Adjusting for Heterogeneity of Malaria Transmission in Longitudinal Studies

Teun Bousema,[1] Benno Kreuels,[2] and Roly Gosling[3,4]

[1]Department of Immunity and Infection, London School of Hygiene & Tropical Medicine, United Kingdom; [2]Section for Tropical Medicine, 1st Medical Department, University Medical Centre Hamburg-Eppendorf, Hamburg, Germany; [3]Department of Disease Control, Faculty of Infectious and Tropical Diseases, London School of Hygiene & Tropical Medicine, United Kingdom; [4]Global Health Group, University of California, San Francisco

(See the article by Bejon et al, on pages 9–18.)

Although the heterogeneity of malaria transmission has been known for many years, it has only recently become recognized as having importance in the analysis of longitudinal studies [1]. Assumptions of homogenous transmission (ie, that every participant in a given study is exposed to the same number of infectious bites) can lead to incorrect conclusions (eg, that placental malaria is a risk factor for childhood malaria or that parasitemia is protective against clinical malaria) [2–3]. In both these cases, associations have been confused with a causal pathway, and the outcomes have been confounded by the heterogeneity of risk in malaria transmission. Persons living in an area of higher transmission are more likely to develop malaria again than are persons living outside that locality. In turn, after repeated attacks, these persons will develop immunity against clinical disease and become asymptomatic carriers of infection. Infection itself is not the cause of the observed association, but rather exposure to repeated infections in the area of residence. In this issue of the *Journal*, 2 original articles have examined the effects of heterogeneity in malaria transmission in great depth, using cohort studies to determine how best to consider variation in risk when analyzing studies. One, an observational cohort [4], and the other, a cohort in a vaccine study [5], use measurements of antibodies as their outcome measures.

ANTIBODY RESPONSES: MARKERS OF PROTECTION AND OF EXPOSURE

There are particular problems when using immune markers as outcome measures, because antibody responses are both correlated with malaria exposure and clinical protection. If variation in malaria exposure is not taken into account during the analysis of data, the paradoxical finding that antibodies, which should be markers of protection, are associated with an increased risk of clinical malaria may occur [4, 6]. The studies described by Greenhouse et al [4] and Bejon et al [5] present exceptionally detailed information on malaria incidence and geographical risk factors for malaria and use repeated assessments of antibody responses to a number of malaria antigens. Despite this level of detail, both studies reveal how difficult it is to adjust for variation in malaria exposure when examining immune correlates of protection.

Variation in malaria exposure is present at micro-epidemiological levels at low [7–8], moderate, and high transmission intensity [9–11]. The causes underlying small-scale variations in malaria exposure are complex and include environmental factors, such as altitude, cultivation practices, urbanization, and distance from bodies of water that are suitable for *Anopheles* propagation; household structure (eg, roofing material, presence of eaves under the roof and wall material); and the use of protective measures against mosquitoes, such as insecticide-treated bed-nets (ITNs) [7, 9, 11–12]. Human genetic factors influence the risk of malaria [12] and potentially other factors (eg, interindividual variation in attractiveness to mosquitoes [13], gametocyte development [14] and gametocyte transmissibility [15]); all of these factors influence the micro-epidemiology of malaria.

ADJUSTING FOR HETEROGENEITY IN MALARIA EXPOSURE

Regardless of the underlying causes, many of which are site specific, practical

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Correspondence: Teun Bousema, PhD, London School of Hygiene & Tropical Medicine, Keppel St, London, WC1E 7HT, UK (Teun.Bousema@lshtm.ac.uk).

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methods to adjust for differential exposure to malaria transmission must be found. Bejon et al [5] and Greenhouse et al [4] initially included previous malaria incidence in their models. This is the most direct manner to adjust for intra-individual variation in malaria exposure. However, both groups observed that this was insufficient to adjust for all heterogeneity in malaria exposure; distance to the swamp [4], living in a hotspot of malaria transmission [5], or baseline antibody concentrations [5] remained significant risk factors for malaria during follow-up, after adjustment for an individual's history of malaria attacks. The number of documented previous malaria episodes did not explain all variation in antibody concentrations. The best-fitting model was identified when the authors restricted their analysis to include only individuals with proven malaria exposure during the evaluation phase by excluding all individuals [5] or person-months [4] during which no parasitemia was detected. This resulted in the effect estimates changing considerably. In both studies, antibody responses against blood stage antigens were initially associated with an increased risk of clinical malaria [4–5]. However, when the analyses were restricted to individuals with parasitemia, both studies showed that higher levels of antibodies were associated with a decreasing risk for clinical episodes of malaria and increasing asymptomatic carriage of malaria. In the article by Bejon et al [5], this analysis allowed the authors to show that vaccination with the pre-erthrocytic vaccine RTS,S/AS01E and sleeping under an ITN were independent risk factors for having clinical malaria attacks, as opposed to having asymptomatic parasitemia. This was interpreted as evidence that both RTS,S/AS01E and ITNs protect against blood-stage infection, and when these children were exposed to blood-stage infection, they lacked protective antibodies and became symptomatic. In contrast, those who were not protected by vaccine and ITNs had more exposure to malaria and, therefore, had protective antibodies and were more likely to remain asymptomatic if infected.

The value of considering individuals who remain parasite free as nonexposed and excluding them from analysis when assessing immune correlates of protection has been shown in several studies [1, 4–6]. This is the most uncomplicated manner to remove some of confounding that arises by differential exposure to malaria and allows a re-analysis of previously conducted and published trials, which we strongly support.

TOWARD A QUANTITATIVE ASSESSMENT OF INDIVIDUAL MALARIA EXPOSURE

There are, however, limitations in the extent to which excluding nonparasitemic individuals from analyses will solve the problem of heterogeneity in malaria exposure. To conclude that all individuals who remain parasite free during follow-up were truly nonexposed, researchers should consider frequent sampling to not miss asymptomatic infections that are cleared between scheduled follow-up visits. Even if participants are examined frequently, not all parasitemic episodes will be detected if microscopy is used for parasite detection [16] and, as Greenhouse et al [4] correctly mention, this qualitative evidence of exposure does not take varied degrees of exposure into account. It will not differentiate between individuals whose parasite carriage is the result of a single inoculum or of multiple infections, although it is clearly relevant to include an individual's level of exposure to malaria. Thus, additional methods to adjust for heterogeneity in exposure should be explored.

The ideal evaluation for studies assessing protection against clinical malaria episodes should determine what proportion of malaria exposures leads to a clinical malaria episode. A direct determination of sporozoite inocula would give this answer but is currently impossible. Intensive sampling of mosquitoes with use of exit traps to sample mosquitoes after they have searched for a blood meal is laborious and costly for all cohort participants but could provide valuable and detailed information on exposure. Another approach would be detection and characterization of each parasite strain causing blood-stage infection with use of molecular methods. The success of this approach would depend on the frequency of sampling and on the sensitivity of molecular methods to detect and distinguish parasite isolates in mixed-clone infections [17–18], with minority variants [19] and submicroscopic infections [16]. Pre-erythrocytic immunity can also make the estimates of exposure incomplete. A third possibility is to use serological markers of exposure. Serological markers of exposure have previously been used to assess small-scale variation in malaria transmission [9–10, 12] but have several disadvantages. First, the strong colinearity between antibody concentrations and clinical immunity makes it difficult to find markers of exposure that do not affect the analysis of correlates of protection. Second, to reliably indicate malaria exposure during a specific period of follow-up, antigens must be highly immunogenic, resulting in measurable antibody concentrations in all exposed individuals, and short lived, indicating exposure in the previous weeks or months. A last approach and one that is perhaps most user friendly would be to define different exposure zones in an area. Malaria exposure may differ between subvillages [5, 12] and by distance to the fringe of the forest [11] or swamp [4]. Incorporating this element in models, together with the exclusion of parasite-free individuals or person-months, may further improve effect estimates.

In conclusion, the studies in Uganda and Kenya reported in this issue show that it is possible to account for part of the heterogeneity in malaria exposure

and allow for conclusions on data to be made. Additional studies need to be performed to clarify the relationships between exposure to infection of malaria and immunity; however, the authors of these studies have shown that some tools exist to investigate this topic. Further developments in this field are needed to find all the important elements of the micro-epidemiology of malaria transmission and resulting immunity.

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References

1. Bejon P, Warimwe G, Mackintosh CL, et al. Analysis of immunity to febrile malaria in children that distinguishes immunity from lack of exposure. Infect Immun **2009**; 77:1917–23.
2. Schwarz NG, Adegnika AA, Breitling LP, et al. Placental malaria increases malaria risk in the first 30 months of life. Clin Infect Dis **2008**; 47:1017–25.
3. Males S, Gaye O, Garcia A. Long-term asymptomatic carriage of Plasmodium falciparum protects from malaria attacks: a prospective study among Senegalese children. Clin Infect Dis **2008**; 46:516–22.
4. Greenhouse B, Ho B, Hubbard A, et al. Antibodies to Plasmodium falciparum antigens predict a higher risk of malaria but protection from symptoms once parasitemic. J Infect Dis **2011.** In press.
5. Bejon P, Cook J, Bergmann-Leitner E, et al. Effect of the pre-erythrocytic candidate malaria vaccine RTS, $S/AS01_E$ on blood stage immunity in young children. J Infect Dis **2011.** In press.
6. Kinyanjui SM, Bejon P, Osier FH, Bull PC, Marsh K. What you see is not what you get: implications of the brevity of antibody responses to malaria antigens and transmission heterogeneity in longitudinal studies of malaria immunity. Malar J **2009**; 8:242.
7. Clark TD, Greenhouse B, Njama-Meya D, et al. Factors determining the heterogeneity of malaria incidence in children in Kampala, Uganda. J Infect Dis **2008**; 198:393–400.
8. Oesterholt MJ, Bousema JT, Mwerinde OK, et al. Spatial and temporal variation in malaria transmission in a low endemicity area in northern Tanzania. Malar.J. **2006**; 5:98.
9. Bejon P, Williams TN, Liljander A, et al. Stable and unstable malaria hotspots in longitudinal cohort studies in Kenya. PLoS Med **2010**; 7:e1000304.
10. Bousema T, Youssef RM, Cook J, et al. Serologic markers for detecting malaria in areas of low endemicity, Somalia, 2008. Emerg Infect Dis **2010**; 16:392–9.
11. Kreuels B, Kobbe R, Adjei S, et al. Spatial variation of malaria incidence in young children from a geographically homogeneous area with high endemicity. J Infect Dis **2008**; 197:85–93.
12. Bousema T, Drakeley C, Gesase S, et al. Identification of hot spots of malaria transmission for targeted malaria control. J Infect Dis **2010**; 201:1764–74.
13. Lindsay SW, Adiamah JH, Miller JE, Pleass RJ, Armstrong JR. Variation in attractiveness of human subjects to malaria mosquitoes (Diptera: Culicidae) in The Gambia. J Med Entomol **1993**; 30:368–73.
14. Lawaly YR, Sakuntabhai A, Marrama L, et al. Heritability of the human infectious reservoir of malaria parasites. PLoS One **2010**; 5:e11358.
15. Gouagna LC, Bancone G, Yao F, et al. Genetic variation in human HBB is associated with Plasmodium falciparum transmission. Nat Genet **2010**; 42:328–31.
16. Okell LC, Ghani AC, Lyons E, Drakeley CJ. Submicroscopic infection in Plasmodium falciparum-endemic populations: a systematic review and meta-analysis. J Infect Dis **2009**; 200:1509–17.
17. Greenhouse B, Myrick A, Dokomajilar C, et al. Validation of microsatellite markers for use in genotyping polyclonal Plasmodium falciparum infections. Am J Trop Med Hyg **2006**; 75:836–42.
18. Bretscher MT, Valsangiacomo F, Owusu-Agyei S, Penny MA, Felger I, Smith T. Detectability of Plasmodium falciparum clones. Malar J **2010**; 9:234.
19. Juliano JJ, Ariey F, Sem R, et al. Misclassification of drug failure in Plasmodium falciparum clinical trials in southeast Asia. J Infect Dis **2009**; 200:624–8.