Long-term outcome of a patient with Transcobalamin deficiency caused by the homozygous c.1115_1116delCA mutation in TCN2 gene: a case report

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Case report

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Abstract

Background: Transcobalamin deficiency is a rare autosomal recessive inborn error of cobalamin transport (prevalence: <1/1000000) which clinically manifests in early infancy.

Case presentation: We describe the case of a 30 year old woman who at the age of 30 days presented with the classical clinical and laboratory signs of an inborn error of vitamin B_{12} metabolism. Family history revealed a sister who died at the age of 3 months with a similar clinical syndrome and with pancytopenia. She was started on empirical intramuscular (IM) cobalamin supplements (injections of hydroxocobalamin 1 mg/day for 1 week and then 1 mg twice a week) and several transfusions of washed and concentrated red blood cells.With these treatments a clear improvement in symptoms was observed, with the disappearance of vomiting, diarrhea and normalization of the full blood count. At 8 years of age injections were stopped for 3 months causing the reappearance of megaloblastic anemia. IM hydroxocobalamin was then restarted *sine die*. The definitive diagnosis could only be established at 29 years of age when a genetic evaluation revealed the homozygous c.1115_1116delCA mutation of TCN2 gene (p.Q373GfsX38).

Currently she is a 30-year old healthy lady taking 1 mg of IM hydroxocobalamin once a week.

Conclusions: Our case report highlights that early detection of TC deficiency and early initiation of aggressive IM treatment is likely associated with disease control and an overall favorable outcome.

Background

Vitamin B_{12} (B_{12}) also known as cobalamin is absorbed at the terminal ileum level, associated with a glycoprotein of gastric origin (intrinsic factor), by means of an endocytosis mechanism, mediated by an enterocyte membrane receptor (1). Subsequently the B_{12} is secreted in the bloodstream where it binds to the vector protein haptocorrin (HC, previously named transcobalamin I). In the enterocyte, the intrinsic factor - cobalamin complex dissociates and cobalamin reaches the portal circulation where it is bound to transcobalamin (formerly known as transcobalamin II [TC]) (1). In this bound form it is recognized by the surface receptors of the user cells (mainly those tissues with proliferative activities such as those of the bone marrow and of the germinative layer of the epithelia) and introduced, through a process of micropinocytosis, inside the cell, where the B_{12} is freed from the vector by lysosomal acid hydrolases and reduced by cytoplasmic enzymes (2).

At this point the reduced B_{12} undergoes two separate metabolic processes to be converted into the two co-enzymatically active forms: in the cytosol, in the form of methyl-cobalamin, it becomes part of the catalytic process of the enzyme methionine synthase; in the mitochondrion undergoes an adenosylation process that forms adenosyl-cobalamin (coenzyme of methylmalonyl-CoA mutase).

Congenital errors of cobalamin metabolism can affect any stage of absorption, blood transport and intracellular use.

The only congenital absorption deficit is due to a genetic alteration of the enterocytic receptor (Imerslund-Grasbeck syndrome), which is characterized by low serum cobalamin levels, a normal intrinsic factor and megaloblastic anemia (3).

TC deficiency is a typical transport error characterized by megaloblastic anemia with normal serum levels of B_{12} (4). In this form there is a combined deficiency of synthesis of both methylcobalamin (methionine synthase coenzyme) and adenosyl cobalamin (methylmalonyl-CoA mutase coenzyme), with the consequent possibility of homocystinuria and methylmalonic aciduria. A massive homocystinuria with methylmalonic aciduria is characteristic of the combined deficits of the common intracellular metabolism of the B_{12} (5).

Isolated deficiencies of the specific metabolic pathways of adenosylcobalamin and methylcobalamin lead, respectively, to methylmalonic aciduria and homocystinuria. In the first case there will be the forms of methylmalonic acidemia due to coenzyme deficiency, which respond in part to therapy with B_{12} (ca. 10–20%), while the methylcobalamin deficiency blocks the methylation of homocysteine, thus causing massive homocystinuria (6). The activity of methionine synthetase also requires the presence of some intermediates of the folate metabolism. However, congenital disorders of folate metabolism exist which, by altering the correct synthesis of these intermediates, cause a deficiency of enzymatic activity.

In particular, in 5–10, methylentetrahydrofolate reductase deficit, there is a lack of synthesis of 5 methyltetrahydrofolate which is the direct donor of the methyl group to homocysteine for the synthesis of methionine (7).

The blockade of methionine synthetase activity causes massive homocystinuria but megaloblastic anemia has never been found.

As for B_{12} there is also a folate malabsorption which is characterized by severe megaloblasticanemia with very low serum folate levels. Albeit rare there are also congenital disorders of folate metabolism, such as the functional deficit of dihydrofolatereductase and the cellular uptake defect of folate, characterized by severe megaloblastic anemia and pancytopenia. These disorders are sensitive to therapy with folate at high doses, in case of normal serum levels of folic acid. In the few cases described it is not clear whether the pathogenesis is due to a primitive metabolic deficit of folate; in any case, aminoaciduria has never been found.

Blood cobalamin levels are not usually low because most of serum cobalamin is bound to HC and not to TC.

TC deficiency (OMIM #275350) is a rare autosomal recessive disorder: the most frequent mutations are deletions or insertions in the TCN2 gene, resulting in frame shifts that predict protein truncation (8, 9). Nonsense mutations and point mutations that activate exonic cryptic splice sites have also been reported (10, 11), as well as, polymorphic variants have also been described (8, 11).

Case Description

Our patient was born to Italian non consanguineous parents. She was born from eutocic birth with a weight of 3,300 g (50th percentile), head circumference 34.5 cm (50th percentile), length 50 cm (50th percentile). She was first evaluated at the age of 30 days when she presented with recurrent vomiting with every meal (exclusive breast feed), diarrhea and weight loss (< 25% percentile). Neurological evaluation was normal.

Family history revealed a sister who died at the age of 3 months with a similar clinical syndrome and with pancytopenia. This sibling was diagnosed an acute myeloid leukemia, but the diagnosis was not confirmed at post-mortem examination.

Complete blood count (CBC) revealed megaloblastic anemia with leukopenia. Peripheral blood film showed hypochromic macrocytic Red Blood Cells (RBC) and hyper-segmented neutrophils, with no reticulocytosis (corrected reticulocyte count was 5%) serum bilirubin was 7 g/dl (< 9.5). Folate, cobalamin and total protein blood concentrations were normal (19.2 mg/mL, 1940 pg/mL and 6 g/dl respectively). Urinary spot methylmalonic acid (qualitative: carried out with thin layer chromatography)(12) was not elevated, while homocystinuria was present (13). The study of lymphocyte subpopulations was normal (lymphocytes: T total 84%, CD3 T activated 0%, T CD4-, CD8 + 22%, T CD4+, CD8 + 0%, T CD4+, CD8- 62%, Total B: DR + 7%, B CD19 7%). No proteinuria was found.

The clinical picture, including family history, megaloblastic anemia and laboratory findings that demonstrate a normal B12 and folate level suggested the diagnostic suspect of deficiency of Transcobalamin II. Therefore, was started a treamtment with several transfusions of washed and concentrated RBC and intramuscular (IM) injections of hydroxocobalamin (1 mg/ day for 1 week and then 1 mg twice a week). The clinical picture progressively improved with disappearance of vomiting and diarrhea, normalization of blood tests and body growth. At the age of 5 months: weight 6,950 g (50th percentile), head circumference 45 cm (50th percentile), length 61,5 cm (50th percentile). Blood analysis: RBC Count 4,270,000/µL, WBC count 7,900/µL, MCV 85 fl.

When she was 8 years old an attempt to suspend treatment (the parents asked to do it under clinical supervision) for three months was unsuccessful causing the reappearance of megaloblastic anemia. IM hydroxocobalamin needed to be restarted *sine die*. Menarche started at the age of 10 years and half. In the last pediatric examination at the age of 16 years, normal growth and development were observed (weight 50 kg, height 150 cm, body mass index 21.65). Cardiovascular and neurological examinations, including ECG and EEG, were normal. Dual-energy X-ray absorptiometry was within normal limits. Blood tests showed a persistent normalization of the CBC and related indices: RBC 4,570,000/µL, Hb 14.8 g/dL, MCV 88 fL, WBC 6,600/µL, platelets 261,000/µL.

To clearly demonstrate the TC deficiency we analyzed TCN2 to assess possible mutations. At the age of 29 the patient underwent genetic diagnosis. The TCN2 gene (NM_000355.3) was analysed by Sanger sequencing using genomic DNA purified from patient blood. A deletion, designed c.1115_1116delCA

(rs1355421014) was identified in homozygosity in exon 8. At protein level, this variation is predicted to cause the frameshift p.Q373GfsX38 resulting in a truncated protein. The mutation has a frequence of 0.000008 (1/125568, TOPMED database) or 0.0000 (0/2188, ALFA Project) and it is scored as pathogenic in ClinVar.

Patient parents screened for the mutation were found both at the heterozygous status.

At present time in 2020 she is a 30-year old healthy lady taking 1 mg of IM hydroxocobalamin once a week.

Discussion And Conclusion

The majority of genes that could explain variation in vitamin B12 concentrations are reported from Caucasian population studies (14).

TC deficiency is a rare and potentially lethal autosomal recessive disease with an early infantile onset and the following clinical and laboratory features: failure to thrive, weakness, diarrhoea, pallor, anemia, pancytopenia, agammaglobulinemia.

This syndrome may resemble a neonatal leukemia or a severe combined immunodeficiency disease (15, 16). Differential diagnosis with these two disease should be warranted because TC deficiency, when treated aggressively, appears to be associated with an overall favorable outcome.

TC deficiency patients usually present with megaloblastic anemia of variable severity. A delay in diagnosis and treatment are associated with life threatening neurological and hematopoietic complications, which can also be fatal (16, 17).

Patients might show isolated elevation in methylmalonic aciduria (MMA), whereas others show combined increase in circulating MMA and homocysteine (18).

In our patient there was a family history of a similar syndrome that included pancytopenia. The blood concentrations of folate and cobalamin were normal excluding the kinds of deficits where they are low (deficiency of the absorption of B_{12}, malabsorption of folate). Aminoaciduria highlighted the presence of homocysteine and the absence of methylmalonic acid, leading to the exclusion of a deficiency of methylmalonyl-CoA mutase. These findings and the presence of anemia lead us to hypothesize the diagnostic suspicion of TC deficiency initially described in 1971 (19).

Our hypothesis was confirmed genetically only at the age of 29 years of age when we found a c.115_116delCA homozigous mutation in TCN2 gene, further confirmed by the parents'genetic analysis that showed the same mutation in the heterozygous status.

About 50 cases have been reported in the literature of TCN2 variants (20), our mutation c.115_116delCA in exon 8 was cited among these, indeed it was reported in only one patient in which this mutation was

present in association with c.501_503delCCA in exon 4. In silico analysis demonstrate that exon 8 is the region involved in cobalamin binding site, whereas mutation in exon 4 should affect TC-TC receptor interaction (15).

For the first time we report the clinical significance of the c.115_116delCA homozigous mutation alone and not in association with other TCN2 mutations.

The TC deficiency suspicion before genetic screening was indirectly confirmed by the clear response to empiric treatment with IM hydroxycobalamin: disappearance of vomiting, diarrhea and normalization of the hematological picture.

At present there are no guidelines on the treatment of TC deficiency regarding the form of B_{12} supplement to use (i.e. hydroxocobalamin *vs* cyanocobalamin), the dose, the administration method (IM *vs* oral), the frequency (weekly *vs* monthly) and the duration of administration, the treatment monitoring period and follow-up.

IM administration of 1 mg of hydroxocobalamin or cyanocobalamin once a week for a lifetime appears to be the most suitable treatment regime according to the observational data reported in a series of 30 patients (16).

The working hypothesis that such a treatment regime can be correct is confirmed *ex adiuvantibus* in our patient by the observed reappearance of megaloblastic anemia following the attempted suspension of IM hydroxycobalamin at 8 years of age. The timely resumption of treatment (3 mg in the first week, 2 mg in the second week and subsequently 1 mg per week *sine die*) normalized the hematological picture.

In conclusion our case report highlights that early detection of TC deficiency and early initiation of aggressive IM treatment is likely associated with disease control and an overall favorable outcome.

Genetic counseling should be provided to affected families. Neonatal screening could be useful in the early diagnosis of the syndrome: some studies suggest that prenatal diagnosis of TC deficiency is possible by means of measuring TC production in amniotic-fluid cells (21–24).

Patient Perspective

The treatment is easily administered, does not give adverse events and overall is well tolerated and safe.

Declarations

Ethical Approval and Consent to partecipate

The parents and the patient (at the age of 29) signed an informed consent for treatment.

Consent for publication

The patient (at the age of 29) signed an informed consent for the publication of data.

Availability of Data and Materials

The data and materials generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interest

The authors declare no conflict of interest

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Authors' contributions

Diagnosis and treatment: FM; Cinical checks during follow-up: FM, EM, CT, PV. Conceptualization: FM, AM, FB. Writing, Review & Editing: FM, AM, MLT, FB. All authors listed, have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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