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CASE SET #22

A Hematology Case:

Megaloblastic Anemia with Neurological Sequelae

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Clinical Laboratory Investigations

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LEARNING OBJECTIVES

Upon completion of reading the case, the learner will be able to:

1. Explain how the laboratory results obtained led to the differential diagnoses considered and how each was eliminated.

2. Interpret laboratory results used to differentiate megaloblastic anemias of different etiologies.

3. Explain the biochemical basis of pernicious anemia and how it differs from other types of Vitamin B_{12} deficiencies.

4. Correlate the physiology of pernicious anemia with its clinical presentation.

Megaloblastic Anemia with Neurological Sequelae

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PATIENT PRESENTATION

The patient, a 52-year-old female, presented to her local emergency room with violent mood fluctuations, retrograde amnesia and an apparent gait disturbance. The three concerned coworkers who escorted this patient to the hospital expressed concern over her recent episode of severe amnesia followed by uncharacteristic aggression. They reported that it was because of one such episode that they brought her to the hospital today.

After questioning the patient's coworkers, the attending ER physician interviewed the patient. The patient reported persistent feelings of depression and fatigue that seemed to have started at least four months ago. Because she was diagnosed with iron deficiency anemia in the past, the patient had suspected that her fatigue was due to an iron deficiency and, as a result, she had been taking daily iron supplements for the past three months. The patient also reported unusual prickling sensations in her extremities (paresthesias) and pain upon swallowing, though she was unable to confidently report when these symptoms began.

When prompted about her physical activity, the patient admitted feelings of exhaustion with even the slightest amount of physical exertion and dizziness, accompanied by nausea, if she stands up too quickly. She was unable to remember the exact date of her last physical exam but guessed that it was in 2010.

PHYSICAL EXAMINATION

Upon her admission to the hospital, the patient appeared pale, thin and haggard with noticeable hair loss. Her sclera and skin were noted to have a faint yellow tinge, a finding that could be indicative of jaundice. When the patient's reflexes were examined, her patellar reflex was completely absent and her triceps reflex appeared to be extremely attenuated. The patient was asked to stick out her tongue, a task that she found painful at the time, and an inflamed, cherry red tongue with few papules was observed.

Palpation of the patient's abdomen revealed a slightly distended large intestine and slight abdominal bloating but no hepatosplenomegaly. The patient seemed to have a difficult time transitioning from a supine position to an upright sitting position following her abdominal exam and even stumbled when trying to take her original seat in the desk chair provided. This latter observation suggested poor spatial coordination.

CLINICAL APPROACH

The physician suspected that her patient had some form of anemia due to her extreme pallor and chronic fatigue. A complete blood count (CBC) with differential was ordered to determine the extent and possible etiology of the patient's anemia. Analyses of serum iron, ferritin, folate and vitamin B_{12} levels were also performed.[1,2]

The slight yellow tinge of the patient's skin and sclera was suggestive of jaundice. Because of this, the patient's total serum bilirubin was quantified. To determine whether the jaundice was of pre-hepatic, intra-hepatic, or post-hepatic origin, the fraction of the total bilirubin attributable to direct bilirubin was also calculated.[1,3] The

concentration of the patient's serum haptoglobin was quantified to gauge levels of extravascular hemolysis. Serum concentrations of clinically significant enzymes (e.g. aspartate aminotransferase [AST], alanine aminotransferase [ALT], alkaline phosphatase [ALP], amylase [AMY], lipase, lactate dehydrogenase [LDH], etc.) were quantified to shed further light on the patient's condition.[1,2,3]

The patient's poor spatial coordination, areflexia, paresthesias, depression and signs of retrograde amnesia were indicative of neurological dysfunction. Whether or not these symptoms stemmed from the same condition causing the patient's anemia and jaundice or from a completely separate condition remained to be discovered. The attending physician was particularly concerned that the patient may have been presenting with early signs of multiple sclerosis (MS), therefore a lumbar puncture and a series of Magnetic Resonance Imaging tests (MRIs) were ordered.[4] Although additional tests could have been ordered to confirm the patient's apparent peripheral neuropathy, her attending physicians deemed these tests to be unnecessary as the patient's areflexia and paresthesias were considered to be diagnostic of peripheral neuropathy.[1]

SIGNIFICANT LABORATORY RESULTS

Results of the CBC and peripheral blood smear can be found in Table I.

Table I: Complete Blood Count (CBC) with Differential

	Patient's Results	Reference Range[1]
Red Blood Cell Count (RBC)	2.1 x 10^6/μL	4.2-5.4 x 10^6/μL
Hemoglobin (HgB)	7.5 g/dL	12-16 g/ dL
Hematocrit (Hct)	22.5%	37-47%
Mean Corpuscular Volume (MCV)	107.14 fL	80-100 fL
Mean Corpuscular Hemoglobin (MCH)	35.7 pg	27-31 pg
Mean Corpuscular Hemoglobin Concentration (MCHC)	33.3 g/dL	32-36 g/dL
Red Cell Distribution Width (RDW)	23%	11.5-14.5%
Reticulocyte Count	15 x 10^3/μL	24-84 x 10^3/μL
Reticulocyte Production Index (RPI)	0.179	0.5-2.5
Platelets	195 x 10^3/μL	150-400 x 10^3/μL
White Blood Cell Count (WBC)	2.9 x 10^3/μL	4.8-10.8 x 10^3/μL
Lymphocytes	39% 1.13 x 10^3/μL	20-40% 1.2-3.4 x 10^3/μL
Neutrophils	50% 1.45 x 10^3/μL	50-70% 1.4-6.5 x 10^3/μL
Bands	1% 0. 03 x 10^3/μL	2-6% 0-0.7 x 10^3/μL
Monocytes	6% 0.17 x 10^3/μL	2-9% 0.11-0.59 x 10^3/μL
Eosinophils	3% 0.09 x 10^3/μL	0-4% 0-0.5 x 10^3/μL
Basophils	1% 0.03 x 10^3/μL	0-2% 0-0.2 x 10^3/μL

The patient's markedly decreased RBC count, hemoglobin (HgB) and hematocrit (Hct) coupled with her elevated mean corpuscular volume (MCV) and normal mean corpuscular hemoglobin concentration (MCHC) were indicative of a megaloblastic anemia. Her significantly elevated RBC width distribution (RDW) was suggestive of anisocytosis, a suspicion that was to be confirmed on her differential.[1] The patient's depressed reticulocyte production index (RPI) suggested that her bone marrow was unable to adequately respond to her severe anemia, a finding indicative of ineffective hematopoiesis.[1,3,5,6] The patient's leukopenia and accompanying lymphopenia were thought to be secondary to the condition causing her megaloblastic anemia.[1]

An examination of the patient's peripheral blood smear revealed macrocytic erythrocytes with marked anisocytosis. Poikilocytosis with schistocytes, target cells, spherocytes and teardrop-shaped erythrocytes was also present. Multiple nucleated RBCs were apparent on the peripheral blood smear (12/ 100 WBCs). Erythrocyte inclusion bodies including Howell-Jolly bodies and basophilic stippling were also observed. Hyper-segmented neutrophils represented forty-two of the one hundred leukocytes counted (42% of WBCs and 87.5% of neutrophils observed). The technologist performing the patient's differential saw approximately 12 platelets per oil immersion field in the ten fields she counted (Normal range: 8-25/OIF)[1] and estimated that the patient's platelet count was within normal limits.

As part of the evaluation of the patient's anemia, a number of chemistry tests were performed (Table II).

Table II: Initial Chemistry Results Relating to Anemia

	Patient's Results	Reference Range[1,3]
Serum Iron	193 μg/ dL	50-170 μg/ dL
Serum Ferritin	139 μg/ dL	10-120 μg/ dL
Total Bilirubin	5.1 mg/dL	0.0-2.0 mg/dL
Direct Bilirubin	0.3 mg/dL	0.0-0.2 mg/dL
Indirect Bilirubin	4.8 mg/dL	0.0-2.0 mg/dL
Haptoglobin	20 mg/dL	30-200 mg/dL
Serum folate	19 ng/mL	5-16 ng/mL
Serum B_{12}	85 ng/L	200-500 ng/L

The patient's elevated serum iron and ferritin levels, coupled with the patient's elevated MCV and normal MCHC ruled out iron deficiency anemia.[1,3,5,6] The patient's acutely elevated total bilirubin and indirect bilirubin and mildly elevated direct bilirubin suggested increased rates of heme catabolism due to increased intravascular or extravascular hemolysis and/or ineffective erythropoiesis.[1] Her reduced haptoglobin levels confirmed increased rates of intravascular hemolysis.[1] The patient's markedly reduced serum B_{12} levels and mildly elevated serum folate levels were consistent with cobalamin (vitamin B_{12}) deficiency.[1,3,5,6] Additional tests were evaluated to confirm this diagnosis (Table III).

Table III: Enzyme Panel

	Patient's Results	**Reference Range[1,3]**
Aspartate aminotransferase (AST)	29 U/L	<31 U/L
Alanine aminotransferase (ALT)	31 U/L	<34 U/L
Alkaline Phosphatase (ALP)	53 U/L	42-98 U/L
Gamma-Glutamyltransferase (GGT)	32 U/L	<38 U/L
Lipase	36 U/L	<38 U/L
Lactate dehydrogenase (LD)	630 U/L *Mostly LD-1 and LD-2	125-220 U/L

The patient's AST, ALT, ALP, and GGT levels all fell within normal parameters, a finding that supported adequate hepatic and hepatobiliary function and tentatively ruled out intra-hepatic and post-hepatic causes of jaundice.[1,3] A jaundice of pre-hepatic etiology (e.g. increased hemolysis) was inferred and confirmed using the aforementioned hematology results.[1,3] Of the serum enzymes investigated, only LD appeared to be elevated. Alkaline protein electrophoresis was used to separate and quantitate the LD isoenzymes present and the LD-1 and LD-2 isoenzymes were found to predominate. Increased concentrations of LD-1 and LD-2 are non-specific indicators of myocardial or renal injury and/or increased levels of hemolysis as these LD isoenzymes are most abundant in erythrocytes, myocardial tissue and the renal parenchyma.[3] These findings supported a diagnosis of megaloblastic anemia with decreased RBC survival and ineffective erythropoiesis.[1,6]

Magnetic Resonance Imaging (MRI)

Contrast-enhanced MRI revealed symmetrical lesions or “hyper-intensities” in the lateral corticospinal tract and the dorsal column of the cervical and thoracic spinal cord. Similar hyper-intensities were observed in the patient’s prefrontal cortex. MRI hyper-intensities are often interpreted as zones of demyelination.[4]

Zones of demyelination always warrant further investigation. Such zones can be seen in a wide range of hereditary, infectious and acquired disorders (including MS). [4] If central nervous system (CNS) demyelination is a form of neurological sequelae secondary to a treatable condition, the primary condition must be treated as quickly as possible to prevent irreversible neurological damage.[1,3,4]

Additional Laboratory Testing

Based upon the MRI findings, cerebrospinal fluid (CSF) was obtained from the patient and analyzed by laboratory staff. Results of these tests can be found in Table IV.

Table IV: Cerebrospinal Fluid (CSF) Results

	Patient's Result	Reference Range[1,3]
Appearance	Clear, non-viscous	Clear, non-viscous
Total Protein	30 mg/dL	15-45 mg/dL
Glucose	73 mg/dL	50-80 mg/dL
IgG	2.3 mg/dL	0.8-4.2 mg/dL
RBC Count	0/μL	<1/μL
WBC Count	3/μL	0-5/μL

All of the patient's CSF values fell within normal parameters. Of special interest was the patient's normal level of CSF IgG. Such a finding indicates normal levels of IgG intrathecal synthesis and is contraindicative of multiple immunologically meditated demyelinating disorders, including MS.[1,3,4] Normal total protein levels indicated that the patient's blood brain barrier retained its selective permeability, a finding that ruled out meningitis and other inflammatory conditions.[1,3,4] Normal levels of CSF glucose and WBCs suggested that the demyelinating disorder was not of infectious etiology. If bacterial or fungal pathogens were present or the numbers of WBCs and RBCs in the CSF were increased, levels of glucose utilization in the CSF would have increased, causing a corresponding decrease in the amount of glucose present.[1,3,4] The clear, colorless appearance of the CSF would have ruled out CNS hemorrhage had that been a part of the patient's differential diagnoses (CSF in CNS hemorrhage, especially subarachnoid hemorrhage, is typically xanthochromic). [1,3]

DIFFERENTIAL DIAGNOSES AND ADDITIONAL TESTING

After a thorough analysis of her test results, the patient's physician strongly suspected that the patient had a vitamin B_{12} deficiency.[1,2,3,5,6] Though there are multiple causes of vitamin B_{12} deficiency, the patient's clinical presentation as well as her demographic profile (a 52 year old women of Northern European descent) were suggestive of pernicious anemia (PA). [1,5,6] Other causes of vitamin B_{12} deficiency, including malabsorption, blind loop syndrome and inadequate dietary intake, still needed to be definitively ruled out however.[6] Though the clinical presentation of folate deficiency often mirrors that of vitamin B_{12} deficiency, folate deficiency could be ruled out based on the laboratory results collected.[1,3,5,6] The patient's attending physicians selected a targeted battery of tests to confirm a diagnosis of PA.

Gastric Antibodies and Indicators of Gastric Atrophy

A qualitative Enzyme Linked Immunosorbent Assay (ELISA) was performed to determine whether or not anti-intrinsic factor (IF) antibodies were present in the patient's serum. The detection of such antibodies is highly specific for PA (95% specificity), though the test lacks sensitivity as less than 70% of those with PA actually develop anti-IF antibodies. [1,3,5,6] Anti-IF antibodies can typically be placed into one of two possible categories based on their biochemical and clinical properties. Anti-IF antibodies that prevent IF from interacting with cobalamin (vitamin B_{12}) after it has been released from R factors in the duodenum are classified as Type I antibodies. The other identified antibody class (Type II) binds to the cobalamin-IF complex, preventing it from

interacting with ileal receptors. [1,6] No anti-IF antibodies were detected in the patient's serum however.

An additional ELISA was performed to identify anti-parietal cell antibodies. The detection of anti-parietal cell antibodies is highly sensitive for PA (80-90%) but lacks specificity as such antibodies are produced in multiple conditions involving immune gastritis. [1,6] Anti-parietal antibodies are generally produced against the hydrogen-potassium adenosine triphosphatase (H-K-ATPase), a transporter that pulls potassium ions from the gastric lumen while simultaneously transporting a hydrogen ion into the lumen. *Helicobacter pylori* infection is thought to precipitate the production of anti-parietal cell antibodies due to the molecular mimicry of *H. pylori* antigens to the H-K-ATPase.[7] Anti-parietal cell antibodies were found in the patient's serum but because such a finding lacks specificity, additional testing was still required to confirm a diagnosis of PA.

The patient's serum gastrin levels were notably elevated, a finding that suggested prolonged achlorhydria (reduced concentration of hydrochloric acid in gastric secretions, resulting in an increased gastric pH). [1,6,7] Because parietal cells secrete both IF and the HCL, achlorhydria with elevated levels of gastrin is a common finding in PA, though this finding is also not specific to PA. [1,6,7]

Related metabolites were tested to confirm a diagnosis of PA in this patient (Table V).

Table V: Related Metabolites

	Patient's Result	**Reference Range**[3,6]
Serum Methylmalonic Acid (MMA)	631 ng/mL	73-271 ng/mL
Urine MMA	16 mg/day	0-3.4 mg/day
Serum Homocysteine	32 μmol/L	4-15 μmol/L

The patient's levels of serum methylmalonic acid (MMA), urine MMA and serum homocysteine were all elevated, a finding consistent with vitamin B_{12} deficiency and thus PA.[1,3,5,6] In vitamin B_{12} deficiency, MMA accumulates in the serum secondary to the inability of methylmalonyl CoA mutase to convert methylmalonyl CoA to succinyl CoA in the absence of its cofactor, adenosylcobalamin (one of the biologically active forms of vitamin B_{12}).[6,8] In this reaction, adenosylcobalamin acts as an oxidizing agent and receives a hydrogen ion from methylmalonyl CoA. If methylmalonyl CoA fails to be oxidized by adenosylcobalamin and methylmalonyl CoA mutase, CoA will dissociate to generate MMA and free CoA. [1,3,6,8]

Homocysteine also requires vitamin B_{12} as a cofactor in its normal metabolism. Vitamin B_{12} receives a methyl group from methyl tetrahydrofolate (CH_3-THF) to generate tetrahydrofolate (THF), the biologically active form of folate, and methylcobalamin. Methylcobalamin then passes a methyl group to homocysteine to generate methionine, restoring vitamin B_{12} to its previous state. When there are inadequate levels of vitamin B_{12} present, CH_3-THF and homocysteine become

“trapped”; as a result, these 2 molecules tend to accumulate in the blood. This rationale also explains the elevated serum folate observed in earlier testing.[1,6]

DIAGNSOSIS: PERNICIOUS ANEMIA (PA)

Following a review of all laboratory tests performed, the patient’s attending physician was able to definitively diagnose the patient with pernicious anemia (PA). PA is a chronic disorder thought to be autoimmune in nature that causes megaloblastic anemia and/or neurological abnormalities. It is the most common cause of vitamin B_{12} deficiency and is especially common in older women of Northern European descent. [1, 2, 3, 5, 6] The pathophysiology of PA stems from an inability of ileal brush border cells to absorb vitamin B_{12} from B_{12}-IF complexes due to either a lack of IF or the inability of IF to function in a productive manner due to the presence of anti-IF antibodies.[1, 3, 6]

Vitamin B_{12} deficiency impairs thymidine triphosphate (TTP) synthesis because vitamin B_{12} is required to generate tetrahydrofolate (THF), one of the intermediates required in de novo TTP (and thus deoxythymidine [dTTP])synthesis. If dTTP is not present in adequate amounts, deoxyuridine (dUTP) may be incorporated into replicating DNA, resulting in a cessation of DNA replication, nuclear fragmentation and ultimately cell death.[1, 3, 8] The pathologically high ratio of dUTP to dTTP present in PA results in ineffective hematopoiesis.[5]

Ineffective hematopoiesis affects all cell lines. However, abnormalities are most pronounced in the erythroid line. The megaloblastic erythroid precursors generated in PA often lyse in the bone marrow before ever becoming functional RBCs. This results in ineffective erythropoiesis related to increased levels of intramedullary hemolysis (as

indicated by a reduced reticulocyte index) and elevated levels of serum iron, LD-1 and LD-2. The low oxygen-carrying capacity of the blood stimulates a flood of erythropoietin (EPO) from renal fibroblasts with hypoxia- inducible transcription factors in an attempt to correct the apparent anemia. Elevated levels of EPO stimulate the differentiation and maturation of erythroid precursors, increasing the cellularity of the bone marrow and driving down the myeloid to erythroid ratio of the bone marrow. [1,5]

The patient had multiple central and peripheral neuropathies secondary to her PA. The lesions of demyelination present on the patient's cervical and thoracic spinal cord can be collectively referred to using the umbrella medical term "subacute combined degeneration of the dorsal and lateral spinal columns".[1,4] The exact pathological mechanisms underlying the neurological disturbances observed in PA have yet to be elucidated, though several hypotheses exist. The most plausible of these hypotheses suggests that myelin formation is impaired due to the inability of methionine synthase to methylate homocysteine in the absence of methylcobalamin, resulting in impaired methionine production. Methionine is used to generate S-adenosyl methionine (SAM), an intermediate required for methylation reactions involved in myelin synthesis. It thus logically follows that an inability to generate methionine results in an inability to generate myelin.[1,6]

TREATMENT PLAN AND PROGNOSIS

The patient was to receive subcutaneous or intramuscular vitamin B_{12} injections for the rest of her life. Injections (100-1,000 µg) were given daily for the first two weeks immediately following diagnosis. The patient then received weekly injections of vitamin

B_{12} until her hematological values fell within normal parameters. At that time, the patient was tapered off to a monthly parenteral injection of vitamin B_{12}.[1,3,6] The patient's RBC indices, especially her reticulocyte counts, were used to monitor her recovery.[1] Though the patient's hematological parameters returned to normal levels within 6 weeks, whether or not she will make a full neurological recovery remains less certain.[1,7,8]

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