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Review

Diagnosis and classification of pernicious anemia

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ABSTRACT

Pernicious anemia (PA) is a complex disorder consisting of hematological, gastric and immunological alterations. Diagnosis of PA relies on histologically proven atrophic body gastritis, peripheral blood examination showing megaloblastic anemia with hypersegmented neutrophils, cobalamin deficiency and antibodies to intrinsic factor and to gastric parietal cells. Anti-parietal cell antibodies are found in 90% of patients with PA, but have low specificity and are seen in atrophic gastritis without megaloblastic anemia as well as in various autoimmune disorders. Anti-intrinsic factor antibodies are less sensitive, being found in only 60% of patients with PA, but are considered highly specific for PA. The incidence of PA increases with age and is rare in persons younger than 30 years of age. The highest prevalence is seen in Northern Europeans, especially those in the United Kingdom and Scandinavia, although PA has been reported in virtually every ethnic group. Because of the complexity of the diagnosis, PA prevalence is probably underestimated and no reliable data are available on the risk of gastric cancer as the end-stage evolution of atrophic gastritis in these patients.

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1. Introduction

Pernicious anemia (PA) is a disease of autoimmune origin in which atrophy of the gastric mucosa which involves the body and fundus of the stomach, reduces the number of parietal cells that produce the intrinsic factor necessary for absorption of vitamin B_{12}, which, in turn, is indispensible for erythropoiesis and myelin synthesis. This condition is progressive over a span of years, from a mild chronic inflammation of the stomach body to an advanced state associated with a lack of vitamin B_{12}.

The symptomatology is dominated by a profound megaloblastic-type anemia and, in the most serious cases, by neurological alterations, which can precede the diagnosis of gastric atrophy by several decades.

The autoimmune nature of the process that brings on gastric atrophy and PA is documented by the presence of autoantibodies against intrinsic factor secreted by the stomach and the gastric parietal cells, and by the frequent coexistence in these patients of other disorders of autoimmune origin. Recent epidemiological studies support the evidence that autoimmune gastritis and PA are found across all continents [1,2] and probably are underdiagnosed [3,4], given that most patients with

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microcytic or macrocytic anemia are treated with iron, folates, and cobalamin, without any more thorough investigation into the cause of the anemia; a biopsy of the gastric mucosa in many cases is not undertaken; even if a biopsy is performed, the pathologists often describe a generic histological pattern of chronic gastritis with intestinal metaplasia [5,6].

2. Historical notes

1860: Austin Flint, connects observations made by Thomas Addison in 1849, describing an important form of anemia associated with a degenerative disease of the tubular glands of the stomach.

1872: Anton Biermer designates this condition 'pernicious anemia'.

1900: Faber and Bloch document for the first time in a patient with PA the presence of a histological pattern of gastric atrophy.

1953: William B. Castle demonstrates that anemia is caused by a concomitant lack of an 'extrinsic factor' metabolized in the liver (cobalamin or vitamin B_{12}) and of an 'intrinsic factor' present in the gastric juice necessary for absorption of cobalamin at the intestinal level.

1960: Michael Schwartz demonstrates the presence in patients with PA of autoantibodies against the intrinsic factor.

1962: James Irvine identifies in patients with PA the presence of another autoantibody against parietal cells of the gastric mucosa [7].

3. Genetics

A genetic susceptibility for PA is suggested by a specific HLA-DR pattern and by blocking experiments with anti-DR and anti-DQ antibodies that have shown that DR antigen represents the HLA restriction element in atrophic body gastritis [8]. By using a DNA-based, sequence-specific oligonucleotide technology, it has been observed that the genotypes HLA-DRB1*03 and DRB1*04, which are known to be associated with other autoimmune disease (such as type1 diabetes and autoimmune thyroid disease) [9], were significantly associated with PA, which further supports the concept that autoimmunity may play a role in PA [10].

4. Clinical features

Pernicious anemia usually manifests itself in persons over age 30 and strikes both sexes equally. It is particularly frequent in northern Europeans, especially Scandinavians. Nevertheless, it is present in other populations but is relatively infrequent in subjects of the oriental races.

Patients usually exhibit symptoms of anemia with pallor, fatigue, lightheadedness, or tachycardia. Often the anemia is of such insidious onset that the severity is not suspected clinically. Inhibition of DNA synthesis due to vitamin B_{12} deficiency causes megaloblastic changes not only in bone marrow but also in other rapidly dividing cells, such as gastrointestinal epithelium. Involvement of small-bowel epithelium may result in malabsorption and diarrhea with weight loss. Anorexia is an additional common complaint. Glossitis is a frequent sign of megaloblastic anemia, with the patient displaying a painful, smooth, red tongue. The elevation in bilirubin levels, caused by ineffective erythropoiesis, manifests as jaundice.

Neurologic abnormalities are seen in PA as a result of vitamin B_{12} deficiency. Demyelination is the initial finding, which progresses to axonal degeneration and neuronal death if left untreated. Peripheral numbness and paresthesias are the initial symptoms, with subsequent development of weakness and ataxia. The appearance of motor symptoms is indicative of subacute combined degeneration involving the dorsal and lateral spinal columns. Mental disturbances may be present, ranging from forgetfulness to psychosis.

In the general population, the prevalence increases with age, from 2.5% to 12% [11] and is, overall, more frequent in carriers of other diseases of immunological pathogenesis, especially endocrine disorders such as Graves' disease, myxedema, thyroiditis, idiopathic adrenal insufficiency, hypoparathyroidism, type 1 diabetes, Addison's disease, inflammatory bowel diseases, acquired agammaglobulinemia, and vitiligo. Anti-thyroid antibodies are present in more than 50% of the subjects with PA and are common in their relatives. Patients with PA may also be at a higher risk for developing gastric cancer as an end-stage evolution of atrophic gastritis.

5. Autoantibodies

Patients with PA have been shown to have two types of antibodies, one to parietal cells (PCA) and the other to intrinsic factor (IFA) or its binding site in the small bowel.

5.1. Parietal cell autoantibodies

The gastric enzyme H^+/K^+-ATPase is the target antigen recognized by PCA [12,13]. This proton pump is responsible for acid secretion in the stomach and is the major protein of the secretory canaliculi of gastric parietal cells. It produces acid by secreting H^+ ions in exchange with K^+ [13,14]. The gastric H^+/K^+-ATPase is formed by a catalytic 100 kDa α subunit and a 60–90 kDa β subunit. The highly conserved catalytic α subunit is phosphorylated during its reaction cycles; the β subunit comprises a heavily glycosylated 35 kDa core protein. The atrophic gastritis is caused by the action of lymphocyte cluster of differentiation T-helper cell-1 inflammatory cells, directed against this enzyme [15]. The β subunit is considered the causal antigen and the source of the autoimmune response responsible for the damage to the gastric mucosa.

PCA are present at a high frequency in PA (80%–90%), especially in early stages of the disease [16] and bind to both α and β subunits of gastric H^+/K^+-ATPase. Antibody reactivity to the α catalytic subunit includes epitopes on the cytosolic side of the secretory membrane. Antibody reactivity to the β subunit requires the antigen to be in a disulfide-bond and glycosylated, suggesting that autoepitopes are located in the luminal domain of the glycoprotein [14,17]. The localization of these molecules may explain why the pathogenetic role of PCA in vivo remains elusive [18]. Circulating PCA belong to IgG, IgA and IgM isotypes. In gastric juice the antibody isotypes are predominantly IgA and IgG [14].

In the later stages of the disease, the incidence of PCA decreases due to the progression of autoimmune gastritis and a loss of gastric parietal cell mass, as a result of the decrease in antigenic rate. In recent studies, an average incidence of 55% of PCA was documented in patients with advanced PA [19]. PCA are, however, not specific as they can be found at low frequency in other autoimmune diseases (e.g., Hashimoto's thyroiditis or type 1 diabetes) or in elderly subjects, even those free of any atrophic gastritis.

5.2. Intrinsic factor autoantibodies

Human intrinsic factor (IF) is a 60 kDa glycoprotein secreted by gastric parietal cells. Its action is high affinity binding and transport of vitamin B_{12}. The complex IF-vitamin B_{12} reaches terminal ileum where it is absorbed after binding to specific receptors in the membranes of cells of ileal lumen. IFA interferes with absorption of intrinsic factor-vitamin B_{12} complex in the terminal ileum.

IFA are considered specific markers for diagnosing PA [20] and are present both in blood serum and in gastric juices. In serum, two specific types of IFA, both of the IgG class, have been described: type 1 (blocking antibody) reacts with the vitamin B_{12} binding site and type 2 (binding o precipitating antibody) recognizes a site away from this binding site and impedes the binding of IF to receptors in the ileal mucosa. Recent studies have reported positivity for IFA in 40%–60% of patients with PA [21,22], which rises to 60%–80% with increasing duration of disease [23]. Autoantibodies directed against the binding site (type I) are found in 70% of patients; autoantibodies directed to

the remote site (type II) are found in about 35–40% of PA patients, and are rarely present in the absence of autoantibodies to type I [24].

In the gastric juices IFA are present in about 80% of patients with PA and are of the IgA type, secreted by plasma cells that infiltrate the mucosa. These antibodies bind to residual IF and impede the absorption of ingested vitamin B_{12}. In addition, the achlorhydria which manifests progressively following damage to the mucosa favors the formation of the antigen–antibody complex, given that the gastric acidity (with consequent reduction in pH) enhances the binding of IF with vitamin B_{12} and therefore the absorption of the vitamin.

5.3. Predictivity of autoantibodies

PCA can precede the clinical symptoms of the gastric disease by several years. Recent studies showed that in PCA-positive patients with type 1 diabetes mellitus, the measurement of other serum biomarkers of gastric damage as gastrin and pepsinogen I, allows the diagnosis of atrophic gastritis many years before the anemia [25]. During a five-year follow-up, 24% of asymptomatic patient's autoimmune thyroiditis without gastric and extragastric symptoms and detectable levels of PCA at baseline, developed clinically overt atrophic gastritis [26].

During the natural course of gastric atrophy, the concentrations of PCA rise progressively over time, reaching a peak level and then falling. These levels correlate with the progressive destruction of gastric mucosa and to the disappearance of target autoantigens (proton pump) [26].

The data on the predictive value of IFA for PA are not conclusive: there are reports describing type 1 IFA in patients with PCA and autoimmune gastritis that antedated development of PA over 1–15 years of follow-up, while other studies reported individuals with type 1 IFA, with or without PCA, that did not progress to PA.

It is likely that the evolution of autoimmune gastritis (associated with PCA with or without circulating IFA) to overt PA requires production of IgA antibodies to intrinsic factor secreted into the stomach, which are able to impair absorption of residual vitamin B_{12} produced by atrophic gastric mucosa. In this context, circulating IFA IgG does not functionally correlate with IFA IgA secreted in the stomach. There are no reports of sequential development of PCA followed by IFA arising as a result of intermolecular epitope spreading.

6. Diagnostic criteria

The diagnosis of PA relies on demonstration of megaloblastic anemia, low serum vitamin B_{12} levels, gastric atrophia, and the presence of antibodies to gastric parietal cell or intrinsic factor (Table 1).

The anemia is macrocytic and normochromic with reduction in the absolute number of reticulocytes. The hemoglogin level may be seriously reduced, even down to 3 g/dL. The patient's red blood cells (RBCs) exhibit marked anisopoikilocytosis and numerous oval macrocytes (megalocytes).

Hypersegmented neutrophils are considered a hallmark of megaloblastic anemia and typically precede the macrocytosis and anemia; in advanced cases, however, they may be rare or even absent, probably due to ineffective granulopoiesis. Although the hypersegmented neutrophils support the diagnosis of megaloblastic anemia, they are not specific, as they also can appear in other types of anemia, such as iron deficiency anemia.

Table 1
Diagnostic criteria for PA.

PA is defined as the presence of:
- Hemoglobin concentration <13 g/dL for men and <12 g/dL for women
- RBC's mean corpuscular volume ≥ 120 fL
- Low levels of serum vitamin B_{12}
- Gastric body mucosal atrophy
- Autoantibodies to intrinsic factor and/or to gastric parietal cells

The differential diagnosis includes: macrocytic anemia, alcohol abuse, liver disease, myelodysplastic syndromes, and additional conditions that may cause vitamin B_{12} deficiency such as a strict vegetarian diet, drugs, infections, and gastric surgery. However, macrocytosis associated with alcoholism or liver disease usually is characterized by a mean corpuscular volume (MCV) ranging from 110 to 115 fL, while the MCV of megaloblastic anemia is extremely variable, ranging from normal to markedly elevated, up to 150 fL. Howell–Jolly bodies and basophilic stippling of RBCs may be present. Pancytopenia is often present but also can be seen in association with alcoholism or liver disease.

A bone marrow examination (usually unnecessary in patients who have classical features of megaloblastic anemia in the peripheral blood) shows a hypercellular bone marrow with a shift toward immaturity and abnormal maturation of erythroid and myeloid cell lines. The erythroid precursors have the morphologic features of megaloblasts and display a predominance of basophilic forms. The immature neutrophil series exhibits nuclear-cytoplasmic asynchrony with numerous giant metamyelocytes. The ineffective erythropoiesis and myelopoiesis are responsible for the pancytopenia in megaloblastic anemia, despite marrow hypercellularity [27].

The biochemical laboratory workup can be accomplished by systematic investigation, starting with an assessment of vitamin B_{12}/folate status. While a deficit of vitamin B_{12} is always present, folates in serum behave variably.

Differentiating between vitamin B_{12} deficiency and folate deficiency is essential to patient management, because treatment of vitamin B_{12} deficient patients with folate alone may reverse the megaloblastic blood picture, but the associated neurologic damage may worsen. Hence, serum vitamin B_{12} and serum folate levels should be determined concurrently to correctly identify patients deficient in either or both [27] (Table 2).

A deficit of intrinsic factor may be demonstrated using the Schilling test, a dynamic multistep investigatory test which involves the ingestion of isotope-labeled vitamin B_{12}, followed by an injection of unlabeled vitamin B_{12}. The levels of labeled vitamin B_{12} are measured in the urine of the patient, collected after 24 h. If the excretion is low, the test is repeated after the oral administration of exogenous intrinsic factor along with an oral dose of labeled vitamin B_{12} which normalizes the absorption of the vitamin. Given its complexity and problems related to the use of radioactive agents, the Schilling test today is employed only occasionally, being no longer held as indispensible for diagnostic purposes when anti-intrinsic-factor antibodies are present.

Other laboratory results include a markedly elevated lactate dehydrogenase level and mildly elevated bilirubin, total iron, and aspartate aminotransferase levels, both expressions of intramedullary erythroblastolisis. Fasting gastrin levels are elevated in 75% of patients, while the levels of somatostatin show as depressed.

A diagnostic workup of megaloblastic anemia also should include evaluation of iron status. During treatment of megaloblastic anemia, the recovering bone marrow will utilize large amounts of iron. Even if a patient with megaloblastic anemia has normal-appearing bone marrow iron stores, the patient actually is deficient because the bone marrow is overloaded with iron that cannot be utilized during the megaloblastic state. Therefore, iron supplementation may be warranted even though the patient has an initial normal serum iron value [27].

Table 2
Laboratory tests to diagnose PA.

- Complete blood cell count
- Examination of peripheral blood smear
- Reticulocyte count
- Blood vitamin B_{12}
- Blood folate
- Blood iron
- Autoantibodies to intrinsic factor and gastric parietal cell
- Bone marrow examination (only in selected cases)

Table 3
Indication to search for antibodies to intrinsic factor and gastric parietal cell.

- Macrocytic anemia with symptoms of vitamin B_{12} deficiency, not responding to oral therapy
- Autoimmune thyroid diseases, type 1 diabetes, Addison's disease or other endocrine autoimmune disorders
- Dyspeptic symptoms not correlated with other gastrointestinal diseases
- Siblings of patients with PA

The need to test for *Helicobacter pylori* is controversial. Recent experimental and clinical data suggest an involvement of long-standing infection in the pathogenesis of atrophic body gastritis and PA [13,28], but it is still under debate whether PA may be included among the long-term consequences of *H. pylori* gastritis [10].

7. Detection of autoantibodies

PCA are usually detected by indirect immunofluorescence (IIF) performed on cryostat sections of rodent stomach; positivity is characterized by a homogeneous and diffuse cytoplasmic staining of only the parietal cells. However, since IIF is not very sensitive, provides only semi-quantitative values, and interpretation of results is highly subjective and depends on the expertise of the observer, ELISA methods, using highly purified H^+/K^+ ATPase antigens from human or porcine stomach have been developed to overcome IIF drawbacks. Several studies reported sensitivity and specificity of the ELISA method of 80–90%, with an excellent agreement with the immunofluorescence method [29].

The analytical methods currently used to detect IFA are immunoblotting, ELISA and chemiluminescent immunoassay (CLIA), detecting both type of IFA using recombinant human antigens. All these methods are characterized by high sensitivity, specificity, and predictive value. In addition, CLIA and ELISA provide quantitative measuring which permits more efficacious monitoring of the disease. The use of a sensitive ELISA method for the determination of autoantibodies has shown that they can be detected more frequently in gastric juice than in serum [29]. Clinical indication to search for PCA and IFA are shown in Table 3.

8. Histopathological features

From the histological point of view, the mucosa of the cardia and fundus is thinned and atrophied, with shrunken glands and containing few principal and parietal cells, while usually the mucosa of the antrum is spared. However, in about 50% of PA patients, antral mucosa is not spared, and in about 27% of PA patients, a concomitant antral atrophic gastritis may be observed. These data strongly suggest that an extension of gastritis to the gastric antrum does not necessarily exclude the diagnosis of PA and the presence of gastric autoimmunity [10].

9. Treatment

Pernicious anemia is caused by inadequate secretion of gastric intrinsic factor necessary for vitamin B_{12} absorption and thus cannot be treated with oral vitamin B_{12} supplements; rather, vitamin B_{12} must be administered parenterally. An intramuscular injection of 1 mg of vitamin B_{12} is generally given every day for 1 week, followed by 1 mg every week for 4 weeks and then 1 mg every month thereafter. PA requires lifelong treatment. Symptoms of vitamin B_{12} deficiency may be improved after just a few days of medical treatment and an increase in the reticulocyte count is the most useful sign of a hematological response to therapy. Gastric atrophy, however, is not cured by cobalamin treatment, but does respond to steroids, with partial regeneration of the mucosa and renewal of IF secretions.

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