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### ► To cite this version:

Ariane Amoura, Anissa Moktefi, Matthieu Halfon, Alexandre Karras, Cédric Rafat, et al.. Imported Malaria, Collapsing Glomerulopathy, and Focal and Segmental Glomerulosclerosis. Clinical Journal of the American Society of Nephrology, 2020, 15 (7), pp.964-972. 10.2215/CJN.00590120 . hal-03160403

**HAL Id: hal-03160403**

**<https://hal.u-pec.fr/hal-03160403>**

Submitted on 5 Mar 2021

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# Imported malaria, collapsing glomerulopathy, and focal and segmental glomerulosclerosis

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## Abstract

**Background and objectives:** Malaria, a potentially life-threatening disease, is the most prevalent endemic infectious disease worldwide. In the modern era, the spectrum of glomerular involvement observed in patients after malarial infections remains poorly described.

**Design, setting, participants, & measurements:** We therefore performed a retrospective multicenter study to assess the clinical, biological, pathological and therapeutic characteristics of patients with glomerular disease demonstrated by kidney biopsy in France within three months of an acute malaria episode

**Results:** We identified 23 patients (12 men), all but one of African ancestry and including 10 patients with concomitant human immunodeficiency virus (HIV) infection. All the imported cases were French citizens, living in France who had recently travelled back to France from an endemic area and developed malaria after their return to France. Eleven patients had to be admitted to an intensive care unit at presentation. *Plasmodium falciparum* was detected in 22 cases and *Plasmodium malariae* in one case. Kidney biopsy was performed after the successful treatment of malaria, a mean of 24 days after initial presentation. At this time, all patients displayed acute kidney injury, requiring kidney replacement therapy in 12 cases. Nephrotic syndrome was diagnosed in 17 cases. Pathological findings included focal and segmental glomerulosclerosis (FSGS) in 21 cases and minimal change nephrotic syndrome in two patients. Among patients with FSGS, 18 had collapsing glomerulopathy (including nine cases of HIV-associated nephropathy). In four patients, immunohistochemistry with an antibody targeting *P. falciparum* histidine-rich protein-2 demonstrated the presence of the malaria antigen in tubular cells, but not in podocytes or parietal epithelial cells. An analysis of the apolipoprotein L1 risk genotype showed that high-risk variants were present in all seven patients tested. After a mean

follow-up of 23 months, eight patients required kidney replacement therapy (kidney transplantation in two cases), and mean eGFR for the other patients was 51 mL/mn/1.73m<sup>2</sup>.

**Conclusions:** In patients of African ancestry, imported *Plasmodium* infection may be a new causal factor for secondary FSGS, particularly for collapsing glomerulopathy variants in an APOL1 high-risk variant background.

## Introduction

Malaria is a major public health concern worldwide, affecting many millions of people living in tropical areas and causing a significant number of deaths annually (1). Endemic malaria has been eradicated in European countries, and the malaria cases notified in France are generally imported cases in travellers returning from or migrants moving to France from countries in which malaria is endemic. Acute kidney injury (AKI) is one of the most feared and severe life-threatening complications, affecting 1% to 4% of all patients, and up to 60% of patients with severe malaria (2,3). A broad spectrum of glomerular lesions has been described in association with malaria, but the pathophysiological relationship between these two conditions has been little investigated (4,5). Early studies, performed in patients with *Plasmodium malariae* (*P. malariae*) infection, found that immune complex-mediated membrano-proliferative glomerulonephritis (MPGN) was one of the most frequent causes of nephrotic syndrome in Africa (5-7). The spectrum of glomerular damages associated with *Plasmodium falciparum* (*P. falciparum*) infection is less common and has not been described in detail (8). The main pathological lesions appear to be immunoglobulin A nephropathy (9), eosinophilic glomerulonephritis (10), minimal change nephrotic syndrome (11) and collapsing glomerulopathy (12-14). We therefore retrospectively assessed the data for 23 patients with biopsy-proven glomerular disease in a context of imported malaria, with the aim of describing the clinical, biological pathological, and therapeutic characteristics of these cases, as well as their outcome. We also performed, in four patients, immunohistochemistry studies with an antibody targeting *P. falciparum* histidine-rich protein-2 (HRP-2), to determine whether *P. falciparum* was present in the kidney tissue at the time of glomerular disease diagnosis.

## Materials and methods

### *Patients and kidney function evaluation*

We conducted this retrospective study by sending a questionnaire to all French nephrology departments, asking them to identify patients with biopsy-proven glomerular disease following acute malaria episode (kidney biopsy performed within three months of *Plasmodium* infection). The main indications of for kidney biopsy were significant proteinuria (urine protein/creatinine ratio (uPCR) > 1.5 g/g) associated with renal impairment. This study was performed in accordance with the ethical standards of the Helsinki Declaration, and was approved by our local institutional review board (IRB 412 Mondor No. 00003835) and by *the Comité de Protection des Personnes d'Ile de France IV* (No. 2016/25NICB). We included 23 adult patients from 10 nephrology departments seen between 1998 and 2019. Demographic, clinical, biological and histological data obtained at the time of malaria diagnosis and at kidney biopsy were assessed for each patient (**supplementary information**). Nephrotic syndrome was defined as an uPCR exceeding 3 g/g and a serum albumin concentration below 3.0g/dL Acute kidney injury (AKI) and chronic kidney disease were defined according to Kidney Disease Improving Global Outcome (KDIGO) criteria (15,16). The genotyping data for two risk alleles (the G1 and G2 variant alleles) of the gene encoding apolipoprotein L1 (APOL1) were systematically noted, when available (17). Follow-up data are listed in **supplementary information**.

### *Plasmodium infection*

The positive diagnosis of malaria was based on blood tests (thick and thin blood smears Giemsa stained and observed by microscopy) (**supplementary information**). The results of

parasitaemia levels and species identification confirmed by polymerase chain reaction (PCR) were noted when available. Patients were considered to have severe malaria if they met the recently updated World Health Organisation (WHO) criteria for severe malaria on admission or during hospitalisation (18).

***Kidney biopsy examination and immunohistochemistry study.***

All patients underwent a kidney biopsy for the exploration of proteinuria with an associated impairment of kidney function, within three months of acute *Plasmodium* infection. Minimal change nephrotic syndrome was diagnosed as previously described (19). Focal and segmental glomerulosclerosis (FSGS) was classified according to the Columbia classification (20). The morphological features of collapsing glomerulopathy (including HIV-associated nephropathy (HIVAN) in HIV-infected patients) were defined as previously described (21). We investigated whether the *Plasmodium* directly infected some kidney cells and the possible presence of parasitised red cells in the kidney parenchyma at the time of biopsy, by performing immunohistochemistry with a monoclonal antibody targeting *P. falciparum* HRP-2 (Meridian Life Science, Inc, Memphis, TN, USA) on frozen kidney biopsy sections fixed in ethanol (22) **(supplementary information)**.



## Results

### *Demographic, clinical and biological characteristics of patients with biopsy-proven glomerular disease occurring after imported Plasmodium infection*

We retrospectively identified 23 patients (12 men and 11 women) with a mean age 47 years (range: 24-66 years). Their demographic, clinical and biologic data at the time of *Plasmodium* infection are summarised in **Table 1**. All but one of the patients were of African ancestry, and 10 patients had concomitant HIV infection, newly diagnosed in three cases and known for some time in the other seven. All the cases were French citizens, living in France who had recently travelled back to France from an endemic area and developed malaria after their return to France. Mean CD4+ T-lymphocyte count in the 10 patients with HIV infection was 345/mm<sup>3</sup>, (range: 17 to 910/mm<sup>3</sup>). At the time of the bout of malaria, four HIV-positive patients were on HAART, which successfully controlled the viral infection, with undetectable levels of HIV in all cases (**Table 1**). Five HIV- positive patients had positive HIV viral load (2.10<sup>4</sup> to 2.10<sup>6</sup> copies/mL). One patient tested positive for hepatitis C virus (viral load: 5.7 viral copies/mL) and one patient was positive for hepatitis B virus (viral load: 260 copies/mL). The *Plasmodium* species responsible for malaria were *P. falciparum* in 22 cases (96%) and *P. malariae* in one case, and mean parasitaemia was 7% (range 0.3 to 29%). Severe malaria infection led to 11 patients (48%) being initially hospitalised in an intensive care unit (ICU) for malaria management. The specific treatments administered for malaria are listed in **Table 1**. The main results for kidney function parameters, immunological findings and other relevant laboratory investigations at the time of kidney biopsy are shown in **Table 2**. All patients displayed AKI (stage 1 in one patient, stage 2 in two patients and stage 3 in 20 patients). AKI required kidney replacement therapy with haemodialysis in 12 patients and the other 11 patients presented significant alterations of kidney function with a mean creatinine level of 4.9 mg/dl (range: 1.3

to 9.6mg/dl). Seventeen of the 19 patients for whom data were available (90%) presented nephrotic syndrome. Mean uPCR and serum albumin levels in the total population were 9.49 g/g (1.7 to 27g/g) and 2.0 g/dl (0.7 to 4g/dl), respectively. Immunological tests, performed in 23 cases, yielded unremarkable results in all but three patients, two of whom had type II cryoglobulinaemia with low levels of complement fractions, whereas the third had a monoclonal immunoglobulin G (IgG) kappa (κ) spike

### ***Underlying glomerular lesions***

The mean time between acute malaria episode and kidney biopsy was 24 days (7 to 80 days). In all patients, kidney biopsy was performed after the successful management of malaria, as demonstrated by an absence of parasitaemia after treatment, in all cases. Kidney pathology findings are shown in **Table 3**. The most frequent glomerular lesion, found in 21 patients (91%), was FSGS, but two patients (9%) had typical features of minimal change nephrotic syndrome (including one patient with *P. malariae* infection). The collapsing glomerulopathy variant was identified in 18 patients (**Figure 1a**) whereas lesions were classified as not otherwise specified (NOS) histological variants in three patients (**Figure 1b**). In the 10 patients with concomitant HIV infection, collapsing glomerular lesions were consistent with HIVAN diagnosis in nine cases, whereas the kidney biopsy specimen from the last HIV patient demonstrated the presence of NOS FSGS lesions. Of note, two patients had a previous history of biopsy-proven HIVAN (13 months and 60 months before the malaria episode) considered to be in remission under HAART until the relapse in a context of *Plasmodium* infection. In one case, HIVAN recurrence was diagnosed in a context of HAART withdrawal. Interestingly, three patients were found to have collapsing glomerulopathy despite good control of HIV infection. In one case, HIVAN lesions were associated with glomerular damage suggestive of additional MPGN lesions with

immunoglobulin monoclonal IgG  $\kappa$  deposits. An electron microscopy study was performed for this patient and revealed non-organized dense subendothelial osmiophilic deposits, suggestive of proliferative glomerulonephritis, with monoclonal IgG deposits but no tubuloreticular inclusion. In one patient, HIVAN was associated with thrombotic microangiopathy lesions in a context of severe hypertension. In two patients with cryoglobulinaemia, no glomerular lesions suggestive of cryoglobulinaemia-related glomerulonephritis were found on biopsy. In HIV-negative patients, collapsing glomerulopathy was the most frequent pattern of glomerular injury (nine patients, 69%). Excluding patients diagnosed with minimal change nephrotic syndrome, the percentage of glomeruli displaying segmental and sclerotic lesions was 64% (from 12.5 to 100%). In patients with collapsing glomerulopathy (regardless of HIV status) the mean percentage of glomeruli displaying segmental or global collapse was 50% (from 4 to 100%). Non-neoplastic lymphoplasmacytic infiltration was found in the interstitium in 18 patients (78%). Acute tubular necrosis (ATN) lesions were found in 15 patients (65%). Microcystic tubular dilatation was observed in 15 patients, including nine diagnosed with HIVAN and six HIV-negative patient (**Figure 1c**). Mild arteriolar hyalinosis was a frequent histological finding, present in 15 patients, mostly in those with collapsing glomerulopathy not associated with HIV infection (eight of nine patients).

### ***Detection of *P. falciparum* protein in kidney parenchyma***

*P. falciparum* was the species potentially implicated in the glomerular diseases occurring after malaria in 96% of cases. We therefore used an anti-HRP-2 antibody to determine whether parasite was present in the kidney parenchyma of 4 of these patients. The positive control, *P. falciparum*-infected red blood cells, is shown in **Figure 2a**. Immunohistochemistry revealed the presence of the parasite antigen in the lumina of the tubules (**Figure 2b**) of the four patients

analysed, and in the tubular cells cytoplasm (**Figure 2c**) in one patient. By contrast, podocytes, parietal epithelial cells and glomerular endothelial cells were negative, in all tested cases (**Figure 2d**). We observed no endocapillary staining suggestive of the presence of circulating infected red blood cells. The negative controls are shown in **Figures 2e and 2f**.

### ***Treatment and follow-up***

Some of the patients therefore underwent additional analyses to assess the likelihood of FSGS being caused by a secondary process (**Table 2**). APOL1 risk allele variants were studied in seven patients, all of whom tested positive (four patients were homozygous for the G1/G1 genotype and three had the compound heterozygous G1/G2 genotype). Serological tests for parvovirus B19 were performed in 14 cases. Four patients tested positive for specific IgM antibodies whereas concomitant serum PCR assays yielded negative results. At the time of kidney biopsy, HAART had been started in three of the HIV-infected patients, reintroduced in one case and modified in six (**Table 4**). Steroid therapy was introduced in 10 patients, including one with HIVAN associated with MPGN and the two patients diagnosed with minimal change nephrotic syndrome. An immunosuppressive regimen was added in two patients. After a mean follow-up of 23 months (0.5 to 220 months), only four of the patients were considered to present a complete remission of nephrotic syndrome. At the end of follow-up, kidney replacement therapy was required in eight patients: maintenance intermittent haemodialysis was performed in six cases and the other two underwent kidney transplantation. All these patients had severe AKI at the time of kidney biopsy (stage 3 of the KDIGO classification in all cases) and seven were already on dialysis at initial nephrological evaluation. One patient died from septic shock one month after kidney biopsy. It was possible to stop kidney replacement therapy in five of

the remaining 15 cases. Mean estimated glomerular filtration rate (eGFR) of these 15 patients was 51 mL/mn/1.73 m<sup>2</sup> at the end of follow-up (ranging from 9.5 to 113 mL/mn/1.73 m<sup>2</sup>).

## Discussion

The main clinical, biological, pathological and therapeutic characteristics of travellers with biopsy-proven glomerular disease after imported malaria have never been investigated in detail. In this retrospective French survey, we found that FSGS was the most frequent glomerular lesion observed in this setting. We also demonstrated that the most frequent morphological variant of FSGS was collapsing glomerulopathy, observed in 18/23 cases.

The mean time between the onset of *Plasmodium* infection and the histological diagnosis of glomerular disease was 24 days. This relatively long interval probably reflects the severity of malaria, as ICU admission was required for 11 patients at initial presentation, and 22 patients were considered to have severe malaria infection according to WHO criteria. AKI is a relatively frequent complication of severe malaria and is associated with higher mortality rates (23). The pathophysiological processes underlying AKI due to malaria seem to be multifactorial, involving haemodynamic instability, immune-mediated kidney injury, metabolic disturbances, and/or the sequestration of parasitised red blood cells in the kidney vasculature leading to ischemic ATN (4). Regardless of the underlying glomerular disease, our pathological study showed that ATN lesions were present in 61% of patients. Given the potential role of ischaemic injury in collapsing glomerulopathy pathogenesis (21), we can hypothesise that ischaemia related to *P. falciparum* infection may act as an additional factor triggering glomerular involvement in these patients. We also observed lymphoplasmocytic cell infiltration into the interstitial area in 78% of cases, suggesting that interstitial inflammation and systemic immune-inflammatory processes may also contribute to AKI (4).

The predominance of collapsing glomerulopathy among the glomerular lesions in these patients may also be a key factor associated with the high frequency of AKI in our cohort (24). Collapsing glomerulopathy, a severe clinicopathologic type of glomerular injury considered to

be a distinctive variant of the FSGS spectrum, was initially described in Afro-Caribbean populations, mostly in patients with HIV infection (classic HIVAN) (24) but also in some patients not infected with HIV (25). The list of known underlying processes associated with collapsing glomerulopathy is continuing to grow (21), but it has rarely been reported in a context of acute malaria (12-14). Several potential confounding causal agents of collapsing glomerulopathy could be considered in our study. In 10 patients, *Plasmodium* infection occurred in a context of HIV infection, and collapsing glomerulopathy is the main glomerular feature in patients diagnosed with HIVAN (21). Compelling evidence has been reported, demonstrating a direct role for HIV viral proteins in affected kidney parenchymal cells, promoting HIVAN (26, 27). Unfortunately, we did not perform in situ hybridisation to detect HIV in kidney tissues. Interestingly, HIV infection was successfully controlled in four patients on HAART therapy at the time of HIVAN diagnosis suggesting that the trigger was not HIV infection but malaria infection. One HIV-infected patient displayed FSGS lesions without the other pathological characteristics of HIVAN, highlighting the specific role of *P. falciparum* in podocyte injury. Infection with parvovirus B19, another agent that has been implicated in FSGS and/or collapsing glomerulopathy (21, 28, 29), was investigated in 14 patients. Four patients (29%) tested positive for IgM antibodies, suggesting recent infection. In our series, we cannot definitively exclude the possibility of a role of parvovirus B19 infection in the pathogenesis of glomerular disease due to the absence of DNA extraction and amplification on kidney tissue. Moreover, only two of our patients displayed clinical or biological features of autoimmune disease. These two patients had cryoglobulinaemia, but their kidney lesions were consistent with collapsing glomerulopathy, associated with TMA lesions in one patient and minimal change lesions in the other.

Our findings therefore suggest that infection with *P. falciparum* (96% of the cases considered here), may be a crucial trigger for FSGS/collapsing glomerulopathy development in patients of

African ancestry. The only patient in this cohort not of African descent was diagnosed with minimal change nephrotic syndrome. Recent studies have demonstrated that Afro-Caribbean individuals carrying APOL1 risk alleles have a higher risk of developing several types of glomerular injury leading to chronic kidney diseases (30-33). We investigated APOL1 risk genotype in seven patients from whom DNA samples were available. Strikingly, all of them were positive for high-risk alleles. There is growing experimental evidence to suggest that the kidney-specific expression of the APOL1-G1/G2 risk variants may interfere with normal podocyte homeostasis (34-36). Unfortunately, the retrospective nature of this study and the limited number of patients tested for APOL1 genetic variants suffers are inherent limitations to the approach used here that weaken our presumptions concerning the potential role of APOL1 polymorphism in the occurrence of glomerular injury. The potential role of malaria in the two patients with *P. falciparum* infection and minimal change nephrotic syndrome remains more speculative. Minimal change nephrotic syndrome is generally considered to be an idiopathic glomerular disorder potentially triggered by immunological stimuli, leading to secondary podocyte dysfunction (37). Electron microscopy analyses are not routinely performed in the context of MCNS at French nephrology centers. Light and immunofluorescence microscopy findings were highly suggestive of this diagnosis, but differential diagnoses could not be definitively ruled out. In our two patients, it remains difficult to determine whether minimal change nephrotic syndrome and malaria should be considered as a related disorder or as a fortuitous association. *Plasmodium* infection is associated with a profound dysregulation of the immune response (38), and we cannot, therefore, exclude the possibility that an increase in the production of pro-inflammatory cytokines interferes with normal podocyte biology, leading to subsequent nephrotic syndrome like it has already been described in other settings such as hemophagocytic syndrome (39).



In order to test the hypothesis of a direct role of the parasitic infection on kidney disease, we searched for *Plasmodium* in the kidney cells of our patients, by performing immunohistochemistry with an antibody specifically targeting a *P. falciparum* protein, in four patients. We detected parasites protein in the tubules, but obtained no evidence of parasitized red blood cell adhesion to the capillary glomerular endothelium or of the presence of parasites in podocytes. Nevertheless, the lack of detection of a *P. falciparum*-specific protein in the glomeruli at the time of kidney biopsy (mean of 24 days between malaria infection and kidney biopsy) does not rule out the possibility of the parasite playing a key role in early stages of glomerular injury. Consistent with this hypothesis, CMV infection may be considered a second hit for de novo collapsing glomerulopathy despite the absence of glomerular staining for CMV (40). The presence of parasite antigens in the tubular lumen and tubular cell cytoplasm after the virus has been eradicated remains surprising. We can hypothesise that, as in HIV infection (41), the kidneys may act as a reservoir of malaria infection despite undetectable parasitaemia. Further studies based on PCR tests on urine should be considered to determine whether this non-invasive method could be used for the diagnosis of persistent *Plasmodium* infection in the kidneys. Furthermore, we cannot exclude the possibility that HRP-2 detection in the kidney parenchyma is related to degradation products resulting from *Plasmodium* lysis.

Collapsing glomerulopathy is a particularly aggressive form of FSGS that responds poorly to steroid therapy (21, 42). In our study, after a mean of 23 months of follow-up, notwithstanding the initiation of steroid treatment in 10 cases, eight of the patients in the total population progressed to end-stage renal disease requiring kidney replacement therapy, and another four patients displayed severe decreases in eGFR. Previous studies in patients with collapsing glomerulopathy showed that the increase in ESRD risk was correlated with the degree of interstitial fibrosis, the number of glomeruli with collapsing lesions, proteinuria and creatinine levels (43,44). Twenty of the patients from our cohort had stage 3 AKI at initial presentation,

and all but one of the patients displayed severe kidney impairment at the time of biopsy. In addition, in 16 patients, more than 20% of the glomeruli presented collapsing lesions, and 12 patients had more than 20% interstitial fibrosis. Overall, these data may explain the poor kidney outcome of patients with glomerular disease after *Plasmodium* infection.

In conclusion, our study suggests that, in patients of African ancestry, imported malaria can be added to the spectrum of underlying processes promoting FSGS lesions. Our immunohistochemistry study results do not support a direct role for *P. falciparum* in glomerular damages. *P. falciparum* infection seems to act as a trigger for FSGS occurrence in patients with genetic susceptibility factors and/or pre-existing viral infection

**Table 1 Demographic and clinical data at the time of malaria**

Mean age (years) (n=23)	47 years (range: 24 to 66 years)
Sex (n= 23)	
Men	12 (52%)
Women	11 (48%)
Race (n= 23)	
Black	22 (96%)
White	1 (4%)
HIV status (n= 23)	
Positive	10 (43%)
Mean HIV viral load (copies/ml) (n= 9/10)	458000 copies/ml (range: undetectable to $2.10^6$ copies/ml)
Mean CD4 count (/mm <sup>3</sup> ) (n= 9/10)	345/mm <sup>3</sup> (range: 17 to 910/mm <sup>3</sup> )
<i>Plasmodium</i> species (n= 23)	
<i>Plasmodium falciparum</i>	22 (96%)
<i>Plasmodium malariae</i>	1 (4%)
Mean Parasitaemia (%) (n=16/23)	7% (range: 0.3 to 29%)
Admission unit (n= 23)	
ICU	11 (48%)
Malaria severity criteria (n= 23)	
None	1 (4.5%)
%P	1 (4.5%)
AKI	15 (64%)
Neurological criteria*	1 (4.5%)
AKI and %P	2 (9%)
AKI and ARDS	1 (4.5%)
AKI and Neurological criteria*	1 (4.5%)
AKI and Neurological criteria* and %P	1 (4.5%)
Specific treatment of malaria episode (n= 22/23)	
Quinine	4 (18%)
Artesunate	10 (45%)
Arteminol piperazine	4 (18%)
Atovaquone proguanil	2 (9%)
Atovaquone proguanil + Artesunate	1 (5%)
Atovaquone proguanil + Artesunate + Quinine	1 (5%)

HIV: human immunodeficiency virus, ICU: intensive care unit, AKI: acute kidney injury, %P: parasitaemia expressed as a percentage, ARDS: acute respiratory distress syndrome

\*Neurological criteria: neurological involvement including impaired consciousness and/or prostration and/or multiple convulsions

**Table 2 Clinical and biological data at the time of kidney biopsy**

AKI according KDIGO criteria (n=23)	
Stage 1	1 (4%)
Stage 2	2 (9%)
Stage 3	20 (87%)
Hypertension (n=23)	10 (43%)
UPCR (g/g) (n=21/23)*	9.49 g/g (range: 1.7-27 g/g)
Serum albumin level (g/dL) (n=21/23)	2.0 g/dL (range: 0.7-4 g/dL)
Microscopic hematuria (n=21/23)*	10 (48%)
Leukocyturia (n=21/23)*	9 (43%)
Time between malaria infection and kidney biopsy (days) (n=23)	24 days (range: 7-80 days)
Immunological findings (n=23)	
None	20 (87%)
IgG k spike	1 (4%)
Cryo (type II)	2 (9%)
Parvovirus B19 serology (n=14/23)	
IgM+, IgG-	4 (29%)
IgM-, IgG+	0 (0%)
IgM+, IgG+ <sub>-</sub>	0 (0%)
IgM-, IgG-	10 (71%)
ApoL1 status (n=7/23)	
G1/G1	4 (57%)
G1/G2	3 (43%)

AKI: acute kidney injury, KDIGO: Kidney Disease Improving Global Outcome, UPCR: urine protein/creatinine ratio, Cryo: cryoglobulinaemia, IgGk: monoclonal immunoglobulin G kappa. ApoL1: apolipoprotein L1

\* Two patients with anuria

**Table 3: Kidney biopsy findings**

Number of glomeruli (n=23)	16 (range 6-30)
Obsolescent glomeruli (%) (n=23)	11% (range 0-50 %)
FSGS lesions (%) (n=21/23)	64% (range 12.5-100%)
Collapsing lesions (%) (n=18/21)	50% (range 4-100%)
Interstitial fibrosis (%) (n=23)	18% (range 0-50%)
Interstitial infiltrate (n=23)	18 (78%)
Interstitial edema (n=23)	7 (30%)
Tubular microcyst dilatation (n=23)	15 (65%)
Acute tubular necrosis (n=23)	15 (65%)
Arteriolar hyalinosis* (n=23)	15 (65%)
Definitive pathological diagnosis**	
FSGS	21 (91%)
Collapsing glomerulopathy	18
(including HIV associated	9
nephropathy)	
Not otherwise specified variant	3
Minimal change disease	2 (9%)

FSGS: focal segmental glomerulosclerosis

\*arteriolar hyalinosis was scored as follows: + mild ++ moderate +++ severe

\*\* In one patient with HIV infection, renal biopsy findings were consistent with not otherwise specified variant. In one case HIV associated nephropathy lesions were associated with additional membranoproliferative glomerulonephritis lesions. In one case, HIV associated nephropathy lesions were associated with thrombotic microangiopathy lesions.

**Table 4: Treatment and follow-up**

HAART initiation in HIV patients (n=10)	10 (100%)
Steroids (n=23)	10 (43%)
RAS inhibitors (n=23)	11 (48%)
Cyclosporine (n=23)	2 (9%)
Mean follow-up (months) (n=23)	23 months (range 0.5-220 months)
Complete remission of nephrotic syndrome (n=22/23)	4 (18%)
Kidney status at last follow-up (n=22/23)* Hemodialysis Kidney Transplant Mean eGFR at last follow-up visit in those not needing KRT (n=15) (ml/min/1.73m <sup>2</sup> )	6 (27%) 2 (9%) 51 ml/min/1.73m <sup>2</sup> (range 9.5-113 ml/min/1.73m <sup>2</sup> )

HAART: highly active antiretroviral therapy, RAS: renin-angiotensin system, eGFR: estimated glomerular filtration rate, KRT: kidney replacement therapy

\* Death occurred in one patient one month after kidney biopsy

**Disclosures :** Dr. Audard reports receiving personal fees from Addmedica outside of the submitted work

**Acknowledgments :** We would like to acknowledge all the nephrologists and kidney pathologists involved in the medical care of the patients included in the present study. We thank Julie Sappa for editorial assistance.

## **Legends to figures**

**Figure 1: Illustration of the main glomerular diseases occurring after imported malaria**

Collapsing glomerulopathy with segmental collapse of the glomerular tuft and overlying epithelial cell hypertrophy and hyperplasia (**1a**, periodic acid-Schiff staining x 400) in an HIV-negative patient

Focal and segmental glomerulosclerosis (NOS variant) in an HIV-negative patient (**1b**, Masson's trichrome staining x400)

Collapsing glomerulopathy was associated with microcystic tubular dilation (**1c**, Masson's trichrome staining x100) in an HIV-negative patient.

**Figure 2: Immunohistochemistry with a monoclonal antibody against *Plasmodium falciparum* HRP-2**

**2a:** *P. falciparum*-infected red blood cells as a positive control (arrow) (x400)

**2b:** *P. falciparum* parasites in the tubule lumen (arrow) (x400)

**2c:** *P. falciparum* parasites in the tubule lumen (arrowheads) and cytoplasm (arrow) (x400)

**2d:** Absence of parasites in FSGS lesions (x400)

**2e:** Negative control, with omission of the primary antibody (x400), for comparison with figure 2 b

**2f:** Absence of staining in a negative control consisting of a HIVAN patient without malaria (x200). Similar results were obtained for patients with acute tubular necrosis unrelated to malaria.

## References

1. Askling HH, Bruneel F, Burchard G, Castelli F, Chiodini PL, Grobusch MP, Lopez-Vélez R, Paul M, Petersen E, Popescu C, Ramharter M, Schlagenhauf P; European Society for Clinical



Microbiology and Infectious Diseases Study Group on Clinical Parasitology. Management of imported malaria in Europe. *Malar. J* 11: 328, 2012

2. Burdmann EA and Jha V. Acute kidney injury due to tropical infectious diseases and animal venoms: a tale of 2 continents. *Kidney Int* 91: 1033–1046, 2017

3. van Wolfswinkel M.E, Koopmans LC, Hesselink DA, Hoorn EJ, Koelewijn R, van Hellemond JJ, van Genderen PJ. Neutrophil gelatinase-associated lipocalin (NGAL) predicts the occurrence of malaria-induced acute kidney injury. *Malar. J* 15: 464, 2016

4. Barsoum RS. Malarial acute renal failure. *J. Am. Soc. Nephrol* 11: 2147–2154, 2000

5. Van Velthuysen ML. Glomerulopathy associated with parasitic infections. *Parasitol. Today* 12: 102–107, 1996

6. Elsheikha HM and Sheashaa HA. Epidemiology, pathophysiology, management and outcome of renal dysfunction associated with plasmodia infection. *Parasitol. Res* 101: 1183–1190, 2007

7. Yashima A, Mizuno M, Yuzawa Y, Shimada K, Suzuki N, Tawada H, Sato W, Tsuboi N, Maruyama S, Ito Y, Matsuo S, Ohno T. Mesangial proliferative glomerulonephritis in murine malaria parasite, *Plasmodium chabaudi* AS, infected NC mice. *Clin. Exp. Nephrol* 21: 589–596, 2017

8. Barsoum R.S. Malarial nephropathies. *Nephrol. Dial. Transplant* 13: 1588–1597, 1998

9. Yoo DE, Kim JH, Kie JH, Park Y, Chang TI, Oh HJ, Kim SJ, Yoo TH, Choi KH, Kang SW, Han SH. Immunoglobulin A nephropathy associated with *Plasmodium falciparum* malaria. *J. Korean Med. Sci* 27: 446–449, 2012.

10. Walker A, Ellis J, Irama M, Senkungu J, Nansera D, Axton J, Coward RJ, Peat DS, Bode HH, Mathieson PW. Eosinophilic glomerulonephritis in children in Southwestern Uganda. *Kidney Int* 71: 569–573, 2007

11. Rangwani N, Facaros, S, Wang J, Agarwal S, Shah P, Raina R. Minimal change disease and malaria. *Clin. Kidney J* 12: 245–27, 2019.

12. Kute VB, Trivedi HL, Vanikar AV, Shah PR, Gumber MR, Kanodia KV. Collapsing glomerulopathy and hemolytic uremic syndrome associated with *falciparum* malaria: completely reversible acute kidney injury. *J. Parasit. Dis* 37: 286–290, 2013

13. Niang A, Niang SE, Ka el HF, Ka MM, Diouf B. Collapsing glomerulopathy and haemophagocytic syndrome related to malaria: a case report. *Nephrol. Dial. Transplant* 23: 3359–3361, 2008

14. Sehar N, Gobran E, and Elsayegh S. Collapsing Focal Segmental Glomerulosclerosis in a Patient with Acute Malaria. *Case Rep. Med* 2015: 420459, 2015

15. Kidney Disease: Improving Global Outcomes (KDIGO) Acute Kidney Injury Work Group: KDIGO clinical practice guideline for acute kidney injury. *Kidney Int Suppl* 12: 1–138, 2012

16. Kidney Disease. Improving global outcomes (KDIGO) CKD work group: KDIGO clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl* 3: 1–150, 2013
17. Freedman BI, and Skorecki K. Gene-gene and gene-environment interactions in apolipoprotein L1 gene-associated nephropathy. *Clin. J. Am. Soc. Nephrol* 9: 2006–2013, 2014
18. World Health Organization. Guidelines for the Treatment of Malaria. Third edition. Geneva; WHO Press; 2015
19. Vivarelli M, Massella L, Ruggiero B, and Emma F. Minimal Change Disease. *Clin. J. Am. Soc. Nephrol* 12: 332–345, 2017
20. D'Agati VD, Fogo AB, Bruijn JA, and Jennette JC. Pathologic classification of focal segmental glomerulosclerosis: a working proposal. *Am. J. Kidney Dis* 43: 368–382, 2004
21. Alqaumi M and Barisoni L. Current views on collapsing glomerulopathy. *J. Am. Soc. Nephrol* 19: 1276–1281, 2008
22. Genrich GL, Guarner J, Paddock CD, Shieh WJ, Greer PW, Barnwell JW, Zaki SR. Fatal malaria infection in travelers: novel immunohistochemical assays for the detection of *Plasmodium falciparum* in tissues and implications for pathogenesis. *Am J Trop Med Hyg* 76: 251–259, 2007
23. Trang TT, Phu, NH, Vinh H, Hien TT, Cuong BM, Chau TT, Mai NT, Waller DJ, White NJ. Acute Renal Failure in Patients with Severe *Falciparum* Malaria. *Clin. Infect. Dis* 15: 874–880, 1992
24. Schwimmer JA, Markowitz GS, Valeri A and Appel G.B. Collapsing glomerulopathy. *Semin. Nephrol* 23: 209–218, 2003
25. Weiss MA, Daquiaoag E, Margolin EG and Pollak VE. Nephrotic syndrome, progressive irreversible renal failure, and glomerular “collapse”: a new clinicopathologic entity? *Am. J. Kidney Dis* 7: 20–28, 1986
26. Ross M.J. Advances in the pathogenesis of HIV-associated kidney diseases. *Kidney Int* 86: 266–274, 2014
27. Swanepoel CR, Atta MG, D'Agati VD, Estrella MM, Fogo AB, Naicker S, Post FA, Wearne N, Winkler CA, Cheung M, Wheeler DC, Winkelmayer WC, Wyatt CM. Kidney disease in the setting of HIV infection: conclusions from a Kidney Disease: Improving Global Outcomes (KDIGO) Controversies Conference. *Kidney Int* 93: 545–559, 2018
28. Moudgil A, Nast CC, Bagga A, Wei L, Nurmamet A, Cohen AH, Jordan SC, Toyoda M. Association of parvovirus B19 infection with idiopathic collapsing glomerulopathy. *Kidney Int* 59: 2126–2133, 2001
29. Tanawattanacharoen S, Falk RJ, Jennette JC and Kopp JB. Parvovirus B19 DNA in kidney tissue of patients with focal segmental glomerulosclerosis. *Am. J. Kidney Dis* 35: 1166–1174, 2000

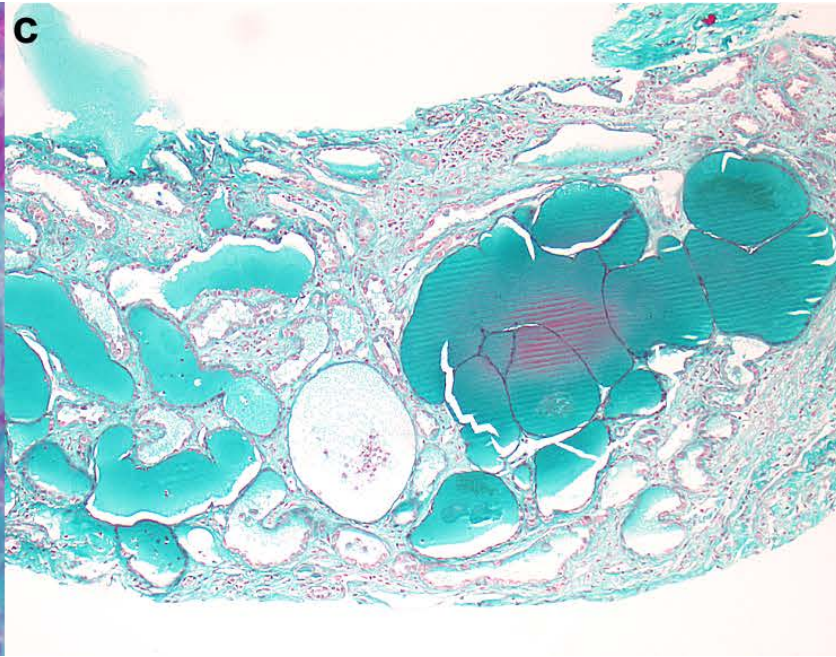
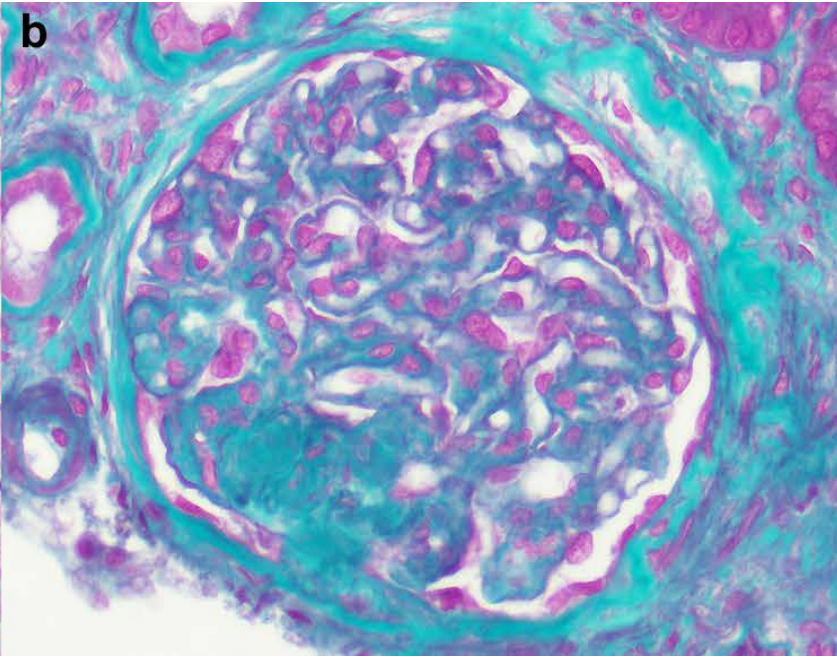
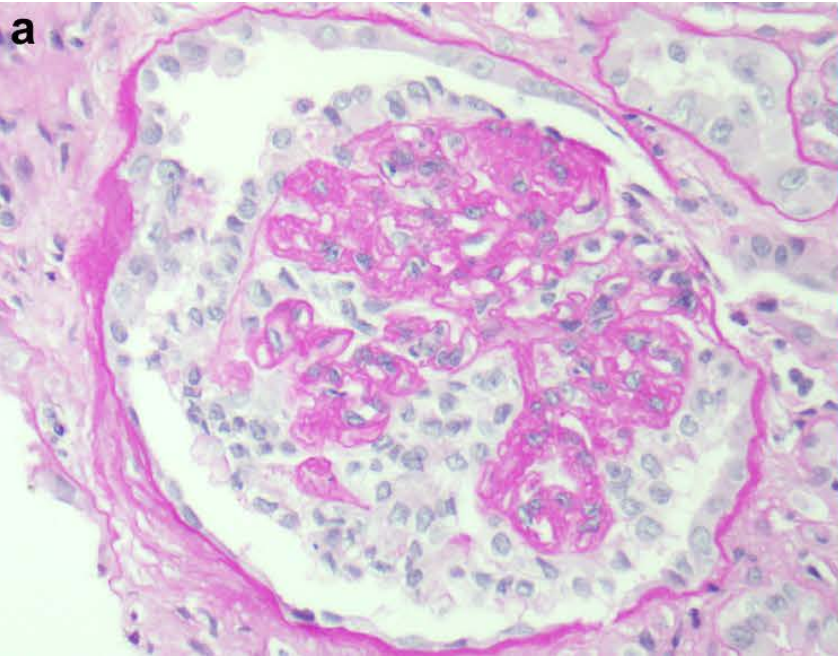
30. Genovese G, Friedman DJ, Ross MD, Lecordier L, Uzureau P, Freedman BI, Bowden DW, Langefeld CD, Oleksyk TK, Uscinski Knob AL, Bernhardt AJ, Hicks PJ, Nelson GW, Vanhollebeke B, Winkler CA, Kopp JB, Pays E, Pollak MR. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. *Science* 329: 841–845, 2010
31. Kopp JB, Nelson GW, Sampath K, Johnson RC, Genovese G, An P, Friedman D, Briggs W, Dart R, Korbet S, Mokrzycki MH, Kimmel PL, Limou S, Ahuja TS, Berns JS, Fryc J, Simon EE, Smith MC, Trachtman H, Michel DM, Schelling JR, Vlahov D, Pollak M, Winkler CA. APOL1 genetic variants in focal segmental glomerulosclerosis and HIV-associated nephropathy. *J. Am. Soc. Nephrol* 22: 2129–2137, 2011
32. Kormann R, Jannot AS, Narjoz C, Ribeil JA, Manceau S, Delville M, Joste V, Prié D, Pouchot J, Thervet E, Courbebaisse M, Arlet JB. Roles of APOL1 G1 and G2 variants in sickle cell disease patients: kidney is the main target. *Br. J. Haematol* 179: 323–335, 2017
33. Larsen CP, Beggs ML, Saeed M and Walker PD. Apolipoprotein L1 risk variants associate with systemic lupus erythematosus-associated collapsing glomerulopathy. *J. Am. Soc. Nephrol* 24: 722–725, 2013
34. Ma L, Shelness GS, Snipes JA, Murea M, Antinozzi PA, Cheng D, Saleem MA, Satchell SC, Banas B, Mathieson PW, Kretzler M, Hemal AK, Rudel LL, Petrovic S, Weckerle A, Pollak MR, Ross MD, Parks JS, Freedman BI. Localization of APOL1 protein and mRNA in the human kidney: nondiseased tissue, primary cells, and immortalized cell lines. *J. Am. Soc. Nephrol* 26: 339–348, 2015
35. Beckerman P, Bi-Karchin J, Park AS, Murea M, Antinozzi PA, Cheng D, Saleem MA, Satchell SC, Banas B, Mathieson PW, Kretzler M, Hemal AK, Rudel LL, Petrovic S, Weckerle A, Pollak MR, Ross MD, Parks JS, Freedman BI. Transgenic expression of human APOL1 risk variants in podocytes induces kidney disease in mice. *Nat. Med* 23: 429–438, 2017
36. Kumar V, Paliwal N, Ayasolla K, Vashistha H, Jha A, Chandel N, Chowdhary S, Saleem MA, Malhotra A, Chander PN, Skorecki K, Singhal PC. Disruption of APOL1-miR193a Axis Induces Disorganization of Podocyte Actin Cytoskeleton. *Sci. Rep* 9: 3582, 2019
37. Sahali D, Sendeyo K, Mangier M, Audard V, Zhang SY, Lang P, Ollero M, Pawlak A. Immunopathogenesis of idiopathic nephrotic syndrome with relapse. *Semin. Immunopathol* 36: 421–429, 2014
38. Ortega-Pajares A, and Rogerson S. The Rough Guide to Monocytes in Malaria Infection. *Front. Immunol* 9: 2888, 2018
39. Thaunat O, Delahousse M, Fakhouri F, Martinez F, Stephan JL, Noël LH, Karras A. Nephrotic syndrome associated with hemophagocytic syndrome. *Kidney Int* 69: 1892-1898, 2006
40. Chang JH, Husain SA, Santoriello D, Stokes MB, Miles CD, Foster KW, Li Y, Dale LA, Crew RJ, Cohen DJ, Kiryluk K, Gharavi AG, Mohan S. Donor's APOL1 Risk Genotype and "Second Hits" Associated With De Novo Collapsing Glomerulopathy in Deceased Donor Kidney Transplant Recipients: A Report of 5 Cases. *Am. J. Kidney Dis* 73: 134–139, 2019
41. Canaud G, Dejucq-Rainsford N, Avettand-Fenoël V, Viard JP, Anglicheau D, Bienaimé F, Muorah M, Galmiche L, Gribouval O, Noël LH, Satie AP, Martinez F, Sberro-Soussan R,

Scemla A, Gubler MC, Friedlander G, Antignac C, Timsit MO, Onetti Muda A, Terzi F, Rouzioux C, Legendre C. The kidney as a reservoir for HIV-1 after renal transplantation. *J Am Soc Nephrol* 25:407–19, 2014

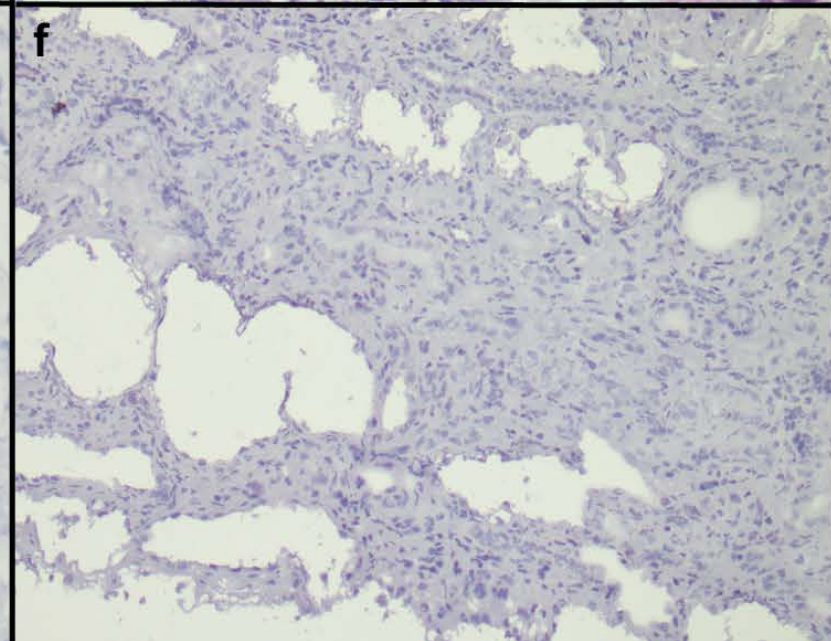
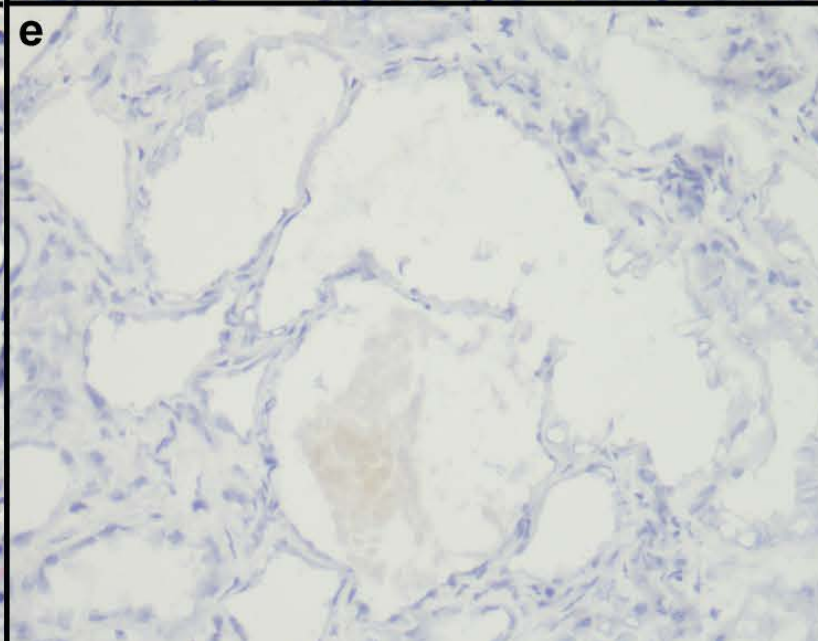
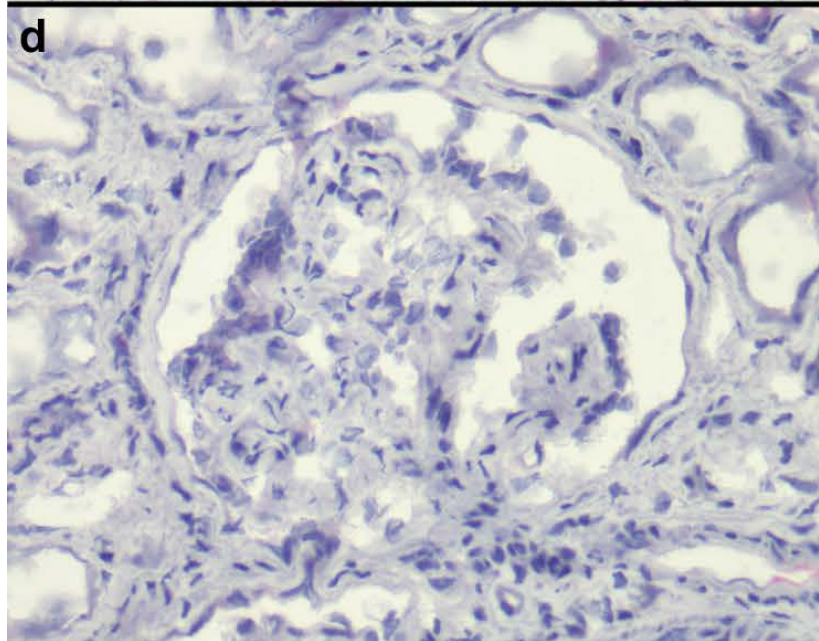
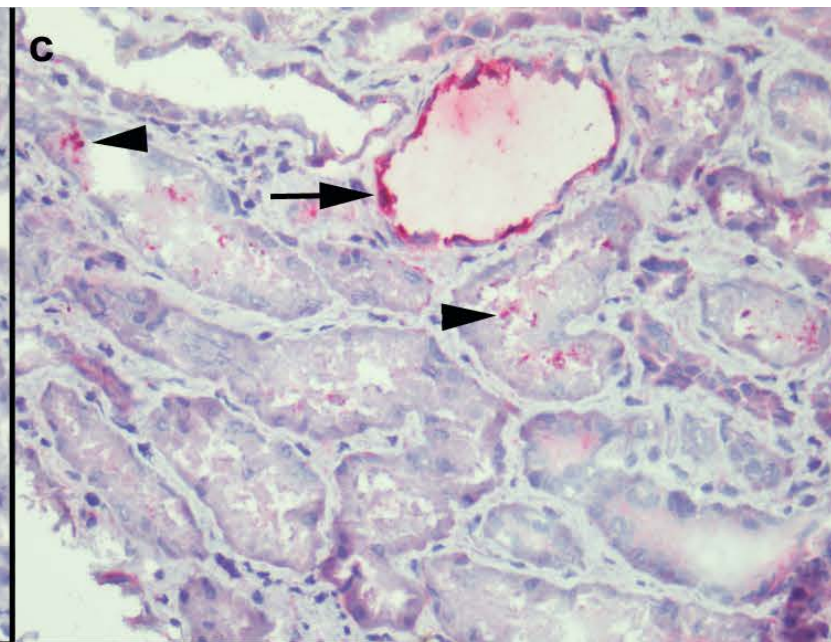
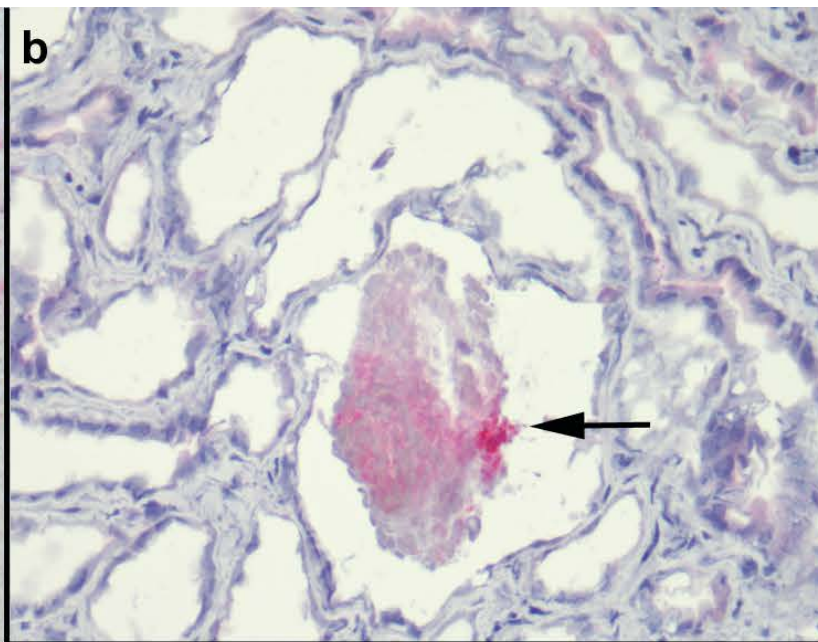
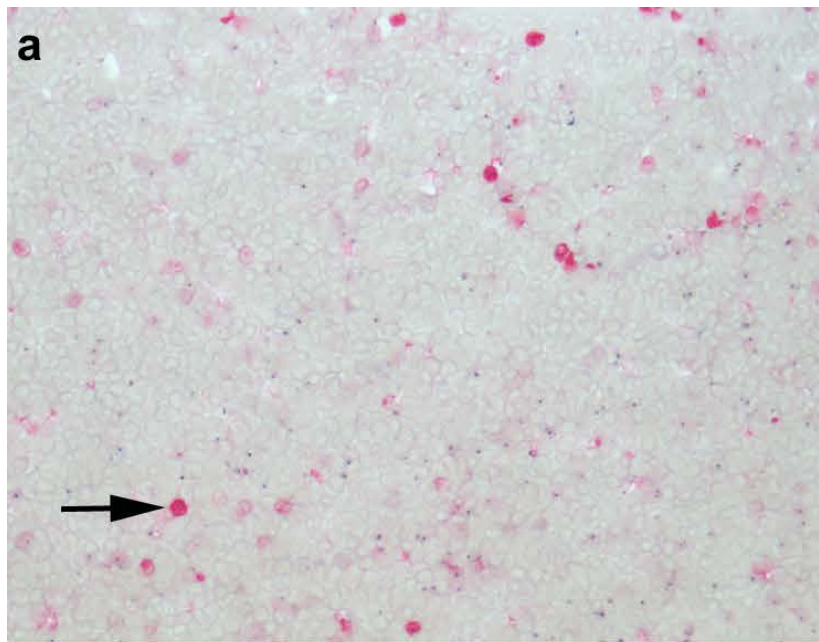
42. Thomas DB, Franceschini N, Hogan SL, Ten Holder S, Jennette CE, Falk RJ, Jennette JC. Clinical and pathologic characteristics of focal segmental glomerulosclerosis pathologic variants. *Kidney Int* 69: 920–926, 2006

43. Laurinavicius A, Hurwitz S, and Rennke HG. Collapsing glomerulopathy in HIV and non-HIV patients: a clinicopathological and follow-up study. *Kidney Int* 56: 2203–2213, 1999

44. Valeri A, Barisoni L, Appel GB, Seigle R, D'Agati V. Idiopathic collapsing focal segmental glomerulosclerosis: a clinicopathologic study. *Kidney Int* 50: 1734–1746, 1996







## **Supplementary information**

### **Material and Methods**

#### ***Patients and renal function evaluation***

##### *Identification of patients, demographic clinical and biological data*

We included 23 adult patients from 10 nephrology departments (Henri Mondor Hospital (Créteil), Tenon Hospital, Necker Hospital, Georges Pompidou European Hospital, Bichat Hospital, *Association pour l'utilisation du Rein Artificiel* (Paris), Foch Hospital (Suresnes), André Grégoire Hospital (Montreuil), Conception Hospital (Marseille), Huriez Hospital (Lille)) seen between 1998 and 2019. The patients at each hospital were identified on the basis of electronic medical records, including the renal disease and clinical diagnosis databases. For all patients, the demographic data recorded included age, sex, ethnicity and coinfections with hepatitis B virus, hepatitis C virus and human immunodeficiency virus (HIV). For patients with HIV infection, HIV viral load (copies/mL), CD4<sup>+</sup> T-lymphocyte counts, and the use of highly active antiretroviral therapy (HAART) were reported. Levels of complement fractions C3, C4 and the total hemolytic activity (CH50) of the serum were determined for all patients. Serum protein electrophoresis and immunological tests, including tests for anti-neutrophil cytoplasm antibodies, anti-nuclear antibodies and anti-DNA antibodies were systematically performed.. High-risk APOL1 genotypes were defined as two risk alleles in any combination (homozygous G1/G1, homozygous G2/G2, or compound heterozygous G1/G2). All patients tested for APOL1 risk alleles gave written informed consent.

##### *Follow-up*

Follow-up data included renal function evaluations (estimated glomerular filtration rate (eGFR) or the need for renal replacement therapy and the outcome of underlying glomerular disease,

based on proteinuria at the last follow-up visit. Complete remission of nephrotic syndrome was defined as the normalisation of urinary protein-creatinine ratio (uPCR) ( $< 0.3 \text{ g/g}$ ), and an albumin concentration  $>3.0\text{g/dL}$ . Specific treatments for underlying glomerular disease (steroid therapy and/or immunosuppressive agents) were specified for all patients.

### ***Plasmodium infection***

The results of parasitaemia levels and species identification confirmed by polymerase chain reaction (PCR) were noted when available. The antimalarial drugs administered and the type of hospitalisation unit to which patients were initially admitted (conventional medicine unit or intensive care unit (ICU)) were recorded for all patients.

### ***Renal biopsy examination and immunohistochemistry study.***

#### ***Diagnosis of underlying glomerular diseases***

Minimal change nephrotic syndrome was diagnosed on the basis of an absence of visible alterations on light microscopy examination, and an absence of immunoglobulin and/or complement deposits in immunofluorescence studies (19). Focal and segmental glomerulosclerosis (FSGS) diagnosis required the presence of segmentally collapsed glomerular capillaries with areas of glomerular scarring associated with focal and segmental granular deposition of IgM and/or C3 within the areas of segmental glomerular sclerosis; all cases of FSGS were classified according to the Columbia classification (20). The morphological features of collapsing glomerulopathy (including HIV-associated nephropathy (HIVAN) in HIV-infected patients) were segmental and/or global collapse of the glomerular capillary tufts,



with overlying epithelial cell hypertrophy and hyperplasia, with or without microcystic tubular dilation and tubulointerstitial lesions (21)

*Immunohistochemistry with a monoclonal antibody targeting P. falciparum HRP-2*

We used *P. falciparum*-infected red blood cells fixed in ethanol as the positive control, and the primary antibody was omitted as a negative control. We also assessed the specificity of staining on clinically relevant negative controls: two kidney biopsy specimens from patients diagnosed with HIVAN diagnosis in the absence of malaria and two kidney biopsy specimens from patients with acute tubular necrosis (ATN) not related to malaria infection.