Spotlight on hematology

Megaloblastic anemia
Vitamin B-12/folic acid deficiency

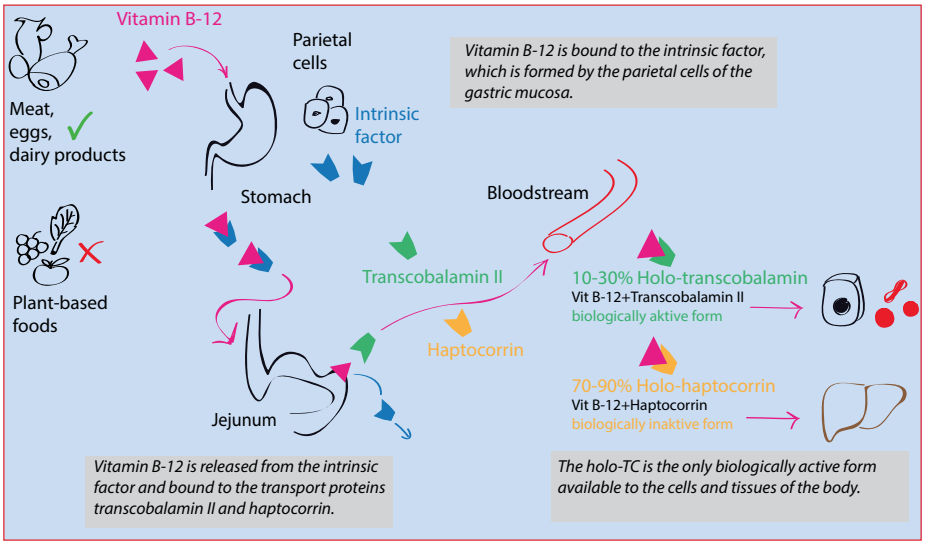
MQZH 2015-01

Introduction

Megaloblastic anemia is caused by vitamin B-12 or folic acid deficiency. Both substances are essential for the body to synthesize DNA. The absence of the two substances affects hematopoiesis; lack of vitamin B-12 also affects the nervous system. In the bone marrow, ineffective cell formation with premature cell death develops. The blood picture shows macrocytic anemia. Frequently pancytopenia is present (reduced numbers of all three cell lineages) with morphological abnormalities.
The suspicion of vitamin B-12/folic acid deficiency can be raised based on the peripheral blood picture findings. The definitive diagnosis is carried out by additional clinical chemistry laboratory analyzes (vitamin B-12 levels, holotranscobalamin, possibly methylmalonic acid and/or folic acid) and a therapeutic response to vitamin B-12/folic acid supplementation.
Our survey specimen 2015-1 H3B is derived from a 58-year-old patient with pernicious anemia. The cause of vitamin B-12 deficiency is a lack of the intrinsic factor.

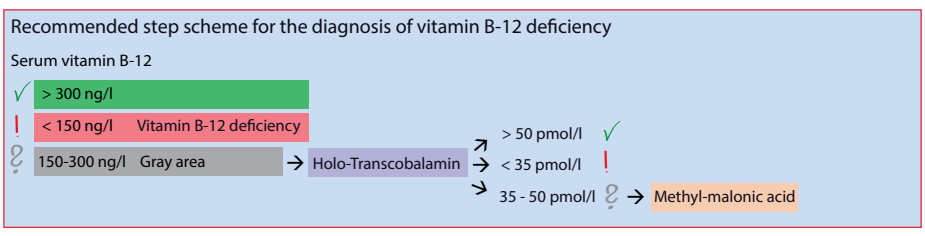
Vitamin B-12 (cobalamin) - Biochemistry and biological function

Vitamin B-12 is synthesized by bacteria and is found in meat, dairy products and eggs, but not in purely plant-based foods. Vitamin B-12 has important functions in the formation of blood and myelin nerve cell sheaths.
Vitamin B-12 can be readily absorbed in the small intestines when it is previously coupled to the intrinsic factor produced by parietal cells of the gastric mucosa. This bond is disrupted following resorption and vitamin B-12 is bound to transport proteins for circulation in the bloodstream.
By binding to transcobalamin II, holotranscobalamin, the biologically active form of vitamin B-12, is formed. Only this form can be delivered to the body's cells and tissues which express corresponding receptors. However, 70-90% is bound to haptocorrin and represents the biologically inactive form, holo-haptocorrin that is delivered to the liver only.
Due to the biological functions of vitamin B-12 in the cell, in cases of deficiency the incorporation of folic acid during erythropoiesis is disturbed, among other events, and the levels of homocysteine and methylmalonic acid increase.



Laboratory diagnosis of vitamin B-12 deficiency

Hematologic changes may provide an indication of megaloblastic anemia, whereby the earliest recognizable character is over-segmentation of neutrophilic granulocytes. This occurs at an earlier time point than macrocytic anemia.
The definitive diagnosis is made by determining clinical chemical parameters.
With the classic vitamin B-12 serum level analysis around 60% of subjects fall into a so-called gray zone. The determination of the holotranscobalamin that is possible today (biologically active part of the vitamin B-12) has significantly improved specificity and sensitivity so that further diagnostics is required in only about 20% of subjects examined.



Effects of vitamin B-12 deficiency

Vitamin B-12 deficiency leads to impaired DNA synthesis, less so of RNA. This results in abnormal cell formation in the bone marrow. The nuclei and the cytoplasm of the cells mature asynchronously. The cells are larger than normal (megaloblastic erythropoiesis) and they frequently die prematurely (ineffective erythropoiesis). This ineffective erythropoiesis can be clinically measured chemically, e.g. in form of elevated LDH values.

In the peripheral blood count macrocytes and isolated megalocytes are found. In addition to erythropoiesis, leukopoiesis and thrombopoiesis are also affected, often resulting in the presence of pancytopenia (decreased values of all three cell lineages).

Just as frequently, and often at an earlier time, neurological or psychiatric symptoms appear, such as

- Tingling paresthesia
- Sensory disturbances
- Gait disturbances
- Confusion
- Memory disorders
- Apathy
- Psychotic states
- Dementia

These symptoms are an expression of a disorder of nervous system development, which is still reversible with timely administration of vitamin B-12.

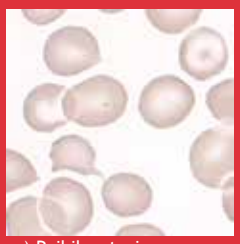
Likewise, atrophic glossitis - Hunter glossitis - with a smooth, crimson and burning tongue may appear.

Causes of vitamin B-12 deficiency

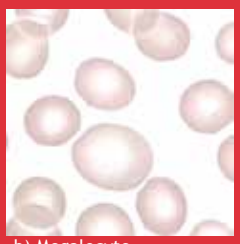
- absent/too low intake (e.g., vegan diet)
- limited resorption in the gastrointestinal tract (e.g., after gastric bypass or partial resection, atrophic gastritis, celiac disease, Crohn's disease)
- absent or non-functional intrinsic factor due to autoimmune disease (anti-parietal cell- or anti-intrinsic factor antibodies), this form of megaloblastic anemia is also known as pernicious anemia or pernicious anemia.

Spotlight on hematology

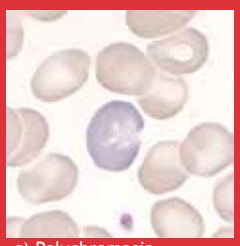
Photos of survey sample specimen



a) Poikilocytosis

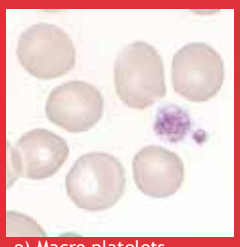


b) Megalocyte



c) Polychromasia

d) Neutrophil, hypersegmentated



e) Macro platelets

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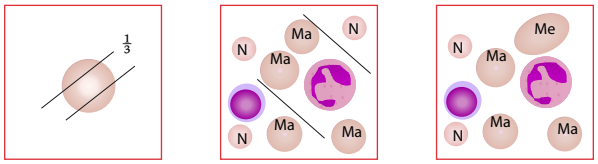
Hematologic findings of megaloblastic anemia

Macrocytosis (MCV)

Round enlarged, macrocytic erythrocytes are found in the blood smear. The hemoglobin content of these erythrocytes is higher than normal relative to their size (MCH ↑). The normal MCHC however shows that the Hb content of these cells is normal relative to their size, so that a central 1/3 brightening is recognizable in the erythrocytes.



Anisocytosis (RDW)

Anisocytosis is understood as an increase in the size variability of a patient's erythrocytes. Morphologically both normo-, micro- and macrocytes are detected simultaneously.

Poikilocytosis

Poikilocytosis is understood as the simultaneous occurrence of different, non-round erythrocyte forms in a blood smear.

Special forms of erythrocytes and erythrocyte inclusions

Megalocytes (syn. macroovalocytes)

Large, highly-filled and slightly oval erythrocytes. Megalocytes can reach the size of neutrophils and, due to their size, frequently no longer exhibit central pallor.

Basophilic stippling and polychromasia

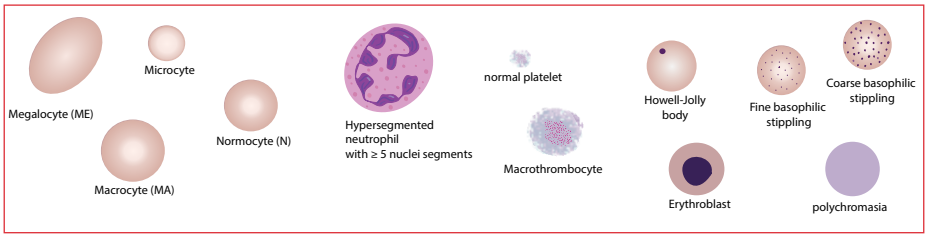
Basophilic stippling is a sign of abnormal cell maturation in megaloblastic anemia.

Howell-Jolly bodies

Dark basophilic round erythrocyte inclusions located mostly as isolated bodies on the cell periphery. In megaloblastic anemia they are chromosomal remnants and signs of abnormal erythropoiesis.

Erythroblasts

Nucleated precursors of mature erythrocytes. In more than 4 erythroblasts per 100 leukocytes the leukocyte measurement in a hematology instrument must be corrected because erythroblasts are erroneously counted as leukocytes (→ *Spotlight on hematology 2012-01)*



Hypersegmented neutrophilic granulocytes

Neutrophilic granulocytes with 5 or more, often strikingly narrow nuclei segments. Hypersegmented neutrophils can be found in the blood prior to the formation of macrocytic anemia. The mechanism of their formation is still unclear.

Macro platelets

Macro platelets are enlarged platelets (>6 µm, normal 1-3 µm).

Differential diagnoses

Macrocytic (not megaloblastic) anemias occur in the context of alcohol abuse-related anemia or with folic acid antagonist therapy (e.g., methotrexate). As a rule the MCV does not exceed 105 fl and megalocytes or hypersegmented neutrophils are not found in the blood count.

Myelodysplastic syndromes are malignant, clonal stem cell diseases, which can also lead to pancytopenia and macrocytic erythropoiesis.