# Nephrotic syndrome associated with hemophagocytic syndrome

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**Hemophagocytic syndrome (HPS) is defined by bone marrow and organ infiltration by activated, nonmalignant macrophages, which phagocytose blood cells. The clinical spectrum of HPS is broad, but renal involvement has rarely been investigated. We report a previously unknown renal manifestation of HPS: nephrotic syndrome. This multicentric retrospective study included patients fulfilling the following criteria: (i) no history of nephropathy; (ii) HPS diagnosis with histologic evidence of hemophagocytosis; (iii) occurrence of nephrotic syndrome during HPS; and (iv) available renal histology. Using the same criteria, we also searched the literature for additional cases. We identified nine patients retrospectively and found two additional cases in the literature (five males and six females, whose mean age was 34±27 years). Black African patients predominated (63.6%). HPS was due to lymphoma (six cases), infectious disease (three cases), and autoimmune disease (one case), and was primary in one patient. Acute renal failure was associated with nephrotic syndrome in 10/11 cases. Renal histology showed acute tubular necrosis associated with collapsing glomerulopathy in five patients (all Africans with negative human immunodeficiency virus serology), minimal change glomerulopathy in four, and thrombotic-microangiopathy with abnormal podocytes in two. Death occurred in seven cases. Nephrotic syndrome should be included among the renal complications of HPS with acute renal failure. We postulate that abnormal T-cell activation and/or high pro-inflammatory cytokine levels during HPS might cause podocyte injuries, especially among African patients with a susceptible genetic background.**

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Hemophagocytic syndrome (HPS) is a clinicopathologic entity caused by excessive activation and proliferation of nonmalignant macrophages. HPS also known as macrophage activation syndrome, may be either primary, as observed in various genetic diseases affecting the immune system, including familial hemophagocytic lymphohistiocytosis, X-linked lymphoproliferative syndrome, Chediak–Higashi syndrome, and Griscelli syndrome, or may be secondary to an underlying disorder, mainly malignancy, severe infection, or autoimmune disease.[1] The manifestations of HPS are the consequences of widespread tissue infiltration by activated histiocytic cells, and also result from the secretion of huge amounts of proinflammatory cytokines by activated T cells and macrophages.[2,3]

Clinically, HPS is usually characterized by the combination of fever, hepatosplenomegaly, lymphadenopathy, and jaundice, and sometimes by skin rash, lung infiltration, and hemorrhagic complications of thrombocytopenia. Laboratory findings frequently include pancytopenia, liver test abnormalities, coagulopathy with low fibrinogen levels, marked hypertriglyceridemia, and elevated serum ferritin.[4] The diagnosis of HPS is confirmed by the presence of diffuse infiltration by well-differentiated macrophages, actively phagocytosing hematopoietic elements in the bone marrow, lymph nodes, liver, or spleen (Table 1).[5]

Since HPS first description in the late 1930s,[6] substantial advances have been achieved in understanding its pathophysiology, leading to the identification of a growing number of cases. However, despite this progress, very few data are available about the renal complications of HPS. Acute renal failure is thought to be the most consistent feature[4,7] and is considered by some authors as a strong prognostic factor,[8] but biopsy-proven glomerular involvement has remained poorly documented.

We recently observed three cases of nephrotic syndrome complicating the course of HPS in the Nephrology Department of Necker Hospital. Secondarily, we undertook a retrospective study and a review of the literature to describe the glomerular lesions associated with HPS.

## RESULTS

Over a 20-year period, nine patients with histologic examination were retrospectively identified as presenting

**Table 1 | Diagnostic guidelines for hemophagocytic syndrome (adapted from Henter *et al.*[5])**

| Clinical criteria | Laboratory criteria | Histologic criteria |
|---|---|---|
| *Fever* | Cytopenias (affecting two or more lineages in the peripheral blood)<br>Hemoglobin $<9$ g/dl<br>Platelets $<100$ G/l<br>Neutrophils $<1$ G/l | Hemophagocytosis on cytology or histology |
| *Splenomegaly* | Hypertriglyceridemia ($>3$ mmol/l) and/or Hypofibrinogenemia ($<1.5$ g/l) | |

Diagnosis of hemophagocytic syndrome is considered if five of the above eight criteria are fulfilled.

with HPS and nephrotic syndrome. In the literature, only two previously reported cases fulfilled the criteria stated in Materials and Methods and were considered for analysis.[9,10] The results given below include these two additional cases, together with the nine new cases that we report.

### Characteristics of HPS

***Clinical features (Table 2).*** The study group included 11 patients (five males and six females), whose mean age was $34 \pm 27$ years (range 2.5–68). Patients of Black African origin predominated ($7/11 = 63.6\%$). Three of the others were Caucasians and in one was Chinese.

Only one of the two pediatric patients had a primary HPS complicating familial hemophagocytic lymphohistiocytosis. The remaining 10 patients (90.9%) therefore had secondary HPS, combined with lymphoma in six cases (Hodgkin's: 1, T-cell: 4, B-cell: 1). In two cases, HPS was secondary to infectious diseases (cytomegalovirus infection: 1, visceral Leishmaniasis: 1), and one case was due to an autoimmune disorder (pediatric Still's disease). For the last case (collected in the literature, patient No. 11), no etiology was identified. HIV serology was negative in all patients.

The frequency of each HPS symptom among these 11 patients is listed in Table 2. Common initial clinical features included fever, chills, weakness or fatigue, anorexia, and weight loss. Six patients (54.5%) exhibited various gastrointestinal symptoms, including diarrhea, abdominal pain, nausea, and vomiting. Four (36.4%) had central nervous system manifestations, and 4 (36.4%) experienced pulmonary signs such as cough or dyspnea.

On physical examination, hepatosplenomegaly was found in four patients (36.4%), superficial lymph node enlargement in 2 (18.2%), and skin rash in 2 (18.2%).

***Laboratory findings.*** Depression of circulating blood element counts was the most common laboratory abnormality. Anemia and thrombocytopenia occurred in 10 patients (90%) and nine (82%) patients, respectively. The mean nadir values for hemoglobin and platelet count were $8.3 \pm 1.7$ g/dl and $88\,000 \pm 11\,0000$ g/l, respectively. All patients (100%) had depression of at least one cell line. Elevated serum liver transaminases (alanine aminotransferase and/or aspartate aminotransferase) and hyperbilirubinemia were present in six (54.5%) and eight (72.7%) patients, respectively. When measured, Lactate dehydrogenase, triglycerides, and ferritin serum were always elevated.

For three patients (No. 2, No. 5, and No. 9), recruited after the initiation of the study, serum samples collected at the admission for cytokine measurement were available. A significant increase in the serum levels of proinflammatory cytokines (interferon $\gamma$, interleukin (IL) 1$\beta$, IL6, and TNF$\alpha$ (tumor necrosis factor alpha)) was observed (Figure 1).

***Histologic features.*** As proved hemophagocytosis was one of the criteria required for inclusion, it was present in all patients (100%). However, the diagnostic test that proven its presence was different from one patient to another.

Bone marrow specimen examination (aspirate and/or biopsy) was performed in 10/11 patients and was contributive in eight cases. In one of these cases, the first aspiration smear was normal, so a second aspiration, performed 2 weeks later, was necessary to confirm the diagnosis of HPS. In three cases, bone marrow examination failed to detect hemophagocytosis, but the diagnosis was confirmed by the biopsy of another organ (skin, lymph node, and kidney, respectively).

### Characteristics of nephrotic syndrome

The clinical features of nephropathy in our patients are listed in Table 2.

In 7/11 patients (63.6%), nephrotic syndrome and HPS were diagnosed simultaneously on admission. In three patients, nephrotic syndrome was diagnosed $25 \pm 30$ days (range: 4–60) after the diagnosis of HPS. In all three cases, HPS was active when the nephrotic syndrome occurred. The remaining patient was initially admitted for nephrotic syndrome with acute renal failure and HPS was only diagnosed 12 days later.

Nephrotic syndrome was severe, as assessed by the mean values for 24-h urine protein and serum albumin, whose respective levels were $11 \pm 9.6$ g/24 h and $17 \pm 1.7$ g/l.

Acute renal failure was present in 10/11 cases (90.9%), and oligoanuria in four patients. Dialysis therapy was required for six patients (54.5%); five of whom had hemodialysis, and one peritoneal dialysis. Among the 10 patients presenting with acute renal failure, 4 (36.4%) presented significant microhematuria.

### Renal histology findings

For the nine patients identified retrospectively, renal tissue for histologic study was obtained from either transcutaneous renal biopsy (7) or necropsy (2). Table 3 shows the detailed

**Table 2 | Clinical and laboratory data for 11 patients with HPS associated with nephrotic syndrome**

| | Retrospective study | | | | | | | | | Literature | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Age/gender/race | 51/M/C | 43/M/Af | 19/M/Af | 60/M/Af | 63/F/C | 16/F/Af | 27/F/Af | 7/F/C | 68/M/Af | 2.5/F/Af | 18/F/As |
| Associated medical conditions | None | Cardiopathy | None | Type II diabetes HCV | Stroke | Malaria | None | 13 relapses of Still's Dis | None | None | None |
| *Hemophagocytic syndrome* | | | | | | | | | | | |
| Etiology of HPS | MNHL-T | MNHL-T | MNHL-T | MNHL-B | CMV | *Leish* | MNHL-T | Still's Dis | Hodgkin | HLH | Unidentified |
| Fever fatigue/anorexia | +/+/+ | +/+/+ | +/+/+ | +/+/+ | +/+/+ | +/+/+ | +/+/+ | +/+/+ | +/+/+ | +/+/+ | +/+/+ |
| Gastrointestinal symptoms | Absent | Absent | + | Absent | + | + | Absent | + | + | + | Absent |
| CNS symptoms | Absent | Absent | Absent | Absent | + | Absent | Absent | + | + | + | Absent |
| Pulmonary symptoms | Absent | + | Absent | Absent | + | Absent | Absent | + | Absent | + | Absent |
| Hepato-splenomegaly | Absent | Absent | Absent | Absent | + | + | Absent | + | Absent | + | Absent |
| Adenopathy | Absent | Absent | Absent | Absent | Absent | Absent | Absent | Absent | + | + | Absent |
| Skin rash | + | Absent | Absent | Absent | Absent | Absent | + | Absent | Absent | + | Absent |
| Hemoglobin (g/dl) | 12 | 5.3 | 8.8 | 9.6 | 8.7 | 6.4 | 9.4 | 8 | 8.5 | 6.7 | 7.9 |
| Platelet count (g/l) | 30 000 | 35 000 | 200 000 | 45 000 | 35 000 | 72 000 | 387 000 | 21 800 | 44 000 | 37 000 | 63 000 |
| Elevated transaminases | + | Absent | + | + | Absent | Absent | + | Absent | + | NA | + |
| Hyperbilirubinemia | + | Absent | + | + | + | + | + | + | + | NA | + |
| LDH (IU/1) | 3115 | 1000 | 1328 | 4160 | 2227 | 1532 | 1775 | 1827 | 1573 | NA | 671 |
| Triglyceride (mmol/1) | NA | NA | NA | 4.9 | 5.8 | 6.4 | 3.6 | 2.3 | 6.3 | 7.6 | 3.5 |
| Ferritin (ng/ml) | NA | NA | NA | 17 200 | 8594 | 552 | 3230 | NA | 2800 | NA | 6640 |
| Hemophagocytosis | + | + | +[a] | + | + | + | +[a] | + | +[a] | + | + |
| *Nephrotic syndrome* | | | | | | | | | | | |
| Oliguria | NA | Absent | + | + | Absent | Absent | Absent | + | + | NA | NA |
| Creatinine clearance (ml/min) | 36 | 23 | 11 | 8 | 6 | 8 | 43 | 7 | 3 | 98 | 51 |
| Dialysis | No | No | HD | HD | HD | HD | No | PD | HD | No | No |
| Proteinuria (g/24 h) | 5.7 | 30 | 21 | 5.1 | 7.3 | 6 | 5.6 | 3.2 | 25 | >3 | 9.6 |
| Serum albumin (g/l) | 26 | 14 | 11 | 17 | 16 | 18 | 17 | NA | 10 | NA | NA |
| Microhematuria | Absent | Absent | Absent | + | + | Absent | Absent | + | + | 0 | + |
| Steroids | Yes | Yes | No | Yes | Yes | No | Yes | Yes | Yes | Yes | Yes |
| *Treatment* | | | | | | | | | | | |
| Cytotoxic agents | No | CY/VP16 | No | VP16 | No | No | CY/VP16 | VP16 | ABVD | VP16 | No |
| Iv Ig | No | No | No | No | Yes | No | No | No | No | No | Yes |
| Outcome | Dead | Neph S* | Dead | Dead | Dead | CRF | Cured | Dead | Dead | Dead | Cured |

Abbreviations: M, male; F, female; C, Caucasian; Af, African; As, Asian; MNHL, malignant non-Hodgkin's lymphoma; Leish, visceral Leishmaniasis; Still's Dis, Still's disease; FLH, familial lymphohistiocytosis; NA, not available; LDH, lactate deshdrogenase.
[a]Negative bone marrow examination; HD, hemodialysis; PD, peritoneal dialysis; CY, cyclophosphamide; VP16, etoposide; ABVD, adriamycin+bleomycin+vinblastine+dacarbazine; Neph S*, patient still nephrotic at 3 months, when lost to follow-up; CRF, chronic renal failure.

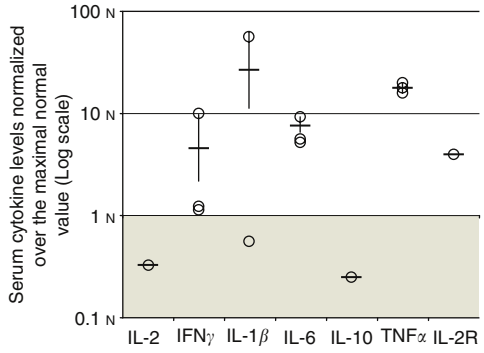


**Figure 1 | Serum levels of cytokine at the admission for three patients with HPS associated with nephrotic syndrome.** Proinflammatory cytokine levels were measured at the admission for three patients (No. 2, No. 5, and No. 9) of the series using commercial immunoenzymatic assay kits (BioSource, Belgium). Each circle represents single individual, horizontal bars indicate the mean, and vertical bars denote s.d. In case of IL-2, IL-10, and IL-2R, samples were available for only one patient. In order to allow the representation of all the data using the same *Y*-axis, we normalized the cytokine levels upon dividing the value measured for each cytokine in each patient by the maximal normal value for the particular cytokine.

histologic features of these patients and of the two reported in the literature.

Although these features were heterogeneous, all patients exhibited injuries of the epithelial side of the glomerular filtration apparatus, which probably accounted for the nephrotic syndrome.

***Glomerular features.*** Glomerular sampling for light microscopy ranged from 5 to 29 glomeruli (mean: 15), and global glomerulosclerosis was observed in <5% of the total number sampled (range 0–16.7%).

Collapsing glomerulopathy was the most frequent light microscopy pattern, and was observed in five patients (45.5%), all black Africans. None of them was seropositive for HIV. Collapsing glomerulopathy was characterized by the global collapse of several capillary loops with hyperplasic, swollen, vacuolated podocytes, which had detached themselves from the glomerular basement membrane and had been shed into the urinary space (Figure 2a).

Four patients (36.4%) displayed optically normal glomerular morphology, without proliferation or deposits (Figure 2b). Immunofluorescence microscopy was always negative. Minimal change glomerulopathy was diagnosed in all four patients. The diagnosis was further confirmed in one of them (patient No. 10, from the literature), for whom electron microscopy was available and showed focal fusion of the podocyte processes. Although we had no access to the electron microscopy analysis to confirm the diagnosis of MCD for the remaining patients, we have biological data to sustain the diagnosis. We have indeed observed a high

**Table 3 | Renal biopsy findings for 11 patients with HPS associated with nephrotic syndrome**

| | Retrospective study | | | | | | | | | Literature | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient number | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| *Light microscopy* | | | | | | | | | | | |
| No. of. glomeruli | Necropsy | 13 | 20 | 22 | 24 | 5 | 8 | Necropsy | 15 | NA | NA |
| No. of sclerotic glomeruli | NA | 1 | 0 | 2 | 4 | 0 | 0 | NA | 2 | NA | NA |
| Pattern of glomerulonephritis | MCD | MCD | CG | CG | TMA+podocytosis | CG | CG | MCD | CG | MCD | TMA+podocytosis |
| Tubular atrophy and interstitial fibrosis | Mild | Absent | Absent | Minor | Mild | Minor | Absent | Absent | Mild | Absent | Minor |
| Interstitial inflammation | Absent | Minor | Mild+[a] | Mild | Mild | Minor | Absent | Minor | Absent | Absent | Minor |
| Tubular damage scale (0 to +++) | | | | | | | | | | | |
| Dilatation | + | ++ | +++ | +++ | ++ | +++ | +++ | ++ | +++ | Absent | NA |
| Necrosis | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | +++ | Absent | NA |
| Protein reabsorption droplets | + | + | +++ | +++ | +++ | +++ | +++ | ++ | +++ | Absent | NA |
| Vascular damages | Absent | Absent | Mild | Mild | TMA | Absent | Absent | Absent | Absent | Absent | TMA |
| *Immuno-fluorescence (scale of 0 to +++)* | | | | | | | | | | | |
| IgG/A/M | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| C3/C1q | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $\kappa/\lambda$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Fibrin | 0 | 0 | 0 | 0 | ++ Glom and capillary thrombi | 0 | 0 | 0 | 0 | 0 | ++ Glom and capillary thrombi |
| Albumin | 0 | + Protein reabsorp droplets | ++ Protein reabsorp droplets | +++ Protein reabsorp droplets | ++ Protein reabsorp droplets | +++ Protein reabsorp droplets | +++ Protein reabsorp droplets | 0 | ++ Protein reabsorp droplets | NA | NA |
| *Electron microscopy (scale of 0 to +++)* | | | | | | | | | | | |
| Mesangial deposits | | | | | | | | | | 0 | 0 |
| Subendothelial deposits | | | | | | | | | | 0 | Fluffy material |
| Subepithelial deposits | | | | | | | | | | 0 | 0 |
| Foot process fusion | | | | | | | | | | +++ | +++ |

Abbreviations: NA, not available; MCG, minimal change glomerulopathy; collapsing G, collapsing glomerulopathy; TMA, thrombotic microangiopathy.
[a]Intrarenal hemophagocytosis; glom, glomerular; reabsorp, reabsorption.

selectivity index for the three patients of the series with optically normal glomerular morphology.

Two patients (18.2%) exhibited a similar complex feature, combining glomerular thrombotic microangiopathy lesions with evident podocyte damage. Light microscopy identified mild endocapillary proliferation and obliteration of some capillary lumina by swollen endothelial cells (Figure 2c). Chromophore silver methenamine staining produced a 'double contour' appearance. These findings were all consistent with thrombotic microangiopathy in a subacute stage. However, light microscopy also showed swollen, vacuolated podocytes, some of which had undergone detachment from the glomerular basement membrane (Figure 2d). Immunofluorescence staining was negative, except for fibrin on glomerular capillary thrombi and albumin on damaged podocytes (Figure 2e). Fusion of some foot processes was also noted in one of these two patients with thrombotic microangiopathy, for whom electronic microscopy was available.

***Tubular and interstitial features.*** The only patient without impairment of renal function (No. 10) exhibited normal tubular histologic features. All the others (10/11, 90.9%) exhibited the same tubulointerstitial histologic pattern.

Interstitial infiltrates ranged from absent to mild and consisted of a few localized clusters of mononuclear cells with interstitial edema (Figure 2g). Immunohistological study showed that they were mainly constituted of T lymphocytes with a few B lymphocytes (Figure 2h) and macrophages (Figure 2i). In only one patient (patient No. 3), intrarenal interstitial hemophagocytosis was noted. At the outset, it appears that macrophage infiltration (CD68-positive) is not necessarily associated with renal involvement during HPS. The degree of cortical scarring in the form of tubular atrophy and interstitial fibrosis ranged from none to mild, thus indicating the acute nature of the renal injury.

In the tubules, severe acute damage was observed by light microscopy, with the presence of tubular casts and microcystic tubular dilatation (Figure 2f). Tubular epithelial cells contained abundant protein reabsorption droplets and showed necrosis with detachment from the tubular basement membrane (Figure 2f).

***Vascular features.*** Two patients (No. 5 and No. 12) (18.2%) exhibited thrombotic microangiopathy lesions affecting both glomeruli and arterioles (Figure 2c). In the other patients, arterio- and arteriolo-sclerosis ranged from none (7) to mild (2).

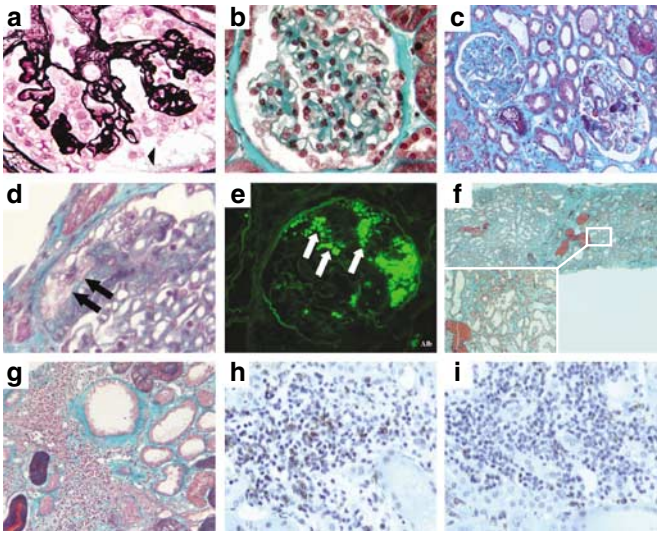


**Figure 2 | Light microscopy and immunofluorescence findings.** (**a**) Silver staining, original magnification × 200: The most common histologic pattern was collapsing glomerulopathy with global collapse of capillary loops and hyperplasic, swollen, vacuolated podocytes, which had undergone detachment from the glomerular basement membrane and had been shed into the urinary space (black arrowhead). (**b**) Masson's trichrome, original magnification × 200: minimal change glomerulopathy was the second histologic pattern displayed by the patients with nephrotic syndrome associated with HPS. It is characterized by optically normal glomerular morphology, without proliferation or deposit. Two patients exhibited a complex similar complex feature comprising a mixture of glomerular thrombotic microangiopathy lesions and severe podocyte damage. (**c**) Masson's trichrome, original magnification × 200: light microscopy identified mild endocapillary proliferation and obliteration of some capillary lumina by swollen endothelial cells and fibrin thrombi, which was consistent with thrombotic microangiopathy in the subacute stage. These two patients also displayed prominent podocyte injuries. (**d**) Masson's trichrome, original magnification × 400: light microscopy showing swollen and vacuolated podocytes (black arrows). (**e**) Immunofluorescence showing strong staining for albumin beyond the glomerular basal membrane, indicating a major defect in the glomerular permeability (white arrows). (**f**) Masson's trichrome, original magnification × 20 and × 40: all patients but one exhibited severe acute tubular damage. Light microscopy showed microcystic tubular dilatation and necrosis of tubular epithelial cells, which underwent detachment from their basement membrane. Interstitial infiltrates ranged from absent to mild and consisted of a few localized clusters of mononuclear cells with interstitial edema. (**g**) Masson's trichrome, original magnification × 40. Immunohistological study showed that these interstitial infiltrate were mainly constituted of polymorphic T lymphocytes with a few B lymphocytes ((**h**) anti-CD20, original magnification × 40) and macrophages ((**i**) anti-CD68, original magnification × 40).

### Clinical outcome

Clinical follow-up data were available for all patients except one (patient No. 2), who was lost to follow-up only after 3 months.

Treatment of HPS was in most cases symptomatic but included steroids in all but two cases. Etoposide (VP16) was used in five cases, sometimes associated with cyclophosphamide (CY). Only one patient with Hodgkin's disease received two full ABVD courses (Table 2).

Death occurred in seven cases (64%). In six of them, it was attributed to an uncontrolled disseminated hemophagocytic process, with multiple organ failure. One patient recovered from HPS after chemotherapy but died 2 months later of severe Hodgkin's disease.

Four patients survived HPS (36.4%). Two of them (18.2%) had no renal sequelae, one patient developed chronic renal failure and underwent subsequent kidney transplantation, and the remaining patient was still nephrotic with normal renal function 3 months after the onset of HPS, when he was lost to follow-up.

In such a small series, it is extremely difficult to find any correlation between treatment modalities and renal or patient survival. Some patients experienced a short remission from HPS after specific treatment but died from malignant lymphoma a few days or a few weeks later, before achieving remission from nephrotic syndrome or recovery from renal failure. Only two patients who achieved definitive remission from HPS recovered from both proteinuria and renal dysfunction, but a third patient, whose leishmaniosis-related HPS improved after specific treatment, progressed to end-stage renal disease and required definitive dialysis therapy. No relationship was found between pathology and outcome.

## DISCUSSION

HPS results from the excessive activation and proliferation of benign macrophages. It can be the consequence of either hereditary dysfunction of the immune system, or a complication of underlying disorder including infectious, neoplastic, or autoimmune diseases. Although the clinical spectrum of HPS is broad, the renal consequences of HPS have rarely been described in the literature. Most of the data on the subject concern the occurrence of acute renal failure,[4,7] sometimes considered as a strong predictor of an unfavorable outcome.[8] The present results are in accordance with these data as they show a high prevalence of this complication (90.9%). Histologic data suggest at least two pathophysiological mechanisms that may be responsible for the impairment of renal function during HPS: acute tubular necrosis and/or reduction of the glomerular filtration flow resulting from the massive collapses of glomerular tufts.

One of the most interesting features of our study was that it documented the occurrence of previously unknown glomerular complications of HPS. Thus, we identified nine cases of nephrotic syndrome associated with HPS, to which we added two isolated cases reported in the literature. The clinical presentation was quite similar in all cases, and consisted of severe proteinuria associated with acute renal failure occurring during the initial phase of a severe HPS.

The small number of patients identified suggests that this complication is rare. On the other hand, the fact that three of the nine cases occurred in a single center over a 6-month period might also indicate that it may be misdiagnosed, leading to underestimation of its frequency.

Although the methodology of our study did not allow us to draw definite conclusions from the results, two arguments

support the hypothesis that HPS was responsible for the development of the nephrotic syndrome in these patients:

(i) The evolution of proteinuria and HPS were closely coordinated.

This finding, observed in all patients, was particularly striking in patient No. 8 (Figure 3), an 8-year-old girl who had been followed for several years in the Necker-Enfants Malades Hospital for systemic juvenile chronic arthritis. Despite prolonged steroid treatment, she had experienced 13 relapses of arthritis between 4 and 8 years of age. During this period, her renal function had always been normal and proteinuria was constantly checked as negative. At the time of the 14th relapse, she simultaneously developed HPS and acute renal failure with important proteinuria, requiring temporary peritoneal dialysis. Remission of the HPS was achieved by an injection of etoposide (VP16), and resulted in rapid normalization of renal function and complete resolution of proteinuria. Nevertheless, HPS recurred 2 weeks later, together with the sudden onset of nephrotic syndrome and acute renal failure, revealing minimal change glomerulopathy and severe acute tubular necrosis.

(ii) In one case published in the literature, the occurrence of nephrotic syndrome was reported during primary HPS.[10] In this particular situation, the absence of underlying disease further support the responsibility of HPS in the genesis of this complication.

Our patients' renal histology exhibited striking features: firstly, in all cases, the glomerular injuries, which ranged from focal fusion of the podocyte processes to the collapse of glomerular tufts with abnormal podocytes detached from the glomerular basement membrane, were concentrated on the epithelial side of the filtration apparatus. Secondly, the severity of these injuries corresponded to the patient's ethnic origin. Thus, only African patients exhibited collapsing glomerulopathy, and all Caucasian patients minimal change glomerulopathy. Although, as stated above, the methodology of this study limited our ability to draw definite conclusions about the pathogenic processes responsible for the occurrence of nephrotic syndrome during HPS, it is tempting to postulate that a common mechanism targeting the podocytes leads to variously severe lesions, depending on the patient's susceptibility as defined by his genetic background.

As our findings indicate that macrophage infiltration is not necessarily associated with renal involvement during HPS, we believe that renal complications result rather from a systemic cytokine burst, as already documented for other clinical manifestations of HPS.[3] This last hypothesis is sustained by the very high amounts of circulating cytokines found in three of the patients (Figure 1). Recent advances in understanding the pathogenesis of HPS have indeed shown that it is characterized by a primary uncontrolled T-cell activation followed by a cytokine burst involving IFN-$\gamma$, TNF$\alpha$, IL-6, IL-1$\beta$, and other proinflammatory cytokines. This physiopathological observation has particular interest in the case of HPS-related nephrotic syndrome, as the role of T lymphocyte dysfunction has also been suggested in the pathogenesis of idiopathic nephrotic syndrome. Experimental data suggest, indeed, that T-cells could be the source of a circulating permeability factor leading to the induction of proteinuria in patients with minimal change glomerulopathy or focal segmental glomerulosclerosis.[11] The nature of this circulating factor remains elusive, but its identification could be guided by the study of HPS mechanisms.

Among many potential culprits, TNF$\alpha$ seems to be of particular interest. TNF$\alpha$ is indeed produced in large amounts during the acute phase of HPS.[1,12] High circulating levels of this cytokine might explain the podocyte injuries observed, as previous experimental studies have shown that TNF$\alpha$ increases the permeability to albumin of isolated glomeruli[13] and induces actin cytoskeleton reorganization in podocytes.[14] The latter phenomenon might account for detachment of podocytes from the glomerular basement membrane. Of note, TNF$\alpha$ has also been shown to be involved in acute tubular necrosis pathogenesis, which is a common histological feature in the patients experiencing nephrotic syndrome during the course of an HPS. Other cytokines could also be, directly or indirectly responsible for modifications of the glomerular barrier permeability, interfering with the podocyte function or its interaction with the glomerular membrane. Future studies will possibly lead us to a common factor involved in both HPS and idiopathic nephrotic syndrome.

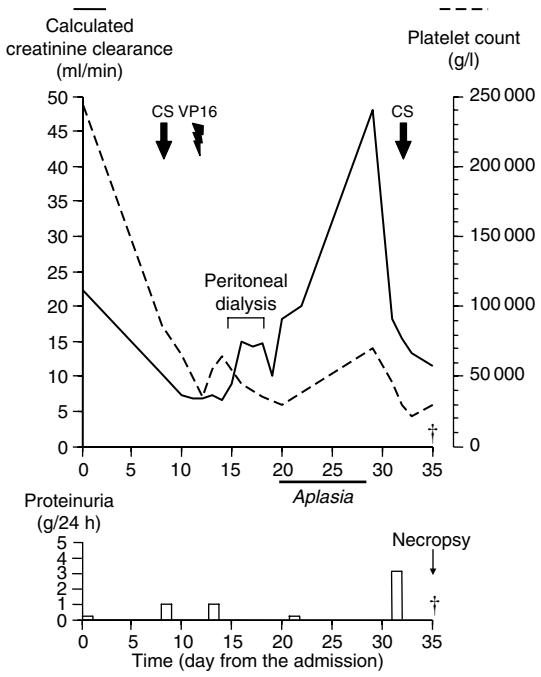


**Figure 3 | Temporal relationship between the HPS and renal abnormalities for one representative patient of the series (patient No. 8).** Abbreviations: CS, corticosteroid; VP16, etoposide; †, death of the patient.

### Conclusion

HPS, a disease resulting from excessive activation of non-malignant macrophages, can be associated with renal manifestations. Besides acute renal failure, glomerular lesions can develop in the course of HPS and can lead to the occurrence of a nephrotic syndrome.

This newly identified severe renal complication of HPS, which mainly seems to affect Africans, results from the development of podocyte injuries.

A prospective study is required to evaluate the frequency of this complication, and the development of animal models of HPS[15] might allow the pathogenesis of the HPS–nephrotic syndrome association to be clarified.

## MATERIALS AND METHODS

### Retrospective study

***Study population.*** This retrospective study was conducted in three Nephrology Departments in the Paris area, over a period of 20 years (from January 1984 to May 2004). To obtain a homogenous population, inclusion criteria were stringent, as follows:

(i) absence of known nephropathy before the diagnosis of HPS;
(ii) presence of typical HPS, assessed by the diagnostic criteria of the Histiocyte Society (Table 1),[5] including biopsy-proven hemophagocytosis;
(iii) occurrence of a nephrotic syndrome during the evolution of HPS; and
(iv) availability of renal histology.

The following definitions were used: nephrotic syndrome, 24 h urine protein $>3.5$ g (or $>50$ mg/body weight for patient under 15 years) and serum albumin $<30$ g/l; renal failure, creatinine clearance (calculated using the Cockcroft and Gault formula) $<60$ ml/min; hematuria, $>5$ red blood cells per high-power field on microscopic examination of the urinary sediment; oliguria, 24 h urine output $<500$ ml.

Patients' medical records were reviewed for age, gender, medical history, clinical presentation, renal function, treatments administered, and final outcome.

***Pathology.*** All renal tissue samples were processed by standard light microscopy techniques. Glass slides were prepared and stained with hematoxylin–eosin and Masson's trichome. Chromophore silver methenamine staining was performed to study glomerular basement membrane structure. Immunofluorescence studies were performed on 3-$\mu$m-thick cryostat sections using polyclonal FITC-conjugated antibodies against IgG, IgM, IgA, C3, C1q, kappa, lambda, fibrin, and albumin (Dako, Denmark).

***Measurement of cytokine serum levels.*** For three patients (No. 2, No. 5, and No. 9), recruited after the initiation of the study, blood sample for cytokine serum analysis were collected at admission. Blood tubes were allowed to clot for 1 h at room temperature, centrifuged at 1500 *g* for 10 min at 20°C, and serum was stored at $-80$°C until analysis. Quantitative measurement of IL2, interferon $\gamma$, IL1$\beta$, IL6, IL10, TNF$\alpha$, and soluble IL2 receptor in patients' serum samples was performed using commercial immunoenzymatic assay kits (BioSource, Belgium).

### Review of the literature

A MEDLINE search was carried out using the following criteria: (1) all MEDLINE listings as of 09-01-2004 with abstracts; (2) English or French language; and (3) Humans.

The following phrases were used to query the database: ('renal' OR 'kidney' OR 'proteinuria' OR 'nephrotic' OR 'glomerulopathy' OR 'nephropathy') AND ('hemophagocytosis' OR 'HPS' OR 'lymphohistiocytosis').

The references of each article identified were carefully reviewed for additional reference.

In all, 50 references were retrieved and screened for the same inclusion criteria as those used in our retrospective study.

### Statistical analysis

Statistical analysis was performed using Stat View software (5.0.1; SAS Inc., Cary, NC, USA). Owing to the small number of patients, only descriptive statistics were applied. Results are expressed as numbers (percentages) for categoric variables, and as means ($\pm$s.d.) for continuous variables.

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