Pernicious anemia: Pathophysiology and diagnostic difficulties

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Abstract
Pernicious anemia (PA) is the most common cause of vitamin B12 (cobalamin) deficiency anemia in the world. It is an autoimmune disease, comprising of salient features of autoimmune chronic atrophic gastritis (CAG) and cobalamin deficiency (CD). Although the anemia was first described as pernicious, it may well be controlled with vitamin B12 replacement. The onset and progression of PA is often insidious. Alternatively, patients may have no anemic symptoms since they become acclimatized to the subtle nature of the disease. Oftentimes, there is a possibility that the underlying disease may be missed unless a full blood count (FBC) is investigated, leading to hindrance in the treatment journey. Diagnostic challenges remain tangible for many practicing clinicians, since there is lack of reliable cobalamin assays to diagnose CD as well as clinical mimics, which simulate many other hematological conditions, such as myelodysplastic syndrome, acute leukemia, sideroblastic anemias, bone marrow failure states, thrombotic microangiopathy, and thromboembolism. Moreover, prompt recognition of the symptoms of CD is also vital, because some neurologic sequelae may become irreversible despite replenishing cobalamin. Herein, we discuss a literature review on the pathophysiology, challenging clinical presentations and diagnostic difficulties of PA. Since the cobalamin replacement therapy for PA is straightforward, it will not be discussed in this review.

KEYWORDS
cobalamin deficiency, macrocytic anemia, pernicious anemia, vitamin B12 deficiency

1 INTRODUCTION

Pernicious anemia (PA) is an autoimmune disease characterized by autoimmune chronic atrophic gastritis (CAG) and cobalamin deficiency (CD). PA is the most common cause of CD throughout the world. The prevalence is hugely affected by age, ranging from 0.1% in the general population to 1.9% in the elderly population above the age of 60 years. PA accounts for 20%-50% of the etiology of CD in adults. Although the incidence of PA rises with age, it varies geographically. It is often described that PA is very common in Scandinavian countries. Nonetheless, many studies have also claimed a high prevalence in the African descendants.

The history of PA began in 1849 when Thomas Addison discovered PA. Austin Flint described the association between the anemia and CAG in 1860 and PA was named afterward. Surprisingly, the anemia was treated successfully with cooked liver, which was later postulated that the extrinsic factor (now identified as cobalamin) was present in the liver and the intrinsic factor (IF) in the gastric secretion. Although the anemia was first described as pernicious, it is now well controlled with cobalamin replacement. The anemia was found to be highly...
associated with underlying autoimmune CAG, which was coupled to the discovery of autoantibodies to gastric parietal cells (PC) and IF.³

2 | PATHOPHYSIOLOGY OF PA

The cobalamin is rich in eggs, meat, and dairy products and the recommended daily intake is 2.4 micrograms.²⁷ Since the absorbed cobalamin is largely stored in the liver, it may take 5-10 years for clinical manifestations to develop if dietary cobalamin is insufficient.² The absorption of cobalamin requires three main proteins: haptocorrin, IF, and transcobalamin.³

The dietary cobalamin binds to haptocorrin in the saliva, forming haptocorrin-cobalamin complex, which is digested by the acidic and the proteolytic activity of gastric pepsin to release around 70% of the cobalamin.⁹ Gastric PCs secrete a glycoprotein called IF which strongly binds to and carries cobalamin to the ileum.⁸,¹⁰ In humans, the cobalamin is absorbed via the cubulin-receptor-mediated endocytosis along the entire ileum. In the serum, cobalamin exists in two forms: holohaptocorrin, the complex of haptocorrin and cobalamin, and holotranscobalamin (HTC), a complex of cobalamin and transcobalamin (TC).¹¹ HTC is transported and metabolized in the liver and cobalamin is stored in the hepatocytes, and a small proportion is secreted back to circulation and is carried by TC to the peripheral tissues so rapidly that < 30% of cobalamin remains as HTC in the plasma at any time.¹² Therefore, the liver self-regulates the metabolism of the absorbed cobalamin for the distribution to the peripheral tissues and storage in the liver.¹³ TC and HTC are filtered through the renal glomeruli and reabsorbed by the renal tubules. Reabsorbed HTC is degraded with the release of cobalamin, which then binds to newly synthesized TC.¹¹ The TC receptors are expressed by various cells in the peripheral tissues for their metabolism. The HTC transported to the peripheral tissues is degraded by the lysosome. The free cobalamin is then converted into methylcobalamin or adenosylcobalamin in the cytosols and mitochondria, respectively. Adenosylation of methionine results in the formation of S-adenosylmethionine (SAM), which is necessary for neuronal methylation of proteins, nucleic acids, neurotransmitters, myelin, and phospholipids. Cobalamin-dependent methylation of homocysteine to methionine needs methylytetrahydrofolate, which donates the methyl group and is converted into tetrahydrofolate which is a precursor for DNA synthesis. Finally, cobalamin is necessary for the conversion of methylmalonic CoA to succinyl CoA by the methylmalonyl CoA mutase in the mitochondria.¹⁴,¹⁵

PA is an autoimmune disorder characterized by the presence of IF autoantibodies (IFA) and PC autoantibodies (PCA) resulting in the mal-absorption of dietary cobalamin.¹⁶ IFA are found in both the gastric fluid and the serum of PA patients. IFA are of immunoglobulin G class and there are two subtypes of antibodies: type I antibody, which can target the binding site of cobalamin and type II antibody which can interfere with binding of IF to the epithelium of ileal mucosa.¹⁶ Small amount of cobalamin can be absorbed independently of IFA in adults. In PA patients, cobalamin level in plasma reach a peak after 3-8 hours after ingestion compared to 2 hours in normal individuals.¹⁷ These findings explain the possibility of an additional nonreceptor assisted absorption of cobalamin in adult humans, which remains an area of interest.¹⁷,¹⁸

Autoimmune CAG is nearly ubiquitous in PA and is explained by destruction of gastric PCs due to CD4 T-cell mediated autoimmune response against the gastric H+/K+/ATPase pump (proton pump).¹⁹,²⁰ PCA in the serum of PA patients are immunoglobulin A, G, and M iso-types directed to both α and β subunits of PC proton pump.²¹ Those PCA and IFA antibodies are produced by plasma cells derived from the B-lymphocytes under the signals from CD4+ T lymphocytes in the paragastric lymphoid tissue. Gastric dendritic cells are responsible for the release of autoreactive CD4+ T lymphocytes.²²

The exact mechanism on how the gastric dendritic cells are activated remains the subject of ongoing studies and debates. There is a controversial hypothesis that PCA, autoimmune CAG, and PA may be attributable to Helicobacter pylori infection based on the theory of sharing similar immunogenicity between the Helicobacter pylori bacteria and the gastric proton pump ATPase.²³–²⁶ PA is commonly associated with various autoimmune disorders, such as Hashimoto thyroiditis, insulin dependent diabetes mellitus, pure red cell aplasia and polyendocrinopathy.²⁷–²⁹ Recent studies highlighted that HLA-DRB1*03 and HLA-DRB1*04 alleles linked to autoimmune gastritis.³⁰

3 | CLINICAL PRESENTATIONS

The onset and progression of PA is often insidious. Alternatively, patients have no anemic symptoms since they become acclimatized to the subtle nature of the disease. Oftentimes, there is a possibility that the underlying disease may be missed unless full blood count (FBC) is investigated, leading to hindrance in the treatment journey. Nevertheless, patients with PA may present with other nonspecific symptoms related to anemia per se, such as lethargy, inability to concentrate, headache, and, cardiac symptoms such as palpitations and chest pain especially in elderly patients. Less often, neurological symptoms may be the first presentation, such as parasthesia, imbalance, and spasticity. In fact, CD may cause damage to the nerves ranging from demyelination to axonal degeneration in the peripheral nerves and in the lateral and posterior columns of the spinal cord called subacute combined degeneration and, in the cerebrum, ultimately causing neuronal death. Furthermore, CD is associated with neuropsychiatric features such as dementia, change in personality, and psychosis.³¹ Characteristic peripheral blood features of CD include hypersegmented neutrophils (HSN) (with more than 5 lobed nuclei or ≥ 5% of 5-lobed neutrophils) and macro-ovalocytes.⁴ The prompt recognition of those symptoms is very crucial, because the neuropahty may be irreversible despite replenishing the cobalamin.³² Patients with PA usually respond very well to cobalamin supplementation, especially in the early course of the disease.³³ Finally, PA may frequently be associated with other autoimmune conditions, for example, Hashimoto thyroiditis, IDDM, and vitiligo.⁴,²⁷–²⁹,³⁵
CHALLENGING CLINICAL PRESENTATIONS WHICH MAKE THE DIAGNOSIS OF PA VERY DIFFICULT

Despite advances in understanding molecular pathophysiology of PA, reaching a timely diagnosis remains challenging for many physicians because of its myriad of clinical presentations and limitations of currently available tests. Herein, we discuss the challenging clinical presentations of PA.

4.1 PA presenting with normal or high cobalamin levels

CD is defined by the serum cobalamin level of less than 200 pg/mL in the appropriate clinical context. Currently, automated competitive-binding chemiluminescence assay is utilized to measure the cobalamin level. Patients with serum cobalamin levels between 200 and 400 ng/L (pg/mL) may have true CD and are considered borderline levels. Serum cobalamin may be falsely normal or even elevated in 22%-35% of patients with PA because of the interaction of IF antibody with IF reagent using the current chemiluminescence assays. Diagnosis of PA in those circumstances may require the demonstration of HSNs and macro-ovalocytes on the peripheral blood smear (PBS), increased serum homocysteine and methylmalonic acid (MMA), any or all positive IFA or parietal cell antibodies, or megaloblastic changes in the bone marrow (BM).

4.2 PA presenting with normocytic or microcytic anemia

Macrocytic anemia with mean corpuscular volume (MCV) of ≥100 fL is the hallmark hematologic feature of CD. Nonetheless, it is not true in all cases and approximately 30% of the cases may not have macrocytosis. Normocytic anemia can be seen in PA if there is concomitant iron deficiency anemia (IDA) or any other diseases that can cause microcytosis. Studies showed that nearly 20.7% of patients with PA have coexisting IDA at presentation. Approximately 22.3% of remaining patients with PA may develop IDA from 4 weeks to more than 10 years later in the future. Macrocytosis may not be a feature in about 42% of African Americans and South Africans. Some African Americans with PA may even present with microcytosis if there is coexisting α-thalassemia which can mask the macrocytosis. Those are the reasons why IDA should be ruled out in patients with PA. PBS review may give some clues, for example, target cells in thalassemia and microcytic erythrocytes and elliptocytes in IDA in addition to characteristic HSNs and macro-ovalocytes in CD.

4.3 PA presenting with nonanemic macrocytosis

Macrocytosis may be the first presentation before the development of anemia in patients with PA. Some PA patients have nonanemic macrocytosis for many months before the diagnosis of PA was established. The first report of PA-related nonanemic macrocytosis dated back to 1964 when a 60-year-old man with neurological symptoms, hemoglobin of 15.1 g/dL and MCV of 110 fL was described by Hattersley in 1964. Carmel described 4 out of 11 patients with PA had macrocytosis only for 2-5 years without anemia before PA was finally diagnosed. The majority of those PA patients with nonanemic macrocytosis developed neurological abnormalities although symptoms improved after treatment.

4.4 PA presenting with warm autoimmune hemolytic anemia (AIHA)

Approximately 1.5% of patients with CD may present with hemolysis due to ineffective erythropoiesis (intramedullary hemolysis). Elevated serum unconjugated bilirubin, lactate dehydrogenase (LDH), and low serum haptoglobin suggest hemolysis in the patients with PA. PA patients may have concomitant warm AIHA although it is not common. Microspherocytes and polychromasia on the PBS would be clues to warm AIHA. Positive direct Coombs test (with IgG ± complement C3) confirms coexisting warm AIHA in PA because Coombs test is negative in ineffective erythropoiesis. There are over 20 reported cases of PA-associated warm AIHA, described in Ref. (47).

4.5 PA presenting with pseudo-thrombotic microangiopathy (pseudo-TMA)

Cytoskeletal fragility of erythroblasts may contribute to schistocyte formation in megaloblastic anemia, depending on the severity of dyserythropoiesis. In a study of 201 patients with CD, pseudo-TMA was reported in 2.5% of patients, which can potentially lead to misdiagnosis of thrombotic thrombocytopenic purpura (TTP) and wrong treatment with plasma exchange. One study depicted that pseudo-TMA patients had higher mean LDH levels, higher mean platelet counts, lower mean reticulocyte count, and lower mean neutrophil counts, compared to patients with true TTP. Other clues on PBS examination pointing against the diagnosis of TTP include the presence of HSNs and macroovalocytes. A recent systematic review by Tran et al discovered that 34% (14/41) of CD-related pseudo-TMA were misdiagnosed as TTP initially and received plasma infusion or exchange. Two patients who underwent plasma infusion or exchange developed complications.
4.6  PA presenting with hyperhomocysteinemia-associated thrombosis

Homocysteine is an amino acid, derived from cobalamin and folate metabolism. CD may be associated with hyperhomocysteinemia, one of the risk factors for atherosclerosis and thromboembolism.54,55 There are several possible mechanisms for the pathogenesis of hyperhomocysteinemia-associated thrombosis. Homocysteine is thought to cause the oxidative stress to the vascular endothelium, impaired endothelium-dependent vasomotor regulation, reduced antithrombin III attachment to endothelium, favored affinity between lipoprotein(s) and fibrin, augmentation of tissue factor expression, and altered platelet activity.54,56–61 Several cases of PA with hyperhomocysteinemia-associated thrombosis have been reported in the Refs. (46), (62), (63). Management of PA-associated thromboembolism requires therapeutic anticoagulation, and cobalamin replacement.

4.7  PA presenting with pseudo-leukemia mimic syndrome

Morphologic changes in hematopoiesis due to CD may mimic those seen in acute leukemia. Both may share pancytopenia, leukoerythroblastic, anisocytosis, macrocytosis, fragmentation, left shift, and nucleated erythrocytes on the PBS.64,65 Furthermore, it will make diagnosis more challenging if patients present with opportunistic infections and severe neurologic complications.64,67 Neutrophil hypersegmentation has not been consistent finding in the literature while it is a hallmark of CD.65,65,66 Other laboratory markers such as high serum LDH and hyperbilirubinemia may overlap between CD and acute leukemia attributable to ineffective erythropoiesis.65 The BM morphological appearances of ineffective hematopoiesis in patients with PA may be variable and overlapping with BM changes in primary myeloid disorders. Some of the overlapping BM changes include hypercellularity with an increased myeloid-erythroid ratio, and arrested maturation of myeloid precursors at various stages.65 Giant and dysmorphic erythroblastic changes in the BM are considered pathognomonic of CD. However, they can also be seen in erythroid leukemia65,67 although some cytoplasmic changes in promyelocytes may help differentiate between benign etiologies and some types of myeloid leukemia.68

4.8  PA presenting with bone marrow failure-like syndrome

Investigation of pancytopenia and BM failure (BMF) are challenging in CD.69 Premkumar et al70 reported that megaloblastic anemia (MA) accounted for > 60% of cases with pancytopenia in a tertiary center in India. Over 90% of cases of MA had severe CD. The differentiation between primary BMF versus cobalamin-associated BMF becomes a diagnostic challenge due to several independent reasons such as concomitant diagnoses, other coexisting iron and folate deficiencies,39,67,70 and the low sensitivity of the serum cobalamin immunoassays.71 A retrospective study of 255 cases diagnosed with myelodysplastic syndrome (MDS) in a large transplantation center showed that approximately 27% of the cases diagnosed as low-risk MDS with normal karyotype had, as a matter of fact, CD and all of them responded well to cobalamin treatment.69

Detection of immunophenotypic and cytogenetic abnormalities due to defective clonal hematopoesis aids in the diagnosis of primary BM disorders. However, those aberrancies are absent in some cases with primary BM disorders. In addition, flowcytometric and cytogenetic changes were reported in severe CD.72,73 The evidence of increased hematogones in severe CD may differentiate nutritional from malignant etiology of BMF.74

4.9  PA presenting with neurological manifestations without anemia or macrocytosis

CD can cause demyelination of the nerves, which can eventually progress to axonal degeneration and neuronal death.75 Approximately 25% of patients with neurologic sequelae may not have anemia and/or macrocytosis. Severe anemia was reported in merely 19% of patients with neurological abnormalities. The white blood cells and platelet counts were normal in most cases.38,75 The researchers claimed that neuropsychiatric abnormalities due to CD without anemia or macrocytosis is not uncommon. Serum MMA and homocysteine should be measured in those patients with inconsistent findings despite normal cobalamin levels. A therapeutic trial of cobalamin replacement should be considered if such metabolites tests are not available.76

4.10  Cobalamin-responsive neurological manifestations in the context of normal plasma homocysteine and serum methylmalonic acid

Although the B12-metabolite testing with fasting homocysteine and MMA have over 95% sensitivity for detecting CD, those tests are not 100% perfect especially when PA patients present with neurological symptoms in the absence of anemia. In the literature, there was a case of subacute combined degeneration of the spinal cord, presenting with nonanemic macrocytosis, mildly low cobalamin level, normal homocysteine and MMA. Interestingly, neurological symptoms and macrocytosis resolved with cobalamin administration, accompanied by lowering of serum MMA level.77 Graber et al78 also described a case of a 62-year-old female who presented with depression, cognitive decline, severe leukoencephalopathy and autonomic dysfunction but had normal blood counts, normal cobalamin, homocysteine, and MMA levels. IF antibody was positive. She made a remarkable neurological recovery with cobalamin injections. Solomon also described a subset of patients with B12-responsive neuropathies who had cobalamin levels > 300 ng/L and normal MMA and homocysteine levels. He concluded that B12-responsive neuropathies can be dissociated from the presence of hematological abnormalities or B12-metabolical
abnormalities and suggested that an empirical trial of parenteral cobalamin should be considered in the appropriate subjects. Therefore, neurological patients with high index of suspicion for PA should be considered for an empirical trial of parenteral cobalamin administration.

5 LIMITATIONS OF CURRENTLY AVAILABLE BLOOD TESTS

5.1 Serum cobalamin assay

In the context of right clinical picture, the level of serum cobalamin less than 200 pg/mL (ng/L) is required for the diagnosis of CD using automated enzymatic chemiluminescence assays. Patients with serum cobalamin levels between 200 and 400 ng/L may have true CD and are considered borderline levels. Serum cobalamin can be falsely elevated in 22%-35% of patients with PA because of the interaction of IF antibody with IF reagent using the current assay. Several cases of PA with falsely normal or elevated serum cobalamin levels were reported in Refs. (35), (36), (46). Occasionally, serum cobalamin levels may be elevated in several other causes such as chronic myeloid leukemia, carcinoma of liver, and other malignancies due to increased synthesis of cobalamin-binding proteins and in renal insufficiency. Pregnancy is a well-known cause of falsely low serum cobalamin levels.

5.2 Serum holotranscobalamin (HTC) assay

Lack of reliable cobalamin assays has posed a challenge to physicians to diagnose CD and PA. HTC is the only active form of cobalamin, which is bound to transcobalamin, and available for cellular metabolism. The level of plasma HTC was first tested as an early marker of CD in late 1980s. Later, plasma holotranscobalamin complex (HTC) level can be measured more accurately by using more reliable assays. The reference range for plasma HTC is 40-200 pmol/L, albeit it largely varied with patient demographics and genotypes of TC. Any level of serum HTC < 35 pmol/L is equivalent in diagnostic accuracy to serum cobalamin level < 148 pmol/L (< 200 ng/L) for screening of CD. False elevation of serum HTC was reported in cases with renal and liver insufficiency. In combination with serum cobalamin results, the diagnostic accuracy is better than the individual assays.

5.3 Serum methylmalonic acid (MMA) and total plasma homocysteine

Serum MMA and total plasma homocysteine are metabolites of the cobalamin and are now available to diagnose patients with CD in patients with borderline or falsely normal/high cobalamin levels. Studies showed that high MMA and total homocysteine levels are reported in > 95% of patients with CD, but those levels usually drop rapidly in response to B12 replacement. The levels of metabolites usually return to normal within 1-2 weeks after the onset of cobalamin therapy. Therefore, measurement of MMA and total homocysteine level has become another welcome addition to the diagnostic tools, which used to ascertain adequate cobalamin replacement. Avoiding large protein meals 6-8 hours before sample collection is recommended to overcome spurious elevation of homocysteine. Homocysteine may be falsely normal or low in patients who are on folic acid-fortified diet. Spurious elevation of MMA level occurred

### Table 1

<table>
<thead>
<tr>
<th>Laboratory tests</th>
<th>Results</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>FBC and PBS</td>
<td>Anemia Macro-ovalocytes Hypersegmented neutrophils</td>
<td>≥ 95%</td>
<td>Not known</td>
<td>May mimic other bone marrow disorders such as myelodysplastic syndromes</td>
</tr>
<tr>
<td>Serum vitamin B12</td>
<td>&lt; 200 ng/L</td>
<td>95%</td>
<td>Not known</td>
<td>Spurious elevation can be seen in hematologic and solid cancers and in renal failure Spuriously normal or high levels due to interference by IF antibodies False low in pregnancy and folate deficiency</td>
</tr>
<tr>
<td>Homocysteine</td>
<td>Higher than upper limit of normal</td>
<td>≥ 95%</td>
<td>Not known</td>
<td>Elevated in renal failure and after large protein meal Spuriously low or normal with folic-acid fortified food</td>
</tr>
<tr>
<td>MMA</td>
<td>Higher than upper limit of normal</td>
<td>≥ 95%</td>
<td>Not known</td>
<td>Elevated in renal failure, old age, volume contraction and intestinal bacterial overgrowth</td>
</tr>
<tr>
<td>Serum HTC</td>
<td>&lt; 35 pmol/L</td>
<td>≥ 95%</td>
<td>Not known</td>
<td>Elevated in renal failure and liver disease</td>
</tr>
<tr>
<td>IF antibodies</td>
<td>positive</td>
<td>40%-80%</td>
<td>Almost 100%</td>
<td>Spuriously positive after vitamin B12 injections</td>
</tr>
<tr>
<td>PC antibodies</td>
<td>positive</td>
<td>55%-80%</td>
<td>90% for CAG</td>
<td>Positive in autoimmune thyroid disease, vitiligo, celiac disease and normal population</td>
</tr>
</tbody>
</table>

Abbreviations: CAG: chronic atrophic gastritis; FBC: full blood count; HTC: holo-transcobalamin; IF: intrinsic factor; MMA: methylmalonic acid; PBS: peripheral blood smear; PC: parietal cells.
in elderly populations, patients with renal disease, dehydration, and bacterial overgrowth syndrome in intestine.\textsuperscript{90,91} Measurement of plasma MMA and homocysteine is very sensitive to detect CD, but a few patients may have elevated levels of only one metabolite either MMA or homocysteine.\textsuperscript{92} Perhaps, elevated levels of plasma homocysteine and MMA are often the first laboratory markers for the diagnosis of CD before the changes in the PBS and level of plasma cobalamin.\textsuperscript{93}

5.4 Parietal cell antibodies (PCA) and intrinsic factor antibodies (IFA)

The diagnosis of PA requires the presence of IFA in the serum in the majority of patients with PA. Presence of IFA can confirm indirectly IF deficiency in clinical practice. IFA is detected in 40%-60% of patients with PA and the rate of positivity of the antibody rises with the disease progression. The specificity of IFA testing is distinctly very high (almost 100%).\textsuperscript{94–96} Autoimmune CAG is caused by antiparietal cell antibodies which can be used as a serological marker to diagnose PA.\textsuperscript{97} Antiparietal cell antibody testing is relatively sensitive in 85% of PA.\textsuperscript{98} However, antiparietal cell antibodies are not specific as they may also be present in patients with autoimmune polyendocrine diseases and 3%-10% of normal healthy populations.\textsuperscript{99} Dual testing of IFAs and PCAs with new ELISA method in PA patients with CAG on biopsy yielded 73% sensitivity and 100% specificity for diagnosis of PA.\textsuperscript{96} It is vital to obtain IFA before cobalamin replacement to overcome false positive results.\textsuperscript{71} Table 1 illustrates the diagnostic performance, interpretations, and limitations of currently available tests in the diagnosis of PA.

6 HOW TO REACH TO THE CORRECT DIAGNOSIS OF PA IN THE TWENTYFIRST CENTURY

A patient suspicious of CD clinically and/or macrocytosis with reticulocytopenia should be investigated for measurement of serum cobalamin, complete iron study, and folate levels. Diagnosis of CD requires serum cobalamin level of $< 200$ pg/mL ($\mu g/L$). Macro-ovalocytes or
HSN are characteristic PBS features of CD. Yet, HSNs are not pathognomonic of CD. HSNs could be observed in up to 98.6% of cases with CD in one study,\textsuperscript{97} and 91% of CD cases in another study.\textsuperscript{100} Thus, the sensitivity of HSNs is very good, and morphological examination of PBS may also reveal other concurrent conditions.

Diagnostic performance may be enhanced by combined measurement of both serum HTC and serum cobalamin levels. Furthermore, plasma homocysteine and serum MMA levels should be measured to establish the CD diagnosis in the setting of cases with borderline serum cobalamin levels.\textsuperscript{101} When there is high clinical suspicion of CD, but for patients with normal or high serum vitamin B12 levels, the measurement of fasting homocysteine and MMA may yield the diagnosis of CD. Diagnosis of PA requires the investigation of PCA and IFA. The combined sensitivity of both PCA and IFA is approximately 73% and hence nearly 30% of PA cases may be negative for PCA and IFA (antibody-negative PA).\textsuperscript{96,102} Histological examination of bone marrow may be required in rare circumstances, which almost always reveals megaloblastic erythropoiesis and maturation arrest of the myeloid precursors. The hallmark of PA is autoimmune CAG characterized by elevated fasting serum gastrin level and therefore fasting serum gastrin assay play a role in establishing the PA diagnosis in difficult cases.\textsuperscript{103} Rarely, gastric biopsy may be necessary to prove CAG in extremely difficult cases.\textsuperscript{103} A therapeutic trial of cobalamin injections should be considered in very difficult cases with a very high index of suspicion. The reticulocyte count usually rises in the first 5 days of cobalamin replacement although it may take up to 2 months for complete normalization of hemoglobin and hematocrit. Figure 1 illustrates the suggested algorithm for the diagnosis of PA.

Schilling test is no longer available and is mainly of historical interest. It is a two-staged test. In the stage I, the patient is administered 2 \( \mu \)g of radiolabeled cobalamin orally. Two hours later, 1000 micrograms of subcutaneous unlabeled cobalamin is given. Then, urine is collected for 24 hours for monitoring of the radioactive cobalamin excretion. If stage I is abnormal (low level of radiolabeled cobalamin in the urine), stage II is performed. Stage II means that the stage I is repeated along with an oral dose of IF. If the stage II shows normal urinary excretion of cobalamin with oral dose of IF, it means that the patient has low IF level and confirms PA.\textsuperscript{104}

7 | CONCLUSION

Despite the entire armamentarium, PA remains diagnostic challenge to many clinicians because of the lack of reliable cobalamin assays to diagnose CD and PA being the great clinical mimicker. Understanding the pathophysiology, and diagnostic conundrum is of paramount importance not only to aid in reaching the correct diagnosis and to initiate proper treatment but also to prevent neurological sequelae from delayed management. Despite the availability of a highly effective and safe therapy, PA continues to represent a significant medical problem. The multiple issues with automated diagnostic methods, the ample spectrum of its clinical presentations, and similarities between CD-related BM morphological findings and those of the primary BM disorders make the PA diagnosis very difficult and challenging. Careful clinical examination, thorough PBS review, appropriate interpretation of the laboratory findings, and precise evaluation of BM biopsy samples (when appropriate) can lead to the definite diagnosis of PA. Despite diagnostic work-up, the PA diagnosis may remain elusive. For such patients who have a high index of suspicion of PA, a therapeutic challenge with adequate cobalamin therapy should be performed.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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