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# Evaluation of some haemostatic parameters in *falciparum* malaria and HIV co-infection

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**ABSTRACT**

**Background:** Studies from sub-Saharan Africa where malaria is endemic have observed high incidences of malaria and HIV co-infection. It has long been accepted that malaria causes alterations in haemostatic parameters and that HIV is associated with a wide range of haematological changes. We assessed the effect of the overlap of these infections on routine haemostatic parameters.

**Method**: The study involved 337 subjects grouped according to their HIV and malaria status: Group 1 'Asymptomatic HIV seropositive, *Plasmodium falciparum* positive' ($n$ = 61); Group 2 'Asymptomatic HIV seropositive, *P. falciparum* negative' ($n$ = 73); Group 3 'Symptomatic HIV seropositive, *P. falciparum* positive' ($n$ = 49); Group 4 'Symptomatic HIV positive *P. falciparum* negative' ($n$ = 56); Group 5 'Control HIV negative, *P. falciparum* positive' ($n$ = 52) and Group 6 'Control HIV negative, *P. falciparum* negative' ($n$ = 46). Blood samples were taken for HIV testing, diagnosis of *falciparum* malaria and malaria parasite density counts. Citrated samples were used within one hour of collection for prothrombin time (PT) and activated partial thromboplastin time (APTT). $CD4^+$ T cell counts, platelet count and haematocrit (Hct) were also performed.

**Results:** Our results demonstrate greater alterations in APTT, PT and platelet count with prolongation of APTT, PT and lower platelet counts in HIV and malaria co-infection. In spite of this, the co-infected subjects with mild to moderate parasitaemia did not show a bleeding tendency; however, the risk is higher in severe malaria.

**Conclusion**: These results suggest that co-infected subjects with severe malaria have a higher risk of bleeding and would require greater monitoring.

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Coagulation; HIV; malaria; HIV/malaria co-infection; asymptomatic; symptomatic

## Introduction

As HIV and malaria have similar global distribution,[1] co-morbidity is likely.[2–4] Either condition alone has been associated with alterations in haemostasis and the interaction between both disease conditions could complicate the clinical picture. High incidences of malaria and HIV co-infection have been observed in Sub-Saharan Africa.[5] That HIV and malaria are often present in the same area has been established and the reason for this may be both environmental and genetic. The human leucocyte antigen HLA-B35 is associated with *Plasmodium falciparum*.[6] According to Ghosh et al., the HLA-B35 allele is common in several population groups where *falciparum* malaria is endemic, suggesting that these populations (exposed for centuries to *falciparum* malaria) were able to acquire resistance and survive by way of positive selection.[7] It is interesting then, to note that other studies from all over the world have shown that HLA B35 is associated with faster disease progression in HIV-1 infection.[8]

There are several potential ways in which malaria and HIV infection could interact. Co-infection could affect progression or clinical manifestation of either condition, and infection with either malarial parasites or HIV could facilitate transmission of the other.[4] Co-infection could affect treatment outcome and there maybe toxicities or interactions between the drugs used to treat different conditions.[9,10] In the malaria endemic region of south-east Nigeria, a co-infection rate of 19% has been reported.[5] In studies carried out throughout sub-Saharan Africa, it has been found that co-infection with HIV approximately doubles the risk of parasitaemia and malaria.[11,12] It has long been accepted that malaria causes alterations in haemostatic parameters and HIV is associated with a wide range of haematological changes. There is increased coagulation activity in malaria.[13] *P. falciparum* infection is often associated with a procoagulant state characterised by thrombocytopenia and activation of the coagulation and fibrinolytic system with systemic endothelial activation and endothelial damage.[14,15] It has also

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been revealed that parasitised red blood cells support assembly of multi-molecular coagulation complexes leading to amplification of the coagulation cascade.[16] However, bleeding and haemorrhage are uncommon events, suggesting that a compensated state of blood coagulation activation occurs in malaria.[17]

The inflammation which is seen in malaria also has a complex relationship with the procoagulant state and may eventually lead to multi-organ failure in severe cases.[13] In studies carried out in HIV positive subjects, increased coagulability and impaired endothelial function are complications often seen in HIV.[18,19] According to Neuhaus et al., there is a growing body of data indicating that the risk of serious non-AIDS conditions such as cardiovascular disease, kidney disease, liver disease and non-AIDS defining malignancies is increased in individuals with HIV infection compared to the general population. HIV-induced activation of inflammatory and coagulation pathways could explain the increased risk.[20] Inflammatory injury of the endothelium, which suggests endothelial dysfunction, has been reported.[18,21]

In view of these, this research was carried out to test the hypothesis that HIV and malaria co-infection is associated with a worse profile than in either infection alone. Thus, this study assessed the combined effect of malaria and HIV co-infection on haemostasis in a malaria endemic environment, where there is also a relatively high HIV burden.

## Materials and methods

Ethical approval for the research design was obtained from the Nnamdi Azikiwe University Teaching Hospital Ethical Committee, NAUTH/CS/66/VOL.3/20. Informed consent was sought and obtained from the subjects.

### *Subjects*

Three hundred and thirty-seven subjects recruited from Nnamdi Azikiwe University teaching hospital, a regional HIV care centre in south-east, Nigeria were studied to assess the effect of HIV/Malaria co-infection on haemostasis. The subjects were grouped according to their HIV and malaria status as: Group 1 'Asymptomatic HIV seropositive, *P. falciparum* positive' ($n$ = 61); Group 2 'Asymptomatic HIV seropositive, *P. falciparum* negative' ($n$ = 73); Group 3 'Symptomatic HIV seropositive, *P. falciparum* positive' ($n$ = 49); Group 4 'Symptomatic HIV seropositive, *P. falciparum* negative' ($n$ = 56); Group 5 'Control HIV seronegative, *P. falciparum* positive' ($n$ = 52) and Group 6 'Control HIV seronegative, *P. falciparum* negative' ($n$ = 46).

The HIV positive subjects were classified according to the CDC and WHO staging. The term 'symptomatic' group was used to describe only HIV positive subjects who had developed some symptoms such as diarrhoea, fever, weight loss, anaemia, bacterial infection and oral candidiasis. The subjects in each group were age and sex matched. Subjects who had commenced on anti-retroviral and anti-malaria drugs were excluded. Citrated blood samples were used within one hour of collection for Prothrombin time (PT) and Activated partial thromboplastin time (APTT) using standard manual methods. CD4 counts, platelet count and haematocrit (Hct) were also performed on the samples using standard automated methods.

### *Laboratory*

Antibodies to HIV-1 and HIV-2 that are determined by two different immunochromatographic methods were used, namely Inverness Determine 1 & 2 (Inverness Medical Japan Co. Ltd, Japan) and STAT-PAK (Chembio Diagnostic system, New York, USA). HIV seropositive results using these two methods are used to classify participants as HIV infected according to national serial algorithm. Tests were carried out according to manufacturer's instructions.

Whole blood was used for the diagnosis of *P. falciparum* malaria using Giemsa stained (pH 6.8) thick blood smear for microscopic detection and *P. falciparum* malaria chromatographic immunoassay (SD Bioline, Standard Diagnostics Inc., Korea), which is for the qualitative detection of circulating *P. falciparum* antigen in whole blood. Only *P. falciparum* positive subjects were recruited, for uniformity as it causes most malaria disease in the study area, and causes the most pathology.

The CD4 counts were measured using the Cyflow SL.3 brand of cytometer counter (Partec, Germany) for automated CD4 counts. This uses green solid-state laser with an excitation light source of 532 nm counter. Partec test kit (Partec, Germany) was used in preparing the samples. The test kit contains mouse monoclonal antibody (mAb) isotype IgG1 clone MEM-241 which recognises the human CD4 antigen and a no lyse buffer.

The PT was carried out manually according to the manufacturer's instruction. Briefly, 0.2 ml of thromboplastin (Diagen, Thame, Oxon, UK) was deposited in clotting tubes in a water bath at 37 °C, left for 2 min, 0.1 ml of plasma was then added; a stop watch started simultaneously. The tube was gently tilted at three-second intervals returning to the water bath at intervals. The time for formation of clot was recorded. The APTT was carried out manually and according to manufacturer's instruction (Diagen, Thame, Oxon, UK). Briefly, 0.02 mol/L calcium chloride was pre-incubated in a glass test tube in a water bath at 37 °C for 10 min. 50 µl of plasma was pipetted into a clean glass tube and incubated in a water bath at 37 °C for 3 min. 50 µl of APTT reagent was added into the tube containing the plasma and the mixture incubated at 37 °C for a further 3 min. 50 µl of the pre-incubated calcium chloride was added and the stop watch started simultaneously. The tube was gently tilted at three-second intervals returning to the water bath at

**Table 1.** Demographic characteristics, CD4 counts and Malaria parasite density counts in the study population.

| Groups | Age Mean age(±SD) | Sex | CD4 (cells/l) Mean (±SD) | MDC (parasites/μl) Median (IQR) |
|---|---|---|---|---|
| AsymHIV$^+$/Pf$^+$ ($n = 61$) | 40 ± 11 | Male:27 Female:34 | 652±137[a] | 9,200 (1,550-11,000) |
| AsymHIV$^+$/Pf$^-$ ($n = 73$) | 42 ± 9 | Male:25 Female:48 | 633±134[b] | Nil |
| SymHIV$^+$/Pf$^+$ ($n = 49$) | 44 ± 12 | Male: 24 Female:25 | 412±149[a] | 11,350 (2,200-15,600) |
| SymHIV$^+$/Pf$^-$ ($n = 56$) | 44 ± 11 | Male:28 Female:28 | 395±167[b] | Nil |
| HIV$^-$/Pf$^+$ ($n = 52$) | 39 ± 13 | Male:24 Female:28 | 715±141[a] | 8,600 (2,890-12,800) |
| HIV$^-$/Pf$^-$ ($n = 46$) | 37 ± 8 | Male: 21 Female:25 | 811±135[b] | Nil |
| ANOVA | 0.875 | 0.511 | **<0.001** | 0.648 |

[a]Significantly different to the other groups infected with *P. falciparum* $P < 0.01$.
[b]Significantly different to the other groups not infected with *P. falciparum* $P < 0.05$.
AsymHIV$^+$/Pf$^+$: Asymptomatic HIV seropositive, *Plasmodium falciparum* positive. AsymHIV$^+$/Pf$^-$: Asymptomatic HIV seropositive, *Plasmodium falciparum* negative.
SymHIV$^+$/Pf$^+$: Symptomatic HIV seropositive, *Plasmodium falciparum* positive.
SymHIV$^+$/Pf$^-$: Symptomatic HIV seropositive, *Plasmodium falciparum* negative.
HIV$^-$/Pf$^+$: Control HIV seronegative, *Plasmodium falciparum* positive.
HIV$^-$/Pf$^-$: Control HIV seronegative, *Plasmodium falciparum* negative.
MDC: Malaria density count. *n* = number. Significant values in bold.

**Table 2.** The means (+/–S.D) of the parameters studied in the various groups.

| Groups | Haematocrit (%) | APTT (s) | PT (s) | Platelet count (× $10^9$/l) |
|---|---|---|---|---|
| AsymHIV$^+$/Pf$^+$ ($n = 61$) | 35.3±4.7 | 43±6 | 16±3 | 205±53 |
| AsymHIV$^+$/Pf$^-$ ($n = 73$) | 35.6±5.0 | 42±5 | 16±2 | 217±54 |
| SymHIV$^+$/Pf$^+$ ($n = 49$) | 31.9±4.6 | 43±5 | 17±2 | 157± 45 |
| SymHIV$^+$/Pf$^-$ ($n = 56$) | 32.4±3.8 | 42±3 | 16±2 | 179± 57 |
| HIV$^-$/Pf$^+$ ($n = 52$) | 35.9±5.1 | 39±5 | 14±2 | 257± 36 |
| HIV$^-$/Pf$^-$ ($n = 46$) | 34.9±4.5 | 38±4 | 14±2 | 260± 61 |
| ANOVA | **0.024** | **0.003** | **<0.001** | **<0.001** |

AsymHIV$^+$/Pf$^+$: Asymptomatic HIV seropositive, *Plasmodium falciparum* positive.
AsymHIV$^+$/Pf$^-$: Asymptomatic HIV seropositive, *Plasmodium falciparum* negative.
SymHIV$^+$/Pf$^+$: Symptomatic HIV seropositive, *Plasmodium falciparum* positive.
SymHIV$^+$/Pf$^-$: Symptomatic HIV seropositive, *Plasmodium falciparum* negative.
HIV$^-$/Pf$^+$: Control HIV seronegative, *Plasmodium falciparum* positive.
HIV$^-$/Pf$^-$: Control HIV seronegative, *Plasmodium falciparum* negative.
APTT: Activated partial thromboplastin time. PT: Prothrombin time MDC-malaria parasite density count. IQR-Interquartile range.
*n* = number, s = seconds.
Mean, median and standard deviation. Level of significance set at $P < 0.05$. Significant values in bold.

intervals and time for formation was recorded. Platelet count and haematocrit (Hct) were carried out using the standard automated SYSMEX KX-21N (Kobe, Japan) on whole blood in EDTA anticoagulant.

### *Statistical analysis*

Data were analysed and comparisons performed using Mean, Standard deviation, Student's *t*-test, ANOVA, and Pearson's correlation (SPSS version 17). Level of significance was set at $P < 0.05$. Sample size was estimated using the formula by Naing et al. [22] with a national HIV prevalence of 3.8%.

## Results

A total of 337 subjects were studied to assess the effect of HIV/Malaria co-infection on haemostasis. Age, sex, CD4 and parasite counts are shown in Table 1. The groups were matched for age and sex, and there was no difference in the parasite load in the particular groups. Unsurprisingly, all HIV patient groups had lower CD4 counts, and levels were lower in symptomatic patients (all between group differences $P < 0.05$).

Table 2 shows haemostatic parameters. There were differences between groups in all four indices. Statistical differences between the malaria-infected groups are shown in Table 3, with overall differences in haematocrit, PT and platelet count. Haematocrit and platelet counts were lower in those whose HIV was symptomatic, and the platelet count was lower in the asymptomatic HIV group. In those symptomatic for HIV, haematocrit and platelet count were lower and the PT prolonged compared to those free of HIV. Table 4 shows statistical differences in patients not infected with *P. falciparum*, and overall, there were differences in all four indices. Patients who were symptomatic for HIV had a lower haematocrit and platelet count than those who were asymptomatic. Patients who were asymptomatic for HIV had a higher haematocrit, a prolonged PT, and a lower platelet count than those free of HIV. Patients who were symptomatic for HIV had a lower haematocrit and platelet count, and prolonged PT and APTT compared to those free of HIV.

There were correlations between the parameters measured. The PT correlated with the CD4 counts ($r = .18$, $P = 0.012$), the APTT correlated with the CD4 counts ($r = 0.22$, $P = 0.028$) and the platelet count correlated with the CD4 count ($r = 0.41$, $P < 0.01$). In addition, there were significant correlations between APTT and parasite count ($r = 0.36$, $P < 0.001$), and correlation between haematocrit and CD4 ($r = 0.21$, $P = 0.024$).

**Table 3.** Inter-group Comparisons for the parameters in *P. falciparum*-infected groups using t-test and ANOVA.

| Groups | Haematocrit | APTT | PT | Platelet count |
|---|---|---|---|---|
| Asym $HIV^+/Pf^+$ vs Sym $HIV^+/Pf^+$ | **0.036** | 0.731 | 0.158 | **0.009** |
| Asym $HIV^+/Pf^+$ vs $HIV^-/Pf^+$ | 0.508 | 0.108 | 0.072 | **0.004** |
| Sym $HIV^+/Pf^+$ vs $HIV^-/Pf^+$ | **0.002** | 0.211 | **0.003** | **<0.001** |
| ANOVA | **0.007** | 0.381 | **0.013** | **<0.001** |

Asym $HIV^+/Pf^+$ vs Sym$HIV^+/Pf^+$ vs $HIV^-/Pf^+$: Asymptomatic HIV seropositive, *Plasmodium falciparum* positive, Symptomatic HIV seropositive, *Plasmodium falciparum* positive and control HIV seronegative, *Plasmodium falciparum* positive compared (using ANOVA).
Asymptomatic HIV seropositive, *Plasmodium falciparum* positive and Symptomatic HIV seropositive, *Plasmodium falciparum* positive compared (using *t*-test).
Asymptomatic HIV seropositive, *Plasmodium falciparum* positive and control HIV seronegative, *Plasmodium falciparum* positive compared (using *t*-test).
Symptomatic HIV seropositive, *Plasmodium falciparum* positive and control HIV seronegative, *Plasmodium falciparum* positive compared (using *t*-test).
Level of significance is set at 0.05. Significant values in bold.

**Table 4.** Inter-group Comparisons for the parameters in groups not infected with *P. falciparum* using ANOVA and t-test.

| Groups | Haematocrit | APTT | PT | Platelet count |
|---|---|---|---|---|
| Asym$HIV^+/Pf^-$ vsSym$HIV^+/Pf^-$ | **0.006** | 0.276 | 0.292 | **0.003** |
| Asym$HIV^+/Pf^-$ vs$HIV^-/Pf^-$ | 0.817 | **<0.001** | **<0.001** | **0.001** |
| Sym$HIV^+/Pf^-$ vs $HIV^-/Pf^-$ | **0.016** | **0.022** | **<0.001** | **<0.001** |
| ANOVA | **0.019** | **0.001** | **<0.001** | **<0.001** |

Asym$HIV^+/Pf^-$ vs Sym$HIV^+/Pf^-$ vs $HIV^-/Pf^-$: Asymptomatic HIV seropositive, *Plasmodium falciparum* negative, Symptomatic HIV seropositive, *Plasmodium falciparum* negative and control HIV seronegative, *Plasmodium falciparum* negative compared (using ANOVA).
Asymptomatic HIV seropositive, *Plasmodium falciparum* negative and Symptomatic HIV seropositive compared (using *t*-test).
Asymptomatic HIV seropositive, *Plasmodium falciparum* negative and control HIV seronegative, *Plasmodium falciparum* negative compared (using *t*-test).
Symptomatic HIV seropositive, *Plasmodium falciparum* Negative and control HIV seronegative, *Plasmodium falciparum* negative compared (using *t*-test).
APTT: Activated partial thromboplastin time. PT: Prothrombin time s = seconds.
Level of significance set at $P < 0.05$. Significant values in bold.

## Discussion

The $CD4^+$ counts obtained show that there were significantly lower $CD4^+$ counts in the test subjects than in the controls. This is not unexpected as $CD4^+$ counts have been accepted as an indicator of immune function in HIV/AIDS. It is however important to note that there is a significant correlation between the $CD4^+$ counts and all the first-line coagulation tests investigated thus implying that the depletion of $CD4^+$ could affect haemostasis. Further work will need to be done to authenticate this (see Table 5).

Increased prevalence of severe malaria in HIV-infected adults was reported by Cohen et al. [23] Three cases of severe malaria were encountered during this study. Though the worst case with a parasite count of 230,000 parasites/µl was seen in a HIV symptomatic patient, there is no proof that severe malaria is more common in HIV/malaria co-infected subjects in this environment. Few cases with severe malaria were encountered in the present study and also, immunity to malaria is quite important. In this region where malaria is endemic,[24] and with stable transmission of malaria all year round, immunity to malaria is developed especially in adults. [25] Non-immune HIV-infected patients are significantly more likely to have severe malaria than non-immune non HIV-infected patients.[23] They reported that HIV serostatus did not affect risk of severe malaria in patients from malaria endemic regions. This seems to agree with results obtained in this study where the severe malaria cases were evenly shared among the groups. However, it is important to note that the symptomatic HIV seropositive, *P. falciparum* positive group had the highest malaria parasite density in this study.

In assessing the results obtained for the first-line coagulation tests, the results show that mean PT were significantly higher in all the HIV-infected and co-infected groups than in the control groups. There was a significant difference in the PT between the symptomatic HIV seropositive, *P. falciparum* positive group and control HIV seronegative, *P. falciparum* positive group. This suggests that though the MP causes some level of derangement in the PT, the combined effect of HIV disease and MP worsens the scenario.

The groups with the most prolonged PT were the HIV/malaria co-infected groups. It can then be inferred that HIV and malaria interaction worsens the haemostatic problem because though the derangement is not much, it is significant at certain levels of comparison. Another important factor is the disease stage. This is seen in the asymptomatic HIV positive groups which also had slight derangements in the PT values. These results suggest that HIV disease stage and HIV/malaria co-infection has a role in the derangement observed.

For the APTT, the results show that the group 1 and group 3 (the co-infected groups) had prolonged APTT while in the HIV, only infected groups, there was slightly less prolongation. When looking at the co-infected groups, however, there was no significant difference observed between the co-infected groups (1 and 3) and even with the control HIV seronegative, *P. falciparum* positive group ($P > 0.05$). This implies that the APTT is not affected as much as the PT by HIV/*P.*

*falciparum* malaria co-infection. Further light is thrown on this when looking at the non-co-infected groups (Table 4). Here, there is a significant difference between the asymptomatic HIV seropositive, *P. falciparum* negative group (2) and the control HIV seronegative, *P. falciparum* negative group (6) and also a significant difference between the symptomatic HIV seropositive, *P. falciparum* negative (group 4) and the control group 6. Thus, HIV alone is likely the driving force of the derangement in the APTT. When looking at the correlation, the APTT shows a correlation with the CD4 which implies the importance in this case as well, of HIV disease stage. Also, a correlation was observed with the PT, the platelet count and the parasite count.

The effect of HIV, HIV/malaria co-infection on the platelet count was also studied. The platelet count showed a correlation with the CD4 count implying that disease staging also has an effect. This is supported by significant difference observed in the platelet counts between the co-infected groups and controls as well as in the non-co-infected groups and controls, suggesting a link with the stage of the infection. There was no significant difference observed when looking at the HIV co-infected and non-co-infected within the same disease stage or control, that is, between group 1 and 2, group 3 and 4 and between group 5 and 6. This suggests that in the HIV infected or controls, the platelet though lower was not significant. HIV infection is reported to be a known trigger of thrombocytopenia.[26,27]

The mean (SD) platelet count in HIV-infected groups carried out in India was 240 (±101).[28] These counts were more than it was observed in this study with the counts in this HIV-infected groups ranging from 157 (±45) in the symptomatic HIV positive group to 217 (±54) in the asymptomatic HIV positive MP negative group. This may not be unrelated to the racial variations in platelet counts which have been observed.[29] The platelet count shows a difference between the co-infected groups and the two control groups but not a significant difference with the non-co-infected groups. The platelet count correlates with the PT and APTT implying that what affects one affects the other.

Co-infection with HIV and malaria worsens the alteration in coagulation parameters which is seen in either disease alone as shown by the results for the PT and platelet counts. The APTT seems less affected by malaria HIV co-infection. Despite these alterations in these haemostatic parameters, the affected subjects have not shown a tendency to bleeding. However, the co-infected subjects with severe malaria are the at-risk group, who would require greater monitoring. This work represents an advance in biomedical science because it has shown that *P. falciparum* malaria causes alterations in haemostasis but HIV co-infection exacerbates it.

**Table 5.** Summary.

What is known about the subjects:

- Co-infection with malaria amongst people living with HIV/AIDS is relatively common in the study population
- Both HIV and malaria can cause derangements in haematologic parameters
- Findings of some coagulation tests may identify patient groups who are at most risk of derangement in haemostasis

What this paper adds:

- The risk degree of platelet reduction, PT and APTT elevation vary according to the severity of the malaria in the subjects
- The platelet count and PT are most sensitive and important in the monitoring of these individuals
- HIV disease exacerbates the alterations seen when assessing these coagulation tests

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## Disclosure statement

The authors declare no conflict of interest.

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