

Appendix

Metabolism of Vitamin B12 and Consequences of Its Distortions

The absorption of vitamin B12 from nutrition is distorted when the mucosa of the corpus area of the stomach is atrophic (atrophic gastritis). This is because an atrophic mucosa of the corpus has deficient secretion of acid (HCl), proteolytic enzymes, intrinsic factor (IF) and haptocorrin (HC). GastroPanel-examination reveals *Helicobacter pylori* infection and atrophic gastritis with related risks. In nearly 90% of the cases atrophic gastritis is caused by *Helicobacter pylori* -infection and in less than 10% of the cases by autoimmune disease. Gastric acid and proteolytic enzymes in the stomach release vitamin B12 from the proteins of food. The haptocorrin (HC) of the gastric juice binds the vitamin B12. The protease enzyme secreted by the pancreas breaks down the HC-B12 complex produced, and the vitamin B12 released is bound by the intrinsic factor. The IFB12 complex is absorbed by the epithelial cells of the small intestine by the receptors of the cell membranes. The vitamin B12, released from the IF-B12 complex, binds with haptocorrin (HC) or transcobalamin (TC). This results in the HCB12 complex (holohaptocorrin or HoloHC) and TCB12 complex (holo-transcobalamin, HoloTC or Active-B12). The complexes are released into the blood stream and the TCB12 portion is subsequently recognized and taken up by specific receptors present in all cell types. This complex, which is physiologically active, includes approx. 20% of the total amount of vitamin B12 present in blood. The remaining 80% of circulating vitamin B12 is HCB12 which has no known function and is cleared by the liver. The concentration of TCB12 decreases rapidly if the absorption of vitamin B12 is distorted. The half-life of the TC-B12 complex in the body is only 0.75 day and that of the HC-B12 complex approx. 9 days. The HC-B12 complex is stored in the liver and kidneys. Vitamin B12 is solely produced by micro-organisms. Thus, vitamin B12 must be received from nutrition, and if the corpus area of the stomach is atrophic (atrophic gastritis), by vitamin B12 treatment. The lack of vitamin B12 in the body is an increasingly growing health problem worldwide and it concerns especially the elderly. The lack of vitamin B12 leads in less than a year to disturbances in the activity of the neural tissues, depression and dementia. These illnesses can become apparent before the development of noticeable haematological changes, and they can become irreversible if the diagnosis and treatment are delayed. Moreover, in connection with the lack of vitamin B12, the concentration of homocysteine (Hcy) in tissues and blood increases, which increases the risk of dementia, atherosclerosis and thromboembolic diseases.

The state-of-the-art, safe and economic GastroPanel examination does not have any of the following serious medical problems:

The 13C urea breath test (UBT), stool antigen test and antibody tests do not detect atrophic gastritis which is caused by *H. pylori* infection or an autoimmune disease.

The diagnosis of atrophic gastritis (AG) is important because of the risks that associate with AG. These are the risks of gastric and oesophageal cancer and malabsorption of vitamin B12, iron, magnesium, calcium and some drugs. Calcium deficiency causes osteoporosis, and vitamin B12 deficiency can cause Alzheimer's disease, dementia, depression and polyneuropathy, as well as high homocysteine content in the body, which in turn is thought to be an independent risk factor for atherosclerosis, heart attacks and strokes. The absorption of dipyridamole, some iron products and antifungals (fluconazole, itraconazole), thyroxine and atazanavir is considerably impaired in an anacidic stomach.

Atrophic gastritis in the gastric corpus and PPI therapy cause anacidity (achlorhydria) of the stomach. The risk of pneumonias and, in senior citizens, even the risk of fatal intestinal infections (such as giardiasis, malaria, *Clostridium difficile* and *E. coli* EHEC) may increase significantly in an anacidic stomach.

H. pylori gastritis may also develop into antral atrophic gastritis, which increases the risk of peptic ulcer disease and gastric cancer. If both antrum and corpus mucosa are atrophic, this condition is the highest risk for gastric cancer known to date.

Furthermore, none of the aforementioned three *H. pylori* tests provides any information on excessive gastric acid secretion (high acid output), which in patients with gastro-oesophageal reflux disease may cause complications of this disease in esophagus. Such complications are often asymptomatic and include ulcerative oesophagitis and Barrett's oesophagus, which may lead to oesophageal cancer if left untreated. In addition, the 13C urea breath test and stool antigen test may give up to 50 % false negative results if the patient has a) atrophic gastritis b) MALT lymphoma or c) bleeding peptic ulcer disease or d) if the patient is currently receiving antibiotics or PPIs.

GastroPanel indications - GastroPanel examination arguments for general practitioners – for huge unmet need

- GastroPanel should be one of the first-line diagnostic tests in examination of all patients with dyspepsia (20-40% of the western population).
- GastroPanel should be used to rule out or confirm the high acid output of reflux patients instead of the trial and error use of PPIs. The long term use of PPIs may increase the risk of stomach and oesophageal cancer.
- GastroPanel is **not** a test for stomach- or oesophageal **cancer**
- GastroPanel biomarkers: Pepsinogen I (PG I), Pepsinogen II (PG II), Gastrin-17 (G-17) and *H.pylori* antibodies reveals:
 - subjects at increased **risk** for stomach- and oesophageal cancer, i.e. those with atrophic gastritis (AG) as well as those with a low risk of cancer; *H.pylori* gastritis with no atrophic gastritis in the antrum and/or corpus
- GastroPanel is also indicated for special target patients, with autoimmune diseases (usually more than one at the same time), including, e.g.:
 - patients with autoimmune thyroiditis who may have autoimmune atrophic gastritis (AAG, 18%) in the corpus with related risks,
 - patients with type 1 diabetes who may have AAG and, e.g., also deficiency of B-12 vitamin (12%) with related risks,
 - patients with celiac disease who may have AAG with related risks, and
 - patients with rheumatoid arthritis who may have AAG with related risks
- In patients with AG or AAG, absorption of vitamin B-12 is reduced.
 - Due to vitamin B-12 deficiency, there is an increased risk of depression, Alzheimer's disease, dementia and polyneuropathy. Consequently, all patients with depression, Alzheimer's disease, dementia and polyneuropathy should be examined by GastroPanel to rule out or confirm those with AG or AAG in the corpus
 - Due to vitamin B-12 deficiency, increased homocysteine levels in the body may be related to:
 - Atherosclerosis – these patients should be examined by GastroPanel to rule out or confirm AG or AAG with related risks
 - Heart attacks – these patients should be examined by GastroPanel
 - Strokes – these patients should be examined by Gastro Panel
- Furthermore, in patients with AG or AAG of the corpus, absorption of Ca, Fe, Mg and Zn is reduced. Low Ca is associated with osteoporosis, while low serum Fe results in anemia.
- All osteoporosis and anemia patients should be examined by GastroPanel to rule out or confirm AG or AAG.
- The risk of pneumonia and, in senior citizens, also the risk of fatal intestinal infections (such as giardiasis, malaria, *Clostridium difficile* and *E. coli* EHEC) may increase significantly due to an anacidic stomach caused by AG, AAG or PPI's. All patients with such infections should be examined by GastroPanel for detection of AG and AAG.
- All subjects diagnosed with AG and AAG in GastroPanel examination need gastroscopic confirmation

Please note that the 13C urea breath test (UBT), stool antigen test or *H. pylori* antibody test alone do not reveal AG. Furthermore, UBT and stool antigen test give 50% of false negative results in *H. pylori* patients, particularly if the patient has AG due to *H. pylori* infection or AAG, bleeding peptic ulcer, chronic use of PPI, antibiotic treatment or MALT lymphoma due to *H. pylori* infection.

GastroPanel is also suitable for screening of healthy (asymptomatic) people, because *H. pylori* infection, AG or AAG with related risks are often asymptomatic.