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How can cobalamin injections be spaced in long-term therapy for inborn errors of vitamin B_{12} absorption?

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ABSTRACT

Inborn errors of cobalamin (Cbl, vitamin B_{12}) absorption include hereditary intrinsic factor deficiency (HIFD) and Imerslund–Gräsbeck disease (IGD). HIFD is secondary to mutations in the *HIF* gene while IGD is due to mutations in one of the 2 subunits of the intrinsic factor receptor that is cubilin (*CUBN*) or amnionless (*AMN*). These disorders lead to intracellular Cbl depletion which in turn causes megaloblastic bone marrow failure, accumulation of homocysteine and methylmalonic acid (MMA), and methionine depletion. The clinical presentation reflects Cbl deficiency, with gastrointestinal symptoms, pancytopenia, and megaloblastic anemia. Mixed proteinuria, when it is present is strongly suggestive of IGD. Accurate diagnosis is always an emergency because early detection and treatment with life-long parenteral pharmacological doses of hydroxocobalamin are life saving and prevent further deterioration. However, the optimal frequency for cobalamin injections as a maintenance therapy is poorly reported. In order to evaluate the optimal maintenance schedule of cobalamin injections, we retrospectively collected clinical, biological, molecular and treatment data on 7 patients affected with congenital Cbl malabsorption. Unlike previous recommendations, we showed that a maintenance dosage of 1 mg cobalamin twice a year was enough to ensure a normal clinical status and keep the hematological and metabolic parameters in the normal range. These data suggest that patients affected with inborn errors of cobalamin absorption may be safely long-term treated with cobalamin injections every 6 months with careful follow-up of hematological and metabolic parameters. This maintenance regime is beneficial because the patients' quality of life improves.

1. Introduction

When pancytopenia presents in infancy, the most important diagnoses to consider are inherited disorders of cobalamin [1] (Cbl, vitamin B_{12}) or folate [2] metabolism and nutritional Cbl deficiency. Accurate immediate diagnosis is mandatory because early detection and treatment are life saving. Whereas malabsorption of vitamin B_{12} is the most frequent cause of vitamin B_{12} deficiency in adults with mainly acquired conditions, pediatric selective vitamin B_{12} malabsorption syndromes are very rare in developed countries and are usually due to hereditary diseases. These include the Imerslund–Gräsbeck syndrome (IGS, first described in 1960 [3,4]) and the much rarer hereditary intrinsic factor (IF) deficiency (HIFD). IGS results from genetic defects of one of the 2 subunits of the ileal receptor of intrinsic factor, amnionless (encoded by *AMN*) and cubilin (encoded by *CUBN*) [5,6]. Both proteins are also involved in renal tubular reabsorption of various ligands providing an explanation for the proteinuria [7] often encountered in IGS patients. In the ileum, these proteins form a heterodimer called cubam responsible for the internalization of the IF–cobalamin complex [6]. The molecular basis of HIFD has been more recently described with up to 20 patients harboring mutations in the gene *GIF* encoding for the intrinsic factor [8–10]. Whatever the genetic cause, there is a selective malabsorption of vitamin B_{12} resulting in low plasma levels of the vitamin. The key pathogenic factors in Cbl deficiency include homocysteine and methylmalonic acid accumulation and methionine depletion. If rapidly diagnosed and treated with parenteral pharmacological doses of vitamin

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B_{12}, patients affected with IGS or HIFD show rapid normalization of hematological and metabolic parameters with subsequent prevention of any further deterioration. However, probably due to the rarity of these conditions, therapeutic recommendations regarding long-term monitoring and follow-up of metabolic parameters as well as schedule of vitamin B_{12} injections in maintenance therapy are poorly reported. Our aim was to specifically review data on 7 patients affected with inborn errors of vitamin B_{12} absorption with special emphasis on long-term maintenance treatment and follow-up of clinical status, hematological and metabolic parameters.

2. Patients and methods

Seven patients with inherited disorders of cobalamin absorption (4 with IGS disease and 3 with HIFD, Table 1a) were reviewed retrospectively for i) clinical signs and age at diagnosis, ii) hematological and metabolic parameters at diagnosis, iii) genotype, iv) treatment options and v) outcome regarding clinical status, hematological and metabolic parameters under treatment.

3. Results

3.1. Clinical presentation (Table 1b)

Patients 1 to 4, affected with IGS presented similarly including failure to thrive, glossitis (3, 4), chronic diarrhea and recurrent vomiting (1, 3, 4) as well as pallor. Patients 2 and 3 were siblings. Age at diagnosis ranged between 14 and 25 months. They all exhibited proteinuria ranging between 0.9 and 2.1 g/L. Patients 5 to 7 were affected with HIFD. One of them (5) presented similarly to the IGS patients with failure to thrive, glossitis and gastro-intestinal symptoms at 18 months of age. Patients 6 (the sister of patient 5) and 7 remained totally asymptomatic until they exhibited subacute anemia at 10 and 6 years of age respectively. Of note, patient 6, because of her affected sister and due to transiently mildly abnormal metabolic parameters (plasma total homocysteine 23 μM [normal <15 μM], plasma methionine 12 μM [normal range 15–30 μM] and urinary methylmalonic acid 47 mmol/mol creatinine [normal <5]) was prophylactively treated with bi-annual Cbl intra-muscular injections from 2.5 years of age. Before starting treatment, blood cell count, and plasma vitamin B_{12} level were in the normal range (not shown). Treatment was stopped at 6.5 years of age. One year later, all the biological (hematological, vitamin B_{12} and metabolic) parameters had remained normal, this made us believe, erroneously that this child was not affected and follow-up was stopped. However, 3.5 years later, at 10 years of age she presented with pallor and tachycardia related to a poorly tolerated anemia requiring transfusion and revealing HIFD (see below). Interestingly, patient 7 presented striking skin and hair reversible abnormalities (Fig. 1) as already reported in patients affected with vitamin B_{12} malabsorption [11,12].

3.2. Hematological and metabolic parameters at diagnosis

All the patients exhibited mild (2, 3, 5) to severe (1, 4, 6, 7) aregenerative and macrocytic (except for patient 7) anemia (Table 2). Four patients (1, 3, 5, 6) had neutropenia and three (1, 6, 7) thrombocytopenia (Table 2). Bone marrow examination (patients 1, 4, 7) showed megaloblastic changes compatible with vitamin B_{12} or folate deficiency (Fig. 2). This was confirmed by a reduced plasma level of vitamin B_{12} for all the patients (Table 2). Red blood cell folate levels were normal for all patients (not shown). We searched for anti-IF antibodies in patients 3, 4, 5 and 7 and the result was negative. In patient 5, the level of IF in gastric juice was undetectable (not shown). In patients where we looked for it, we found that methylmalonic acid (MMA) urinary excretion was moderately increased (Table 2). Plasma methionine level (Table 2) was low in 1 patient (2), at the lower limit of the normal range in 4 patients (3, 5, 6, 7) and normal in 2 patients (1, 4). Total plasma homocysteine (tHCy) level was mildly increased in 5 patients (1, 2, 4, 5, 6) and frankly elevated in one (7)(Table 2). For patient 3, tHCy could not be determined and free HCy was increased to 44 μM (normal value: undetectable).

Table 1a
Diagnostic and epidemiological data on the 7 patients.

Patient #	Sex	Diagnosis	Origin	Consanguinity	Age at diagnosis
1	M	Imerslund–Gräsbeck	Turkey	y	25 mo
2	F	Imerslund–Gräsbeck	Tunisia	n	14 mo
3	M	Imerslund–Gräsbeck	Tunisia	n	17 mo
4	F	Imerslund–Gräsbeck	Tunisia	y	14 mo
5	F	HIF	France	y	18 mo
6	F	HIF	France	y	10 y
7	F	HIF	Mali	y	6 y

Table 1b
Main clinical symptoms at diagnosis.

Patient #	1	2	3	4	5	6	7
Failure to thrive	X	X	X	X	X		
Proteinuria	X	X	X	X			
Glossitis			X	X	X		
Gastro-intestinal symptoms	X		X	X	X		
Pallor	X		X	X	X	X	X
Neurological symptoms							
Hair abnormalities					X		X
Hyperpigmentation							X

3.3. Genotype (Table 3)

Three of the IGS patients (1, 2, 3) exhibited a homozygous mutation of the *AMN* intron 3 acceptor splice site (c.208-2A>G). This mutation was originally reported in families of Tunisian Jewish and Turkish origin as c.208-2A>G, skipping of exon 4; fs [5,13] and causes complete skipping of exon 4 resulting in a frameshift. *In silico* analysis of the mutant transcript using ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) also predicted incorporation of 99 aberrant amino acids before reaching a premature stop codon. In patient 4, a novel missense mutation of *CUBN* exon 24 was identified (c.3335G>A, p.Gly1112Glu). Functional characterization of this mutation is being reported elsewhere (in prep). Patients 5, 6 (siblings) and 7 were homozygous for a new stop codon mutation in exon 5 (c.691C>T, p.Gln231X, patients 5 and 6) and for a previously reported deletion in exon 2 (183_186delGAAT, patient 7) [9] of *GIF*.

3.4. Long-term maintenance cobalamin treatment and outcome (Table 4)

Initially, for patients with acute and severe anemia (1, 4, 6, 7), intramuscular (IM) hydroxocobalamin (OHCbl) was given daily at the dosage of 1 mg. After reticulocyte crisis, OHCbl injections were spaced to once a week. The other patients without severe anemia (2, 3, 5), were directly treated with weekly IM hydroxocobalamin (2, 5) or cyanocobalamin (3). Initial vitamin B_{12} depletion was immediately corrected after the first injection with a normal vitamin B_{12} level (not shown). Cobalamin injections were gradually spaced provided hematological parameters (blood cell count), vitamin B_{12} plasma levels and metabolic (plasma methionine and tHCy levels, urinary MMA) parameters remained in the normal range. Maintenance IM Cbl schedule could be progressively reached thanks to cautious monitoring of hematological and metabolic parameters. As detailed in Table 4, all our patients are being treated with 1 mg OHCbl or CNCbl (4) every 6 months. With such a maintenance schedule, they exhibit a normal clinical status associated with normal hematological and metabolic parameters (plasma methionine and tHCy levels missing for patient 4). Plasma residual vitamin B_{12} determined before the next injection was normal for 4 patients