cowman, A.F., et al., *Malaria: Biology and Disease.* Cell, 2016. **167**(3): p. 610-624.
22. Langhorne, J., et al., *Immunity to malaria: more questions than answers.* Nat Immunol, 2008. **9**(7): p. 725-32.
23. Farnert, A., et al., *Duration of residency in a non-endemic area and risk of severe malaria in African immigrants.* Clin Microbiol Infect, 2015. **21**(5): p. 494-501.
24. Askling, H.H., et al., *Travellers returning to Sweden with falciparum malaria: pre-travel advice, behaviour, chemoprophylaxis and diagnostic delay.* Scand J Infect Dis, 2005. **37**(10): p. 760-5.
25. Shokoples, S.E., et al., *Multiplexed real-time PCR assay for discrimination of Plasmodium species with improved sensitivity for mixed infections.* J Clin Microbiol, 2009. **47**(4): p. 975-80.
26. Rono, J., et al., *Breadth of anti-merozoite antibody responses is associated with the genetic diversity of asymptomatic Plasmodium falciparum infections and protection against clinical malaria.* Clin Infect Dis, 2013. **57**(10): p. 1409-16.
27. Yman, V., et al., *Antibody acquisition models: A new tool for serological surveillance of malaria transmission intensity.* Sci Rep, 2016. **6**: p. 19472.