# Public summary - HIVmalaria project

Background

The rise and spread of drug resistant malaria parasites is one of the major

challenges for malaria control and may soon proof to be one of the biggest obstacles to malaria eradication. Due to extensive geographical overlap of HIV and malaria, the two most serious health problems in the world, co-infections are common in nature. These co-infections may increase the emergence and spread of malaria drug resistance, as a result of intervention strategies and weaker immune systems. However, evidence for this is lacking and discussion is speculative of nature. Using an evolutionary framework, we aimed to unravel (1) the link between HIV infection and frequency of malaria drug resistance and (2) the selective forces that drive this resistance to higher levels in HIV co-infected individuals.

Literature study

Firstly, a statistical analysis was performed with data on prevalence of resistance mutations *pfmdr*86Y, *pfcrt*76T and *pfdhps*540E extracted from published studies. The aim was to determine whether a correlation can be seen between prevalence of these mutations in an area with the prevalence of HIV infection in the same area. The preliminary data suggests a positive correlation between prevalence of HIV infection and prevalence of sulphadoxine-pyrimethamine (SP) resistance markers (*pfdhps*540E) in sub-Saharan Africa. No correlation was found for the other markers of resistance. We are currently working on expanding the range of years of study inclusion to increase our statistical power and on adding certain co-variates to the analysis, most importantly known drug use history per region.

Clinical study

Next, clinical samples of HIV-positive and HIV-negative pregnant women were examined to determine whether HIV carriage as well as other markers of immunity affects the selection on resistant parasites. The women were recruited between 2009 and 2012 as part of the Malaria in Pregnancy Preventive Alternative Drugs (MiPPAD) clinical trials (ClinicalTrials.gov NCT0081121) that evaluated the efficacy and safety of two doses of Intermittent Preventive Treatment in pregnancy (IPTp) with mefloquine (MQ) compared to IPTp with sulphadoxine-pyrimethamine in HIV-uninfected women from Benin, Gabon and Mozambique, and of three doses of IPTp with MQ compared to placebo in HIV-infected women on cotrimoxazole prophylaxis, from Kenya and Mozambique.

For this, we planned to assess (i) the frequency of resistance markers on the genes *pfdhfr*, *pfdhps*, *pfcrt* and *pfmdr* by Ligase Detection Reaction fluorescent microspheres (LDR-fm), (ii) the multiplicity of malaria infection using genetic barcoding and (iii) the densities of the transmission stages using stage-specific qPCR.

Current status of project

A total of 896 samples qPCR positive for *Plasmodium falciparum* were identified (Kenya: 77, Mozambique: 210, Benin: 327, Gabon: 282) and included in the study. Polymorphisms of all involved genes have been determined and additionally we will be evaluating *pfmdr* copy number variation in September 2016. Molecular barcodes have successfully been determined for all women from Mozambique and Kenya. The barcodes from a subset of the women from Gabon and Benin have been analysed, but the multiplicity rate of malaria infections in these samples was at such a high level that this technique was not suitable for these samples. We are planning on utilizing a different molecular approach to determine the multiplicity of infection in these samples in the near future. Unfortunately, the quality of the samples was insufficient to be able to reliable determine transmission stage densities.

Preliminary results

Overall in all the MiPPAD sites the large majority of the infections carry mutations 51I, 59R, 108N, 436S and 437G. 540E is fixed in Kenya and close to fixation in Mozambique, whereas no 540E is observed in Benin and only a minor proportion in Gabon. 164L is absent in all sites. *Dhps*581 and 613 are wildtype in Kenya, Mozambique and Gabon and some mutations found in Benin. The CVMNK (wildtype) and CVIET (resistant) haplotypes of the *pfcrt* gene were the only haplotypes observed. SVMNT was not detected. Both haplotypes are under selection with a small majority of the wildtype haplotype in Kenya and Mozambique and a large proportion of the mutant haplotype CVIET in Gabon and Benin. *Pfmdr*1034, 1042and 1246 are nearly all wildtype in all four sites. *Pfmdr*86 wildtype is in the majority in Kenya and Mozambique, whereas 86Y is in the majority in Gabon and Benin. *Pfmdr*184 mutants are in the small majority at all sites.

No difference between carriage of resistance mutations were found between HIV positive and HIV-negative women. Treatment practices for HIV-positive women have been altered in recent years where HIV-positive women receive three intermittent preventive therapy (IPTp) doses compared to two doses in HIV-negative women. They additionally receive daily cotrimoxazole treatment against opportunistic infections which has some antimalarial activity. Thus, by directly comparing these two groups this treatment effect may bias or obscure a possible immune status effect. To determine an effect of immune depletion on resistance evolution we therefore compared the level of CD4 depletion within the HIV-positive group as well as viral load. Our preliminary data suggest that in Mozambique, in an area with high levels of HIV, women with mutant *pfcrt* infections had significantly higher viral loads and trended towards lower CD4 counts. No significant differences were found for the other measures of immunity; resistant mutants were equally present in the placenta as in the periphery and a weak trend towards increased carriage of *pfmdr86* was seen with increased number of previous pregnancies.

The analysis of resistant markers in the context of this clinical trial allowed us to additionally determine how IPTp affected selection for resistant mutants and how resistant mutants affected the efficacy of IPTp. Here, we found that IPTp with mefloquine in HIV-positive women significantly selected against *pfmdr* and *pfcrt* mutants. In HIV-negative women, those receiving IPTp with mefloquine also had less *pfmdr* and *pfcrt* mutants than those receiving IPTp with sulfadoxine-pyrimethamine. Interestingly, we found that Mozambiquan women with *pfcrt76T* mutants and/or *pfmdr86Y* mutants delivered babies with a lower birth weight than women with wildtype parasites, suggesting that either resistant mutants have a direct or indirect effect on birth outcome or women that deliver babies with lower birth weight or for unknown reasons more likely to carry resistant infections. However, these are preliminary results that are not yet adjusted for covariates and thus need to be verified.

Conclusions thus far

These preliminary results provide new insight into the role of HIV infection and other factors of immunity in the evolution of resistant parasites. These first data do not show a great impact of HIV infection on resistance evolution although there are some indicators that the intensity of HIV infection could play a role. Unfortunately, the sample size of HIV-positive women does not give much statistical power to really understand these complex interactions. Interestingly, we found a negative selection against resistant parasites by IPTp with mefloquine. This is an important finding that provides more insight in how different drugs provide possibly opposing direction of selection; a finding that has important implications for drug selection in prophylactic, clinical and MDA drug choices.

More information, see http://www.huijbenlab.net