Combined Pulmonary Hypertension and Renal Thrombotic Microangiopathy in Cobalamin C Deficiency

abstract

Pulmonary arterial hypertension (PAH) and renal thrombotic microangiopathy (rTMA) are rare diseases in childhood, frequently leading to death and end-stage renal disease, respectively. Their combined occurrence has been reported anecdotally. We investigated the clinical, biochemical, and genetic aspects of 5 children with the rare combination of PAH and rTMA. Onset of disease ranged from 1.5 to 14 years of age. The 2 youngest patients presented with concomitant pulmonary and renal disease; in the older patients, PAH was preceded by rTMA from age 2.5 to 7 years. Three patients presenting at ≤3 years of age died of right ventricular failure secondary to progressive PAH. In 2 patients, cobalamin C (cblC) deficiency was diagnosed postmortem. Three patients were treated with hydroxocobalamin; 1 died 2 weeks after diagnosis, 1 patient exhibited progressive pulmonary vasculopathy, and 1 patient is currently in stable condition. cblC deficiency was diagnosed biochemically 2 days to 18 years after initial presentation. Genetic analysis confirmed mutations in MMACHC in all patients; 4 patients were compound heterozygous, with all having base-pair substitutions (G>A or G>T) at nucleotide 276 in addition to frame-shift mutations. One patient had homozygous nonsense mutations of MMACHC. We established cblC deficiency as the denominator in the rare combination of PAH and rTMA in these children. Early recognition of cblC deficiency and vigorous treatment with hydroxocobalamin may beneficially affect the course of this devastating disease. Pediatrics 2013;132:e540–e544

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KEY WORDS

atypical hemolytic uremic syndrome, cobalamin C deficiency, pulmonary arterial hypertension, renal thrombotic microangiopathy

ABBREVIATIONS

aHUS—atypical hemolytic uremic syndrome

cblC—cobalamin C

PAH—pulmonary arterial hypertension

rTMA—renal thrombotic microangiopathy

TMA—thrombotic microangiopathy

Dr Kömhoff identified patients, conceptualized and designed the study, drafted the initial manuscript and revised it, and approved the final manuscript as submitted; Dr Roothoof coordinated therapy of pulmonary arterial hypertension, drafted the initial manuscript and revised it, and approved the final manuscript as submitted; Dr Berger supervised the therapy of pulmonary arterial hypertension, conceptualized and designed the study, drafted the initial manuscript and revised it, and approved the final manuscript as submitted; Dr Teertstra identified patients, reviewed and revised the manuscript, and approved the final manuscript as submitted; Dr Losito identified a patient, reviewed and revised the manuscript, and approved the final manuscript as submitted; Dr van de Kar supervised the genetic analysis, reviewed and revised the manuscript, and approved the final manuscript as submitted; and Dr Losito supervised the genetic analysis, reviewed and revised the manuscript, and approved the final manuscript as submitted.

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Pulmonary arterial hypertension (PAH) is characterized by obliterative lesions of intra-acinar pulmonary arteries. PAH leads to an increase in pulmonary vascular resistance and pressure and eventually results in right ventricular failure, with a 3-year mortality rate in children of 25%.1,2 In contrast to adult patients with familial PAH who frequently harbor genetic defects in receptors of the transforming growth factor-β receptor family, relevant genetic defects remain elusive in the majority of children.3

Renal thrombotic microangiopathy (rTMA), including the so-called atypical hemolytic uremic syndrome (aHUS), affects the glomerular microvasculature and can lead to renal failure, with aHUS frequently progressing to end-stage renal disease.4 In ~50% of pediatric aHUS cases, genetic defects are detected, mostly in complement regulating genes.5,6

Cobalamin C (cblC) deficiency is the most commonly inherited form of a defect in vitamin B12 metabolism, with ~500 such patients reported worldwide.7,8 This deficiency may lead to a heterogeneous phenotype ranging from severe neonatal hyperammonemia to neuropsychiatric illness in old age. A few patients with cblC deficiency (<20) have been reported to have rTMA, some of them presenting with aHUS.9,10

We previously reported a postmortem diagnosis of cblC deficiency in a child presenting with aHUS and a risk allele of a complement regulating gene (MCP) known to promote aHUS, who subsequently developed PAH.11 We then specifically screened patients presenting at the Dutch national referral center for pediatric pulmonary hypertension with the rare combination of PAH and rTMA for serum levels of homocysteine and methylmalonic acid.

**CASE SERIES**

**Clinical and Biochemical Investigation**

From 2009 to 2011, a total of 4 patients with combined PAH/ rTMA were identified at the Dutch national referral center for pediatric pulmonary hypertension (with 3–4 newly diagnosed PAH patients per year). A fifth patient with combined PAH/ rTMA was contacted (via his physician) to collect clinical data and to perform genetic analysis of MMACHC. rTMA was determined by renal biopsy findings and/or the presence of HUS. PAH was confirmed by echocardiography, right heart catheterization, and histologic assessments. Laboratory investigations included serum levels of homocysteine and methylmalonic acid in patients 2 through 5 in addition to routine biochemical analysis in all patients.

The study was approved by the institutional medical ethical committee, and patients and/or their parents gave informed written consent for publication of the data.

**Genetic Investigation**

Genomic DNA was amplified for MMACHC (NCBI RefSeq NM_015506.2) by means of polymerase chain reaction. Amplimers, including the individual exons and the splice donor and acceptor site, were subjected to double-stranded DNA sequence analysis on an ABI 3130 xl Genetic Analyzer (Applied Biosystems, Carlsbad, CA). Potential pathogenicity and evolutionary conservation of genetic alterations were checked in the literature and several in silico prediction programs (SIFT: Sorting Intolerant From Tolerant; http://sift.jcvi.org/; PolyPhen-2: Polymorphism Phenotyping v2; http://genetics.bwh.harvard.edu/pph2/; and Align GVGD: http://agvgd.iarc.fr/index.php). Influence on splicing was assessed by using the splice site prediction software Human Splicing Finder (www.umd.be/HSF) and SpliceSiteFinder (www.genet.sickkids.on.ca/~ali/splicesitefinder.html).

Genetic analysis of the complement genes involved in aHUS (CFB, CFI, CFH, MCP, and C5) was performed in patients 1 through 4.

**RESULTS**

In total, 5 pediatric patients with the combination of PAH and rTMA were identified. Clinical characteristics and biochemical and genetic analyses are summarized in Tables 1 and 2.

**Clinical Investigation**

Patient 1 presented at 1.5 years of age with failure to thrive, cyanosis, gallop rhythm, and right ventricular failure. Echocardiography revealed PAH, with no identifiable cause. Hematuria and proteinuria prompted a renal biopsy, the results of which revealed thrombotic microangiopathy (TMA). The renal biopsy procedure induced a pulmonary hypertensive crisis, which was fatal 12 days after admission. CblC deficiency was diagnosed genetically postmortem.

Patient 2 was admitted at 2.5 years of age because of longstanding fatigue, coughing, and failure to thrive. He was in a subcomatose state, with pallor, cyanosis, tachydyspnea, hepatomegaly, and systemic hypertension. Echocardiography revealed biventricular hypertrophy, dilated right ventricle with impaired function, increased pressure in the right ventricle, pulmonary arteries (maximal velocity of tricuspid regurgitation jet, 4.5 m/s), and pericardial effusion. Dialysis was initiated because of aHUS with renal failure. Despite aggressive treatment with PAH-targeted drugs and immediate recognition and treatment of cblC deficiency with hydroxocobalamin (1 mg/day), the patient died of right ventricular failure 2 weeks after diagnosis.
Patient 3 presented at 3 years of age with aHUS (with rTMA confirmed by biopsy findings), which ultimately led to renal transplantation.12 The latter was uneventful except for persistent intravascular hemolysis. Seven years after initial presentation, he developed progressive fatigue, tachypnea, and hypoxia and was diagnosed with PAH (right heart catheterization). Despite treatment with PAH-targeted drugs, the patient died 2 months later of progressive right ventricular failure. cblC deficiency was diagnosed postmortem.

Patient 4 (sister of patient 1) was evaluated at 4 years of age for malaise and failure to thrive. Echocardiography revealed no signs of PAH. Elevated blood pressure, intravascular hemolysis, hematuria, and proteinuria led to renal biopsy, the results of which revealed rTMA. She was managed conservatively after a trial with plasma infusions, which produced no improvement in her renal abnormalities. After 2.5 years, progressive fatigue and dyspnea at exertion led to a cardiologic reevaluation, with right heart catheterization demonstrating mild PAH. cblC deficiency was diagnosed, and treatment with intramuscular hydroxocobalamin (1 mg/day, 3 times a week) led to normalization of haptoglobin levels, indicating that intravascular hemolysis responded well to hydroxocobalamin. Despite concomitant treatment with

**TABLE 1**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at initial presentation, y</td>
<td>1.5</td>
<td>2.5</td>
<td>3</td>
<td>4</td>
<td>14</td>
</tr>
<tr>
<td>Presenting symptom</td>
<td>Tachydyspnea</td>
<td>Tachydyspnea</td>
<td>Fatigue</td>
<td>Fatigue</td>
<td>Fatigue</td>
</tr>
<tr>
<td>Blood pressure, mm Hg</td>
<td>102/50</td>
<td>130/80</td>
<td>160/105</td>
<td>142/91</td>
<td>160/100</td>
</tr>
<tr>
<td>Outcome</td>
<td>Died of RVF</td>
<td>Died of RVF</td>
<td>Renal Tx, died of RVF</td>
<td>CKD II/WHO FC IV</td>
<td>CKD II/WHO FC II</td>
</tr>
<tr>
<td>Time from TMA to PAH</td>
<td>Simultaneous</td>
<td>Simultaneous</td>
<td>7 y</td>
<td>2.5 y</td>
<td>5 y</td>
</tr>
<tr>
<td>Follow-up</td>
<td>12 d</td>
<td>14 d</td>
<td>7 y</td>
<td>3.7 y</td>
<td>14 y</td>
</tr>
<tr>
<td>Follow-up PAH</td>
<td>12 d</td>
<td>14 d</td>
<td>4 mo</td>
<td>17 mo</td>
<td>9 y</td>
</tr>
<tr>
<td>eGFR (90–120 mL/min/1.73 m²)</td>
<td>117</td>
<td>31</td>
<td>98</td>
<td>130</td>
<td>84</td>
</tr>
<tr>
<td>Platelets (150–350 × 10⁹/L)</td>
<td>7.0</td>
<td>4.7</td>
<td>9.0</td>
<td>7.1</td>
<td>5.7</td>
</tr>
<tr>
<td>Homocysteine (4–12 μmol/L)</td>
<td>—</td>
<td>123</td>
<td>185</td>
<td>142</td>
<td>147</td>
</tr>
<tr>
<td>MMA (90–340 nmol/L)</td>
<td>135</td>
<td>144</td>
<td>250</td>
<td>365</td>
<td>257</td>
</tr>
<tr>
<td>Hematuria</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteinuria</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Type of TMA</td>
<td>TMA</td>
<td>aHUS (aFH)</td>
<td>aHUS (MCP)</td>
<td>TMA</td>
<td>TMA</td>
</tr>
</tbody>
</table>

Clinical data revealed heterogeneity of combined PAH/rTMA in terms of age at onset, presenting symptoms, disease progression, and outcome. At presentation with PAH, elevated lactate dehydrogenase and reduced haptoglobin levels revealed intravascular hemolysis, indicating renal TMA. Patients 2 and 3 presented with aHUS. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; MMA, methylmalonic acid; RVF, right ventricular failure; Tx, transplant; WHO FC, PAH World Health Organization functional class. +, urine dipstick positive; −, urine dipstick negative.

**TABLE 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Patient 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right heart catheterization</td>
<td>—</td>
<td>—</td>
<td>4.9</td>
<td>5.2</td>
<td>7.6</td>
</tr>
<tr>
<td>PVR (RU: &lt;2.5)</td>
<td>—</td>
<td>—</td>
<td>30</td>
<td>26</td>
<td>68</td>
</tr>
<tr>
<td>mPAP (&lt;20 mm Hg)</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Renal biopsy</td>
<td>TMA</td>
<td>—</td>
<td>TMA</td>
<td>TMA</td>
<td>TMA</td>
</tr>
<tr>
<td>Lung autopsy</td>
<td>PAHV</td>
<td>PAHV/ PVOD</td>
<td>PAHV/ PVOD</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Genetic evaluation</td>
<td>Spanish/Turkish (c.276G&gt;T/ c.271dupA)</td>
<td>Dutch (c.464G&gt;A/ c.464G&gt;A)</td>
<td>Dutch (c.276G&gt;T/ c.442_444delinsA)</td>
<td>Italian (c.276G&gt;T/A/ c.14_24del11)</td>
<td>MMACHC (p.Glu92Asp/p. Arg91LysfsX14)</td>
</tr>
</tbody>
</table>
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Clinical and Biochemical Characteristics at Presentation With PAH

Clinical data revealed heterogeneity of combined PAH/rTMA in terms of age at onset, presenting symptoms, disease progression, and outcome. At presentation with PAH, elevated lactate dehydrogenase and reduced haptoglobin levels revealed intravascular hemolysis, indicating renal TMA. Patients 2 and 3 presented with aHUS. CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; MMA, methylmalonic acid; RVF, right ventricular failure; Tx, transplant; WHO FC, PAH World Health Organization functional class. +, urine dipstick positive; −, urine dipstick negative.

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PAH-targeted drugs, the patient exhibits progressive pulmonary vascularopathy.

Patient 5 presented at 14 years of age with pallor, fatigue, and elevated systemic blood pressure.\textsuperscript{13} He was diagnosed with rTMA, 5 years later with PAH, and was treated with PAH-targeted drugs. Eighteen years after initial presentation, the patient was diagnosed with cblC deficiency, and treatment with hydroxocobalamin was instituted.

**Biochemical and Genetic Evaluation**

Elevated homocysteine and methylmalonic blood levels suggested cblC deficiency in patients 2 through 5 (Table 1). Mutational analysis of \textit{MMACHC} confirmed cblC deficiency in all patients, with 4 patients harboring heterozygous mutations at nucleotide 276 (c.276G>A and c.276G>T), which is located at the end of exon 2, adjacent to intron 2 (Table 2). The mutation c.276G>T has, to the best of our knowledge, not been described previously. In addition, these patients were heterozygous for various frame-shift mutations. Patient 2 had a homozygous missense mutation (c.464G>A, p.Gly155Glu), which also has not been described previously. Complement analysis revealed postmortem anti-complement factor H autoantibodies (αFH) in patient 2.

Analysis of the complement genes (C3, CFB, CFH, CFI, and MCP [data not shown]) was normal except for MCP in patient 3.\textsuperscript{12}

**DISCUSSION**

To our knowledge, this case series is the first description of children with the rare combination of PAH and rTMA with cblC deficiency established as the denominator. Combined PAH and rTMA in patients with cblC deficiency is heterogeneous with regard to age of onset, initial presentation, and disease progression and is (as with the other phenotypes of cblC deficiency) thus difficult to recognize. Recognition of cblC deficiency also may be hampered by detection of elevated concentrations of vitamin B\textsubscript{12}, which can be misinterpreted as a finding excluding defects in vitamin B\textsubscript{12} metabolism.

Pediatric PAH is known to have a poor prognosis. Although the clinical course varied in the current patient series, 3 of the 5 study patients died within 2 months of diagnosis of PAH, suggesting a rapidly progressive course in these patients despite the start of aggressive PAH-targeted treatment. Patients 1 and 3 were not treated with hydroxocobalamin, and patient 2 was treated with hydroxocobalamin for 2 weeks only.

In patient 4, therapy with hydroxocobalamin and PAH-targeted drugs was instituted at an early stage of PAH but did not halt its progression. Whether earlier therapy with hydroxocobalamin would be more effective is unknown. In patient 5, who had stable disease before the diagnosis of cblC deficiency, therapy with hydroxocobalamin did not induce significant changes in his clinical condition.

The specific type of rTMA depends on the individual genetic makeup; defects in complement-related factors, were only detected in those 2 patients presenting with aHUS (patient 2: αFH; patient 3: heterozygous mutation in MCP\textsubscript{12}). Complement C3 was reduced only in patient 2 with αFH.

As in other patients with cblC deficiency, early onset was associated with worse outcome.\textsuperscript{14} In addition to potential differences in residual enzymatic function of \textit{MMACHC}, other factors play a role as exemplified by the different clinical course in the sibling.

Genetic analysis of \textit{MMACHC} revealed that patients from 3 of the 4 unrelated families (with 4 parental nationalities) had base-pair substitutions at c.276, which is the last nucleotide from exon 2. These changes should result in a missense and silent mutation, respectively (Table 2). Because at least the latter is incompatible with significantly elevated homocysteine levels and hence significantly reduced enzymatic activity (Table 1), we assumed that the base-pair changes at c.276 lead to aberrant splicing and ultimately to deletion of the erroneous pre-mRNA via nonsense-mediated decay. Usually, the last exonic nucleotide at an exon-intron boundary is guanine, and mutations at this position have been shown to result in splicing errors.\textsuperscript{15}

Given that mutations at nucleotide 276 were found solely in 4 of our patients with combined PAH/rTMA and cblC deficiency but not in ~500 patients with cblC deficiency with other phenotypes\textsuperscript{8} or 200 control individuals, we assume that these mutations hold specific vascular pathogenicity in addition to compromising enzymatic function. The specific effects of mutations at nucleotide 276 on the renal and pulmonary vasculature remain elusive. They apparently arise only in conjunction with cblC deficiency as exemplified by identification of 2 parents harboring these heterozygous mutations next to a wild-type allele without renal or pulmonary disease (data not shown).

We speculate that the combination of PAH and rTMA is associated with cblC deficiency due to specific mutations at nucleotide 276 or accompanying sufficiently pathogenic abnormalities of the complement system (ie, anti-factor H autoantibodies).

We conclude that the combination of PAH and renal rTMA in these study children was associated with cblC deficiency and that patients with rTMA and combined disease need to be screened for high concentrations of homocysteine and methylmalonic acid to
allow for timely recognition of cblC deficiency. Further study is needed to determine whether early treatment with hydroxocobalamin beneficially affects the course of this devastating disease.

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