

Educational Case: AA Amyloidosis Complicating Common Variable Immunodeficiency

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The following fictional case is intended as a learning tool within the Pathology Competencies for Medical Education (PCME), a set of national standards for teaching pathology. These are divided into three basic competencies: Disease Mechanisms and Processes, Organ System Pathology, and Diagnostic Medicine and Therapeutic Pathology. For additional information, and a full list of learning objectives for all three competencies, see <http://journals.sagepub.com/doi/10.1177/2374289521994236>.¹

Keywords

pathology competencies, disease mechanisms, immunological mechanisms, immune dysfunction, immunodeficiencies, common variable immunodeficiency, acute phase proteins, amyloidosis

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Primary Objective

Objective IM1.9: Immunodeficiencies. Compare and contrast the genetic basis and inheritance patterns of the well-defined primary immunodeficiency syndromes, discuss the pathogenesis and clinical sequelae of these disorders, and describe therapeutic interventions that can mitigate or correct them.

Competency 1: Disease Mechanisms and Processes; Topic IM: Immunological Mechanisms; Learning Goal 1: Immune Dysfunction.

Secondary Objective

Objective SP1.2: Differential Diagnosis. List the major differential diagnoses for each type of cytology or surgical pathology specimen derived from a lesion or mass and describe appropriate further studies, both special stains and immunohistochemistry.

Competency 3: Diagnostic Medicine and Therapeutic Pathology; Topic SP: Surgical Pathology; Learning Goal 1: Role in Diagnosis.

Patient Presentation

A 60-year-old man presents to his primary care doctor for evaluation of new onset lower extremity swelling and fatigue of several weeks duration. His past medical history is significant for hypertension and common variable immune deficiency (CVID) and bronchiectasis complicated by frequent pneumonia (approximately 1 episode per year). In addition, he receives monthly intravenous immunoglobulin (IVIg) therapy and has a long history of nonsteroidal antiinflammatory drug (NSAID) use.

Physical examination reveals a man in no acute distress, speaking without shortness of breath. The vital signs are a temperature of 97.7 °F, heart rate of 73 beats per minute, blood pressure of 146/90 mm Hg, and respiratory rate of 20 breaths

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per minute with pulse oximeter showing 94%. There is pitting edema (peripheral edema of the lower extremity demonstrates tissue depression after pressure is applied to the edematous legs for 5 seconds). There is no increase in jugular venous pressure, and the auscultation of the heart reveals a normally placed apex beat and no murmur is appreciated. Lung sounds are clear bilaterally without rales or crackles. Hepatomegaly is not appreciated on palpation of the mildly distended soft abdomen.

Diagnostic Findings, Part I

Initial laboratory work up in this patient demonstrates plasma natriuretic peptide 41 pg/mL (expected < 100 pg/mL) and troponin 0.1 ng/mL (expected <0.4 ng/mL). Complete metabolic panel reveals aspartate aminotransferase (AST) 10 U/L (expected 5-40 U/L), alanine aminotransferase (ALT) 7 U/L (expected 7-56 U/L), albumin 2.5 g/dL (expected 3.4-5.4 g/dL), and creatinine 4.39 mg/dL (expected 0.8-1.2 mg/dL). Complete blood count is significant for platelet count of 115 000 (expected 150 000-350 000) and lymphocyte count of $0.65 \times 10^9/L$ (expected $1.0-4.0 \times 10^9/L$). Urinalysis shows 3+ protein and trace hematuria.

Questions/Discussion Points, Part I

What Is the Differential Etiology for an Increase in Interstitial Fluid Volume Leading to Edema?

An increase in interstitial fluid volume leading to edema may result from heart, liver, or kidney disease. Normal plasma natriuretic peptide and troponin levels support normal heart function. Note that albumin is low, which may be a consequence of decreased production or increased loss. Albumin is produced in the liver; however, AST and ALT were within normal range indicating a lack of damage to the liver cells. Urinalysis demonstrates 3+ proteinuria, supporting an increased loss of albumin (quantification of proteinuria can be performed via a 24-hour urine protein collection). Thus, in this patient with CVID, the most likely underlying etiology for his edema is kidney injury, as further supported by the increase in serum creatinine.

What Is the Hallmark Immune Defect in Common Variable Immune Deficiency?

This primary immunodeficiency is characterized by defective B cell terminal differentiation into plasma cells and memory B cells with impaired secretion of immunoglobulin. Underlying the clinical diagnosis of CVID is a heterogeneous mix of genetic mechanisms, with examination by high resolution sequencing of the B cell receptor showing a range of multiple abnormalities including aberrant gene rearrangement, impaired somatic hypermutation, decreased diversity of the naïve B cell repertoire, and abnormal expansion of unmutated B cell clones.^{2,3}

What Are the Clinical Manifestations of Common Variable Immune Deficiency?

As its name implies, CVID is the most common form of primary immunodeficiency with approximately 1 in 25 000 individuals estimated to be affected, and its clinical manifestations are variable and heterogeneous with the vast majority of individuals experiencing recurrent infections, autoimmunity, chronic lung disease/bronchiectasis, gastrointestinal disease, and a heightened susceptibility to lymphoma.^{4,5}

What Infections Are Individuals With CVID Most Susceptible to and Can We Protect Them With Vaccination?

Patients are particularly susceptible to encapsulated bacteria including *Streptococcus pneumoniae* and *Hemophilus influenzae*, due to the importance of a robust immunoglobulin response in countering infections by these organisms. While both of these pathogens have effective vaccines, vaccination success requires the ability to produce memory B cells and antibodies, which patients with CVID cannot accomplish. Thus, although vaccines recommended for the general population are generally recommended for patients with severe antibody deficiency, live vaccines (MMR, VZV) are contraindicated (potentially lethal adverse reactions may occur) and partial/incomplete immune protection has to be expected. Additionally, antibodies contained in immunoglobulin preparations interfere with the development of active immunity after vaccination.⁶ Therefore, the mainstay of treatment is immunoglobulin replacement (IVIg) to reduce the burden of recurrent infection and subsequent complications. To protect those who cannot respond to vaccination, household members should be vaccinated and well-informed about the risk for the patient by viral/bacterial shedding in the course of infection/vaccination of close contacts.

What Is the Cause of Common Variable Immune Deficiency?

The cause of CVID is unknown for most patients and is likely attributable to both environmental and genetic factors. Mutations in at least 13 genes involved in the development and function of B cells have been associated with CVID. The most frequent mutations occur in the *TNFRSF13B* gene, which is normally responsible for survival and maturation of B cells and in the production of antibodies. Most mutations are sporadic without a family history of disease, but in rare cases, CVID is inherited in an autosomal recessive pattern (both copies of the gene have mutations) and occasionally CVID is inherited in an autosomal dominant pattern (one copy of the altered gene is sufficient to cause disease).

Compare the genetic inheritance pattern of CVID with that of the 2 other best-recognized and most common B-cell immunodeficiencies, X-linked agammaglobulinemia and selective immunoglobulin A (IgA) deficiency.

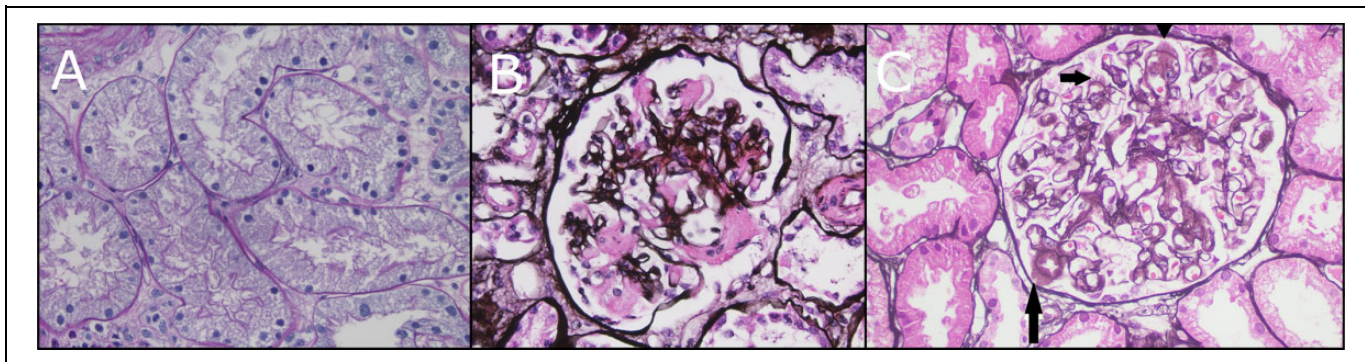


Figure 1. Light microscopy shows tubular epithelial cells with cytoplasmic isometric vacuolization and intact brush borders on Periodic Acid-Schiff (PAS) stain (A; $\times 400$). Glomeruli show mild expansion of mesangial matrix by amorphous, eosinophilic, acellular material on silver stain with involvement of adjacent arteriole (B; $\times 400$) and segmental expansion of glomerular basement membranes by similar material resulting in the appearance of feathery spikes on silver stain (C with arrows; $\times 200$).

X-linked agammaglobulinemia results from mutations in the Bruton's tyrosine kinase (BTK) gene that makes the BTK protein. Absence of functional BTK protein precludes the development of B cells and leads to a lack of antibody production. As its name implies, this condition is inherited in an X-linked recessive pattern. The gene is located on the X chromosome. In females with 2 X chromosomes, a mutation will have to occur in both copies of the genes to cause the disorder, whereas in males with one X chromosome, only one altered copy is necessary. A characteristic of X-linked inheritance is that fathers cannot pass X-linked traits to their sons.

Selective IgA deficiency is a primary immune deficiency disorder in which an individual has complete absence or severe deficiencies of IgA. Immunoglobulin A is essential in the respiratory and gastrointestinal tract for mucosal immunity. Similar to CVID, it is typically inherited as either an autosomal dominant or autosomal recessive trait, but the underlying cause is unknown.

Diagnostic Findings, Part 2

The findings of elevated serum creatinine, proteinuria, and hematuria with normal liver and cardiac function suggest kidney disease as the underlying etiology of our patient's edema. The 24-hour urine collection demonstrated 1.1 g/d of proteinuria. A kidney biopsy was performed and is shown in Figures 1, 2, and 3.

Questions/Discussion Points, Part 2

*What Does the Kidney Biopsy Show on Light Microscopy?
What Is the Differential Diagnosis for These Findings?*

Figure 1A shows swelling of tubular epithelial cells with cytoplasmic isometric vacuolization, and preservation of the luminal brush border on PAS stain. These findings are consistent with osmotic tubular injury, which is a cause of acute kidney injury (increase in serum creatinine). Osmotic tubular injury is most commonly associated with therapeutic volume expanders,

such as dextran, mannitol, and hydroxyethylstarch intravenous infusion, and can also be seen with high-osmolar intravascular radiocontrast agents and IVIg infusion. Persistent acute kidney injury lasting months after initial injury can occur in up to 20% of IVIg recipients. This may explain our patient's elevated serum creatinine, but does not fully account for the extent of his proteinuria.

The glomerulus in Figure 1B shows mild expansion of mesangial matrix by acellular, amorphous, eosinophilic material without increased mesangial cellularity. Glomerular basement membranes show segmental deposition of the same material resulting in segmental feathery spikes on silver stain (Figure 1C, arrows). In addition, Figure 1B shows focal deposition of this material in the tunica media of arterioles at the glomerular vascular pole. This appearance by light microscopy suggests amyloidosis. Other rare causes of similar glomerular findings by light microscopy, like fibrillary glomerulonephritis, are rendered unlikely by the pattern of extraglomerular deposition.

What Does the Kidney Biopsy Show on Electron Microscopy?

Electron microscopy demonstrates cytoplasmic vacuoles and lysosomes in proximal tubule epithelium with preserved brush border (Figure 2A). Within glomeruli, there are randomly arranged, nonbranching fibrils within the mesangium (Figure 2B) and glomerular basement membranes (Figure 2C). The average diameter of the fibrils, about 10 nm, (Figure 2D) is consistent with amyloid fibrils. Fibrillary glomerulonephritis would show similarly randomly arranged, nonbranching fibrils, but with an average diameter of 20 nm.

Light and Electron Microscopy Favor Amyloidosis. What Stain, if Positive, Is Diagnostic for Amyloidosis?

Congo red special stain positivity is the gold standard for the diagnosis of amyloidosis (Figure 3). The characteristic apple-green birefringence under polarized light is due to the parallel

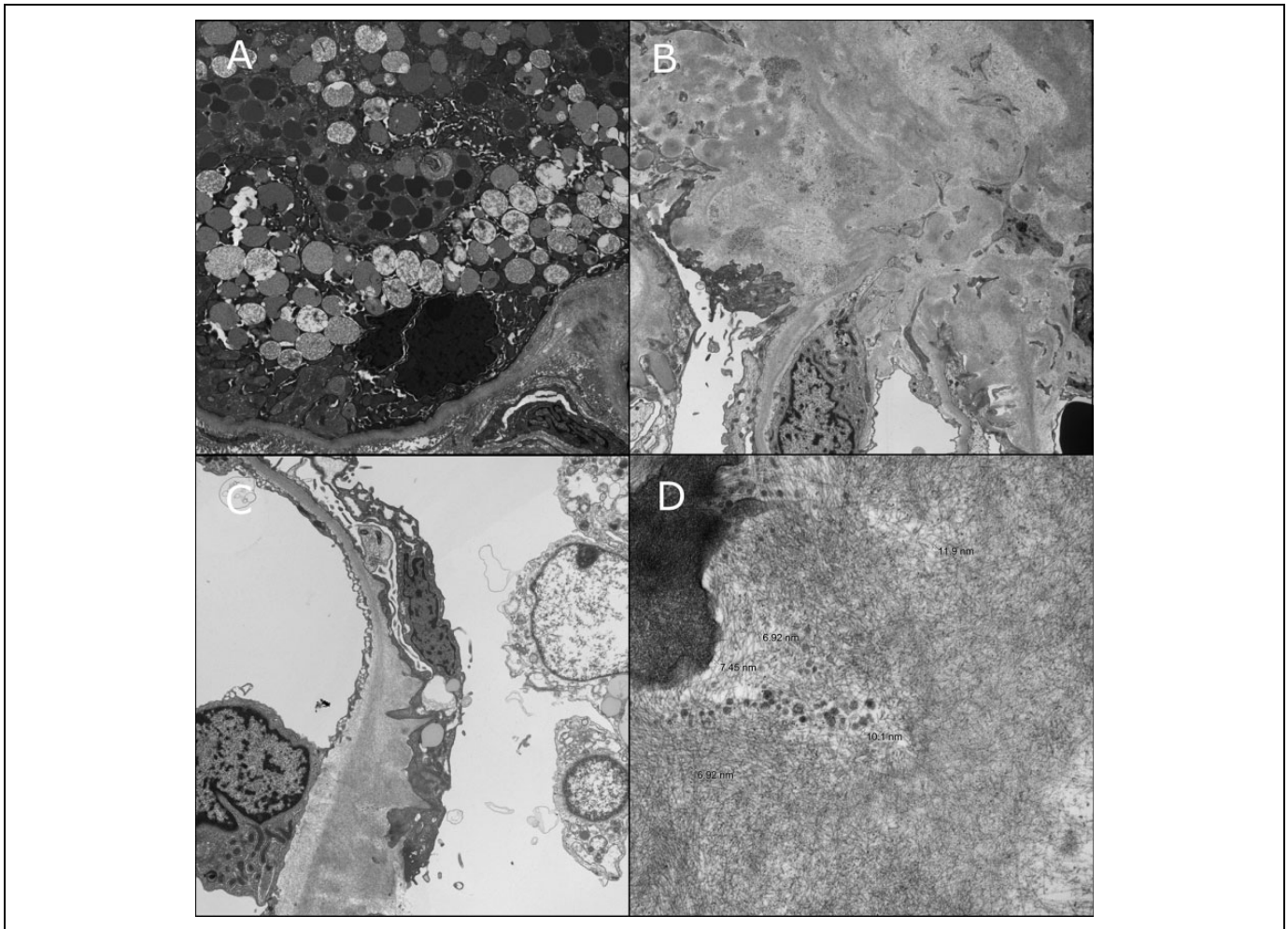


Figure 2. Electron microscopy shows isometric vacuoles and pronounced lysosomes within tubular epithelial cells (A; $\times 3500$). Corresponding to the amorphous material noted by light microscopy, there is deposition of randomly arranged, nonbranching fibrils within the mesangium (B; $\times 3500$) and along glomerular basement membranes (C; $\times 4400$) which measure approximately 7 to 12 nm in diameter (D; $\times 28000$).

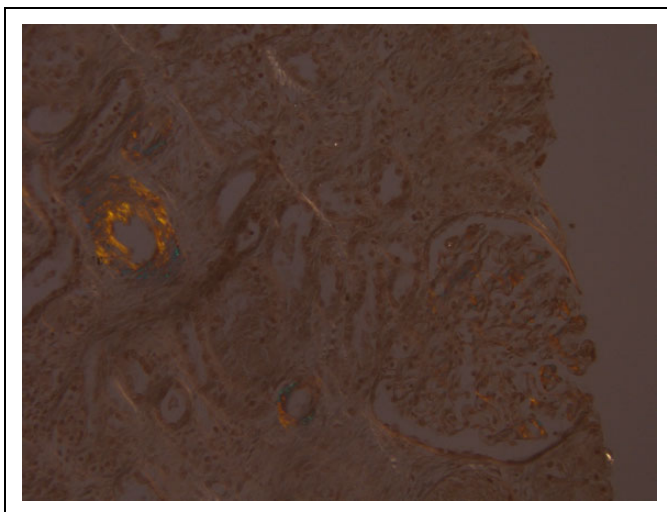


Figure 3. A positive Congo red special stain for amyloid shows apple-green birefringence under polarized light within interlobular arteries, arterioles, and glomeruli ($\times 200$).

alignment of dye molecules along the beta-pleated sheet conformation of the amyloid fibril.

What Additional Studies Can be Performed to Identify the Type of Amyloid Present?

Over 30 human proteins have been recognized as causative agents in systemic and/or localized amyloidosis⁷; thus, the classification of the amyloid protein is critical for diagnosis of the underlying disease process to provide appropriate treatment and more accurate prognosis (Figure 4). The 5 most frequent classes of amyloid proteins (see Table 1) account for up to 95% of cases, all of which have commercially available antibodies for identification.⁸

Immunofluorescence microscopy (IF) is a routine staple of the renal pathologists' diagnostic toolkit. In this case, immunofluorescence for kappa and lambda light chains were both negative, helping to eliminate AL amyloidosis from the

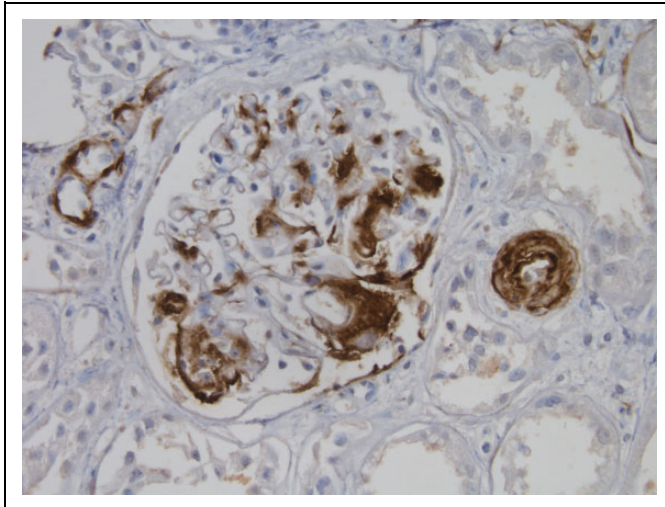


Figure 4. An immunohistochemical stain for AA amyloid is positive within the glomerular tuft and arterioles ($\times 400$).

Table 1. Five Most Frequent Classes of Amyloid.

Protein	Abbreviation
Light chains (kappa and lambda)	AL
Transthyretin	ATTR
Serum amyloid A	AA
$\beta 2$ -microglobulin	A β 2M
Leukocyte chemotactic factor-2	ALECT2

differential. An immunohistochemical stain (IHC) for AA amyloid was positive (Figure 4).

Another technique that can be used for amyloid typing is liquid chromatography (LC) combined with mass spectrometry (MS) following laser microdissection (LMD) of areas that are positive on Congo red stain on formalin-fixed paraffin-embedded tissue. This LMD-LC-MS technique results in a signature proteomic profile that identifies the type of amyloid with great sensitivity and specificity, and is predominantly used when more readily available techniques (IF and IHC) are inconclusive.⁹

What Is the Connection Between the Patient's Clinical History and Deposition of AA Amyloid?

Serum amyloid A (SAA) is an acute-phase protein produced in the liver and released in response to inflammation or infection stimulated by pro-inflammatory cytokines such as interleukin-6, tumor necrosis factor, and interferon- γ . It is also the precursor of amyloid A protein, a fibrillar, insoluble product. In this patient with a primary immunodeficiency and a history of bronchiectasis (permanent dilation of bronchi and bronchioles due to destruction of elastic tissue and muscle by chronic necrotizing infections), chronic inflammation and infection put him at risk for AA amyloidosis due to the increased synthesis of SAA.

What Is Your Diagnosis, Based on the Clinical Information and Microscopic Findings?

This patient's renal biopsy shows AA amyloidosis involving glomeruli and arteries, and diffuse acute tubular injury with isometric vacuolization. A history of chronic inflammation and immune activation leads to upregulation of pro-inflammatory markers, which can lead to an increased risk of amyloid deposition. In addition, the patient receives chronic IVIg therapy for his underlying CVID. This hyperosmotic solution is filtered and absorbed by proximal tubules through pinocytosis. The solute is retained in endosomes creating an intracellular oncotic gradient. Water absorption causes hydropic swelling of the cytoplasm. In summary, both findings are attributable to the patient's underlying CVID due to recurrent infections and IVIg therapy, respectively. AA amyloidosis was the result of increased production of SAA in this patient whose underlying immunodeficiency caused frequent infections and chronic inflammatory state. They amyloidosis resulted in loss of protein, including albumin, which resulted in peripheral edema due to decreased oncotic pressure. His acute kidney injury, as evidenced by the rise in creatinine, was mostly attributable to the acute tubular injury from his IVIg therapy.

Teaching Points

- Common variable immunodeficiency is a common primary immunodeficiency defined by markedly reduced serum concentrations of immunoglobulin G (IgG), IgA, and/or IgM; poor or absent response to immunizations; and an absence of any other defined immunodeficiency state.
- Primary immunodeficiencies are caused by genetic abnormalities that prevent the development of normal immune responses. Several types of inherited mutations can cause primary immunodeficiencies.
 - Autosomal dominant: An abnormal gene is inherited from one parent and disease is manifested.
 - Autosomal recessive: Two abnormal genes must be present for disease to develop.
 - X-linked recessive: The gene is located on the X chromosome. Males inheriting a single abnormal X chromosome will manifest the disease. In contrast, females who have 2 X chromosomes require 2 abnormal genes to be affected.
- Recurrent infections are a common complication of those affected, in addition to chronic lung disease, gastrointestinal disease, autoimmune disease, and an increased risk of lymphoma.
- Serum amyloid A is an acute-phase protein. Any chronic inflammatory state that elevates SAA protein for a long time has potential to lead to AA amyloidosis.
- Serum amyloid A is one of over 30 proteins known to cause amyloidosis.

- The most common organ involved by AA amyloid is the kidney, typically with glomerular amyloid deposition leading to nephrotic syndrome.
- Amyloid appears as deposition of acellular, amorphous, eosinophilic material by light microscopy which is comprised of randomly arranged, nonbranching fibrils measuring an average of 10 nm in diameter by electron microscopy. Amyloid shows Congo red positive staining with apple-green birefringence under polarized light, the gold standard for the histologic diagnosis of amyloid.

Declaration of Conflicting Interests

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