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COBALAMIN C DEFICIENCY - EXPERIENCE OF A REFERENCE CENTER OF HEREDITARY METABOLISM DISEASES

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ORIGINAL SCIENTIFIC ARTICLE

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Índex

Abstract

The cobalamin C deficiency (CblCD) is the most common inborn error of the cobalamin metabolism. It is characterized by inability to convert dietary vitamin B_{12} into the coenzymes adenosylcobalamin and methylcobalamin, in cell. The former is crucial for the activity of methylmalonyl CoA mutase and the later for methionine synthase. Their deficit leads to accumulation of methylmalonic acid (MMA) and homocysteine, respectively. They have a toxic effect in various systems, including the nervous system and eye. Besides these products, disease can be detected by an increase of propionylcarnitine and diagnosed by identification of biallelic pathogenic variants in the *MMACHC* gene. Treatment consists in a combination of vitamin B_{12} and betaine. The benefits of folic acid supplementation and protein restricted diet are limited. Despite therapy, prognosis is poor, especially in the early onset phenotype.

The goals of the present work were to describe the clinical presentation, diagnosis, treatment and disease progression of children (less than 18 years old) with an established diagnosis of CblCD, followed at Centro de Referência de Doenças Hereditárias do Metabolismo do Centro Hospitalar Universitário de Coimbra, between 2002 and 2021 (20 years).

In the defined period, CblCD was diagnosed in five children, one of them presented symptoms before birth, while the other four were diagnosed by the Programa Nacional de Rastreio Neonatal. All presented the same mutation, (c.271dupA), in *MMACHC* gene.

All the patients of our cohort presented clinic in the first weeks of life. The first manifestation of the disease was hypotonia in all patients. Some of them, also presented feeding difficulties, metabolic acidosis and seizures. With increasing age, all manifested ophthalmological and neurological features and three presented cardiovascular manifestations. All children presented global development delay, which is very common in this disorder. One child presented concomitant Type 1 Diabetes Mellitus, which to our knowledge, this association had never been reported. Two children have died.

No correlation was found between MMA levels and date of onset or severity of the clinical manifestations. Methionine and homocysteine levels at presentation did not correlate with clinical severity. In spite of early treatment normal amino acids levels were never achieved, except for methionine.

The current base of CblCD treatment is hydroxocobalamin and betaine. Folic acid and oral metronidazole were also used, despite the fact that their effectivity in CblCD is not consensual.

Although the disease has progressed in all cases, the three living patients remain apparently stable. The youngest has a significant global development delay/intellectual disability. The others, who attend regular school with good performance, although needing visual aids, live a close to normal life.

Keywords
Vitamin B_{12}, Methylmalonic Acidemia, Homocystinuria

Resumo

A deficiência de cobalamina C (CblCD) é a doença congénita mais frequente do metabolismo da cobalamina. Caracteriza-se pela incapacidade de converter vitamina B_{12} da dieta em coenzimas, adenosilcobalamina e metilcobalamina, nas células. Estas são cruciais para a atividade da metilmalonil CoA mutase e da metionina sintase, respetivamente. O seu défice induz acumulação de ácido metilmalónico (AMM) e homocisteína, respetivamente. Os produtos acumulados são tóxicos para o organismo, nomeadamente, para o sistema nervoso e ocular. Para além destes metabolitos, a doença pode ser suspeitada por um aumento de propionilcarnitina e confirmada pela identificação das variantes patogénicas bi-alélicas no gene *MMACHC*. O tratamento consiste numa combinação de vitamina B_{12} e betaína. A suplementação com ácido fólico e a restrição proteica dietética apresentam benefícios limitados. Apesar da terapia, o prognóstico é reservado, especialmente no fenótipo de início precoce.

Os objetivos deste trabalho foram descrever a apresentação clínica, o diagnóstico, o tratamento e a progressão da doença nas crianças (idade inferior a 18 anos) com o diagnóstico de CblCD, seguidas no Centro de Referência de Doenças Hereditárias do Metabolismo do Centro Hospitalar Universitário de Coimbra, entre 2002 e 2021 (20 anos).

No período definido, a CblCD foi diagnosticada em cinco crianças, uma delas apresentou sintomas antes do nascimento, enquanto as restantes quatro foram diagnosticadas pelo Programa Nacional de Rastreio Neonatal. Todas apresentaram a mesma mutação (c.271dupA), no gene *MMACHC*.

Todos os pacientes da nossa coorte apresentaram manifestações clínicas na primeira semana de vida. A primeira manifestação da doença foi a hipotonia em todas as crianças. Alguns deles, apresentaram, concomitantemente, dificuldade de alimentação, acidose metabólica e convulsões. Ao longo do tempo, todos apresentaram manifestações oftalmológicas e neurológicas e três delas tiveram manifestações cardiovasculares. Todos os pacientes apresentaram atraso global de desenvolvimento, uma manifestação muito comum nesta doença. Uma das crianças apresentou concomitantemente Diabetes Mellitus Tipo 1, que à luz no nosso conhecimento, nunca tinha sido associada como manifestação desta patologia. Duas das crianças morreram.

Não foi encontrada uma correlação entre os níveis de AMM e o início ou severidade das manifestações clínicas. Os níveis de metionina e de homocisteína, ao diagnóstico, não aparentam correlação com a severidade das manifestações clínicas.

Apesar do início precoce do tratamento, os níveis dos aminoácidos nunca normalizaram, à exceção da metionina.

O tratamento atual da CblCD assenta em hidroxicobalamina e betaína. O ácido fólico e o metronidazol oral também são utilizados, apesar da sua eficácia na CblCD não serem consensuais.

Apesar da doença ter progredido em todos os casos, os três pacientes vivos permanecem, aparentemente, estáveis. O mais novo apresenta atraso global do desenvolvimento/ défice cognitivo. Os outros, que frequentam o ensino regular com bom desempenho, apesar das adaptações visuais, vivem uma vida aproximadamente normal.

Palavras-Chave
Vitamina B_{12}, Acidémia Metilmalónica, Homocistinúria

Illustration Index

List of Abbreviations

- AdoCbl: Adenosylcobalamin
- BHMT: Betaine-homocysteine methyltransferase
- C3: Propionylcarnitine
- CblCD: Cobalamin C deficiency
- CHUC: Centro Hospitalar e Universitário de Coimbra
- CR-DHM: Centro de Referência de Doenças Hereditárias do Metabolismo
- D: Day
- DOB: Date of birth
- ER: Emergency Room
- Hcy: Homocysteine
- IAC: Interatrial communication
- IUGR: Intrauterine Growth Restriction
- M: Month
- MAT: Methionine Adenosyltransferase
- MeCbl: Methylcobalamin
- Met: Methionine
- MMA: Methylmalonic Acid
- MMA mutase: Methylmalonyl-CoA Mutase
- MRI: Magnetic Resonance Imaging
- OTR: Osteotendinous reflexes
- PFO: Patent foramen ovale
- PNRN: Programa Nacional de Rastreio Neonatal
- R.v.: Reference value
- RPE: Retinal Pigment Epithelium
- SAH: S-adenosylhomocysteine
- SAHH: S-adenosylhomocysteine hydrolase
- SAM: S-adenosylmethionine
- Y: Year

Introduction

The cobalamin C deficiency (CblCD) is the most common inborn error of the cobalamin metabolism. Its incidence varies from 1:46000 to 1:200000 in American countries and Europe to 1:3220 to 1:21488 in China.[1]

The disease is characterized by the inability to convert dietary vitamin B_{12} into adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl), the two metabolically active forms, which are coenzymes of methylmalonyl CoA mutase (mitochondrial) and methionine synthase (cytoplasmic), respectively. Their deficit leads to accumulation of methylmalonic acid (MMA) and homocysteine (Hcy) (Figure 1).[2-5]

High levels of Hcy and MMA have a toxic effect in various systems, especially the nervous system and eye. There are several clinical manifestations of the disease, including hematologic, cardiovascular, ophthalmologic, neurologic, and other (Table 1).[2,3,6,7]

Although there is a spectrum of clinical severity, some authors distinguish two major phenotypes according to the age of onset and severity. Early onset patients, who have symptoms in the first year of life, represent 90% of reported cases. They are more severely affected with multisystemic disease, including failure to thrive, cardiovascular, gastrointestinal, kidney, liver, eye, blood and/or skin manifestations. Signs and symptoms can even manifest in utero. Late onset patients present, at any age, acute or slowly progressive neurological symptoms (extrapyramidal symptoms and gait abnormalities) and behavior disturbances, along with thromboembolic complications. [2,3,6,7]

In 2006, Lerner-Ellis et al., have identified the *MMACHC* gene, located at 1p34.1.[8] There are around 42 different pathogenic variants in this gene, but three have a higher prevalence: c.271dupA, c.394C>T and c.331C>T. The c.271dupA and c.331C>T are more frequently associated with early presentation, whereas the c.394C>T is often found in the late phenotype.[9]

The disease can be detected by an increase of propionylcarnitine (C3) in blood spots and is therefore included in the Programa Nacional de Rastreio Neonatal (PNRN). The subsequent investigation showing high Hcy and MMA and low methionine (Met) levels in biological fluids (plasma and/or urine) are highly suggestive of a vitamin B_{12} deficiency.[10,11] The diagnosis is confirmed by identification of biallelic pathogenic variants on the *MMACHC* gene.[7]

The treatment consists in a combination of vitamin B_{12} and betaine. The benefits of folic acid supplementation and protein restricted diet are limited.[3,4,12]

Despite therapy, prognosis is poor, especially in early onset phenotypes. The course of retinal degeneration is unaltered, resulting in blindness, in spite early treatment. Also, several children end up dying from metabolic decompensation, especially associated with neurological manifestations.[13]

The main goal of the present work is to describe the clinical presentation, diagnosis, treatment and disease progression of pediatric patients with CblCD followed at the Centro de Referência de Doenças Hereditárias do Metabolismo (CR-DHM) of Centro Hospitalar e Universitário de Coimbra (CHUC). Through this report, we aim to alert clinicians and investigators to this rare disorder, in order to foster diagnosis and investigation of new therapies.

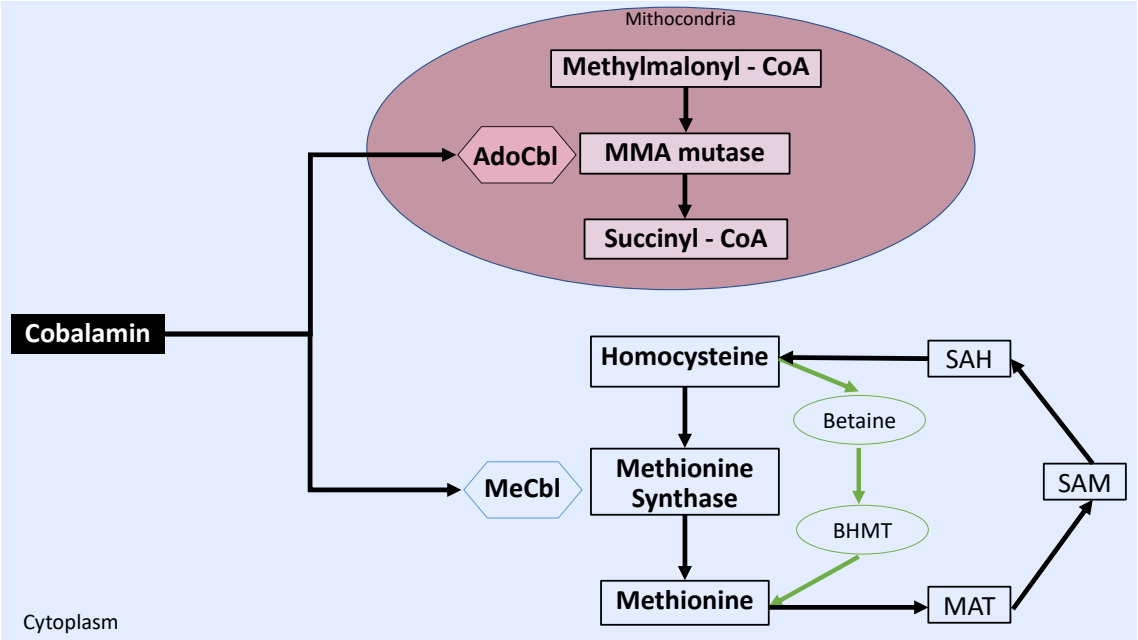

Figure 1. Intracellular Cobalamin Metabolism. Cobalamin is converted into adenosylcobalamin (AdoCbl) and methylcobalamin (MeCbl). AdoCbl is the cofactor of the methylmalonyl-CoA mutase (MMA mutase) which converts methylmalonyl-CoA into succinyl-CoA; MeCbl is the cofactor of methionine synthase in the remethylation of homocysteine (Hcy). Hcy is a sulfur-containing amino acid derived from the metabolism of methionine (Met): Met is adenylated by methionine adenosyltransferase (MAT) into S-adenosylmethionine (SAM), the methyl donor for most methylation reactions; after donating a methyl group, SAM is converted to S-adenosylhomocysteine (SAH), which is hydrolyzed to Hcy by S-adenosylhomocysteine hydrolase (SAHH); Met synthesis can also occur by the alternative betaine-homocysteine methyltransferase (BHMT) reaction, which doesn't require cobalamin.[3]

Table 1. Clinical manifestations of the CblCD[2,3,7]

	Prenatal	Early Onset	Late Onset
General signs			
Nonimmune hydrops	+		
IUGR	+		
Blood cytopenia		+	+/-
Acidosis		+	
Hemolytic uremic syndrome		+	+/-
Renal failure		+/-	+/-
Dysmorphic features		+	
Cardiopulmonary signs	+	+	
Thromboembolic events			+/-
Atrophic gastritis		+/-	
Neurological signs			
Hypotonia		++	
Developmental delay/ intellectual deficit		++	+/-
Seizures		+	
Psychiatric symptoms			++
Microcephaly		+	-
Spasticity			+
Myelopathy			++
Gait abnormalities			++
Ophthalmologic signs			
Nystagmus		++	
Visual impairment		++	+/-
Optic atrophy		+	
Pigmentary retinopathy		+	+/-
Gastrointestinal signs			
Feeding difficulties		++	
Failure to thrive		++	
Recurrent vomiting		+	
Constipation		+	+/-
Pancreatitis		+	+/-
Brain MRI			
Hydrocephalus		+	
White matter alterations		+	+
Brain atrophy		++	+
Basal ganglia lesions		+	

Subtitle 1: Clinical and radiological manifestations of the CblCD, by age group.
IUGR: Intrauterine Growth Restriction; MRI: Magnetic Resonance Imaging.
++: very frequent; + frequent; +/-: rare.

Patients and Methods

A retrospective and descriptive analysis of the clinical files of children (less than 18 years old) with an established diagnosis of CblCD followed at CR-DHM of CHUC between January 2002 and December 2021 (20 years) was carried out.

Based on those criteria, we obtained a sample of five children.

The following data were collected by the pediatricians, through consultation of the clinical records of each patient: current age; gender; age at first disease manifestations; age at diagnosis; general manifestations; cardiovascular manifestations; neurological manifestations; hematological manifestations; ophthalmologic manifestations; complementary exams carried out, including brain Magnetic Resonance Imaging (MRI) and their results; measurements of plasma amino acid and urinary organic acids; neurodevelopmental/cognitive assessment; therapy and disease progression.

The study was approved by the Comissão de Ética do Centro Hospitalar e Universitário de Coimbra.

Results

Case 1

Case 1 (Tables 2,3) refers to a boy, born in 2002 and deceased in 2004, in whom intrauterine growth restriction (IUGR) was identified during pregnancy. One day after birth, he presented generalized hypotonia, severe feeding difficulties, refractory seizures and apneas (needing mechanical ventilation). Metabolic acidosis, neutropenia, thrombocytopenia and several episodes of hyper and hypoglycemia were noticed.

Plasma amino acids disclosed an increase of Hcy and a normal Met and urinary organic acids presented high excretion of MMA, suggesting a CblCD (Table 2).

Decreased Met synthesis, low total content of radioactive cobalamins and deficient production of both methyl- and adenosyl- forms of cobalamin coenzymes and deficient propionate fixation in fibroblasts allowed the diagnosis of CblCD (Table 3).

Besides symptomatic treatment, specific therapy was started soon after diagnosis suspicion, at 13 days old at Intensive Unit Care and consisted of intramuscular hydroxocobalamin, oral betaine, folic acid, levocarnitine and metronidazole (intermittently, 10 days/month) and a protein restricted diet: 1g/kg/d.

At the first biochemical re-evaluation on 22 days of treatment, urine MMA and plasma Hcy decreased and plasma Met increased.

During follow-up, other manifestations emerged: cardiovascular (mitral and tricuspid valves prolapse and enlargement of the aortic root), hematologic (besides persistent neutropenia and thrombocytopenia, a chronic anemia that required several red cell transfusions), gastrointestinal (chronic diarrhea with perianal ulcerations, chronic hypoalbuminemia with edema and deficiency of coagulation factors V and VII), ophthalmologic (severe retinopathy) and neurologic (seizures, microcephaly and severe global developmental delay). He also presented persistent low weight and height and multiple severe infectious disorders that forced several prolonged hospitalizations.

He died at 23 months of Severe Acute Respiratory Syndrome.

Case 2

Case 2 refers to a currently 15-year-old boy (born in 2007), who was referred from the PNRN with a CblCD suspicion, which was confirmed (Tables 2, 3). Plasma amino acids disclosed an increase of Hcy and a normal Met and urinary organic acids presented high excretion of MMA, suggesting a CblCD (Table 2).

Treatment was started at 19 days of life at Emergency Room (ER), after clinical presentation of axial hypotonia.

At the first biochemical re-evaluation on 11 days of treatment (hydroxocobalamin, betaine, folic acid, levocarnitine, metronidazole and a restricted protein diet), urine MMA and plasma Hcy and Met decreased.

Over the years, other symptoms have emerged affecting mostly neurological (global development delay/intellectual disability) and ophthalmic systems (horizontal nystagmus at four years, Bull's eye maculopathy and decreased visual acuity (1/10) with astigmatism and myopia bilaterally at five years, macular pseudocobolomas and modifications in retinal pigment epithelium at the macular and outside the arcades level at seven years old).

He attends 8th grade of regular school with reasonable performance, with adaptations for the visual deficit.

Case 3

Case 3 refers to a girl, born in 2009 and deceased in 2016, who was diagnosed with a CblCD, after referral by the PNRN (Tables 2,3).

Plasma amino acids disclosed a high Hcy and normal Met and urinary organic acids presented high excretion of MMA, suggesting a CblCD (Table 2).

Treatment was started at 11 days of life at ER, where she presented axial hypotonia and metabolic acidosis.

At the first biochemical re-evaluation after two months of treatment (hydroxocobalamin, betaine, folic acid, levocarnitine, metronidazole and a restricted protein diet: 1 g/kg/d), urine MMA and plasma Hcy decreased and plasma Met increased.

During follow-up, other manifestations emerged: neurologic features included microcephaly, epileptic encephalopathy and severe global development delay; ophthalmologic manifestations included horizontal nystagmus at one-month, alternating strabismus (bilateral epicanthus) at six months, stable pigmentary retinopathy at 11 months, granular alteration of the macula and decreased visual acuity at two years.

MRI at two years disclosed a slight hypersignal in long TR sequences at the peri/paraventricular white matter, with greater expression in parietal topography, bilaterally and substantially symmetrical, which could be related to areas undermyelinated. It also presented a slight cortico-subcortical atrophy of subcortical predominance.

At 26 months old, she was diagnosed with Type 1 Diabetes Mellitus, after an episode of metabolic acidosis with severe hyperglycemia, glucosuria and ketonuria and started insulin treatment.

She died at seven years of multiorgan failure secondary to toxic megacolon.

Case 4

Case 4 refers to a 10-year-old girl, born in 2012, who was referred from the PNRN with a CblCD suspicion, which was confirmed (Tables 2, 3).

Plasma amino acids disclosed an increase of Hcy and a low Met and urinary organic acids presented high excretion of MMA, suggesting a CblCD (Table 2).

Treatment was started at 19 days of life at ER, where she presented axial hypotonia.

At the first biochemical re-evaluation on 18 days of treatment (hydroxocobalamin, betaine, folic acid, levocarnitine, metronidazole and a restricted protein diet: 0.75 g/kg/d), plasma Hcy and urinary MMA decreased and plasma Met increased.

Over the years, other symptoms have emerged. Neurologic manifestations included global development delay/intellectual disability. Cardiovascular features included a restrict interatrial communication and a globular and hypertrophic left ventricle diagnosed at two months and a patent foramen ovale with a left-right shunt diagnosed at one year. Ophthalmologic features comprised horizontal nystagmus that blocks in convergence at three months, convergent strabismus at one-year, visual deficit (3/10) without refractive error, severe central maculopathy with pseudocolobomatous changes and nonspecific midperipheral RPE changes at two years, pale papilla with mild vascular narrowing bilaterally and enophthalmos at five years.

She attends 4th grade, with adaptations for the visual deficit.

Case 5

Case 5 refers to a 7-year-old boy (born in 2014) who was diagnosed with a CblCD due to neonatal screening PNRN (Tables 2, 3).

Plasma amino acids disclosed an increase of Hcy and a low Met and urinary organic acids presented high excretion of MMA, suggesting a CblCD (Table 2).

Treatment was started at 13 days of life at ER, where he presented hypotonia, severe feeding difficulties, metabolic acidosis and failure to thrive.

At the first biochemical re-evaluation on one month of treatment (hydroxocobalamin, betaine, folic acid, levocarnitine, metronidazole), plasma Hcy decreased and urine MMA and plasma Met increased. After this evaluation he started a restricted protein diet: 2,88 g/kg/dia.

During follow-up, other manifestations emerged. Neurologic features included an axial hypotonia with poor head control at five months old, an increased peripherical tonus and increased osteotendinous reflexes at six months, an ataxic and broad-based gait at five years-old and global development delay/intellectual disability. Cardiovascular

features included: restrict interatrial communication (ostium secundum-like) at one month and a tortuous aortic cross with turbulent flow at three months. Ophthalmologic system was the most affected, with fixation/search nystagmus at two months, stable pigmentary retinopathy with macular changes (retinoschisis) and pale papilla at six months and a macular cyst with hard exudate deposits in both eyes at two years.

Table 2. Demographic parameters, mutation, plasma and urinary amino acids and treatment of patients.

Patient	*Gender DOB*	*Death*	*Current Age*	*Mutation/ Gene*	*MMA, Hcy & Met at diagnosis*	*MMA, Hcy & Me under treatment (first re-evaluation)*	*MMA, Hcy & Met at last determination*	*Treatment **
1	Male 2002	+ 23 m	--	c.271dupA homozygosity *MMACHC* gene	Hcy: 130.1 (r.v.7-14); Met: 4 (r.v.4-24); MMA: 56.9 (r.v.0-15.6)	Hcy: 42.9 (r.v.7-14); Met: 6 (r.v.4-24); MMA: 48 (r.v.0-15.6)	Hcy: 44 (r.v.7-14); Met: 10.2 (r.v.8.7-40.9); MMA: 102.6 (r.v.0-15.6)	Hydroxocobalamin Betaine Folic acid Levocarnitine Metronidazole Low protein diet
2	Male 2007		15 y	c.271dupA homozygosity *MMACHC* gene	Hcy: 81 (r.v.5-15); Met: 25 (r.v.11-33); MMA: 6948 (r.v.0-7.3)	Hcy: 45 (r.v.5-15); Met: 21 (r.v.11-33); MMA: 6048 (r.v.0-7.3)	Hcy: 74 (r.v.5-15); Met: 33 (r.v.4-44); MMA: 164 (r.v.0-3.3)	Hydroxocobalamin Betaine Folic acid Levocarnitine Metronidazole Low protein diet
3	Female 2009	+ 7y	--	c.271dupA homozygosity *MMACHC* gene	Hcy: High** Met: 8.4 (r.v.7-57); MMA: 30428 (r.v.0-15.6)	Hcy: 47 (r.v.5-15); Met: 21 (r.v.7-57); MMA: 48 (r.v.0-15.6)	Hcy: 44.3 (r.v.7-14); Met: 42 (r.v.4-44); MMA: 102.6 (r.v.0-15.6)	Hydroxocobalamin Betaine Folic acid Levocarnitine Metronidazole Low protein diet
4	Female 2012		10 y	c.271dupA homozygosity *MMACHC* gene	Hcy: 122 (r.v.5-15); Met: 7 (r.v.11-33); MMA: 3626 (r.v.0-15.6)	Hcy: 29 (r.v.5-15); Met: 18 (r.v.11-33); MMA: 80 (r.v.0-15.6)	Hcy: 45 (5-15); Met: 18 (r.v.11-33); MMA: 95 (0-3.3)	Hydroxocobalamin Betaine Folic acid Levocarnitine Metronidazole Low protein diet
5	Male 2014		7 y	c.271dupA homozygosity *MMACHC* gene	Hcy: 191 (5-15); Met: 4 (9-41); MMA: 73 (0-15.6)	Hcy: 39 (5-15); Met: 26 (9-41); MMA: 76 (0-15.6)	Hcy: 41.1 (5-15); Met: 13 (9-41); MMA: 16.3 (0-3.3)	Hydroxocobalamin Betaine Folic acid Levocarnitine Metronidazole Low protein diet

Subtitle 2: Demographic parameters, current age, type of mutation and gene, initial presentation, clinical manifestations -cardiovascular, ophthalmologic, central nervous system or others-, plasma/urinary amino acids at diagnosis, after treatment and last evaluation and treatment instituted in patients with early onset of CblCD.

DOB: date of birth; m: month; y: year; r.v.: reference value; Hcy: Homocysteine; Met: Methionine; MMA: Methylmalonic Acid.
Units: Homocysteine (umol/l); Methionine (umol/l); Methylmalonic Acid (umol/mmol creatinine).
* Treatment protocol was updated after the publication of 2017 Guidelines: doses of hydroxocobalamin were increased and routine protein restriction and metronidazole ceased.[17]
** High levels of Hcy, according to clinical registry.

Table 3. Age of onset, diagnosis and clinical manifestations of patients.

Patient	*Age at onset*	*Suspicion / Initial Presentation*	*Diagnosis*	*Cardiovascular*	*Ophthalmologic*	*Neurologic*	*Other Manifestations*
1	1 d	IUGR + Generalized hypotonia Feeding difficulties Seizures Respiratory apneas Metabolic acidosis Neutropenia Thrombocytopenia Hyper and hypoglycemia	Study of the methionine synthesis 0.03, (r.v 1-4) + Uptake and synthesis of MeCbl 5.5% (r.v 40-76%) and AdoCbl 15%, (r.v 14-27%) + Measurement of propionate incorporation in fibroblasts (1.2 nmol/mg, (r.v 3.5-24.4 nmol/mg) + Genetic study	Mitral /tricuspid valves prolapse, Enlargement of the aortic root	Severe retinopathy	Severe global development delay Microcephaly Seizures	Multiple severe infectious episodes Anemia Chronic diarrhea with chronic perianal ulcerations Chronic hypoalbuminemia Deficiency of coagulation factors V and VII Persistent low weight and height
2	19 d	Axial hypotonia	C3: High* (PNSP data) + Genetic study		Horizontal nystagmus Decreased visual acuity Bull's eye maculopathy Macular pseudocobolomas Modifications in RPE	Global development delay/intellectual disability Unbalanced tandem gait	

3	11 d	Axial hypotonia, Metabolic acidosis	C3: 7.45 umol/mol (r.v.: 0-2.44 umol/mol) (PNSP data) + Genetic study		Horizontal nystagmus Alternating strabismus Pigmentary retinopathy Granular alteration of the macula Decreased visual acuity	Severe global development delay Microcephaly Epileptic encephalopathy	Multiple severe infectious episodes Type 1 Diabetes Mellitus
4	19 d	Axial hypotonia	C3: 7.5 umol/mol (r.v.: 0-2.44 umol/mol) (PNSP data) + Genetic study	IAC, Globular/ hypertrophic left ventricle PFO with shunt	Horizontal nystagmus, Convergent strabismus Visual deficit Maculopathy with pseudocobolomas RPE changes Enophthalmos Pale papilla	Global development delay/intellectual disability	Anemia
5	13 d	Hypotonia Feeding difficulties Metabolic acidosis Failure to thrive	C3: 6.08 umol/mol (r.v.: 0-2.44 umol/mol) (PNSP data) + Genetic study	Restrict IAC Tortuous aortic cross	Fixation/search nystagmus Pigmentary retinopathy Macular changes (retinoschisis) Pale papilla Macular cyst with hard exudate deposits	Global development delay/intellectual disability Axial hypotonia with poor head control Increased peripherical tonus and OTR Ataxic/ broad-based gait	

Subtitle 3: Age at onset, initial presentation, diagnosis, clinical manifestations: cardiovascular, ophthalmologic, neurologic system or others.
D: day; m: month; IUGR: Intrauterine Growth Restriction; MeCbl: Methylcobalamin; AdoCbl: Adenosylcobalamin; r.v.: reference value; C3: Propionylcarnitine; PNRN: Programa Nacional de Rastreio Neonatal; PFO: Patent foramen ovale; IAC: Interatrial communication; RPE: Retinal Pigment Epithelium; OTR: Osteotendinous reflexes.
* High levels of C3, according to clinical registry.

Discussion

In the last two decades, five patients with CblCD were followed at Hospital Pediátrico of CR-DHM of CHUC.

Except for patient 1, all cases were detected by the PNRN. This program was broadened in 2006 to other inherited metabolic disease, including CblCD.[11] Since then, 27 patients with CblCD were screened (~ incidence 1:52700). [11] Three of the four cases detected by PNRN were still asymptomatic when referred to our center and the other had just entered ER of our hospital.

All patients presented the same pathogenic variant (c.271dupA) in the gene *MMACHC*, one with the highest prevalence in literature and associated with an early-onset phenotype. In fact, all of patients of our cohort presented clinic in the first weeks of life.[9]

The first manifestation of the disease was hypotonia in all children. Some of them, also presented feeding difficulties, metabolic acidosis and seizures. All these symptoms are described in literature as being associated with this disorder.[3,5,7] The oldest patient, boy born in 2002 was the only one who presented prenatal manifestations, although nonspecific (IUGR).

Despite early treatment, it was not possible to avoid some typical manifestations of the disease. With increasing age, all children manifested ophthalmological and neurological alterations. Most children had nystagmus, pigmentary retinopathy, maculopathy and decreased visual acuity. The pathophysiology behind retinal dysfunction associated with CblCD remains unknown, although it has been attributed to pathogenicity of organic acid metabolites.[5,14]

All children presented global development delay, which is very common in this disorder.[3,15] The children with more severe manifestations and with a greater developmental delay had microcephaly. Although microcephaly might have other causes, in this case it has special relevance as it is a manifestation of severity.[16]

The girl born in 2009 (case 3) developed concomitant Type 1 Diabetes Mellitus, with positive autoantibodies at 26 months of age. She presented a severe metabolic acidosis episode of ketoacidosis and methylmalonic aciduria, associated with hyperglycemia, which lead to that diagnosis. To our knowledge, this association had never been reported. This girl presented cortico-subcortical atrophy, with subcortical predominance, a consistent neuroradiologic feature of CblCD.[15]

There seems to be no relationship between MMA urinary excretion and the severity of manifestations. In fact, the child born in 2007 (case 2) showed the highest MMA values in urine: 6948 umol/mmol creatinine, but presented at 19 days, later than

the other patients, except patient 4. At 15-years of age, he attends regular school, although with the need of visual aids due to retinopathy.

Case 1 and 3 were the ones who presented earlier, at day one and 11, respectively, and with more severe manifestations of the disease, including methylmalonic acidemia, and both died. Case 1 presented IUGR, which, in spite of the placenta protective effect, might be caused by the intracellular cobalamin abnormalities. Again, clinical severity seems to be not related to MMA in urine, since case 1 had 56.9 and case 3 had 30428 umol/mmol creatinine in urine at presentation.

CblCD can led to low levels of Met, due to methylcobalamin deficiency, the cofactor of methionine synthase. In fact, at presentation patients 4 and 5 had low levels, while patient 1 had low/normal. However, Met levels at presentation did not correlate with clinical severity. Homocysteine levels were high at diagnosis in all patients, as expected.

Starting the treatment as soon as the diagnosis is established is crucial to the prognosis. The main goal of the treatment is to accomplish normal levels of Hcy, Met and MMA. [3,4,7,12] In our cohort, all children complied with the appropriate treatment. Over the years, evaluations of Hcy, Met and MMA levels have been carried out to adjust the dose of the treatment. In spite of early and through treatment, according to international guidelines, that objective was never achieved, except for Met levels. Again, we found no relation between control levels of Hcy and MMA and evolution.[17]

The current base of CblCD treatment is hydroxocobalamin and betaine.[17] With high doses of intramuscular vitamin B_{12} we aim to increase intracellular production of cobalamin cofactors, needed for adequate enzyme activities. Hydroxo- and not cyano- or other forms of cobalamin should be used, as it is more effective in these patients. [17] We had the unfortunate experience of confirming it, since cyanocobalamin was used in the first few days in patient 1 with no clinical response. Of note is that the biochemical control in table 2 was done on hydroxocobalamin treatment.

Betaine, an oral orphan drug, promotes conversion of Hcy to Met through and alternative pathway (betaine Hcy methyltransferase). In our patients, although Met levels normalized, Hcy remained elevated, although lower than at presentation. High Hcy is a known risk factor for cardiovascular disease. Probably due the low age range of our patients, ischemic events were not observed. However it is of note that complex cardiovascular manifestations were present in three.

Folic acid, which effectivity in CblCD is not as consensual as the formers, was used in all our patients, aiming to increase remethylation and control Hcy and Met levels.[17]

Patients with CblCD can have severe MMA acidosis episodes, similar to classic methylmalonic aciduria. So, we have used a diet restricted in propionic precursors (protein restricted), oral metronidazole (to decrease the production of propionyl-CoA derived from anaerobic bacterial fermentation of carbohydrates in the gut and levocarnitine supplementation (to avoid carnitine depletion due to acylcarnitine urinary excretion) in our patients.[4] Nevertheless, our treatment protocol was updated after the publication of 2017 Guidelines: doses of hydroxocobalamin were increased and routine protein restriction and metronidazole ceased; levocarnitine currently is used only to maintain normal plasma levels, which analysis is now available.[17]

Although the disease has progressed in all cases, the three living patients remain apparently stable. The youngest has a significant global development delay/intellectual disability. The others, who attend regular school with good performance, although needing visual aids, live a close to normal life.

Conclusion

CblCD is a rare inborn metabolic disease. The mainstain of treatment is high doses of parental hydroxocobalamin and betaine. Although newborn screening programs may allow an early diagnosis and treatment implementation, disease prognosis is still poor, with high mortality in infancy and development delay and/or visual deficit in the surviving patients.

Despite the small cohort of this rare disorder and the retrospective nature of our work, which are limitations, we think it is nevertheless relevant, since it reflects the experience of a CR-DHM. A multicenter national/international study with a much larger series is warranted.

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