3 Megaloblastic Anemia and Dual Nutritional Deficiency Anemia

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3.1 Megaloblastic Anemia

3.1.1 Definition

Megaloblastic anemia is a general term used to describe a group of anemias caused by impaired DNA synthesis and characterized by macro-ovalocytes in peripheral blood smear and megaloblastic erythroid hyperplasia in the bone marrow.

3.1.2 Pathophysiology of Megaloblast Morphology

Megaloblasts, the hallmark of these anemias, are caused by asynchronous maturation between the nucleus and the cytoplasm due to impairment of DNA synthesis. When DNA synthesis is impaired, the cell cannot proceed from the G2 growth stage to the mitosis (M) stage. Hence, the DNA synthesis is retarded, whereas the RNA and protein synthesis of the cell occurs at a normal pace. This leads to continuing cell growth without division, resulting in a mature cytoplasm with an immature nucleus.

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3.1.3 History

1849—Addison described this condition as anemia, general languor, and debility

1877—Osler and Gardner found its association with neuropathy

1880—Ehrlich first described megaloblasts

1926—Minot and Murphy identified that this condition could be reversed by ingestion of large quantities of liver

1929—Castle established that gastric acid contains an "intrinsic factor" (IF) that combines with an "extrinsic factor" to allow the latter to be absorbed

1934—Dorothy Hodgkin described the structure of vitamin B_{12} for which she received the Nobel Prize

1948—Herbert discovered the structure of folic acid and its association with megaloblastic anemia

3.2 Folic Acid Metabolism

The synthetic form of folate is more commonly known as folic acid. The metabolically active form of folic acid is known as tetrahydrofolic acid. The main dietary sources include green leafy vegetables, lemons, oranges, banana, cereals, fish, liver, kidneys, etc. Folate in food can be lost on prolonged storage or overcooking. The daily requirement varies from 50 to 100 μg; however,

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recommended daily allowances are kept at a higher level of around 400 μg. This is because the bioavailability depends on the conversion of the polyglutamated form in the diet into monoglutamated form for absorption [1]. The body stores are found in the liver. They range from 3 to 5 mg and last for about 3–5 months [2].

3.2.1 Folate Absorption

Folate is absorbed in the small intestine, primarily the duodenum and the upper part of jejunum. Natural folates are absorbed faster than folic acid. The polyglutamated folates are converted to the monoglutamated form by the enzymes carboxypeptidase or polyglutamate hydrolase (also known as intestinal conjugase). The monoglutamated form is then absorbed either passively along the concentration gradient or actively by binding to the transporters RFT-1, RFT-2, and folate-binding protein (FBP). Once within the enterocyte, they are further converted to dihydro- and tetrahydro-folate by a reductase enzyme and further converted to methyltetrahydrofolate which then passes into the systemic circulation freely or bound to albumin. This form (5-MTHF) then enters the target cell membrane through the reduced folate carrier and is demethylated (Fig. 3.1).

Physiological function of THF: The most important role of THF is in the synthesis of thymidylate (a pyrimidine base of DNA). In the absence of thymidylate, uracil is incorporated which results in deranged DNA function [4]. Folic acid deficiency in pregnant women may cause neural tube defects in the fetus, underlining its role in neural development of fetus.

3.2.2 Causes of Folic Acid Deficiency [5]

Deficiency of folic acid is usually due to low folate in food or imbalance between demand and intake.

Causes of Reduced Availability

- Dietary deficiency can occur in generalized malnutrition states, elderly and in association with alcohol intake.
- Impaired absorption usually occurs in primary gastrointestinal conditions such as tropical sprue, celiac disease, Crohn's disease, extensive small bowel resection, infiltrative disorders, Whipple's disease, scleroderma, amyloidosis, and diabetes mellitus.
- Genetic defects of enzymes involved in their uptake such as intestinal conjugase deficiency, resulting in malabsorption, dihydrofolate reductase, methylenetetrahydrofolate, and glutamate formiminotransferase deficiencies.
- Drugs can also reduce absorption by several mechanisms:
  (a) Trimethoprim, pyrimethamine, methotrexate—inhibit dihydrofolate reductase
  (b) Phenytoin and valproic acid—reduce absorption and also affect its metabolism

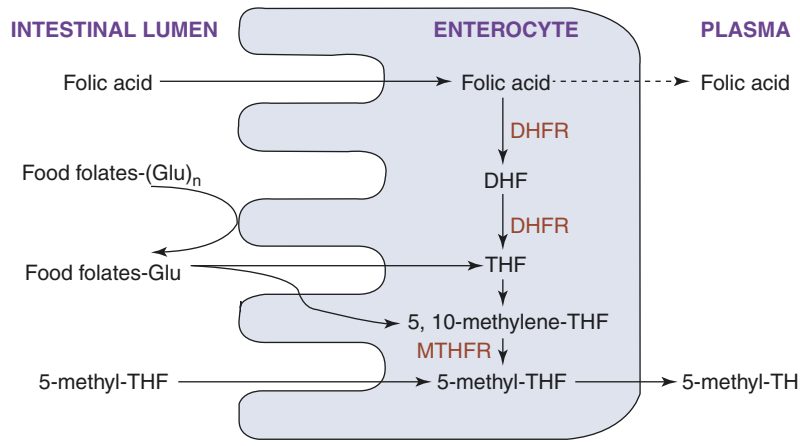

Fig. 3.1 Absorption of folate in the intestine [3]

Causes of Increased Demand

- Increased requirements can occur in physiological and pathological states such as pregnancy, breastfeeding, chronic hemolytic anemia, and chronic exfoliative dermatitis.

Chronic hemolytic anemias cause rapid depletion of the folate reserves due to the associated erythroid hyperplasia. This can further result in megaloblastic crisis in the presence of exacerbating factors such as fever and illness, which can impede oral intake. Hence, prophylactic folic acid should be given to patients with hemolytic anemias such as hereditary spherocytosis and sickle cell anemia.

3.3 Vitamin B_{12} Metabolism

The chemical name of vitamin B_{12} is cyanocobalamin. There are also other forms depending upon the radical to which they are bound such as adenosylcobalamin and methylcobalamin. The source of vitamin B_{12} includes animal foods such as fish, liver, and dairy products. Plant foods do not contain vitamin B_{12}. The RDA of vitamin B_{12} is around 2 μg though requirements may increase in pregnant and breastfeeding women. Similar to folic acid, vitamin B_{12} is stored in the liver, the storage lasting for about 3–5 months.

3.3.1 Absorption of Vitamin B_{12}

When vitamin B_{12} is ingested in diet, it is normally found bound to proteins. These proteins are degraded by the enzyme pepsin released by the parietal cells of the stomach. The free vitamin B_{12} binds to R-binder (also known as haptocorrin or transcobalamin I [TC-I]) which protects the molecule and carries it till the second part of duodenum. The R-binder is secreted by the salivary glands of the oropharynx and is also found in neutrophils and monocytes. Here, the proteases in the pancreatic juice (trypsin, chymotrypsin, elastase) degrade TC-I and release vitamin B_{12} from the complex [6].

The free vitamin B_{12} now binds to IF which is also released from the parietal cells in the fundus and cardia of the stomach. The IF-B_{12} complex finally reaches the ileum. The ileal enterocytes possess the IF receptor (also known as cubilin-amnionless complex). The complex binds to this receptor and is internalized. Once within the enterocyte, the IF is degraded and cobalamin released. It then binds to transcobalamin II (TC-II) which carries vitamin B_{12} in the systemic circulation. The target cells take up this bound cobalamin by the virtue of TC-II receptors on their surface [6] (Fig. 3.2).

Physiological function of vitamin B_{12}: It acts as a coenzyme in the synthesis of methionine and tetrahydrofolate from methyltetrahydrofolate and homocysteine. This process is known as "folate trapping" since folate needs to be demethylated to remain in the cell, failing which they escape the cell without being used. This demethylation is brought about by the above reaction. Thus, it is hypothesized that manifestations of cobalamin deficiency is primarily due to folate deficiency. Cobalamin also acts as a coenzyme in the conversion of methylmalonic acid (MMA) to succinic acid, the latter being an important substrate in the Krebs cycle [6].

3.3.2 Causes of Cobalamin Deficiency [5, 8]

Vitamin B_{12} deficiency is most commonly due to impaired absorption rather than due to reduced uptake. Reduced uptake occurs mostly in strict vegetarians, due to dietary deficiency. Impaired absorption can be caused by the following disorders:

- Gastric disorders—pernicious anemia, gastritis, old age, atrophic gastritis, use of proton pump inhibitors or H2 antagonists, *Helicobacter pylori* infection, total or partial gastrectomy, Zollinger–Ellison syndrome
- Intestinal disorders—extensive ileal resection, ileitis, leukemic/lymphomatous infiltration, tuberculosis, Crohn's disease, postradiation ileitis, tropical sprue or celiac disease, scleroderma, amyloidosis, blind loop syndrome with bacterial overgrowth, parasitic infections such as *Diphyllobothrium latum*, *Giardia lamblia*, and *Strongyloides stercoralis*.

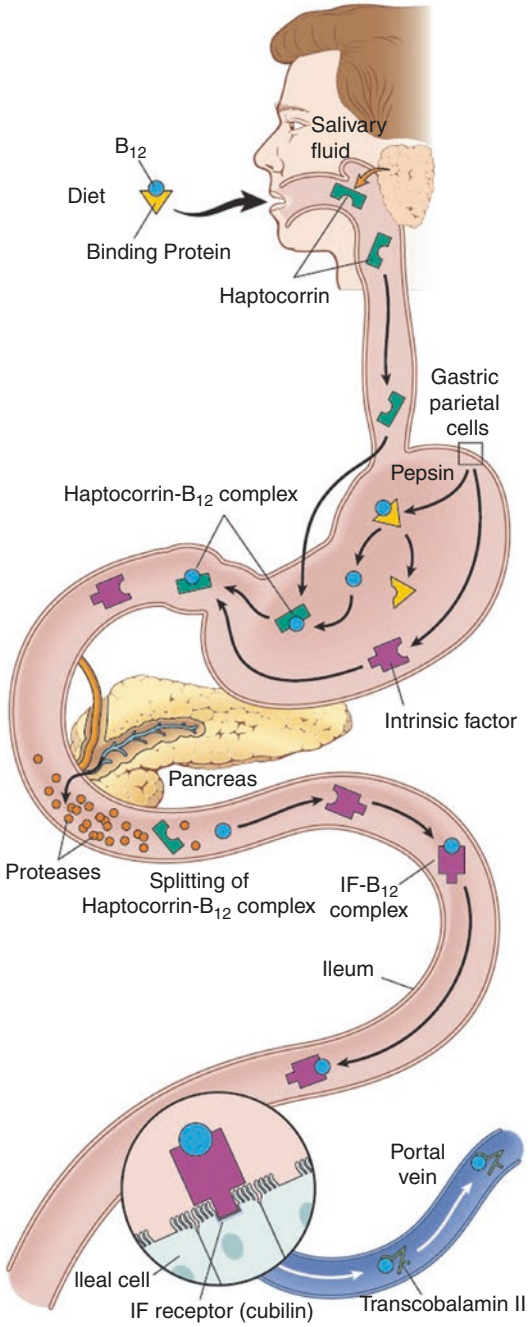

Fig. 3.2 Schematic illustration of vitamin B_{12} absorption [7]

- Pancreatic disorders—hereditary chronic pancreatitis, Imerslund–Gräsbeck disease (inherited cubilin deficiency)
- Genetic defects—TC-II deficiency, functional impairment of TC-II, haptocorrin or TC-I deficiency, homocystinuria, methylmalonicacidemia, methylenetetrahydrofolate deficiency collectively known as congenital megaloblastic anemias
- Drugs—cholestyramine, metformin, colchicine

3.4 Pernicious Anemia

Pernicious anemia is caused due to impaired IF secretion by the gastric parietal cells, resulting in impaired vitamin B_{12} absorption. It can be inherited or acquired. The inherited form is a rare autosomal recessive disorder causing deficient secretion of IF in the absence of gastric atrophy and is seen in children below 2 years of age. The acquired form is more common and occurs in adults. It is an autoimmune disorder with genetic predisposition and is associated with HLA A2, A3, B7, patients with type A blood group and other autoimmune disorders. Patients with pernicious anemia have a higher risk of irreversible gastric atrophy, achlorhydria with concomitant iron deficiency, and gastric carcinoma.

The antibodies in pernicious anemia are of two types: anti-parietal cell and anti-IF antibodies. Anti-parietal cell antibodies are the most common and present in 90% of patients with pernicious anemia but in only 5% of healthy adults. Similarly, antibodies to IF are also found in most patients with pernicious anemia. IF antibodies, type 1 and type 2, are present in 50% of patients with pernicious anemia and are highly specific. Therefore, they can be used as a confirmatory test. Treatment requires parenteral administration of vitamin B_{12} to circumvent the impaired absorption of oral vitamin B_{12}.

3.5 Congenital Megaloblastic Anemia

Congenital megaloblastic anemia is due to deficiency or altered activity of the enzymes, carrier proteins, or receptors involved in the metabolism (absorption, transport, and conversion to active form) of vitamin B_{12} or folate, resulting in impaired utilization of these nutrients. This group

has been explored in detail in the context of vitamin B_{12} deficiency. These disorders need to be suspected when clinical manifestations of cobalamin deficiency are observed in infancy or childhood. Three disorders where absorption and transport of cobalamin are affected have been identified, and another seven alter cellular use and coenzyme production. The disorders of absorption and transport are TC-II deficiency, IF deficiency (also known as inherited pernicious anemia), and IF receptor deficiency (Imerslund–Gräsbeck syndrome). These defects cause developmental delay and megaloblastic anemia since birth, which can be treated with pharmacologic doses of parenteral cobalamin. Serum cobalamin values are reduced in the IF defects and normal in TC-II deficiency.

The defects of cellular use, commonly denoted by letters CblA to G, can be detected by the presence or absence of methylmalonic aciduria and homocystinuria. The presence of only methylmalonic aciduria indicates a block in conversion of methylmalonic CoA to succinyl-CoA and results due to defect in the methylmalonyl-CoA mutase enzyme that catalyzes the reaction or a defect in the synthesis of its cobalamin coenzyme (cobalamin A and cobalamin B deficiency).

The presence of only homocystinuria can result either from poor binding of cobalamin to methionine synthase due to cobalamin E deficiency or defect in producing methylcobalamin from cobalamin and *S*-adenosylmethionine due to cobalamin G deficiency. This results in a reduction in methionine synthesis, with accumulation of the enzyme substrates causing homocystinemia and consequent homocystinuria.

Methylmalonic aciduria and homocystinuria occur when the metabolic defect impairs conversion of cobalamin III to cobalamin II (cobalamin C, cobalamin D, and cobalamin F deficiency). This reaction is common for the formation of both MMA and homocystinuria. Early detection of these disorders is important because most patients respond favorably to supra-pharmacological doses of cobalamin, thereby preventing the neurological abnormalities associated with them.

3.6 Imerslund–Gräsbeck Syndrome

This is an autosomal recessive disorder caused due to biallelic mutations in the IF receptor also known as cubilin-amnionless receptor. This receptor is essential not only for intestinal vitamin B_{12} absorption but also for renal reabsorption of proteins. The deficiency of this receptor (either cubilin or amnionless) therefore results in selective vitamin B_{12} malabsorption and proteinuria. The diagnosis of this disorder is suspected by a decreased excretion of cobalamin-IF complex in urine and is confirmed by mutation study of the involved genes. The treatment involves lifelong administration of parenteral vitamin B_{12}.

3.7 Pathophysiology of Megaloblastic Anemia

The pathophysiology of megaloblastic anemia is due to ineffective erythropoiesis resulting from intramedullary apoptosis of hematopoietic precursors (rapidly dividing cells). This apoptosis is not solely due to deficiency of thymidylate. In the absence of thymidylate, it is replaced by uracil which activates the DNA repair pathways resulting in p53-mediated apoptosis. To some extent, RNA and protein synthesis is also affected. This results in asynchronous maturation of the nucleus and cytoplasm. The nucleus devoid of DNA does not mature, whereas the cytoplasm matures at a normal rate due to normal RNA and hemoglobin synthesis, thus resulting in megaloblastosis [5]. It is also important to keep in mind that DNA synthesis can also be impaired directly in HIV and myelodysplastic syndromes in the absence of vitamin B_{12} and folate deficiency.

3.8 Pathophysiology of Neurological Changes

Neurological symptoms are confined to cobalamin deficiency. Two factors contribute to these symptoms. The first factor is the impaired synthesis of the amino acid methionine which is

essential for myelin synthesis. The second factor is the accumulation of MMA, a neurotoxic intermediate. These factors together cause degeneration of myelin fibers of the posterior and lateral grey horn of the spinal cord, resulting in subacute combined degeneration [5]. Increased levels of cytokines such as TGF-α and EGF have also been identified in these patients, which may also contribute to the pathogenesis of neurodegeneration [9].

3.9 Clinical Features

When patients present with the symptoms of chronic anemia, there may be mild jaundice due to the associated intramedullary hemolysis. The combination of pallor and jaundice gives the skin a lemon yellow color, characteristic of megaloblastic anemia. There may also be glossitis (beefy tongue), nail pigmentation, change of hair color (due to increased melanin synthesis), etc. The neurological symptoms of cobalamin deficiency include loss of joint position sense, loss of vibration sense in toes and fingers, paresthesia, hypoesthesia, tingling, gait abnormalities, loss of coordination, muscle weakness, spasticity, optic neuropathy, urinary and fecal incontinence, erectile dysfunction, dementia, and memory loss. Neuropathy is symmetric and mainly affects the lower extremities (stock and glove distribution). Clinically, patients with subacute combined degeneration have a positive Romberg's test. Patients with pernicious anemia have symptoms of other associated autoimmune disorders such as type 1 diabetes, thyroid disorders, and Addison's disease.

3.10 Laboratory Findings

3.10.1 Hemogram and Peripheral Smear

The characteristic peripheral smear finding is macrocytic anemia (increased mean corpuscular volume) with macro-ovalocytes and reticulocytopenia. There may also be leukopenia and thrombocytopenia in severe cases. Hypersegmented neutrophils are another characteristic finding in megaloblastic anemia. Hypersegmented neutrophils refer to the presence of >5% of neutrophils with five segments, or >1% with six segments. Macrocytosis is defined as increased mean corpuscular volume, which may be masked in patients with iron deficiency. However, hypersegmented neutrophils persist in iron deficiency and aid in the diagnosis of megaloblastic anemia. Other findings include anisocytosis, basophilic stippling, Howell–Jolly bodies, Cabot rings (remnants of mitotic spindle), etc. Increased bilirubin and LDH reflect ongoing intramedullary hemolysis.

3.10.2 Bone Marrow Examination

Bone marrow aspiration is usually done to rule out myelodysplastic syndromes and to assess iron stores. There is panmyelosis with megaloblastic changes in the erythroid and myeloid series. There is erythroid hyperplasia with increase in orthochromatic erythroid precursors showing megaloblastic change. The cells are enlarged with immature nucleus and a sieve like chromatin, whereas the cytoplasm is mature and adequately hemoglobinized (Fig. 3.3). In the myeloid series, there are giant metamyelocytes and band forms. A less common finding is the presence of hyperlobated and hyperdiploid megakaryocytes and giant platelets. Lymphocytes and plasma cells are spared from the cellular gigantism and cytoplasmic asynchrony observed in other cell lineages. Bone marrow megaloblastosis is reversed within 24 h of vitamin B_{12}/folate therapy and returns to normal in 2–3 days [5, 10].

3.11 Primary Tests for B_{12} and Folate Deficiencies [10]

3.11.1 Serum B_{12} (Cobalamin)

Reference range: 200–900 pg/mL

Anemia and neuropathy can be seen at levels of <180 mg/L; however, levels <150 mg/L are diagnostic of B_{12} deficiency. The serum cobalamin

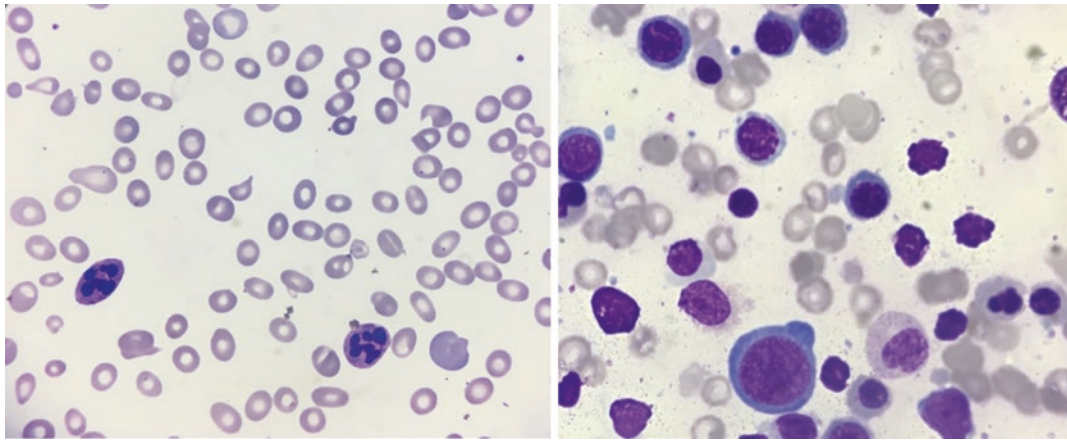

Fig. 3.3 Peripheral smear showing macro-ovalocytes and hypersegmented neutrophils and occasional polychromatophils (left) and bone marrow aspirate smear showing megaloblasts with erythroid hyperplasia (right)

level can however be within normal range in the following circumstances:

- TC-II deficiency
- Acute cobalamin deficiency due to nitrous oxide (prevalent in older times)

Serum cobalamin levels may be physiologically low in pregnancy, intake of oral contraceptives, severe folic acid deficiency, and patients taking large doses of ascorbic acid.

3.11.2 Serum Folate

Reference range: 2.5–20 ng/mL

A diagnosis of folate deficiency can be made when the folate levels are less than 2.5 ng/mL. However, in some cases of folate deficiency, there can be an overlap with the normal range (2.5–5 ng/mL).

The following precautions need to be considered when assessing serum folate values:

- A single dose of folate either by medication or meal may falsely elevate serum folate levels to normal. Hence, blood sample should be drawn prior to any intervention (transfusions, meals, and therapy) to achieve accurate results.
- Hemolysis can cause false results due to release of red cell folate.

3.11.3 RBC Folate

Reference range: >140 ng/mL

- Not affected by diet and reflects tissue stores since folate content is established early in RBCs.
- Red cell folate levels may be affected by hemolysis.
- Low in severe B_{12} deficiency.
- Test is complex and expensive.

Serum for folate and cobalamin should be collected prior to meals or therapy, frozen and then stored if the tests cannot be performed immediately.

3.12 Lab Tests to Confirm and Distinguish Vitamin B_{12} and Folate Deficiencies

Serum homocysteine and MMA levels together are helpful to distinguish between cobalamin and folate deficiencies. Both are increased in cobalamine deficiency. Homocysteine is increased in folate deficiency; however, MMA is normal in these cases. These should be used if the clinical presentation and serum vitamin B_{12} and folate levels do not correlate. However, homocysteine and vitamin B_{12} levels may be altered in

conditions other than megaloblastic anemia such as the following:

The MMA level can be elevated in

- End-stage renal disease
- Inborn error of MMA metabolism

Serum homocysteine can be increased in

- Homocystinuria
- Hyperhomocysteinemia
- Certain MTHFR polymorphisms

3.13 Testing for Parietal Cell/IF Antibodies

Antibodies against both IF and gastric parietal cells can be demonstrated in patients with pernicious anemia. The former is seen in 50% of cases and is specific. The latter though seen in 90% of patients is not specific as it can be positive in other autoimmune and thyroid disorders.

3.14 Diagnostic Therapeutic Trial

In under-resourced laboratories where serum studies are unavailable, a therapeutic trial of vitamin B_{12}/folate can be given and the reticulocyte response can be assessed after 72 h. However, a trial of folate therapy alone is contraindicated since this may exacerbate neurological symptoms in patients with coexisting cobalamin deficiency. Therapeutic trial may also be useful in elderly patients with subclinical deficiency in whom laboratory tests are within normal limits.

3.15 Treatment

3.15.1 Folic Acid Deficiency

Folic acid therapy in a patient with coexisting cobalamin deficiency may alleviate symptoms of anemia; however, neurological symptoms worsen. Hence, they need to be given together. Folic acid is given at a dosage of 1–5 mg/day for 3–4 months along with cyanocobalamin. Treatment should be ideally continued till blood counts normalize. Treat underlying cause if any. If no secondary cause is identified, continue therapy indefinitely. Folate should be administered prophylactically when there is increased physiological demand such as during pregnancy, lactation, and in the perinatal period during breastfeeding. Folate is also indicated in patients with chronic hemolytic anemias, psoriasis, and exfoliative dermatitis and during extensive renal dialysis. Folate therapy has been recommended in patients with hyperhomocysteinemia, who are at risk for thromboembolic complications [11].

3.15.2 Cobalamin Deficiency

Cyanocobalamin is given at a dosage of 1000 μg intramuscularly daily for 2 weeks, followed by weekly once until blood count normalizes, then continued monthly once for life. Prophylaxis for life is especially important in strict vegetarians, elderly, and patients with prior gastric surgery. Oral route is usually preferred in patients with contraindications to intramuscular injections such as hemophilias. Patients with genetic causes such as TC-II deficiency may require higher doses.

3.16 Monitoring of Therapy

- 1–2 days: Reduction of serum iron, indirect bilirubin, and lactate dehydrogenase
- 3–4 days: Reticulocytosis
- 10 days: Hemoglobin starts to increase, reduction of MCV
- 14 days: Hypersegmented neutrophils start to disappear though they may persist for longer
- 2 months: Resolution of anemia
- 3–12 months: Resolution of neurological symptoms

3.17 Dual Nutritional Deficiency Anemia

Megaloblastic anemia may sometimes be masked by coexistent iron deficiency. Thus, the macrocytic anemia may be masked by the microcytic

hypochromic anemia of iron deficiency. This clinical picture is known as dimorphic anemia or dual nutritional deficiency anemia. According to NHANES III data, dual nutritional deficiency anemia constitutes for about 10% of nutritional anemias [12]. This is commonly seen in elderly patients, pregnancy, extensive bowel resection, pernicious anemia, malnutrition, etc. Iron deficiency may also be precipitated by the treatment of megaloblastic anemia (associated with accelerated erythropoiesis). Combined deficiency may also be suspected in patients who do not show appropriate response to iron/vitamin B_{12}/folate monotherapy. Peripheral smear characteristically shows two distinct red cell populations. There is one population of hypochromic, microcytic cells and another of macrocytic cells with reticulocytopenia. There may also be hypochromic macro-ovalocytes. The MCV may be normal though the RDW is increased. In dimorphic anemia, all three types of erythropoiesis can usually be detected—hypochromic, megaloblastic, and normoblastic—the latter, however, usually predominating. Variability in red blood cell morphologic characteristics in this setting reflects the relative degree of deficiency of each of these substrates. The diagnosis relies upon a high degree of suspicion along with proper clinical examination and specific investigations. A bone marrow examination may be done to assess iron stores. Serum ferritin levels may not truly reflect the degree of iron deficiency in patients with coexisting megaloblastic anemia. Treatment is by administration of iron and vitamin B_{12}/folate either orally or parenterally depending on the cause of deficiency. Clinical course may be monitored by the reticulocyte response.

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