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LOW COBALAMIN LEVELS ASSOCIATED WITH SICKLE CELL DISEASE: CONTRASTING ORIGINS AND CLINICAL MEANINGS IN TWO INSTRUCTIVE PATIENTS

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Keywords

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Patient 1 (Table I)

A 24-year old black man with hemoglobin SC was hospitalized in 2003 because of a 5-day history of fever, headache, and diffuse joint pains; a longer history of tiredness; and an unexplained 30-lb weight loss in the past year. He had been minimally symptomatic all his life, other than one presumed crisis with pneumonia at the age of 12 years. In 2002 and 2003, however, he required hospitalization and antibiotics elsewhere twice for presumed crises with pneumonia. He was taking no medications other than daily folic acid and denied drug or alcohol abuse.

Physical examination in 2003 was remarkable only for fever that remitted spontaneously and a soft, apical, holosytolic murmur. No neurological abnormalities were described but he admitted to depression and his behavior was uncooperative and erratic. Chest radiography showed a small pleural effusion. Laboratory findings included a hemoglobin of 8.5 g/dl, MCV 115 fl, reticulocytes 83,200/μl, and white cells 15,200/μl with 64% neutrophils (hemoglobin had been 9.8 g/dl, MCV 106 fl, and white cell count 9500/μl at a brief first visit to our clinic 6 weeks earlier; blood smear was not examined on either occasion). Serum lactate dehydrogenase was 432 U/l. Cultures of blood and urine were negative. Studies done because of his worsened macrocytosis and anemia showed a low serum cobalamin (111 pmol/l) and high red cell folate. Further testing uncovered very high plasma methylmalonic acid (1840 nmol/l), homocysteine (125.9 μmol/l), and gastrin levels (631 ng/l), and a positive serum antibody to intrinsic factor.

The patient's striking metabolic changes confirmed that his low cobalamin level and progressive macrocytic anemia represented clinically relevant cobalamin deficiency. His positive intrinsic factor antibody, supported by a high gastrin level, established pernicious anemia (PA; defined as cobalamin malabsorption resulting from loss of gastric intrinsic

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factor) as its cause. This diagnosis should be considered whenever anemia worsens, MCV rises, or neurologic or mental status changes in a patient with sickle cell disease.

The patient received 100-μg cyanocobalamin injections on two consecutive days and was discharged without folic acid. He was lost to follow-up thereafter, but contact reestablished in 2008 elicited the following information. He had continued his monthly cobalamin injections and restarted folic acid after leaving our hospital. Despite hospitalization elsewhere for one more crisis in 2003 and again in 2004, he regained weight (weight loss is not uncommon in PA),1 was no longer depressed, and soon recovered his premorbid crisis-free existence. On his doctor's advice in 2007, he changed from injections to weekly oral doses of 500 μg cobalamin (the popularity of oral cobalamin treatment has come with reduced precision about doses, schedules, and durations appropriate to the cause of deficiency).2 His mother was told she too was cobalamin-deficient and received monthly cobalamin injections. At his visit to us in 2008, his hemoglobin level was 12.3 g/dl and MCV was 79 fl (iron status was normal), which are typical for uncomplicated SC hemoglobinopathy.3 Neurological examination was normal; he was bright, cheerful, and alert. He was given a cobalamin injection and advised to increase his oral cobalamin doses to 1000 μg daily or restart monthly injections(the popularity of oral cobalamin treatment has increased imprecision among physicians and patients about appropriate doses, schedules, and durations). He was also advised to obtain referral from his physician for endoscopy, because of the risk of gastric cancer and carcinoid tumors associated with PA, and for annual thyroid function testing because thyroid antibody was detected (thyroid function was normal) and the risk of thyroid dysfunction is increased in PA.1,2

This is the fourth documented case of PA occurring with sickle cell disease4–6 (the third seen by one of us, and the second at our hospital in recent years), and it suggests that the two diseases coexist often enough to warrant serious clinical notice. A retrospective survey of cobalamin levels in sickle cell disease claimed another 4 cases of PA7 but did not provide criteria or details. Greater awareness may uncover more cases of PA among patients with sickle cell disorders in the future because PA affects young adults proportionately more frequently among blacks than whites (19 of 100 black patients with PA were <40 years old vs only 5 of 115 white patients; $p<0.01$).8 The frequency of PA in blacks, especially young women, approaches even that in elderly whites,8,9 and there is no reason to think blacks with sickle cell disease are exempt. However, frequencies of PA in blacks with and without sickle cell disease have not been compared prospectively, a task made more difficult nowadays because the Schilling test is no longer available.10

The diagnosis of PA has often been delayed in patients with sickle cell disease, whose anemia is itself often macrocytic or becomes actually megaloblastic after hydroxyurea therapy. Diagnostic delay increases the risk of neurological irreversibility,1 and it may be exacerbated by the nearly universal use of folic acid supplements in sickle cell disease which was predicated on the now increasingly untenable presumption that such patients were not at risk for cobalamin deficiency because PA was rare in blacks and predominated in old age which was infrequently attained in sickle cell disease despite improving life span. 11 Two earlier patients with sickle cell disease and PA had neurological symptoms.4,6 Our patient 1 was depressed, erratic, and uncooperative when deficient but not afterwards, although that could reflect reaction to circumstances. Routine folate supplement use in sickle cell disease merits rethinking, as suggested previously.6,12 Indeed, no benefits were apparent in a clinical trial.13 In any event, folic acid fortification of the American diet14 has probably rendered supplementation superfluous. At the least, periodic screening of cobalamin levels in patients taking folic acid is prudent.

The patient's history of several sickle cell crises in the 9 months preceding his hospitalization and the return to his lifelong, virtually crisis-free state after cobalamin treatment is also noteworthy. This and the even more striking flurry of crises accompanying cobalamin deficiency and subsidence after cobalamin therapy in another such patient6 suggest that worsening of crises should raise suspicions of superimposed PA.

Patient 2 (Table I)

A 32 year-old black man had recurrent hospitalizations over more than a decade for crises, transfusions, and other complications of his hemoglobin SS disease. His past history included frequent otitis media when younger, depression, cholecystectomy, aplastic crisis, and acute chest syndrome that required exchange transfusion. There was no known family history of cobalamin problems. He took folic acid daily and required frequent pain medications. In 1998, hydroxyurea was started but he frequently resisted medical testing and advice.

During an admission for pain crisis in 1999, brief work up for macrocytosis (hemoglobin 5.6 g/dl, MCV 113 fl) revealed serum cobalamin to be low (125 pmol/l) while serum and red cell folate levels were elevated. Physical examination was consistent with pain crisis, and he refused more than cursory neurological examination. Normal methylmalonic acid (179 nmol/l) and homocysteine levels (4.9-5.5 μmol/l) ruled out cobalamin deficiency (Table I). Serum ferritin was elevated (2922 μg/l), probably reflecting his history of many blood transfusions. Antibody to intrinsic factor was absent and serum gastrin was normal, which made PA unlikely. Radioimmunoassay[15-17] showed his plasma total transcobalamin I (TC I; also called haptocorrin) concentration to be 68 pmol/l (normal = 165-454 pmol/l); salivary TC I content was normal. These findings are typical for mild TC I deficiency.17 Two injections of 1000 μg of cyanocobalamin were given before TC I assays were completed and he was discharged, taking folic acid, hydroxyurea, and pain medications. The patient refused further cobalamin injections and added oral multivitamins containing cobalamin to his folic acid regimen.

TC I is a cobalamin-binding glycoprotein of uncertain function found in plasma and secretions, such as saliva and breast milk.18 Because it does not undergo specific uptake by cells and turns over slowly, TC I carries >70% of circulating cobalamin at any time.19 TC I deficiency therefore causes low plasma cobalamin levels without impairing cellular cobalamin status, which depends on the smaller, rapidly cleared TC II-cobalamin pool.18 The asymptomatic nature explains why TC I deficiency typically escapes diagnosis. Its low cobalamin level allows it to be mistaken for cobalamin deficiency and treated with cobalamin without benefit,[15,17,20-22] sometimes for years.

The underdiagnosis of TC I deficiency is further abetted by the limited availability of reliable assays of total TC I. Such an immunoassay showed that patient 2 had the diminished levels of total TC I of mild TC I deficiency rather than the undetectable TC I levels of the rarer, severe form of TC I deficiency.[17,20,22,23]

Monitoring later that year showed occasional improved cobalamin levels (297-370 pmol/l) as a result of the cobalamin injections but no signs of clinical, psychological, or laboratory improvement. His hydroxyurea-induced macrocytosis and hypersegmented neutrophils persisted despite cobalamin injections. Plasma TC I remained low (59-131 pmol/l). The genetic nature of his TC I deficiency was later confirmed by DNA sequencing, which uncovered heterozygosity for a nonsense mutation (315C>T) of the *TCN1* gene that is associated with TC I deficiency (he was included in that genetic study of 3 families).24

Hydroxyurea was discontinued because of apparent ineffectiveness in 2001; transfusion use, which had diminished somewhat after 2000, returned in 2004-2005 to its 1997-1999 levels. By 2006, he had chronic hepatic dysfunction, probably primarily from iron overload caused by his heavy transfusion history (serum ferritin >8000 μg/l). Pulmonary hypertension was noted in 2008. He takes folic acid and multivitamins and has had no cobalamin injections since 1999 but continues resisting most medical advice. In 2009, off hydroxyurea since 2001, his hemoglobin level was 5.2 g/dl and MCV was 93 fl.

Although blacks have significantly higher cobalamin levels and TC I levels in general than whites,16،25-27 it is striking and perhaps paradoxical that all 8 patients with severe TC I deficiency reported to date, except one Asian Indian,28 have had at least partial African ancestry.15،17،20،22،23 Moreover, patients with sickle cell disease tend to have lower or more often subnormal cobalamin levels;7،29،30 the causes are often unexplained. Most interestingly and relevantly in that context, TC I deficiency coexists remarkably frequently with hemoglobin S: 4 of 7 (57%) tested patients with severe TC I deficiency have had sickle cell trait (Table II; and a fifth28 had ß-thalassemia minor), whereas the prevalence of sickle cell trait is approximately 8% among American blacks and that of sickle cell anemia is < 0.2%.3 Patient 2 extends the association, although his TC I deficiency is mild and he has sickle cell disease, not the trait. Both the TC I and ß-globin genes are located on chromosome 11, but linkage dysequilibrium is unlikely because their locations are far apart (11q12-13 and 11p15, respectively) and their mutation dosages do not match in many cases (Table II). Pending clarification of TC I function and organization of large prospective surveys applying appropriate assays17 and genetic tools,24،31 the association between TC I deficiency and sickle cell disease may turn out to be indirect, perhaps involving an unidentified selection pressure.

Distinguishing TC I deficiency from cobalamin deficiency

As long as TC I deficiency was considered a rare curiosity, diagnosing it was not a high medical priority. However, its mimicry of cobalamin deficiency may have substantive medical relevance if TC I deficiency is relatively common. Diverse findings have combined to suggest that is likely, especially in heterozygotes with mild TC deficiency. Whereas severe TC I deficiency, thus far associated with mutation in both TCN1 alleles,24 is indeed rare,17 mild hereditary TC I deficiency, thus far associated with the heterozygous state,31 is likely to be more common simply because heterozygous states always outnumber homozygous (or compound heterozygous) ones. A prospective immunoassay survey found mildly low TC I levels in 15% of all cases with unexplained low cobalamin levels, most of them in whites, whereas severe TC I deficiency accounted for only 0.6%, most of them in blacks.17 The 15% rate of TC I deficiency is compatible with the observation that 21% (Pfeffer CM, personal communication) to 22%32 of all low cobalamin levels lack the metabolic indicators of cobalamin deficiency (such as methylmalonic acid and homocysteine abnormality), suggesting that many low cobalamin levels have nothing to do with cobalamin deficiency.

TC I deficiency should be considered whenever low cobalamin levels are not accompanied by clinical or metabolic signs of cobalamin deficiency. Reliable testing involves assay of total TC I, but must use rapidly separated plasma; serum, with its artifactual release of TC I from granulocytes in vitro,33 must be avoided. However, because TC I levels can be borderline in some patients with mild TC I deficiency,17 genotyping for *TCN1* mutations may prove useful.24،31 As mutations are being identified, they seem thus far to differ between nonwhites and whites.24،31 Prospective genetic surveys can address the frequency of TC I deficiency, how often it is hereditary, and its precise association with sickle cell and other hemoglobinopathies.

CLOSING COMMENTS

Despite similarly low cobalamin levels (Table I), the cobalamin-related conditions in the two cases have distinctively different origins, clinical impacts, prognoses, and management strategies. The clinical relevance of distinguishing the low cobalamin levels of PA from those of TC I deficiency extends beyond sickle cell disease but evidence of a special resonance for sickle cell disease is accumulating. Patient 1 is the fourth reported case of PA complicating sickle cell disease, suggesting the combination is not rare. Recognition of superimposed PA and other irreversible causes of cobalamin malabsorption has special urgency in the face of the presently routine folate supplementation in sickle cell disease in the era of folic acid fortification.

Cobalamin levels are also often low for reasons other than PA, however, and many may not require cobalamin treatment. The task of distinguishing them can be difficult, but it must be pursued in order to avoid unnecessary cobalamin treatment in many cases while assuring that the few who need cobalamin urgently receive proper treatment. TC I deficiency, which might be disproportionately associated with sickle cell trait and disease (Table II), is one such cause of a low cobalamin level mimicking that of PA but not requiring cobalamin treatment.

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TABLE I

Comparison of the relevant clinical and laboratory findings in the two patients with sickle cell disease and a subnormal serum cobalamin level.

	PATIENT 1	PATIENT 2
Cobalamin-related diagnosis	Pernicious anemia	Mild (heterozygous) transcobalamin I deficiency
Age at diagnosis (years)	24	32
Serum cobalamin (pmol/l and, in parnetheses, ng/l)	111 (150)	125 (170)
Hemoglobin (gm/dl)	8.5	5.6[a]
MCV (fl)	115	113 [a]
Methylmalonic acid (nmol/l)[b]	1840	179
Homocysteine (μmol/l)[c]	125.9	4.9
Neurologic abnormalities	None	None obvious (patient refused full examination)
Mental status	Depression Uncooperativeness; erratic behavior	Depression Uncooperativeness
Clinical change after cobalamin therapy	Hematologic and mental changes and frequency of sickle cell crises improved	None

[a] The patient was taking hydroxyurea, which causes macrocytosis and megaloblastic changes, but also received frequent transfusions, which tend to blunt macrocytosis.

[b] Normal, <271 nm ol/l.

[c] Normal, <14 μmol/l.

TABLE II

Association of severe transcobalamin I (TC I) deficiency with sickle cell and other ß-globin hemoglobinopathies, along with the present patient 2, who had mild TC I deficiency.

Patient reference	Hgb type	Ethnicity	TC I Deficiency	Serum cobalamin (pmol/l)[a]	Plasma total TC I (pmol/l)[b]	Age at diagnosis (yrs)
Ref 17,20 Propositus 1 (1969)	AS[c]	Mixed[d]	Severe	0	Undetectable	47
Ref 17,20 Propositus 2 (1969)	AA[c]	Mixed[d]	Severe	39	Undetectable	46
Ref 22 (1982)	AS	African-American	Severe	<37	Undetectable	77
Ref 15 (1983)	AS	African-American	Severe	145	Undetectable	64
Ref 28 (1983)	ß-thal. minor	Asian Indian	Severe	52[e]	Undetectable	34
Ref 23 (1988)	AA	North African Arab	Severe	37-41[f]	Undetectable	14
Ref 17 (2003)	AS	Mixed[g]	Severe	65	Undetectable	57
Present report; patient 2	***SS***	***African-American***	***Mild***	***125***	***59-131***	***32***

Abbreviations: Hgb, hemoglobin; TC I, transcobalamin I; thal, thalassemia.

[a] Assay methods differed among the original reports; <150 pmol/l was generally considered low.

[b] Reference interval: 165-454 pmol/l (plasma)

[c] Whereas all other patients in the Table were unrelated to each other, both these affected brothers from one family are shown: one had Hgb AS and the other had Hgb AA but also had a suspected α-thalassemia. Propositus 1 also had undergone partial gastrectomy. Propositus 2 had an unidentified neurological syndrome unrelated to TC I deficiency.

[d] The brothers were Corsican-Puerto Rican, with partial Caribbean Indian,and African roots.

[e] The patient was lactovegetarian, in addition to her TCI deficiency.

[f] The patient had hereditary intrinsic factor deficiency in addition to TC I deficiency.

[g] The patient was Puerto Rican, with partial Caribbean Indian and African ancestry.