

Milder form of cobalamin C disease could be missed by newborn screening: the importance of methylmalonic acid assessment



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Background

Cobalamin C (CblC) disease is the most common inborn error of intracellular cobalamin metabolism and expanded newborn screening (NBS) allows for its early diagnosis. The key reactions of intracellular cobalamin metabolism are the conversion of dietary vitamin B12 (cobalamin, Cbl) to its active cofactors, methylcobalamin (MeCbl) and adenosylcobalamin (AdoCbl), both of these are respectively essential cofactors of the enzyme methionine synthase in cytoplasm and methylmalonyl-CoA mutase in mitochondrion. Methionine synthase catalyzes the conversion of homocysteine to methionine while methylmalonyl-CoA mutase the conversion of methylmalonyl-CoA to succinyl-CoA. The biochemical impairment in CblC (as well as CblF and CblD) can results therefore to an elevation of circulating and urinary levels of homocysteine (HCY) and methylmalonic acid (MMA).

Propionylcarnitine (C3) is the primary marker, although with a low specificity, for different disorders of propionate metabolism such as methylmalonic acidemia, propionic acidemia, congenital cobalamin defects, and secondary maternal vitamin B12 deficiency. To increase positive predictive value, we use C3/C2, C3/C0 and C3/Met ratios and measure methylmalonic acid (MMA), methylcitric acid (MCA) and homocysteine (tHCY) in DBS as second-tier test. Here we report the diagnosis of 3 CblC patients with persistent MMA elevation, despite normalization of C3 and its ratios.

Methods

Data from our NBS center has been collected since 2010. The XEVO/TQD MS/MS system (Waters, MA, United States) and Neogram derivatized MS/MS kit (Perkin Elmer, United States) was used; the cut off values for C3, C3/C2, C3/C0, C3/Met were 4.38 $\mu\text{mol/L}$, 0.13, 0.14, 0.22 respectively. Newborns with abnormal levels of even only one of these analytes were analyzed further by LC-MS/MS to evaluate MMA, MCA and by HPLC to evaluate tHCY concentration in the same DBS. In newborns with alteration of MMA level on the first DBS a second DBS was requested. The analysis of MMA was performed in the second DBS even if C3, C3/C0 C3/C2 and C3/Met levels were within normal values. The algorithm utilized is shown in **Figure 1**.

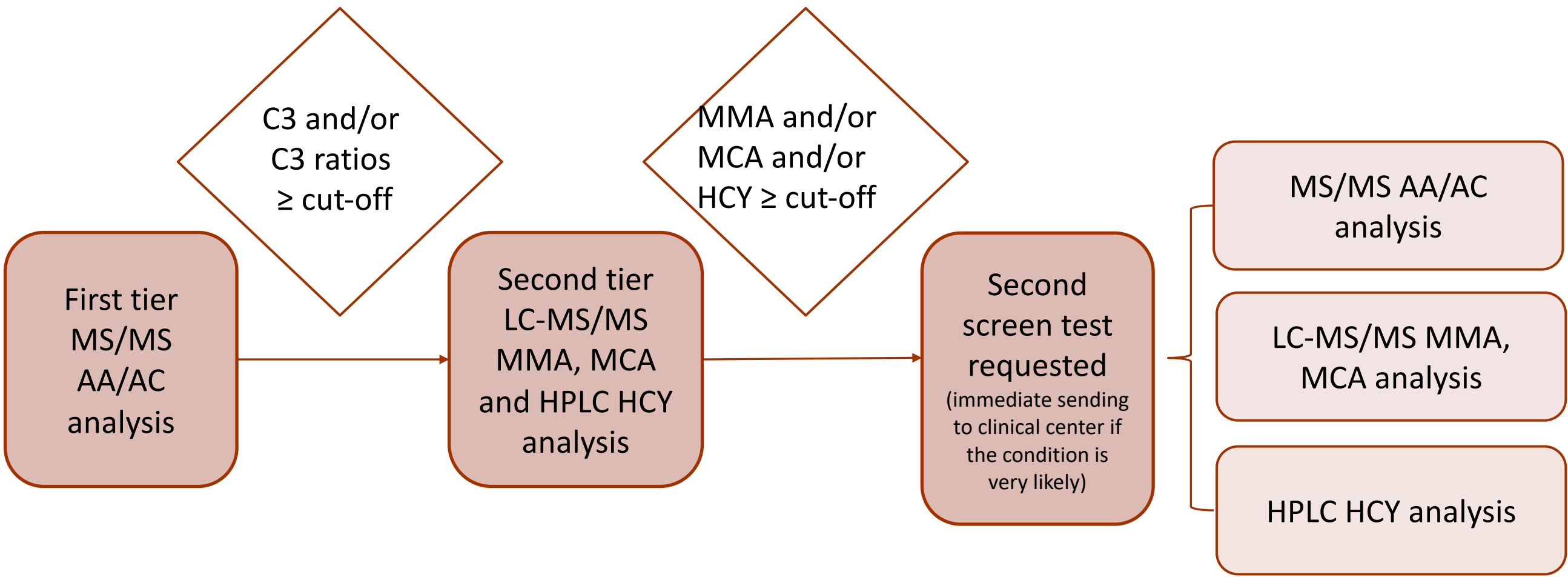


Figure 1. Algorithm used after a C3, C3/C2, C3/C0, C3/Met positive results from NBS test.

Results

Nine out of 308964 screened newborns were diagnosed with CblC (**Table 1**). Three of them had a complete normal C3 and C3 ratios at the second screen test (between 8 and 20 days of life), while MMA was above (between 3.1 and 7.6 $\mu\text{mol/L}$) the cut-off value (2 $\mu\text{mol/L}$) (**Table 2**, ID5, ID6, ID8). Molecular analysis confirmed CblC deficiency. Patients were supplemented with hydroxocobalamine alone allowing for a normalization of homocysteine and urinary MMA. Furthermore, an older sibling of a CblC patient who was not submitted to expanded NBS was diagnosed with CblC at 4 years of age with normal C3. All patients have a normal development.

ID	Age (h)	C3 ($\mu\text{mol/L}$)	C3/Met	C3/C2	C3/C0	Met ($\mu\text{mol/L}$)	C16:1OH	MMA ($\mu\text{mol/L}$)	MCA ($\mu\text{mol/L}$)	tHcy ($\mu\text{mol/L}$)
cut-off 2-15 days		4,38	0,219	0,18	0,14	<10.5	0,18	2	1	3,7
1	48	8,5	1,25	0,43	0,24	7,0	0,34			
2	72	6,4	0,45	0,13	0,09	14,1	0,03	8		
3	72	10,3	1,08	0,22	0,15	9,6	0,13	38		25
4	72	8,6	1,5	0,28	0,27	5,7	0,18	49		31
5	72	4,3	0,23	0,14	0,2	18,7	0,06	24,6	<LOQ	7,4
6	72	4,9	0,22	0,14	0,14	21,9	0,06	5,81	<LOQ	4,3
7	48	9,0	1,19	0,27	0,25	5,6	0,19	59	<LOQ	49,4
8	48	3,9	0,14	0,11	0,14	27,5	0,04	14,4	0,72	7,14
9	72	11,8	1,93	0,32	0,26	6,1	0,18	80	2	68,2

Table 1. NBS results. Legend. LOQ: limit of quantification. In bold values $\geq$ cut-off.

ID	Age (h)	Age (days)	C3 ($\mu\text{mol/L}$)	C3/Met	C3/C2	C3/C0	Met ($\mu\text{mol/L}$)	C16:1OH	MMA ($\mu\text{mol/L}$)
cut-off 2-15 days			4,38	0,219	0,18	0,14	<10.5	0,18	2
cut-off >15 days			3,13	0,118	0,14	0,057	<11.5		2
5	240	10	1,8	0,09	0,08	0,08	20,4	0,03	8,01
6	480	20	1,4	0,05	0,07	0,04	30,2	0,02	3,11
8	192	8	1,7	0,09	0,08	0,07	18,9	0,02	7,6

Table 2. Second screen test. In bold values $\geq$ cut-off.

Discussion

Early diagnosis and treatment of CblC through NBS has improved patients' outcome but it could not prevent all the symptoms in early-onset forms while it can be particularly beneficial in late-onset forms. These data underlie the difficulty of a correct CblC diagnosis through NBS, especially for the milder form, those who would really benefit of an early diagnosis and that respond to a simplified treatment.

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