Operational Evaluation of the Effectiveness of Long-lasting Insecticidal Nets on Human-Vector Contact in an African Urban Malaria Context

Dipomin F. Traoré,1,2,3 André B. Sagna,1,2 Serge B. Assi,1 Bertin N’Cho Tchiekoi,1 Akré M. Adja,1,4 Mamadou Dagnogo,3 Alphonsine A. Koffi,1 Christophe Rogier,5 and Franck Remoue1,2

1Institut Pierre Richet (IPR), Institut National de la Santé Publique (INSP), Bouaké, Côte d’Ivoire, 2MIVEGEC, University of Montpellier, IRD, CNRS, Montpellier, France, 3Unité de Formation et de Recherche en Sciences de la Nature (UFR SN), Université Nangui Abrogoua, Abidjan, Côte d’Ivoire, 4Unité de Formation et de Recherche en Biosciences (UFR Biosciences), Université Félix Houphouët Boigny, Abidjan, Côte d’Ivoire, and 5Service de Santé des Armées, Hexagone Balard, Paris, France

Background. Malaria is still a major public health concern in Côte d’Ivoire despite mass distribution of long-lasting insecticidal nets (LLINs) as a key preventive strategy. This study intended to evaluate the operational effectiveness of LLINs on the level of human-vector contact using 1 antibody-based biomarker of exposure to Anopheles in urban areas.

Methods. This cross-sectional study collected socio-demographic data and use of LLINs from 9 neighborhoods in the city of Bouaké (Côte d’Ivoire). Dry blood spots performed in children aged >6 months and adults were used to evaluate immunoglobulin G (IgG) responses to the Anopheles gSG6-P1 salivary peptide.

Results. IgG response levels to the salivary peptide were significantly lower in individuals who declared having “always” (n = 270) slept under an LLIN compared with those who had “often” (n = 2087) and “never” (n = 88) slept under an LLIN (P < .0001). IgG response levels to gSG6-P1 between those who declared having “always” and “not always” slept under an LLIN varied according to neighborhood, socio-professional category, and age group.

Conclusions. The human IgG level to this gSG6-P1 salivary peptide could be a useful tool to evaluate the actual effectiveness of LLINs and help design behavioral change interventions that are crucial for sustaining universal coverage.

Keywords: urban areas; salivary biomarkers; gSG6-P1; Anopheles; LLIN use.

In Africa, malaria remains a serious public health problem. Although urbanization is generally expected to reduce malaria transmission, the disease persists in African cities, sometimes at high levels [1]. A study carried out in Bouaké in 2019 showed that human exposure to Anopheles bites remained similar between urban and nearby rural areas, whatever the season [2]. City dwellers could be then at high risk of severe malaria infections, and owing to their low acquired immunity to malaria parasites, they would be more likely to develop severe malaria [3, 4].

The distribution of long-lasting insecticidal nets (LLINs) is one of the key intervention strategies for preventing malaria in Côte d’Ivoire [5]. Increasing the coverage and use of LLINs is also the preferred malaria vector control strategy in malaria-endemic countries, according to World Health Organization recommendations. Therefore, since 2011, the National Malaria Control Program (NMCP) has been carrying out a large-scale distribution campaign every 3 years throughout the country [6]. However, an evaluation of the actual use of LLINs in operational conditions is critical to better guide and help the NMCP identify high-risk malaria transmission areas (hot spots) where behavioral change interventions must be achieved.

To evaluate the effectiveness of vector control strategies on human-vector contact, entomological methods are currently used. However, these methods are labor-intensive and difficult to sustain on a large scale [7, 8]. As an alternative to entomological surveys, the level of exposure to Anopheles vector bites can be assessed by using the measure of human Antibody (Ab) response to gSG6-P1 salivary peptide of Anopheles saliva [9]. Immunoglobulin G (IgG) Ab titers to this salivary peptide are an indirect proxy for the intensity of Anopheles bites received by the individual [10, 11]. This biomarker has been used to (i) assess the heterogeneity of exposure to Anopheles bites and malaria risk according to urban districts [12, 13] and (ii) evaluate the effectiveness of various vector control strategies [14, 15].

In Bouaké, it has been shown, using this biomarker, that city dwellers were highly exposed to Anopheles bites as populations...
living in rural areas; this exposure varied significantly by neighborhood [2]. In light of these observations and after a national campaign of universal LLIN distribution by the NMCP, the next study step is to understand how LLIN use affects human-vector contact between neighborhoods within the same urban area in Africa, while people continue to be bitten outside of sleeping hours under LLINs.

The present study aims to evaluate under operational conditions the effectiveness of LLIN use on human-vector contact and its heterogeneity by urban neighborhood using this new biomarker of exposure in a population living in urban areas of Bouaké.

Two specific objectives are targeted: (i) specific IgG response to *Anopheles* exposure in the whole population according to dwelling place and socio-epidemiological factors to evaluate the factors of variation of specific IgG responses and (ii) specific IgG responses according to “declared” LLIN use and its factors of variations.

**METHODS**

**Patient Consent Statement**

This study followed the ethical principles recommended by the Edinburgh revision of the Declaration of Helsinki and was approved by the Ethics Committee of the Ministry of Health of Côte d’Ivoire (June 2015; No. 029/MSLS/CNER-dkna). Written informed consent of all parents or guardians of children who participated in the study was obtained before inclusion.

**Study Area**

This study was carried out in 9 neighborhoods randomly selected in 3 health districts (Bouaké North West, North East, and South), located in central Côte d’Ivoire in a climatic transition area that has 2 contrasting seasons: a dry season and a rainy season. The annual average rainfall ranges between 1000 and 1200 mm [16]. The Bouaké urban area is crossed by many small watercourses located 500–800 m apart and used by the local populations as paddy field areas and vegetable gardens.

**Study Design and Participants**

This study was part of the PALEV ALUT project, which was a multidisciplinary and multicentric project aimed to develop, validate, and publish integrated methodologies for evaluating malaria control means (www.palevalut.org). The study was funded as a multidisciplinary and multicentric project aimed to develop, validate, and publish integrated methodologies for evaluating malaria control means (www.palevalut.org). The study was carried out in August 2016 (rainy season). Overall, 2447 individuals were analyzed and distributed as follows: health district Bouaké North West: 232 in Dar-es-salam (DAR), 239 in Djeoukouaméako (DJ), and 259 in Ngattakro (NGA); health district Bouaké North East: 370 in Sokoura (SOK), 332 in Belle ville (BLV), and 326 in Attienkro (ATK); health district Bouaké South: 119 in Kennedy (KEN), 213 in Air France (AIF), and 357 in Ngouatanoukro (NGO). All children aged >6 months and adults usually living in the study area for >6 months were included. Sociological, epidemiological, and LLIN use information was collected from the head of household. If the head of household was not present at the time of the interview, the information was collected from his wife. Sera from blood were collected from all participants for immunological tests.

**Salivary Peptide gSG6-P1**

The gSG6-P1 peptide was designed using bioinformatics to maximize its *Anopheles* specificity and immunogenicity, as previously described [9]. It was synthesized and purified (>95%) by Genepep SA (Saint Jean de Védas, France). The peptide was shipped in lyophilized form and then resuspended in 0.22-µm ultrafiltered water and stored at −20°C until use.

**Evaluation of Human IgG Antibody Levels by Enzyme-Linked Immunosorbent Assay**

Enzyme-linked immunosorbent assays (ELISAs) were carried out on individual sera to measure IgG levels to gSG6-P1 peptide as previously described [17, 13]. Maxisorp plates (Nunc, Roskilde, Denmark) were coated with gSG6-P1 (20 µg/mL) in phosphate-buffered saline (PBS). After washing (deionized water + Tween 0.1%), plates were saturated with Protein-Free (TBS) Blocking Buffer (Thermo Scientific, Courtaboeuf, France), and each serum was incubated in duplicate at 4°C overnight at a 1/320 dilution in PBS + Tween 1%. A biotinylated mouse antihuman IgG (BD Biosciences, Le Pont de Claix, France) was incubated at a 1/2000 dilution for 1 hour at 37°C, and peroxidase-conjugated streptavidin (GE Healthcare, Velizy, France) was then added (1/2000; 1 hour at 37°C). Colorimetric analysis was carried out using ABTS (2,2-azino-bis (3 ethylbenzthiazoline 6-sulfonic acid) diammonium; Thermo Scientific) in 50 mM of citrate buffer (Sigma, Ph = 4, containing 0.003% H2O2), and the absorbance (optical density [OD]) was measured at 405 nm. Individual results were expressed as ΔOD = ODX − ODN, where ODX represents the mean of the individual OD value in both wells with gSG6-P1 antigen and ODN, the individual OD value in a blank well containing no gSG6-P1 antigen. The positivity threshold (PT) of the IgG level to gSG6-P1 was calculated using the following formula: PT = mean (ΔODneg) + 3SD. The ΔODneg mean of unexposed individuals to *Anopheles* mosquito (n = 12, France) was 0. As PT = 0, an individual was classified as an immune IgG responder if the ΔOD was >0.

**Statistical Analysis**

All data were digitized in Excel and then transferred to GraphPad Prism (San Diego, CA, USA) for analysis. After confirming that specific IgG response data (expressed in ΔOD) did not fit a Gaussian distribution, the nonparametric Mann-Whitney *U* test was used for comparison of the Ab levels of 2 independent groups, and the nonparametric Kruskal-Wallis test
was used to compare ΔOD between >2 independent groups. The Dunn post-test was used for all multiple comparison tests. All differences were considered significant at \( P < .05 \).

**RESULTS**

**Specific IgG Response According to Neighborhoods and Socio-epidemiological Factors: IgG Responses to gSG6-P1 Peptide According to Neighborhoods**

Median IgG responses to gSG6-P1 in the whole population (children and adults, \( n = 2447 \)) were highly heterogeneous between the 9 studied neighborhoods (\( P < .0001 \), Kruskal-Wallis test) (Figure 1). Among the 9 neighborhoods (\( n = 239, 370, 119, 213 \); median, 0.699, 1.123, 0.817, 0.738, 0.694 for DJZ, SOK, KEN, AIF, NGO, respectively), DJZ, SOK, KEN, AIF, and NGO had a high IgG median >0.500 while the 4 remaining neighborhoods, DAR, NGA, BLV, ATK (\( n = 232, 259, 332, 326 \); median, 0.202, 0.103, 0.123, 0.068 for DAR, NGA, BLV, ATK, respectively), displayed a low median of specific IgG (<0.500). These results indicate that human exposure to *Anopheles* bites varied considerably within the same city, with some neighborhoods being highly exposed compared with others. Even within the 2 group of neighborhoods, which could be considered more and less exposed to *Anopheles* bites, the heterogeneity of human exposure to *Anopheles* between neighborhoods was also very pronounced in both groups (high-exposure group, \( P < .0001 \); low-exposure group, \( P = .005 \)) (Supplementary Figure 1).

**IgG Responses to gSG6-P1 Peptide According to Age Groups**

For the whole population, the level of specific IgG responses showed a high variation according to age when delineated into 5 age groups (<5, 6–15, 16–25, 26–49, and >50 years; \( P < .0001 \)) (Supplementary Figure 2A). The median IgG value was 0.367 in children aged <5 years; it increased to reach a peak in individuals aged 6–15 and 16–25 years (median, 0.507 and 0.506, respectively) and decreased progressively in individuals group aged 26–49 years (median, 0.323) until the >50 years age group (median, 0.135). The same trend of IgG level variation between age groups was observed according to gender of individuals; that is, in the female group (median, 0.372, 0.422, 0.617, 0.278, 0.135 for the age <5 group, respectively; \( P = .0009 \)) (Supplementary Figure 2B) and in male group (median, 0.367, 0.551, 0.424, 0.386, 0.132 for the age <5 group, respectively; \( P = .006 \)) (Supplementary Figure 2C). No difference was observed regarding the comparison of specific IgG levels between the male and female groups by age group (Supplementary Figure 2D).

**IgG Responses to gSG-P1 Peptide According to Socio-professional Categories**

Participants were categorized according to their professional situation into 4 groups: employees, students, craftsmen and traders, and unemployed persons. Analyses of the median IgG responses to gSG6-P1 peptide showed significant differences

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**Figure 1.** Immunoglobulin G (IgG) response level to gSG6-P1 according to neighborhoods. Statistical difference of the level of IgG responses between neighborhoods is indicated by \( P \) values, estimated using the Kruskal-Wallis test. Abbreviations: AIF, Air France; ATK, Attienkro; BLV, Belle ville; DAR, Dar-es-Salam; DJZ, Djézoukouamékro; KEN, Kennedy; NGA, N’gattakro; NGO, N’gouatanoukro; SOK, Sokoura; ΔOD, optical density variation.

**Figure 2.** Immunoglobulin G (IgG) response level to gSG6-P1 by socio-professional category. Statistical difference of the level of IgG responses between different socio-professional categories was indicated by \( P \) values, estimated using the Kruskal-Wallis test, and between all pairs of socio-professional categories, using the Dunn post hoc test.
The median IgG responses to gSG6-P1 salivary peptide were significantly lower in employees than in students or craftsmen and traders. The Dunn multiple comparison test showed no significant differences between all other socio-professional categories.

**Specific IgG Response According to “Declared” LLIN Use; IgG Responses to gSG6-P1 Peptide According to LLIN Use in the Whole Bouaké Area**

To evaluate the actual effectiveness of implemented LLINs in the city of Bouaké, the individuals were categorized into 3 groups according their use of LLINs: those who declared having always slept under an LLIN (always), those who declared having often slept under an LLIN (often), and those who declared having never slept under an LLIN (never). Analyses of IgG responses to the gSG6-P1 peptide showed a gradual and significant increase of IgG median between these 3 groups ($P<.0001$, Kruskall-Wallis test) (Figure 3A). IgG responses were significantly lower in individuals who declared having always slept under an LLIN (median, 0.158) than in those who declared having often (median, 0.401; $P<.0001$) or never (median, 0.620; $P=.0006$) slept under an LLIN. As no difference was observed between individuals who declared having often and never slept under an LLIN, these 2 groups were then combined and considered “not always” (individuals who declared not always having slept under an LLIN) (Figure 3B). Then, the specific IgG responses of individuals who declared having always slept under an LLIN remained significantly low compared with those who declared having not always slept under an LLIN ($P<.0001$, Mann-Whitney test). All further comparisons of LLIN use were done with these 2 groups: always vs not always.

**IgG Responses to gSG6-P1 Peptide and LLIN Use According to Neighborhoods**

The IgG responses to the gSG6-P1 peptide were compared between individuals who declared having always and not always slept under an LLIN in each neighborhood of the city of Bouaké. In 6 neighborhoods (DAR, NGA, SOK, BLV, ATK, and NGO), the IgG responses were significantly lower in individuals who declared having always slept under an LLIN compared with those who did not (Table 1). In the 3 remaining neighborhoods (KEN, AIF, and DJZ), no difference of specific IgG responses was observed between these 2 groups (Table 1).

**IgG Responses to gSG6-P1 Peptide and LLIN Use According to Epidemiological Factors**

The comparison of specific IgG levels between “always” and “not always” LLIN use was significant in the female and male groups ($P= .007$ and $P= .0001$, respectively, Mann-Whitney test) (Supplementary Figure 3). According to age groups, results showed that in 2 age groups (16–25 and >50 years) the level of specific IgG responses was lower in individuals who always used an LLIN compared with those who did not always use an LLIN ($P = .030$ and $P = .007$, respectively) (Supplementary Figure 4).

**IgG Responses to gSG6-P1 Peptide and LLIN Use According to Socio-professional Categories**

In each socio-professional category previously defined, the specific IgG responses were compared between individuals who

![Figure 3](image-url)
declared having always vs not always slept under an LLIN. Results showed that only employees who had always slept under an LLIN had a lower IgG level compared with those who had not (P = .012, Mann-Whitney test) (Table 2). For other socio-professional categories, no significant difference was observed between the 2 groups.

**DISCUSSION**

The evaluation of human exposure to *Anopheles* bites in African urban populations (children and adults) using such a biomarker showed a high variation of IgG levels to gSG6-P1 salivary peptide and so to the risk of malaria transmission between neighborhoods in the Bouaké sanitary district. The diversity of socio-cultural behavior of Bouaké inhabitants could be a considerable factor that may modify the ecological environment and *Anopheles* density of Bouaké neighborhoods and hence the specific IgG variation between neighborhoods [18, 2]. In addition, the evaluation of LLIN use in operational conditions in the field within neighborhoods of Bouake could also help us understand whether LLIN use is also involved in variation of malaria risk between neighborhoods and thus evaluate LLIN use’s effectiveness on human-vector contact. Our results showed a gradual and significant increase of the specific IgG responses between these 3 groups of individuals (sleep always, often, and never under LLINs). IgG responses were lower in individuals who declared having always slept under an LLIN than in those who declared having often slept under an LLIN, suggesting that LLINs confer real protection against *Anopheles* bites in individuals who sleep always under LLINs compared with those do not. The use of this biomarker, which directly assesses human–*Anopheles* contact, shows that the declared response "often" does not seem relevant to evaluate LLIN use. These results confirm those of a study conducted in Dakar in 2010 showing that good use of LLINs (always sleeping under LLINs in good physical condition, ie, without holes) significantly improved the protection of populations against the mosquito bite vector. The specific IgG responses in these populations significantly decreased compared with those who did not use LLINs [19].

The level of specific IgG in studied individuals also showed high variations according to age groups. The median IgG was high in children and young adults and decreased in adults aged >50 years. Results on LLIN use have shown that adults aged >50 years and aged 16–25 years who always sleep under LLINs present lower IgG responses to gSG6-P1 compared with those who not always sleep under LLINs. Adults aged >50 are less active in the evenings, and they tend to go to bed earlier under LLINs compared with young adults and children, which avoids high exposure to *Anopheles* bites.
before sleeping under LLINs. In contrast, young adults are more active in the evenings for various sociological reasons (learning lessons for students, meeting with friends at night in entertainment venues). A study carried out in 2019 showed that in some Bouaké neighborhoods individuals go to sleep under LLINs late in the evening for different sociological reasons [2].

The present study showed significant differences in IgG response to gSG6-P1 by socio-professional group. The specific IgG level in employees was lower than in students, craftsmen and traders, and unemployed persons. But no difference was observed between craftsmen and traders and unemployed persons. In addition, a significant difference was observed between individuals who declared having always slept under LLINs and those who did not always use LLINs only in the category of employees. A previous study showed that sleeping always under a mosquito net in good physical condition (without holes) greatly diminished the mosquito bite vector [19], which confirms the good use of LLINs category. As already explained in terms of age groups, the effectiveness of LLIN use on the human contact vector varies also according to socio-professional categories. Populations according to their socio-professional activity would not go to sleep at the same time in the evening. Some individuals go to bed early, and others go to bed late. Some use LLINs, and others do not. In Bouaké, most craftsmen and traders (home care workers, food sellers, barmen, and others) work late into the evening. Students stay up late to study. Unemployed individuals may also meet friends at night in public spaces to converse. Common nighttime activities across settings included household chores and entertainment during the evening hours, as well as livelihood and large-scale socio-cultural events that can take place at night. It has been also shown that in areas where individuals typically stay outside later in the evenings, the protective effect of using an LLIN or protection inside houses is nullified if they are exposed during the evening hours outside the home [20, 21]. The increase of bites outside the household and LLINs reduces the effectiveness of LLINs to protect individuals against the mosquito bite vector of malaria in communities, even for those who use LLINs [21, 22].

In conclusion, the biomarker tool of human exposure to malaria vectors (IgG Ab to gSG6-P1 salivary peptide) showed that human exposure to bites outside households and LLINs can affect LLIN use’s effectiveness on human-vector contact, which generates variations in malaria risk between neighborhoods and socio-professional categories and age groups. This immunological indicator could be a relevant tool to assist the NMCP to assess vector control strategies on human-vector contact in African urban settings in the operational context. The benefit of such a biomarker is that it can also take into account the potential residual transmission (indoors and outside) occurring during the non-LLIN-protected period of time (before and after sleep). By consequence, the NMCP could use this new immune-epidemiological indicator for early detection of the risk of malaria transmission in urban areas and to make other additional control efforts including behavioral change in people and integrated vector control measures.

**Supplementary Data**

Supplementary materials are available at *Open Forum Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyrighted and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

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**Author contributions.** F.R., C.R., A.A.K., S.B.A., B.N.T., A.M.A., and M.D. conceived the study; D.F.T., T.B.N., and A.B.S. collected data in field surveys. D.F.T. and A.B.S. carried out the immunological assessments and analyzed data. D.F.T. drafted the first manuscript. F.R. participated in preparing and writing the manuscript and revised the manuscript. A.B.S., A.M.A., and M.D. provided guidance on revision of the manuscript. F.R. and D.F.T. are the guarantors of the paper. All authors read and approved the final manuscript.

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