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Cobalamin C Deficiency Induces a Typical Histopathological Pattern of Renal Arteriolar and Glomerular Thrombotic Microangiopathy

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Introduction: Cobalamin C (cblC) deficiency is the most common inborn error of vitamin B₁₂ metabolism. Renal failure attributed to thrombotic microangiopathy (TMA) has occasionally been described in the late-onset presentation of cblC deficiency, but kidney lesions associated with cblC deficiency remain poorly defined. This study aims to describe the characteristics of kidney disease in cblC deficiency, and to provide a comparative histological analysis with cblC-independent renal TMA.

Methods: We performed a multicenter retrospective study including 7 patients with cblC deficiency and 16 matched controls with cblC-independent TMA. The patients included were aged 6 to 26 years at the time of the first manifestations. All patients presented with acute renal failure, proteinuria, and hemolysis; 5 patients required dialysis.

Results: The histological study revealed arteriolar and glomerular TMA in all patients. After comparison with the cblC-independent TMA control group, a vacuolated aspect of the glomerular basement membrane and the intensity of glomerular capillary wall IgM deposits were more present in cblC deficiency patients than in controls. Six patients were treated with hydroxycobalamin. All of them improved, with disappearance of hemolysis, and 3 of the 4 patients requiring renal replacement therapy were weaned off dialysis.

Conclusion: This study provides a precise description of kidney pathology in cblC deficiency. Due to major therapeutic implications, we suggest that patients with renal TMA be screened for cblC deficiency regardless of age, particularly when the kidney biopsy provides evidence of long-lasting TMA, including a vacuolated aspect of the glomerular basement membrane and glomerular capillary wall IgM deposition.

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KEYWORDS: cobalamin C deficiency; genetic kidney disease; renal pathology; thrombotic microangiopathy

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Thrombotic microangiopathy syndromes are characterized by the association of hemolytic anemia, thrombocytopenia, and organ injury due to arteriolar and capillary thrombosis. Typical hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) are the 2 most frequent causes of TMA.

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Typical HUS is due to a verocytotoxin commonly secreted by *Escherichia coli* O157:H7, whereas TTP is caused by deficiency of von Willebrand factor protease (ADAMTS 13). Other causes of TMA include atypical HUS, resulting from uncontrolled activation of the alternative pathway of complement, malignant hypertension, drugs, or malignancies.^{1,2} Familial TMA has been identified in patients with cobalamin C deficiency.

Methylmalonic aciduria (MMA) and homocystinuria cblC type, also named cblC deficiency, is the most

common inborn error of intracellular vitamin B₁₂ metabolism. This autosomal recessive disease, due to mutations in *MMACHC* (methylmalonic aciduria cblC type with homocystinuria) gene,³ leads to a defect in the synthesis of the 2 active coenzyme derivatives of cobalamin (Cbl), adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl). The substance AdoCbl acts as a cofactor for the mitochondrial L-methylmalonyl-coenzyme A (CoA) mutase (MMA mutase) during the transformation of methylmalonyl-CoA in succinyl-CoA, whereas MeCbl is required for the intracytoplasmic methylation of homocysteine in methionine by the methionine synthase (Figure 1). Two distinct phenotypes of cblC disease have been described, differing in age at presentation, symptoms, and outcomes.⁴ The most frequent is the early-onset disease, characterized by failure to thrive, neurologic deterioration, and hematologic abnormalities during the first year of life.⁵ The late-onset forms are rarer, usually less severe, and revealed by neuropsychiatric symptoms, pulmonary arterial hypertension (PAH), HUS, or myelopathy.

Although timely recognition of cblC deficiency is of the utmost importance because it has specific therapeutic implications, the kidney lesions in this setting are poorly described, due to the limited number of cases available and the absence of a specific study.⁶ In this context, the identification of characteristic kidney lesions encountered in cblC deficiency would be helpful for pathologists and nephrologists to identify this frequently misdiagnosed entity.

The present study provides a systematic retrospective description of the histopathological findings on kidney biopsy in cblC deficiency, and a comparative analysis of renal lesions in cblC deficiency patients and in matched TMA control patients.

METHODS

Study Population and Data Collection

We performed a multicenter retrospective study in 7 patients with proven cblC deficiency and available kidney biopsy. The cases were selected in 6 different centers in France and Belgium. The diagnosis of cblC deficiency was made by complementation studies or genetic testing. The data collected included clinical (age, hypertension, edema, PAH, neurologic symptoms, and thromboembolic events), biological (hemoglobin, platelets, schizocytes, lactate dehydrogenase [LDH], haptoglobin, plasma creatinine, estimated glomerular filtration rate (eGFR), proteinuria, and complement system), and biochemical (vitamin B₁₂, total homocystinemia, MMA, and methioninemia) characteristics at initial presentation. The management and the outcome of each patient were also recorded.

The cblC cases were compared with controls with TMA of cblC-independent origin. The controls were selected in 2 different centers (Rouen University Hospital, France, and Necker Hospital, Paris, France). We included all of the patients aged 4 to 28 years who presented between 1999 and 2015 with available kidney biopsy findings revealing renal TMA. Patients with TMA lesions associated with IgA nephropathy or lupus nephritis were excluded. Data on age, proteinuria, eGFR, and cause of TMA were collected for each control.

The eGFR was calculated based on the Modification of Diet in Renal Disease (MDRD) formula for adults and the Schwartz formula for children and adolescents.

All of the research procedures were approved by the local ethics committee of Rouen, France (E2015-36).

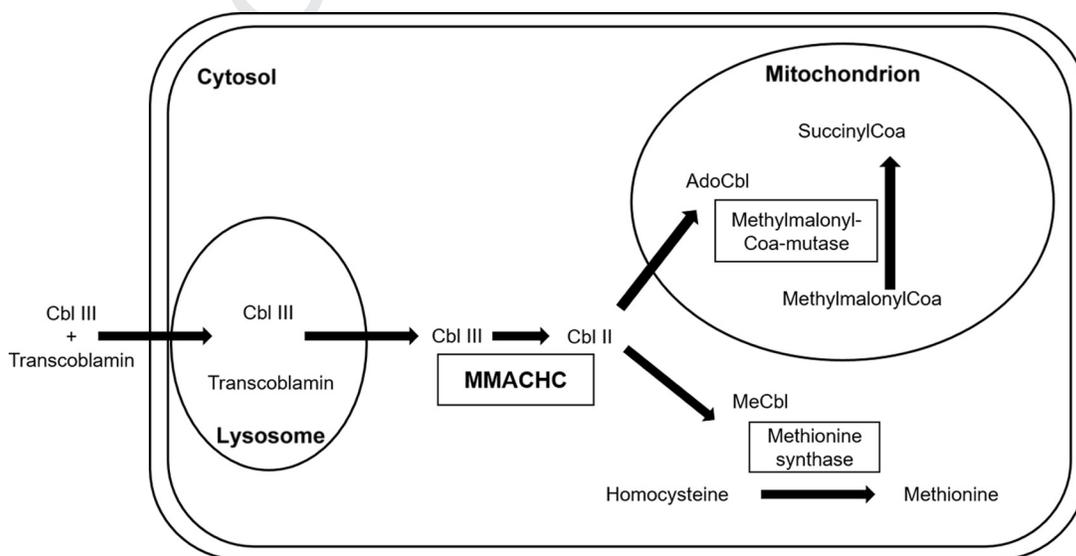


Figure 1. Intracellular cobalamin metabolism. AdoCbl, adenosylcobalamin; Cbl, cobalamin; MeCbl, methylcobalamin; MMACHC, ■■■■.

Kidney Pathology

Kidney biopsy specimens from the cblC patients and control patients were collected. A centralized review of all the biopsy findings was performed in a blinded manner by an experienced pathologist and a nephrologist of Rouen University Hospital. Masson trichrome, hematoxylin/eosin/saffron, and Jones methenamine silver stainings were used to assess glomerular, vascular (interlobular arteries and juxtaglomerular arterioles) and tubulo-interstitial lesions. The severity of each lesion was quantified as following: 0 (no lesion), 1 (light), 2 (moderate), and 3 (severe). The reports of the immunofluorescence study were collected from each center. Intensity of immunofluorescence was quoted from 0 to 3. Ultrastructural electron microscopy was performed in 1 patient.

Statistical Analysis

Quantitative variables are expressed as mean \pm SD and were compared with the Mann–Whitney *U* test, whereas qualitative variables are expressed in numbers and percentages and were compared with the Fisher exact test. A *P* value of <0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

Seven patients with cblC deficiency and available kidney biopsy were included. Five of them were previously reported.^{7–9} Their baseline characteristics are described in Table 1. All the patients presented with late-onset disease and the age at initial presentation varied from 6 to 26 years. Familial disease was noted in 2 sets of siblings (patients 1 and 2⁷ and patients 4 and 5⁸). All but 1 patient had hypertension, and 4 had edema. Only 1 patient had a history of neurological disorder (language delay), 1 had experienced a thromboembolic event, and 1 had PAH.

All of the patients presented with hemolysis, but 4 of them had a normal platelet level. Five patients required renal replacement therapy, and only 1 patient had an eGFR >30 ml/min per 1.73 m². Proteinuria was present in all tested patients and ranged from 1.3 to 9.0 g/d. All of the patients had normal C3 and C4 levels, but patient 4 had a heterozygous mutation in complement factor H.

Total homocysteine varied from 44 to 230 μ mol/l (normal <13 μ mol/l¹⁰) and urine methylmalonic acid from 19 to 244 μ mol/mmol creatinine (normal <4 μ mol/mmol¹⁰).

Table 1. Clinical, biological, and biochemical characteristics of the 7 cases at initial presentation

	Patient 1 (7)	Patient 2 (7)	Patient 3 (9)	Patient 4 (8)	Patient 5 (8)	Patient 6	Patient 7
Clinical presentation							
Age (yr)	18	18	20	6	8.5	15	26
Hypertension	Yes	Yes	Yes	Yes	No	Yes	Yes
Edema	Yes	No	Yes	Yes	No	No	Yes
PAH	No	Yes	No	No	No	No	No
Neurologic symptoms	Yes	No	No	No	No	No	No
Thromboembolic event	No	No	No	Yes	No	No	No
Biology							
Hemoglobin (g/dl)	7.8	12.5	11.6	7.2	11.5	8.9	7.0
Platelets (g/l)	141	191	101	330	440	128	74
Schizocytes	Yes	Yes	Yes	No	No	No	No
LDH (U/l)	787	444	1044	NA	NA	1021	3000
Haptoglobin (μ mol/l)	< 0.1	NA	< 0.1	0.1	0.2	< 0.2	< 0.1
Creatinine (μ mol/l)	Dialysis	Dialysis	Dialysis	Dialysis	55	Dialysis	290
eGFR (ml/min per 1.73 m ²)					110		18
Proteinuria (g/24 h)	6	2	1.3	1.6	4.8	9	7
C3	Normal	NA	Normal	Normal	Normal	Normal	Normal
C4	Normal	NA	Normal	Normal	Normal	Normal	Normal
Complement alternative pathway	Normal	NA	Normal	Factor H mutation	Normal	Normal	Normal
Biochemistry							
Vitamin B ₁₂ (pg/ml)	558	NA	500	600	550	580	1082
Total plasma homocysteine (μ mol/l)	73	NA	185	104	44	97	230
Urine MMA (μ mol/mmol)	NA	NA	244	19	22.5	91	83
Plasma methionine (μ mol/l)	17	NA	4	30	30	40	NA
Complementation study	No	No	No	Yes	Yes	No	Yes
Genetic study of <i>MMACHC</i>							
Mutation 1	c.271dupA	c.271dupA	c.271dupA	c.271dupA	c.271dupA	c.271dupA	c.271dupA
Mutation 2	c.82-9_12delTTTC	c.82-9_12delTTTC	c.389A>G	c.82-9_12delTTTC	c.82-9_12delTTTC	c.82-9_12delTTTC	c.389A>G

eGFR, estimated glomerular filtration rate; LDH, lactate dehydrogenase; MMA, methylmalonic acid; NA, not available; PAH, pulmonary hypertension.

Plasma methionine was decreased in 1 tested patient (patient 3) (normal 11–37 $\mu\text{mol/l}$). Patient 5, who had the lowest homocysteine concentration, was diagnosed early, when she had only proteinuria without kidney failure or extrarenal manifestation.

Genetic analysis of *MMACHC* was available in all patients, revealing compound heterozygosity for the frameshift mutation c.271dupA, together with the splice mutation c.82-9_12delTTTC in patients 1, 2, 4, 5, and 6, and the missense mutation c.389A>G (p.Tyr130Cys) in patients 3 and 7. In 3 patients, the diagnosis was also based on complementation analysis in fibroblasts (patients 4, 5, and 7).

Management and Outcomes

Patient management and outcomes are presented in **Table 2**. Time between the first presentation and the treatment initiation varied from 10 days to 7 years. One patient was not treated and died of pulmonary vein-occlusive disease at 18 years of age (patient 2). Autopsy showed pulmonary capillary hemangiomas and renal TMA. The diagnosis was made 15 years later, when his brother (patient 1) was diagnosed. The 6 other patients were treated with parenteral hydroxycobalamin (OHCbl), betaine, and folic acid. All of them improved, with disappearance of hemolysis. Three of the 4 patients requiring renal replacement therapy were weaned off dialysis. The fourth received a kidney transplant 1 year after the diagnosis.

Four patients were treated by plasma exchanges before diagnosis, and 3 of them improved temporarily

and partially. One patient was treated with eculizumab without any improvement.

Patient 7 had an atypical history. She was diagnosed with a renal TMA during pregnancy and received a kidney transplant 5 years later. The diagnosis of *cbfC* deficiency was made 9 months later, after the recurrence of TMA on the graft. She received a second kidney transplant 6 years after the diagnosis.

One patient relapsed 5 years after treatment initiation because of inobservance. None of the 2 patients who had undergone transplantation relapsed on the kidney graft under treatment.

Histopathological Characteristics of Renal Disease

Nine biopsies were available for the 7 patients (**Supplementary Table S1**). Light microscopic evaluation revealed glomerular and arteriolar TMA in all patients.

Most of the glomeruli presented typical lesions of TMA (**Figure 2**), with thickening of the capillary wall (**Figure 2a**) in all patients and intraglomerular thrombi in 3 patients (**Figure 2b**). Remodeling of the glomerular basement membrane (GBM), defined as duplication and/or a vacuolar aspect of the GBM, was seen in 6 patients altogether. Typical duplication of the GBM was seen in 2 patients at diagnosis, but was also noted in the 2 biopsies performed during the follow-up (**Figure 2c**). The GBM vacuoles (**Figure 3a**) were particularly frequent, as they were present in 6 patients. Microaneurysm, mesangial sclerosis, endocapillary proliferation, and glomerular ischemia were

Table 2. Management and outcomes of the 7 cases

	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7
Management							
Time before treatment (mo)	0.3	N/A	12	18	4	1	84
Hydroxycobalamin	i.m.	No	IM	SC	SC	IM	IM
Betaine	Yes	No	Yes	Yes	Yes	Yes	Yes
Folic acid	Yes	No	Yes	Yes	Yes	Yes	Yes
Other treatment	Plasma exchange Solumedrol	No	Eculizumab	Plasma exchange	No	Plasma exchange	Plasma exchange
Outcome after treatment							
Stop hemolysis	Yes	N/A	Yes	Yes	Yes	Yes	Yes
Time (days)	7		0	30	30	NA	NA
Weaning off dialysis	Yes	No	Yes	Yes	N/A	No	N/A
Time (mo)	5		3	0.8			
Relapse	No	N/A	No	Yes	No	No	No
Time (yr)				5			
Dialysis	No	N/A	No	No	No	Yes	Yes
Time (yr)						0	5
Kidney transplantation	No	N/A	No	No	No	Yes	Yes
Time (yr)						1	6
Relapse on graft						No	No
Death	No	Yes	No	No	No	No	No
Time (yr)		1					

i.m., intramuscular; N/A, not applicable; NA, not available; SC, subcutaneous.

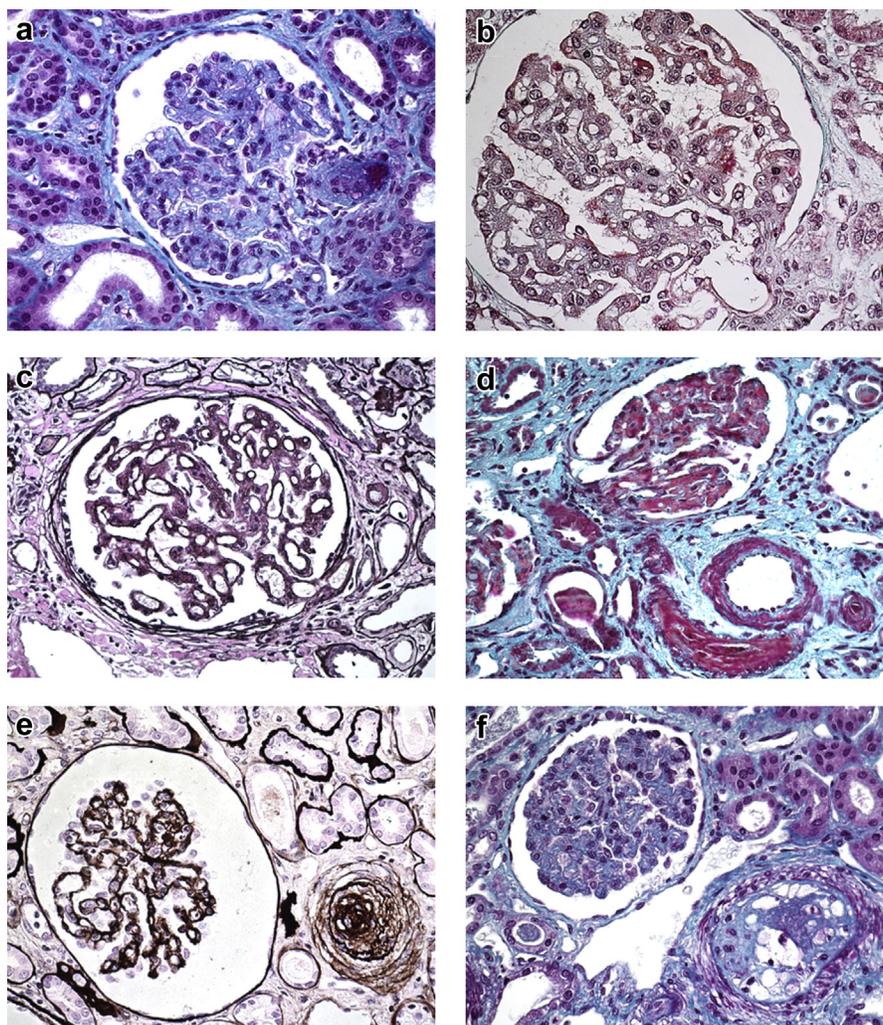


Figure 2. Glomerular and arteriolar lesions of thrombotic microangiopathy in patients with cobalamin C (cbIC) deficiency. (a–f) Kidney biopsy. Light microscopy. (a) Arteriolar and glomerular thrombotic microangiopathy with thickening of capillary walls and arteriolar thrombus (patient 1; Masson trichrome staining; original magnification, $\times 400$). (b) Global thickening of glomerular capillary walls and endocapillary glomerular thrombi (patient 5; Masson trichrome staining; original magnification, $\times 400$). (c) Global thickening of glomerular capillary walls and duplication of the glomerular basement membrane (patient 5²; Jones staining; original magnification, $\times 400$). (d) Massive juxtaglomerular arteriolar and glomerular thrombi (patient 6; Masson trichrome staining; original magnification, $\times 400$). (e) Glomerular ischemia and arteriolar fibrous plug (patient 2; Jones staining; original magnification, $\times 400$). (f) Glomerular TMA and arteriole occluded by fibrosis infiltrated by foamy cells (patient 1; Masson trichrome staining; original magnification, $\times 400$).

seen in 2, 5, 3, and 4 patients, respectively. Synechiae and extracapillary proliferation were not seen.

All the patients had vascular lesions of TMA. Intravascular thrombi were present in all but one patient (Figure 2d). Fibrous plugs (Figure 2e), fibrous endarteritis (Figure 2f), and hyalinization were seen in 4, 3, and 3 patients, respectively.

Five patients had interstitial fibrosis with tubular atrophy at diagnosis and all of them presented acute tubular necrosis. The most severe lesions of interstitial fibrosis with tubular atrophy were found in the 2 patients who were not weaned off dialysis.

Two patients had a second biopsy during follow up, 1 patient a few months after treatment with eculizumab, revealing an increase in tubulo-interstitial lesions, and 1

patient 5 years after treatment by intramuscular OHcbl, showing disappearance of thrombi but arteriolar hyalinization. The kidney lesions found in the patient presenting with a heterozygous mutation in complement factor H were similar to those of the other patients.

Immunofluorescence reports are detailed in Supplementary Table S2. All but 1 patient had light-to-severe glomerular capillary wall IgM deposits (Figure 3b). One patient had moderate endomembranous C3 deposits, and no patient had IgG deposition.

Electron microscopy was performed in 1 patient (patient 1). It showed thickening of the GBM with vacuolated aspect (Figure 3c) due to intramembranous granular deposits and mesangial extensions (Figure 3d).

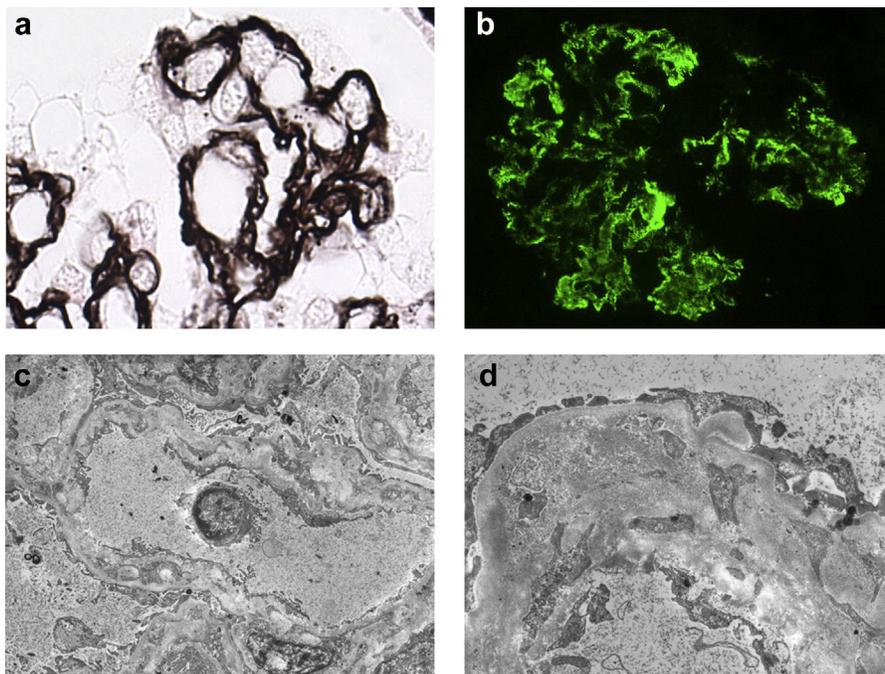


Figure 3. Vacuolated aspect of the glomerular basement membrane (GBM) due to IgM deposits in patients with cobalamin C deficiency. (a) Light microscopy. Glomerular capillary wall thickening with vacuoles and spikes (patient 3; Jones staining; original magnification, $\times 2000$). (b) Immunofluorescence microscopy. Glomerular capillary wall granular IgM deposits (patient 1; immunofluorescence staining for IgM; original magnification, $\times 400$). (c,d) Electron microscopy. Patient 1. (c) Thickening of the glomerular capillary wall with intramembranous granular deposits and material leading to the vacuolated aspect of the GBM (original magnification, $\times 16,000$). (d) Intramembranous granular deposits and mesangial cell expansion in vacuolated GBM (original magnification, $\times 50,000$).

Comparison of Renal Histopathological Findings Between cblC Deficiency and Other Causes of TMA

The most frequent causes of cblC-independent TMA were typical HUS, atypical HUS, and malignant hypertension (Supplementary Table S3). There was no difference in age (cblC 15.9 ± 6.8 years vs. control 12.7 ± 7.2 years, $P = 0.3$), dialysis (cblC, $n = 5$ [71%] vs. control, $n = 5$ [33%], $P = 0.2$) or proteinuria (cblC 4.5 ± 2.9 g/d vs. control 3.9 ± 8.7 g/d, $P = 0.07$) between the 2 groups.

A comparison of the histopathological characteristics between the 2 groups is reported in Table 3. There was no difference in vascular and tubulo-interstitial lesions between the 2 groups. It is interesting that among the GBM remodeling lesions, we found that the vacuolated appearance of the GBM was significantly more frequent in patients with cblC deficiency than in controls (cblC, $n = 6$ [86%] vs. controls, $n = 1$ [7%], $P < 0.001$).

Immunofluorescence study revealed that glomerular IgM deposits were significantly more frequent and abundant among patients with cblC deficiency (cblC 1.8 ± 0.8 vs. controls 0.4 ± 0.5 , $P = 0.003$).

DISCUSSION

In this study, we found that cblC patients, as opposed to renal TMA control patients, presented with a

peculiar histopathological picture of the GBM associated with a vacuolated aspect and IgM deposits, in addition to arteriolar and glomerular lesions of TMA.

Cobalamin C deficiency is the most common inherited disorder of vitamin B₁₂ metabolism. Pediatricians are usually aware of the multisystem disorders associated with early-onset cblC deficiency in infants. On the contrary, the presentation in adults is generally not recognized at an early stage due to limited knowledge of this disorder, and patients with cblC deficiency represent a diagnostic conundrum for the physicians involved. Renal complications in patients with cblC disease are a relatively rare entity, with 40 cases described to date.^{7,11} Among them, the majority (24 of 40) presented with late-onset disease, including 12 adolescents or young adults. The most frequently reported renal manifestation was TMA, although a few cases describe an atypical glomerulopathy, considered as glomerular consequences of TMA.^{12,13} Biological screening of cblC deficiency is classically recommended in children with TMA, but not in adults.¹⁴ However, recent data highlight the interest for clinicians to consider the diagnosis of cblC deficiency when renal TMA is diagnosed in adolescents or young adults.¹ Recently, Fakhouri *et al.* have proposed a diagnostic algorithm suggesting that cblC deficiency must be ruled out in STEC-negative HUS, by the measurement

Table 3. Comparison of histopathological findings between TMA during cobalamin C (cbIC) deficiency and other causes of TMA

Histological findings	cbIC Deficiency (n = 7)	Control TMA (n = 16)	P value
Glomerulus			
Glomeruli with TMA (% of glomeruli)	97.6 ± 6.4	95.3 ± 10.1	0.67
Sclerotic glomeruli (% of glomeruli)	1.6 ± 4.2	12.6 ± 21.2	0.12
Thrombi (score)	0.7 ± 1.1	0.6 ± 1.0	0.72
Thickening of capillary wall	2 ± 0.8	1.7 ± 1.1	0.59
Microaneurysm	0.2 ± 0.4	0.3 ± 0.4	0.65
GBM remodeling			
Duplication of GBM	n = 4 (57%)	n = 7 (44%)	0.34
GBM vacuoles	n = 6 (86%)	n = 1 (7%)	<0.001
Mesangial sclerosis	1.3 ± 0.9	1.2 ± 1.0	0.72
Endocapillary proliferation	0.6 ± 0.8	0.7 ± 0.7	0.71
Synechiae	0	0.3 ± 0.6	0.27
Ischemia	0.6 ± 0.9	0.6 ± 0.9	0.88
Extracapillary proliferation (n of patients, %)	n = 0 (0%)	n = 1 (6.3%)	0.9
MPGN aspect	n = 2 (29%)	n = 1 (6%)	0.21
Vessels			
Interlobular arteries (score)			
Thrombi	0.3 ± 0.5	0.5 ± 1.1	0.84
Fibrous endarteritis	0.3 ± 0.5	0.7 ± 1.2	0.9
Bulbiform FE	0.8 ± 1.3	0	0.07
Juxtaglomerular arterioles (score)			
Thrombi	1.1 ± 0.7	0.8 ± 0.9	0.29
Bulbiform FE	1 ± 1.4	0.4 ± 0.8	0.38
Fibrous plugs	1.3 ± 1.4	0.5 ± 0.9	0.19
Hyalinization (n of patients, %)	n = 1 (14%)	n = 1 (6%)	0.53
Tubules and interstitium (score)			
Interstitial fibrosis	1.6 ± 0.9	1.3 ± 1.0	0.59
Tubular atrophy	1.3 ± 1.1	0.6 ± 0.9	0.17
Acute tubular necrosis	1.6 ± 0.9	0.8 ± 1.2	0.12
IgM deposits (score)	1.8 ± 0.8	0.4 ± 0.5	0.003

Results are expressed as mean ± SD for qualitative variables (comparison with Mann–Whitney *U* test) or as number (percentage) for quantitative variables (comparison with Fisher exact test).

FE, fibrous endarteritis; GBM, glomerular basement membrane; MPGN, membranoproliferative glomerulonephritis; n, number; TMA, thrombotic microangiopathy.

of plasma homocysteine and urine or plasma methylmalonic acid, with or without MMACHC direct sequencing.¹⁵ In our study, all but 1 patient were weaned off dialysis after treatment initiation, which supports the benefit of identifying the diagnosis to rapidly start the specific treatment.

Cobalamin C deficiency is due to mutations in *MMACHC* gene, which is located on chromosome 1p34³ and encodes a protein acting as a cyanocobalamin decyanase.¹⁶ Among the 87 mutations identified in *MMACHC*,¹¹ c.271dupA, resulting in a frame shift, is the most frequently encountered (40%–55%), and was present in all patients analyzed in our study.^{3,17} Several studies suggest a phenotype–genotype correlation, and the homozygous c.271dupA mutation appears to be associated with early-onset disease.¹⁷ In contrast, in heterozygous patients, phenotype appears to be

determined by the less deleterious mutation.¹⁸ Indeed, in this study, all patients presented with late-onset disease and they all had at least 1 partially functional allele (splice or missense mutation). The 2 other mutations found in our work were the missense mutation c.389A>G,¹⁷ which has rarely been reported, and the splice mutation c.82-9_12delTTTC, which has been described only in patients with renal TMA.¹⁸

The pathophysiology of TMA during cbIC deficiency is not completely understood. Hyperhomocysteinemia may play a crucial role in renal vascular endothelial toxicity.^{19,20} It also alters the antithrombotic properties of the endothelium, possibly through impairing the nitric oxide–mediated inhibition of platelet aggregation,^{21,22} and leads to increased coagulation.^{23,24} However, the absence of reported cases of TMA during cystathionine synthase deficiency,²⁵ characterized by hyperhomocysteinemia with hypermethioninemia, highlights the existence of additional factors. Thus, hypomethioninemia may play a role in small vessel injury. In our population, plasma exchange partially improved hemolysis and kidney function in 3 patients, which may suggest a beneficial role of the clearance of a potential circulating factor. Another interesting point is the atypical history of patient 7, in whom the diagnosis was made after a first kidney transplantation. To our knowledge, cbIC-related HUS had not yet been reported after kidney transplantation. This case holds important pathophysiological implications, as it strongly supports the role of plasma disturbances in TMA, as opposed to the hypothesis of a primitive involvement of cbIC deficiency in vascular endothelial injury at the cellular level. HUS have also been described in cbIG²⁶ and cbIE disease,²⁷ characterized by hyperhomocysteinemia without MMA, whereas only tubulo-interstitial injuries have been reported during MMA without hyperhomocysteinemia.²⁸ These 2 points remove the potential role of urine or plasma methylmalonic acid. Associated factors of TMA should also be searched, such as complement alternative pathway abnormalities,^{8,29} which may participate in endothelial injury in selected cases.

The pathophysiology of TMA during cbIC deficiency is supposed to be similar to the pathophysiology of PAH and communicating hydrocephalus.³⁰ However, in our population, no patient presented with hydrocephaly, and only 1 patient had a history of neurological abnormality, revealed by a language delay. The clinical features are atypical in our study because neurological signs are usually the most frequent symptoms at onset or during course of cbIC deficiency.³¹ This confirms the wide heterogeneity of cbIC disease, and that the diagnosis has to be evoked

751 even in the absence of neurologic damage.³² Like- 805
 752 wise, only 1 patient presented with PAH in our 806
 753 study, but several cases of PAH during cblC defi- 807
 754 ciency have been reported, frequently associated 808
 755 with TMA.^{33,34} 809

756 Our histopathological study revealed arteriolar and 810
 757 glomerular TMA in all cblC patients described, with 811
 758 typical acute and chronic lesions. In their recent review, 812
 759 Beck *et al.* retrospectively reported the results of 16 813
 760 biopsies of cblC patients.¹¹ The authors described 814
 761 glomerular TMA lesions with endothelial swelling, 815
 762 glomerular thrombi, and/or duplication of the GBM. In 816
 763 the latter study, there was no immunofluorescence 817
 764 analysis, centralized review or control population. In the 818
 765 present study, we showed that GBM vacuoles and IgM 819
 766 deposition were significantly more frequent in patients 820
 767 with cblC disease, compared to a control population 821
 768 with cblC-independent TMA. Vacuolated GBM has not 822
 769 yet been reported in cblC patients, but IgM deposits 823
 770 have already been described in 3 patients.^{12,35} GBM 824
 771 remodelling, including vacuoles, can be seen in chronic 825
 772 TMA. In this study, there was no difference regarding 826
 773 the time from clinical diagnosis of TMA to biopsy be- 827
 774 tween the 2 groups. However, due to the genetically 828
 775 driven pathophysiology of the disease, we can suppose 829
 776 that, as opposed to some cblC-independent TMA pa- 830
 777 tients, the cblC patients presented ongoing lesions years 831
 778 before the diagnosis was made. These findings correlate 832
 779 with the late-onset presentation of the disease in our 833
 780 patients, in which diagnosis is made late because of 834
 781 subacute presentation. This chronic evolution could also 835
 782 explain that cblC patient had relatively normal platelets 836
 783 level, which has already been described in several case 837
 784 reports.^{33,35,36} Red blood cell count is also almost normal 838
 785 in our study, and schizocytes, LDH elevation, or low 839
 786 haptoglobin is the only hint to TMA. Cobalamin C 840
 787 deficiency is not an immune-related disease, and no 841
 788 study has yet focused on immune deposits in this dis- 842
 789 order. In the few cases reporting the results of autopsy in 843
 790 patients with cblC defect, there are no data on immu- 844
 791 nofluorescence in other organs.^{37,38} Due to the similar 845
 792 histopathological pattern, we can speculate that the 846
 793 vacuolated aspect is secondary to deposits, as occurs in 847
 794 membranous nephropathy. This is supported by the 848
 795 ultrastructural study revealing intramembranous gran- 849
 796 ular deposits, although electron microscopy was avail- 850
 797 able in only 1 patient. In cblC-dependent TMA, 851
 798 endothelial cell injury, especially oxidative stress 852
 799 mediated by hyperhomocysteinemia, may involve 853
 800 complement activation,³⁹ and leucocyte aggregation,⁴⁰ 854
 801 leading to deposits of IgM on the external face of the 855
 802 GBM. We can also speculate that circulating IgM, due to 856
 803 its biochemical characteristics, could be nonspecifically 857
 804 trapped within the glomerular lesions. 858

This retrospective study is limited mainly by the 805
 relatively small number of patients included, due to the 806
 rarity of the disease. The strength of this study lies 807
 both in the detailed systematic histopathological anal- 808
 ysis and in the comparison with cblC-independent 809
 TMA, which identifies typical renal lesions occurring 810
 in late-onset cblC deficiency. These original results 811
 provide novel information for pathologists, who should 812
 specifically evoke the diagnosis of cblC deficiency 813
 when kidney biopsy reveals TMA lesions associated 814
 with a vacuolated aspect of the GBM and/or IgM de- 815
 posits. Dosage of plasma homocysteine, methylmalonic 816
 acid, and methionine are easy and inexpensive pro- 817
 cedures that can highlight a misdiagnosed entity in 818
 which prognosis is highly linked to the rapidity of 819
 treatment initiation. When the diagnosis of cblC defi- 820
 ciency is biochemically suspected, *MMACHC* direct 821
 sequencing allows one to affirm the diagnosis, but the 822
 specific treatment, including intramuscular injections 823
 of OHCbl, betaine, and folinic acid,⁵ must be rapidly 824
 started, without waiting for confirmatory testing. 825

In conclusion, we suggest that all the patients with 826
 renal TMA be screened for cobalamin metabolism dis- 827
 order with plasma homocysteine measurement, 828
 regardless of age at presentation, particularly when 829
 microscopy reveals a vacuolated aspect of the GBM and 830
 glomerular capillary wall IgM deposition. 831

832 DISCLOSURE

All the authors declared no competing interests. 833
 834

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850 SUPPLEMENTARY MATERIAL

Table S1. Review of the histopathological characteristics of 851
 the 9 biopsies. 852

Table S2. Glomerular immunofluorescence reports for 6 853
 patients. 854

Table S3. Etiologies of control thrombotic micro- 855
 angiopathies. 856

Supplementary material is linked to the online version of 857
 the paper at www.kireports.org. 858

REFERENCES

1. George JN, Nester CM. Syndromes of thrombotic microangiopathy. *N Engl J Med*. 2014;371:654–666.
2. Rosove MH. Thrombotic microangiopathies. *Semin Arthritis Rheum*. 2014;43:797–805.
3. Lerner-Ellis JP, Tirone JC, Pawelek PD, et al. Identification of the gene responsible for methylmalonic aciduria and homocystinuria, cblC type. *Nat Genet*. 2006;38:93–100.
4. Rosenblatt DS, Aspler AL, Shevell MI, et al. Clinical heterogeneity and prognosis in combined methylmalonic aciduria and homocystinuria (cblC). *J Inherit Metab Dis*. 1997;20:528–538.
5. Carrillo-Carrasco N, Chandler RJ, Venditti CP. Combined methylmalonic acidemia and homocystinuria, cblC type. I. Clinical presentations, diagnosis and management. *J Inherit Metab Dis*. 2012;35:91–102.
6. Carrillo-Carrasco N, Venditti CP. Combined methylmalonic acidemia and homocystinuria, cblC type. II. Complications, pathophysiology, and outcomes. *J Inherit Metab Dis*. 2012;35:103–114.
7. Grangé S, Bekri S, Artaud-Macari E, et al. Adult-onset renal thrombotic microangiopathy and pulmonary arterial hypertension in cobalamin C deficiency. *Lancet*. 2015;386:1011–1012.
8. Guignon V, Frémeaux-Bacchi V, Giraudier S, et al. Late-onset thrombotic microangiopathy caused by cblC disease: association with a factor H mutation. *Am J Kidney Dis*. 2005;45:588–595.
9. Cornec-Le Gall E, Delmas Y, De Parscau L, et al. Adult-onset eculizumab-resistant hemolytic uremic syndrome associated with cobalamin C deficiency. *Am J Kidney Dis*. 2014;63:119–123.
10. Carrillo N, Adams D, Venditti CP. Disorders of intracellular cobalamin metabolism. Available at: <http://www.ncbi.nlm.nih.gov/books/NBK1328/>. Accessed December 21, 2016.
11. Beck BB, van Spronsen F, Diepstra A, et al. Renal thrombotic microangiopathy in patients with cblC defect: review of an under-recognized entity. *Pediatr Nephrol*. 2017;32:733–741.
12. Brunelli S, Meyers K, Guttenberg M, et al. Cobalamin C deficiency complicated by an atypical glomerulopathy. *Pediatr Nephrol*. 2002;17:800–803.
13. Brandstetter Y, Weinhouse E, Splaingard ML, et al. Cor pulmonale as a complication of methylmalonic acidemia and homocystinuria (Cb1-C type). *Am J Med Genet*. 1990;36:167–171.
14. Loirat C, Saland J, Bitzan M. Management of hemolytic uremic syndrome. *Presse Méd*. 2012;41:e115–e135.
15. Fakhouri F, Zuber J, Frémeaux-Bacchi V, et al. Haemolytic uraemic syndrome. *Lancet*. 2017;12(390):681–696.
16. Kim J, Gherasim C, Banerjee R. Decyanation of vitamin B12 by a trafficking chaperone. *Proc Natl Acad Sci*. 2008;105:14551–14554.
17. Lerner-Ellis JP, Anastasio N, Liu J, et al. Spectrum of mutations in *MMACHC*, allelic expression, and evidence for genotype-phenotype correlations. *Hum Mutat*. 2009;30:1072–1081.
18. Morel CF, Lerner-Ellis JP, Rosenblatt DS. Combined methylmalonic aciduria and homocystinuria (cblC): phenotype-genotype correlations and ethnic-specific observations. *Mol Genet Metab*. 2006;88:315–321.
19. Weiss N. Mechanisms of increased vascular oxidant stress in hyperhomocysteinemia and its impact on endothelial function. *Curr Drug Metab*. 2005;6:27–36.
20. Starkebaum G, Harlan JM. Endothelial cell injury due to copper-catalyzed hydrogen peroxide generation from homocysteine. *J Clin Invest*. 1986;77:1370–1376.
21. Stamler JS, Osborne JA, Jaraki O, et al. Adverse vascular effects of homocysteine are modulated by endothelium-derived relaxing factor and related oxides of nitrogen. *J Clin Invest*. 1993;91:308.
22. Upchurch GR, Welch GN, Fabian AJ, et al. Homocysteine decreases bioavailable nitric oxide by a mechanism involving glutathione peroxidase. *J Biol Chem*. 1997;272:17012–17017.
23. Lentz SR, Sadler JE. Inhibition of thrombomodulin surface expression and protein C activation by the thrombogenic agent homocysteine. *J Clin Invest*. 1991;88:1906.
24. Hajjar KA, Mauri L, Jacovina AT, et al. Tissue plasminogen activator binding to the annexin II tail domain. Direct modulation by homocysteine. *J Biol Chem*. 1998;273:9987–9993.
25. Mudd SH, Skovby F, Levy HL, et al. The natural history of homocystinuria due to cystathionine β -synthase deficiency. *Am J Hum Genet*. 1985;37:1.
26. Vaisbich MH, Braga A, Gabrielle M, et al. Thrombotic microangiopathy caused by methionine synthase deficiency: diagnosis and treatment pitfalls. *Pediatr Nephrol*. 2017;32:1089–1092.
27. Labrune P, Zittoun J, Duvaltier I, et al. Haemolytic uraemic syndrome and pulmonary hypertension in a patient with methionine synthase deficiency. *Eur J Pediatr*. 1999;158:734–739.
28. Morath MA, Hörster F, Sauer SW. Renal dysfunction in methylmalonic acidurias: review for the pediatric nephrologist. *Pediatr Nephrol*. 2013;28:227–235.
29. Bouts AH, Roofthoof MTR, Salomons GS, et al. CD46-associated atypical hemolytic uremic syndrome with uncommon course caused by cblC deficiency. *Pediatr Nephrol*. 2010;25:2547–2548.
30. Greitz D. The hydrodynamic hypothesis versus the bulk flow hypothesis. *Neurosurg Rev*. 2004;27:299–300.
31. Fischer S, Huemer M, Baumgartner M, et al. Clinical presentation and outcome in a series of 88 patients with the cblC defect. *J Inherit Metab Dis*. 2014;37:831–840.
32. Li Q-L, Song W-Q, Peng X-X, et al. Clinical characteristics of hemolytic uremic syndrome secondary to cobalamin C disorder in Chinese children. *World J Pediatr*. 2015;11:276–280.
33. Kömhoff M, Roofthoof MT, Westra D, et al. Combined pulmonary hypertension and renal thrombotic microangiopathy in cobalamin C deficiency. *Pediatrics*. 2013;132:e540–e544.
34. Gündüz M, Keci F, Özaydin E, et al. Reversible pulmonary arterial hypertension in cobalamin-dependent cobalamin C disease due to a novel mutation in the *MMACHC* gene. *Eur J Pediatr*. 2014;173:1707–1710.
35. Van Hove JLK, Damme-Lombaerts RV, Grünwald S, et al. Cobalamin disorder Cbl-C presenting with late-onset thrombotic microangiopathy: late-onset cobalamin C disorder. *Am J Med Genet*. 2002;111:195–201.

967	36. Baumgartner ER, Wick H, Maurer R, et al. Congenital defect in intracellular cobalamin metabolism resulting in homocysteinuria and methylmalonic aciduria. I. Case report and histopathology. <i>Helv Paediatr Acta</i> . 1979;34:465–482.	1021
968		1022
969		1023
970		1024
971	37. Russo P, Doyon J, Sonsino E, et al. A congenital anomaly of vitamin B12 metabolism: a study of three cases. <i>Hum Pathol</i> . 1992;23:504–512.	1025
972		1026
973		1027
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