QUESTION 82
A 69-year-old woman presents with severe pancytopenia and peripheral blood macrocytosis associated with a low serum vitamin B\textsubscript{12} level.
Which one of the following investigation results most strongly supports a diagnosis of pernicious anaemia?
A. Positive anti-parietal cell antibody.
B. Positive intrinsic factor antibody.
C. Elevated fasting plasma homocysteine level.
D. Elevated fasting serum gastrin level.
E. Reduced red cell folate level.

Pernicious anaemia
Pernicious anemia, a sequela of autoimmune chronic atrophic gastritis directed against hydrogen-potassium ATPase in the gastric parietal cells, is associated with an increased risk of intestinal-type gastric cancer. A two- to threefold excess risk has been reported but, as with other predisposing conditions, the actual degree of risk varies with the duration of disease and geographic location. Pernicious anemia is also associated with an increased risk of gastric carcinoid tumors, presumably due to prolonged achlorhydria resulting from parietal cell loss, compensatory hypergastrinemia, and argyrophilic cell hyperplasia.

In the "classic" case of vitamin B12 (cobalamin, cbl) or folic acid deficiency, the patient presents with a severe macrocytic anemia (red blood cell mean corpuscular volume (MCV) >100 fL, and often >115 fL), a low to low-normal absolute reticulocyte count, and a characteristic blood smear showing macroovalocytes occasional megaloblasts, and hypersegmented neutrophils (greater than five percent of neutrophils with five or more lobes or 1 percent with six or more lobes).

However, many patients with B12 deficiency present with no or only mild anemia, and the macrocytosis may be masked by a concurrent disorder such as iron deficiency or one of the thalassemias (eg, alpha thalassemia trait).

EVALUATION — The evaluation of suspected Cbl or folate deficiency generally proceeds in two stages:
- documenting the presence of the vitamin deficiency and then
- determining its cause (eg, pernicious anemia, malabsorption, dietary lack).

Diagnosis
- Evaluation of the peripheral blood smear for the presence of oval macrocytes and hypersegmented neutrophils.
- Measurement of the serum Cbl and the red blood cell (or serum) folate concentrations
- Evaluation of specific metabolites (eg, methylmalonate and homocysteine)
- For the diagnosis of pernicious anemia, the presence of antibodies to intrinsic factor is a helpful finding.
- Malabsorption of Cbl, and its correction by the addition of intrinsic factor, can be established through use of the Schilling test, when available
- Bone marrow examination to demonstrate megaloblastic erythropoiesis is usually unnecessary. Even if performed, this examination will not distinguish Cbl deficiency from folate deficiency.
- Red blood cell MCV — An elevation of the red blood cell mean corpuscular volume (MCV) is one of the hallmarks of Cbl and folate deficiency, although other causes are possible
- The degree of elevation of the MCV is often a clue as to whether a vitamin deficiency is present. Thus, the probability of a deficiency of folate and/or Cbl being present when the MCV is normal (ie, 80 to 100 fL), 115 to 129, or >130 fL has been estimated at <25, 50, and 100 percent, respectively [2]. Unless a combined deficiency (eg, iron deficiency plus a deficiency of Cbl or folate) is suspected, routine testing for Cbl or folate deficiency in an anemic patient in the presence of a MCV <80 fL is not likely to be productive.
- Folate levels —
  - The serum folate concentration, although typically low in patients with folate-deficient megaloblastic anemia, is primarily a reflection of short-term folate balance, as can be illustrated by the following observations: One hospital meal can normalize the serum folate in patients who are folate deficient Pregnancy, alcohol intake, certain anticonvulsants, or a few days of decreased dietary intake can lower the serum folate concentration, despite the presence of adequate tissue stores.
  - The red cell folate concentration is theoretically a more reliable indicator of tissue folate adequacy, since it reflects a time-averaged value of folic acid availability, and is therefore not
subject to the short-term fluctuations noted above. However, it is not entirely without its own problems of interpretation.

- As a result, it has been suggested that the less expensive serum folate concentration should be obtained as an initial screening test, and that there is no basis for the routine testing of all samples for both serum folate and red cell folate. If the serum folate concentration is >4 ng/mL (9.1 nanomol/L), folate deficiency is effectively ruled out. The more expensive metabolite testing should be reserved for patients with borderline values (folate concentration 2 to 4 ng/mL), in those suspected of having a combined deficiency of both Cbl and folate, and for patients in whom the serum folate level may not be easily interpreted (eg, recent hospital meal or recent anorexia). In the absence of recent anorexia or fasting, a serum concentration <2 ng/mL (4.5 nM/L) is diagnostic of folate deficiency.

- Cobalamin levels — Several commercial laboratories use different methods (chemiluminescence or radioassay) for measuring Cbl. As a result, there are different normal ranges and no "gold standard"

- Methylmalonic acid and homocysteine — Patients with low-normal or even normal serum Cbl values may be truly Cbl deficient and respond to replacement therapy. Therefore, measurement of the serum concentrations of homocysteine and methylmalonic acid is helpful in clarifying the diagnosis when serum Cbl or folate concentrations are equivocal, or are low in the pregnant subject

- Serum concentrations of homocysteine (HC) as well as serum and urinary concentrations of methylmalonic acid (MMA) are elevated in Cbl deficiency, due to a decreased rate of metabolism

Diagnosis of PA — Several laboratory studies can suggest the diagnosis of pernicious anemia (PA). Patients with PA are achlorhydric, but confirming this by measurement of gastric acid secretion is highly invasive and rarely needed. Bone marrow examination may confirm the diagnosis of megaloblastic anemia, but this finding is not specific for any cause of Cbl or folate deficiency, and is also highly invasive.

- Antibodies to IF — The presence of anti-intrinsic factor (IF) antibodies is highly confirmatory for the diagnosis of PA, with a sensitivity varying from 50 to 84 percent, depending upon the population tested, and a specificity approaching 100 percent. On the other hand, anti-parietal cell antibodies are much less specific, and may even be less sensitive (50 percent); as a result, the utility of anti-parietal cell antibodies in diagnosing PA has been called into question.

- Schilling test — A classic procedure for diagnosing pernicious anemia is the two stage Schilling test

  - In the first stage of the test, one to two microg of radiolabeled crystalline cyano-Cbl is given orally, followed by an intramuscular injection of 1000 microg of Cbl one hour later, in order to saturate the transcobalamines and to "flush" any absorbed radiolabeled Cbl from its tissue and blood binding sites into the urine. A 24-hour urine is then collected for determination of the percent excretion of the oral dose. An accurately timed and complete urine collection is critical.
    - Normal subjects excrete 8 to 35 percent of the administered radiolabeled dose in the urine over the ensuing 24 hours.
    - A low percent excretion (usually <8 percent, depending upon the laboratory) of radiolabeled Cbl indicates Cbl malabsorption, due to pernicious anemia or other causes of intestinal malabsorption.
    - Renal insufficiency is a cause of falsely low values. On the other hand, the first stage Schilling test may be spuriously normal in patients with B12 deficiency due to atrophic gastritis or gastrectomy.

  - In the second stage of the Schilling test, performed only if the first stage result shows reduced excretion, the test is repeated with added oral intrinsic factor. This should normalize Cbl absorption in patients with pernicious anemia, but not in those with intestinal malabsorption. It is important to recognize, however, that Cbl deficiency adversely affects intestinal mucosal cells and can cause malabsorption. Thus, the second stage should only be performed after at least four weeks of Cbl replacement.
    - If the second stage of the Schilling test is abnormally low, this suggests the presence of generalized malabsorption, such as may occur in sprue, pancreatic insufficiency, or blind loop syndromes. While these conditions can be diagnosed by other means, a "third stage" Schilling test can be performed following appropriate treatment, in order to further confirm the operative mechanism.

Recommendation — Vitamin B12 deficiency, pernicious anemia, malabsorption, blind loop syndromes, and ileal disease can be reliably and quickly diagnosed using methods other than the Schilling test. Difficulties with the
radiolabeled reagent used in the Schilling test, as well as certification issues, make this test generally unavailable in many parts of the United States. Accordingly, although the Schilling test has historical importance in understanding abnormalities of vitamin B12 absorption, it is not commonly employed, and has potential usefulness only when more simple tests (e.g., anti-IF antibodies) are normal (see "Diagnostic strategy" below).

TREATMENT

Folate deficiency — Folate deficiency is treated with folic acid (1 to 5 mg/day PO) for one to four months, or until complete hematologic recovery occurs. A dose of 1 mg/day is usually sufficient, even if malabsorption is present. These doses are in excess of those recommended for disease prevention (e.g., recommended daily allowance in normal adults, alcoholics, the elderly, prevention of neural tube defects).

Cobalamin deficiency — Pernicious anemia (PA) is typically treated with parenteral (i.e., intramuscular) Cbl, in a dose of 1000 µg (1 mg) every day for one week, followed by 1 mg every week for four weeks and then, if the underlying disorder persists, as in PA, 1 mg every month for the remainder of the patient's life.

Oral and nasal formulations — An alternative that appears to be as or more effective than parenteral therapy, but which requires much greater patient compliance, is high dose oral cobalamin. The rationale for this approach in patients with impaired intrinsic factor function is the presence of a second, lower efficiency transport system for Cbl that does not require intrinsic factor or a functioning terminal ileum. This system consistently produces adequate long-term vitamin replacement at doses of 1000 to 2000 microg/day. Because of variability in absorption, lower doses are not completely effective in some patients with pernicious anemia. Similarly, use of "timed release" oral Cbl preparations should be avoided.

Blood transfusion — In patients who are severely anemic at presentation, the decision to transfuse can be a difficult one, particularly in elderly patients at risk for congestive heart failure due to volume overload. If the anemia is extreme and the patient is critically ill, one unit can be given initially at a slow rate, in combination with a diuretic, if fluid status is a concern. In extreme circumstances, isovolemic exchange can be performed, in which one unit of the patient's blood (with a low hematocrit) is removed at the same time as a unit of packed cells (with a hematocrit of 60 to 80 percent) is infusing.

MONITORING FOR MALIGNANCY — Patients with PA may have an increased risk of developing gastric or colorectal adenocarcinoma, but the data are not entirely conclusive. Nevertheless, it is prudent to periodically monitor stools in these patients for the presence of blood.

A. Positive anti-parietal cell antibody.

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B. Positive intrinsic factor antibody. Correct

The presence of anti-intrinsic factor (IF) antibodies is highly confirmatory for the diagnosis of PA, with a sensitivity varying from 50 to 84 percent, depending upon the population tested, and a specificity approaching 100 percent.

C. Elevated fasting plasma homocysteine level.

Homocysteine is an intermediary amino acid formed by the conversion of methionine to cysteine. Homocystinuria or severe hyperhomocysteinemia is a rare autosomal recessive disorder characterized by severe elevations in plasma and urine homocysteine concentrations. Clinical manifestations of homocystinuria include developmental delay, osteoporosis, ocular abnormalities, thromboembolic disease, and severe premature atherosclerosis.

Increased blood levels of homocysteine may reflect deficiency of folate, vitamin B6, and/or vitamin B12. Plasma folate and B12 levels, in particular, are strong determinants of the homocysteine concentration. Homocysteine levels are inversely related to folate consumption, reaching a stable baseline level when folate intake exceeds 400 µg/day. Vitamin B6 is a weaker determinant.

D. Elevated fasting serum gastrin level.
The chronic atrophic gastritis in PA is also associated with an increased risk of intestinal-type gastric cancer and of gastric carcinoid tumors. The latter are presumably due to prolonged achlorhydria resulting from parietal cell loss, compensatory hypergastrinemia, and argyrophilic cell hyperplasia.

Causes of hypergastrinemia: Antisecretory therapy, Atrophic gastritis, Diabetes mellitus, Gastrin cell hyperplasia or hyperfunction, Massive small bowel resection, Ovarian cancer, Pheochromocytoma, Renal insufficiency, Retained gastric antrum, Rheumatoid arthritis, Sjögren’s syndrome, Vitiligo, Zollinger-Ellison syndrome

E. Reduced red cell folate level.
See discussion above about the problems with measurement

In Carol’s lecture she said for PA
1. IF antibodies
2. Fasting serum gastrin