Amyloidosis of the Lung

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- **Context.**—Amyloidosis is a heterogeneous group of diseases characterized by the deposition of congophilic amyloid fibrils in the extracellular matrix of tissues and organs. To date, 31 fibril proteins have been identified in humans, and it is now recommended that amyloidoses be named after these fibril proteins. Based on this classification scheme, the most common forms of amyloidosis include systemic AL (formerly primary), systemic AA (formerly secondary), systemic wild-type ATTR (formerly age-related or senile systemic), and systemic hereditary ATTR amyloidosis (formerly familial amyloid polyneuropathy). Three different clinicopathologic forms of amyloidosis can be seen in the lungs: diffuse alveolar-septal amyloidosis, nodular pulmonary amyloidosis, and tracheobronchial amyloidosis.

**Objective.**—To clarify the relationship between the fibril protein–based amyloidosis classification system and the clinicopathologic forms of pulmonary amyloidosis and to provide a useful guide for diagnosing these entities for the practicing pathologist.

**Data Sources.**—This is a narrative review based on PubMed searches and the authors’ own experiences.

**Conclusions.**—Diffuse alveolar-septal amyloidosis is usually caused by systemic AL amyloidosis, whereas nodular pulmonary amyloidosis and tracheobronchial amyloidosis usually represent localized AL amyloidosis. However, these generalized scenarios cannot always be applied to individual cases. Because the treatment options for amyloidosis are dependent on the fibril protein–based classifications and whether the process is systemic or localized, the workup of new clinically relevant cases should include amyloid subtyping (preferably with mass spectrometry–based proteomic analysis) and further clinical investigation.


The term *amyloid* (starchlike) was introduced into the medical literature by Rudolph Virchow in 1854, when he made the observation that corpora amylacea in the brain stained similarly to starch, that is, stained pale blue after treatment with iodine, and violet upon the subsequent addition of sulfuric acid.1,2 The name *amyloid* now encompasses a wide variety of fibrillar proteins that exhibit similar tinctorial, ultrastructural, and x-ray diffraction properties.

In hematoxylin-eosin–stained sections, amyloid appears as homogeneous eosinophilic material. Congo red–stained deposits are orange-red with bright field microscopy and display apple-green birefringence under polarized light (Figure 1, A and B). Ideally, Congo red staining is performed on 10-μm sections, and the apple-green birefringence is often best appreciated with the ambient room lights off. This apple-green birefringence under polarized light is considered the gold standard for identifying a substance as amyloid in histologic sections. In addition to Congo red, amyloid can also be stained by thioflavin T and metachromatic dyes, such as crystal violet. Electron microscopy scans of amyloid typically reveal haphazardly arranged nonbranching fibrils that measure 8 to 10 nm in diameter2 (Figure 2), and x-ray diffraction demonstrates a characteristic cross-B pattern.3

Approximately 95% of amyloid is composed of fibril proteins, and the remaining 5% consists of serum amyloid P component and other glycoproteins. The insoluble fibril proteins originate from improper folding of soluble precursors because some proteins with a normal amino acid sequence are prone to misfolding when they are produced in an excessive amount (eg, immunoglobulin light chains, serum amyloid A, and wild-type transthyretin). Misfolding may also result from an abnormal amino acid sequence (eg, transthyretin variants). To date, 31 human fibril proteins have met the criteria from the International Society of Amyloidosis (ISA) Nomenclature Committee.4 These criteria include unambiguous characterization of the amyloid fibril protein by protein sequence analysis and publication of the findings in a peer-reviewed journal. The fibril proteins most commonly encountered by pathologists include AL, AA, and ATTR. Precursor proteins for AL, AA, and ATTR fibril proteins are immunoglobulin light chains, (apo) serum amyloid A, and transthyretin (wild type and variants), respectively.

Major amyloid subtypes can be identified by immunohistochemistry. Antibodies are readily available for κ and λ light chains, serum amyloid A, and transthyretin (prealbumin). Immunohistochemistry can be performed on

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frozen or paraffin sections with immunofluorescence or immunoperoxidase techniques, respectively. However, the classification of amyloid by immunohistochemistry is often challenging owing to abundant background staining. Laser microdissection followed by mass spectrometry–based proteomic analysis can be performed on formalin-fixed, paraffin-embedded tissue. This technique offers high sensitivity and specificity and is now considered the preferred method for amyloid subtyping.

The deposition of amyloid in the extracellular matrix of various tissues and organs is called amyloidosis. It is a heterogeneous group of diseases caused by a variety of fibril proteins deposited under a variety of conditions. Amyloidosis can be acquired or hereditary, systemic or localized. Because treatment options and the prognosis of various forms of amyloidosis can differ, precise identification of the disease process is pivotal. The ISA recommends that names of various forms of amyloidosis follow the names of fibril proteins. Whether the disease is systemic or localized should also be noted. The use of historical names such as primary and secondary amyloidosis is now discouraged and should be avoided.

The most common forms of amyloidosis include systemic AL amyloidosis (formerly primary amyloidosis), systemic AA amyloidosis (formerly secondary amyloidosis), systemic wild-type ATTR amyloidosis (formerly age-related or senile systemic amyloidosis), systemic hereditary ATTR amyloidosis (formerly familial amyloid polyneuropathy), and localized AL amyloidosis (Table 1). Involvement of the lungs is relatively common but rarely symptomatic. From the pathologists’ perspective, amyloidosis can appear in the lungs in 3 distinct forms: diffuse alveolar-septal amyloidosis, nodular pulmonary amyloidosis, and tracheobronchial amyloidosis.

**DIFFUSE ALVEOLAR-SEPTAL AMYLOIDOSIS**

**Definition**

Diffuse alveolar-septal amyloidosis, also known as diffuse parenchymal amyloidosis, is characterized by the presence of amyloid deposits in the alveolar septa and vessel walls. As a rule, it is a manifestation of systemic amyloidosis, but unusual cases of diffuse alveolar-septal amyloidosis with no evidence of a systemic disease have been described. The most common association is systemic AL amyloidosis, but cases of diffuse alveolar-septal amyloidosis that are caused by systemic AA, systemic wild-type ATTR, and systemic hereditary ATTR amyloidosis have also been reported (Table 2).

**Clinical Features**

Diffuse alveolar-septal amyloidosis is rarely symptomatic. The patient’s symptoms are usually related to the deposition of amyloid in other organs. The treatment options and prognosis are dependent on the amyloid subtype.

**Systemic AL amyloidosis** (formerly primary amyloidosis) is a monoclonal plasma cell proliferative disorder in which monoclonal immunoglobulin light chains are deposited in tissues. It may be preceded by monoclonal gammopathy of undetermined significance and may occur in association with other plasma cell dyscrasias, such as multiple myeloma and Waldenström macroglobulinemia. Elderly men are more commonly affected than other demographic groups. Affected patients present with nonspecific symptoms, such as fatigue and unintentional weight loss. Clinical signs depend on the organs involved and may include nephrotic syndrome, restrictive cardiomyopathy, peripheral neuropathy, hepatomegaly with elevated liver
enzymes, macroglossia, purpura, and an unexplained bleeding diathesis. The International Myeloma Working Group requires the following criteria for a diagnosis of systemic AL amyloidosis: (1) the presence of a systemic amyloid-related syndrome; (2) proof of amyloid deposition in any tissue by a Congo red stain; (3) proof that the deposits are composed of immunoglobulin light chains; and (4) evidence of a monoclonal plasma cell proliferative disorder. Systemic AL amyloidosis is usually treated with chemotherapy followed by autologous stem cell transplant.

Systemic AA amyloidosis (formerly secondary amyloidosis) is due to tissue deposition of serum amyloid A, which is an acute phase reactant produced by the liver. It is usually “secondary” to a chronic inflammatory condition, such as rheumatoid arthritis, juvenile chronic polyarthritis, ankyllosing spondylitis, inflammatory bowel disease, familial Mediterranean fever, or a chronic infection. It can affect a variety of organs, including the kidneys. The development of nephrotic syndrome in a patient with a chronic inflammatory condition is often suggestive of AA amyloidosis, but biopsy confirmation is required for the diagnosis. AA amyloidosis is preferably managed by treating the underlying disease. Colchicine has been used widely for both prophylactic and therapeutic purposes. If left untreated, AA amyloidosis has significant mortality due to end-stage renal disease, infection, heart failure, bowel perforation, or gastrointestinal bleeding. However, successful treatment of the underlying condition can lead to stabilization or improvement of renal function.

Systemic wild-type ATTR amyloidosis (formerly age-related or senile systemic amyloidosis) refers to the deposition of unmutated transthyretin in tissues, often in the myocardium, in elderly individuals. Affected patients present with heart failure or arrhythmia. Significant renal involvement is rare. Recognition is important because survival is better than that associated with AL amyloidosis, and chemotherapy or autologous stem cell transplant is contraindicated.

Systemic hereditary ATTR amyloidosis (formerly familial amyloid polyneuropathy) is caused by mutations in the transthyretin gene. The most common mutation in the United States and the United Kingdom is Thr60Ala (T60A). Clinically, there may be considerable overlap between wild-type ATTR amyloidosis and hereditary ATTR amyloidosis. Because a family history may not be apparent, DNA sequencing may be necessary to distinguish these 2 causes of restrictive cardiomyopathy in elderly individuals. Because transthyretin is made by the liver, liver transplant with a donor liver that produces unmutated transthyretin may lead to regression of hereditary ATTR amyloidosis. Chemotherapy and autologous stem cell transplant have no role in the treatment of hereditary amyloidosis.

**Pathology**

With rare exceptions, diffuse alveolar-septal amyloidosis is a manifestation of systemic AL, AA, wild-type ATTR, or hereditary ATTR amyloidosis. Because pulmonary impairment rarely dominates the clinical picture, pathologists most often encounter diffuse alveolar-septal amyloidosis as a postmortem finding. On the autopsy table, the lungs are rubbery, and their cut sections have a uniform spongelike appearance. Typically, all lobes are involved.
Table 2. The Etiology of Pulmonary Amyloidosis

<table>
<thead>
<tr>
<th>Subtype of Amyloidosis</th>
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<tr>
<td>Diffuse alveolar-septal amyloidosis</td>
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<tr>
<td>Typical scenario</td>
<td>Cordier,¹⁰ 2005; Utz et al,⁹ 1996</td>
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<td>Case reports</td>
<td>Utz et al,⁹ 1996</td>
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<tr>
<td>- Systemic AL</td>
<td>Utz et al,⁹ 1996</td>
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<tr>
<td>- Systemic wild-type ATTR</td>
<td>Utz et al,⁹ 1996; Authier et al,¹¹ 1999; Ueda et al,¹² 2006</td>
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<td>- Systemic hereditary ATTR</td>
<td>BoydKing et al,² 2009; Hui et al,⁸ 1986; Rajagopala et al,⁹ 2010</td>
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<td>- Localized AL</td>
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<td>Nodular pulmonary amyloidosis</td>
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<td>Typical scenario</td>
<td>Grogg et al,³³ 2013; Kaplan et al,³⁴ 2005</td>
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<tr>
<td>Case reports</td>
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<tr>
<td>- Localized AL or AL/AH</td>
<td>Ikeda et al,³⁵ 1999; Okuda et al,³⁶ 2004</td>
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<tr>
<td>- Localized AL</td>
<td>Beer and Edwards,³⁷ 1993; Calatayud et al,³⁸ 2007</td>
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<tr>
<td>- Localized wild-type ATTR</td>
<td>Roden et al,⁴⁰ 2010</td>
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<tr>
<td>- Localized Aβ₂M/AL</td>
<td>Yang et al,⁴¹ 2009</td>
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<td>Tracheobronchial amyloidosis</td>
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<td>Case reports</td>
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<td>- Localized AL</td>
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<td>- Systemic AL</td>
<td>Celli et al,³⁹ 1978; Kirbas et al,⁴⁰ 2009</td>
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<tr>
<td>- Systemic AA</td>
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Abbreviations: AL/AH, mixed immunoglobulin light chain/heavy chain; Aβ₂M/AL, mixed β₂-microglobulin/immunoglobulin light chain.

Figure 3. Diffuse alveolar-septal amyloidosis. Amyloid deposits can be seen in the alveolar septa, particularly around arterioles and venules (hematoxylin-eosin, original magnifications ×80 [A] and ×150 [B]).

Figure 4. Nonspecific interstitial pneumonia, fibrosing pattern. A, Low-magnification view shows uniform thickening of alveolar septa. B, At higher magnification, the alveolar septal thickening is due to collagen deposition (hematoxylin-eosin, original magnifications ×40 [A] and ×200 [B]).
The visceral pleura may also be affected; pleural involvement correlates with pleural effusion. Histologically, at low magnification, the pulmonary architecture appears to be well preserved. However, at higher magnification, the alveolar septa are thickened by glassy eosinophilic material (Figure 3, A and B). The vessel walls are often involved, and small nodules may be formed. Amyloid material may also be seen in the visceral pleura. The lesions are typically hypocellular, but scant plasma cells may be present. Giant cells are not usually seen with diffuse alveolar-septal amyloidosis.

Congoophilia with apple-green birefringence under polarized light is diagnostic of amyloidosis. As mentioned above, various forms of systemic amyloidosis are treated differently. Therefore, if diffuse alveolar-septal amyloidosis is diagnosed in a biopsy specimen, amyloid subtyping is pivotal. It can be done by immunohistochemistry, but mass spectrometry-based proteomic analysis has a higher sensitivity and specificity and is considered the preferred method for amyloid subtyping.5

In hematoxylin-eosin–stained sections, amyloid and collagen are somewhat similar in appearance. As a result, diffuse alveolar-septal amyloidosis is sometimes confused with a fibrosing interstitial pneumonia, such as usual interstitial pneumonia or fibrosing nonspecific interstitial pneumonia (Figure 4, A and B). The presence of perivascular glassy eosinophilic deposits may be a tip-off, and a Congo red stain can be used to confirm the diagnosis. Pathologists should have a low threshold for ordering a Congo red stain, if amyloidosis is suspected.

Similar to systemic AL amyloidosis, light chain deposition disease is a monoclonal plasma cell proliferative disorder. Lung involvement by light chain deposition disease may mimic either diffuse alveolar-septal amyloidosis or nodular pulmonary amyloidosis (see below).30 The diffuse form is histologically indistinguishable from diffuse alveolar-septal amyloidosis. However, nonamyloid light-chain deposits are Congo red negative. Furthermore, electron microscopy reveals a granular material instead of the typical fibrils seen in amyloidosis. Light chain deposition disease produces κ light chains as a rule, whereas λ light chains are more common in systemic AL amyloidosis and diffuse alveolar-septal amyloidosis.31,32

**NODULAR PULMONARY AMYLOIDOSIS**

**Definition**

Nodular pulmonary amyloidosis, also known as nodular parenchymal amyloidosis or nodular amyloidoma, is defined as 1 or more tumefactive amyloid deposits involving the lungs. It usually represents localized AL or AL/AH (mixed immunoglobulin light chain/heavy chain) amyloidosis,33,34 but rare cases of systemic AL, localized AA, localized wild-type ATTR, and localized AL β₂-M/AL (mixed β₂-microglobulin/immunoglobulin light chain) amyloidosis have been reported (Table 2).35–41 Of note, localized AL amyloidosis is not unique to the lungs and the tracheobronchial tree (see below). It can also occur at other sites including the larynx,32 urinary bladder,33 and colon.44

In the past, nodular pulmonary amyloidosis and primary pulmonary lymphoma with amyloid production were thought to be 2 fundamentally different processes.45,46 However, many experts now believe that most cases of nodular pulmonary amyloidosis are the result of an underlying lymphoproliferative disorder in the spectrum of extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma).33 The underlying lymphoproliferative disorder might be subtle, but sensitive methods reveal a clonal B-cell population in most cases.33,47,48
Clinical Features

Similar to other forms of amyloidosis, nodular pulmonary amyloidosis is also rare. The mean age of patients is 67 years, and the male to female ratio is 3:2.9,49 The nodules are usually solitary and asymptomatic and are discovered incidentally during chest imaging studies performed for unrelated reasons. Unusual cystic radiologic features have been described.50 Because most cases of nodular pulmonary amyloidosis are localized, conservative excision is usually curative, and the long-term prognosis is excellent. Nevertheless, it is important to confirm the absence of systemic amyloidosis, which requires additional treatment.

Pathology

Gross examination of the lungs reveals 1 or rarely more nodules, which typically measure 0.4 to 5 cm in greatest dimension.9 However, larger masses measuring up to 15 cm in greatest dimension have been reported.6 The cut surface of the lesions is waxy, gray-tan (Figure 5). Histologically, the nodules are well circumscribed and are composed of homogeneous, densely eosinophilic material (Figure 6, A). Small aggregates of lymphocytes and plasma cells are usually found within or adjacent to the nodules. Foreign body giant cells, calcifications, and bony or cartilaginous areas may also be seen (Figure 6, B).

If amyloid is suspected, a Congo red stain should be performed. Congophilia with apple-green birefringence under polarized light is diagnostic of amyloidosis. Additional studies should include amyloid subtyping (preferably with mass spectrometry-based proteomic analysis), an attempt to reveal an underlying localized lymphoproliferative disorder, and, although it is exceptionally rare in this context, exclusion of a plasma cell dyscrasia. Amyloid subtyping usually reveals monoclonal immunoglobulin light chains. Interestingly, the light chains in nodular pulmonary amyloidosis are more frequently of κ than of λ type, with a ratio of 3:1, in contrast to the λ predominance noted in most cases of systemic AL amyloidosis.33 In rare cases of nodular pulmonary amyloidosis, serum amyloid A or transthyretin may be detected.8,37,38,40 The clonality of the lymphoplasmacytic component can be evaluated by immunohistochemistry for κ and λ light chains or immunoglobulin gene rearrangement analysis. Exclusion of a plasma cell dyscrasia requires further clinical consideration and laboratory testing.

Differential diagnoses of nodular pulmonary amyloidosis include pulmonary hyalinizing granuloma and amyloid-like nodules, particularly light chain deposition disease.51 Similar to nodular pulmonary amyloidosis, pulmonary hyalinizing granuloma often presents as incidental solitary or multiple nodules on chest imaging studies performed for unrelated reasons. The histologic appearance of the 2 entities, however, is somewhat different. Unlike amyloid, which is homogeneous in appearance, pulmonary hyalinizing granuloma is composed of thick collagen bundles arranged in lamellae (Figure 7, A and B). Bony and

Figure 7. Pulmonary hyalinizing granuloma. A, Low-magnification view shows a well-demarcated, eosinophilic nodule. B, Higher magnification reveals thick collagen bundles arranged in lamellae (hematoxylin-eosin, original magnifications ×20 [A] and ×270 [B]).

Figure 8. Light chain deposition disease is histologically indistinguishable from nodular pulmonary amyloidosis; however, Congo red staining is negative (not shown) (hematoxylin-eosin, original magnifications ×20 [A] and ×400 [B]).

252 Arch Pathol Lab Med—Vol 141, February 2017

Amyloidosis of the Lung—Khoor & Colby
cartilaginous areas are not seen in pulmonary hyalinizing granuloma. Most importantly, Congo red staining is negative.

Amyloid-like nodules are histologically indistinguishable from nodular pulmonary amyloidosis, but Congo red negative (Figure 8, A and B). They are usually composed of nonamyloid light chains (typically κ), which means that the light chain fragments do not form fibrils and electron microscopy shows a granular material. Although a localized form has been reported, nonamyloid light-chain deposition in the lungs is usually associated with systemic light chain deposition disease. Since most patients with light chain deposition disease show evidence of renal involvement and an underlying monoclonal plasma cell proliferative disorder, the presence of appropriate clinical features may also be helpful in separating light chain deposition disease from nodular pulmonary amyloidosis.

TRACHEOBRONCHIAL AMYLOIDOSIS

Definition

Tracheobronchial amyloidosis is characterized by amyloid deposition in various segments of the tracheobronchial tree. Most cases represent localized AL amyloidosis and are restricted to the tracheobronchial tree (Table 2). The alveolated parenchyma is typically not involved, but colocalization of laryngeal and tracheal amyloidosis has been described. Furthermore, rare cases of tracheobronchial amyloidosis caused by systemic AL and systemic AA amyloidosis have been reported.

Clinical Features

Tracheobronchial amyloidosis is the least common form of pulmonary amyloidosis; approximately 100 cases have been reported. The mean age of patients is somewhere between 48 and 57 years. There is no sex predilection. In individual cases, various segments of the tracheobronchial tree are involved to various extents. Three patterns of involvement have been described: proximal, mid, and distal airway disease. Patients usually present with dyspnea, cough, hemoptysis, or hoarseness. Bronchoscopy with transbronchial biopsy is most useful for establishing the diagnosis of tracheobronchial amyloidosis, whereas computed tomography is very helpful for determining the extent of the disease. On pulmonary function tests, patients with proximal airway disease have decreased airflows, whereas patients with distal airway disease have normal airflows. Proximal and severe mid airway disease can lead to airway compromise, which is usually treated with laser or forceps debridement, or external beam radiation therapy. Recurrence is common after laser or forceps debridement, and approximately 30% of these patients eventually die of the disease. However, external beam radiation therapy may offer better outcomes.

Pathology

In tracheobronchial amyloidosis, the walls of the affected airways are thickened, and there is luminal narrowing. If grossly visible, the deposits are located in the submucosa (Figure 9). Histologically, the deposits are composed of homogeneous eosinophilic material and surround seromucous glands and cartilage plates (Figure 10, A). Small submucosal vessels are involved in most cases. Plasma cells, foreign body–type giant cells, calcifications, and ossification are common findings (Figure 10, B).

Congophilia with apple-green birefringence under polarized light is diagnostic of amyloidosis. If amyloid subtyping is performed, it should reveal monoclonal immunoglobulin light chains. Lambda light chains are more commonly detected than κ light chains. Polymerase chain reaction may detect a localized clonal expansion of B cells.
References


