



Amyloidosis hiding in plain sight



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Cover Story

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If examining tissue sometimes seems akin to looking for the proverbial needle in a haystack, then trying to identify amyloidosis can make pathologists feel like they're looking through every field of hay in the Great Plains. On foot. Amyloidosis is that hard to find.

Or is it? Maybe the real problem of finding a needle in a haystack is that no one actually ever looks for one. The task sounds impossible, so why begin? A small but vocal number of pathologists, clinicians, and patients, however, are urging physicians to start looking for amyloidosis, suggesting they may be surprised by what they find.

No one is saying the disease isn't rare, because it is. But they contend it may not be as rare as previously thought, and that misdiagnosis and underdiagnosis may be warping perceptions. Moreover, failing to make a correct diagnosis causes unconscionable harm—costly and damaging procedures, failure to treat, and early death. It's time, they say, to take a fresh look at amyloidosis.

For many physicians, the first—and sometimes only—look comes in medical school, where they're shown the most obvious presentation of the disease and told that amyloid is rare, that no treatment exists, and that they'll never see a case anyway. Listen to the experts, however, and it quickly becomes obvious such views are as outdated as taxi dancers.

"These are misconceptions that have been carried over from 15, 20 years ago," says Morie Gertz, MD, chair, Division of Hematology, Mayo Clinic, and professor of medicine, Mayo Clinic College of Medicine. "It's wrong to say there's nothing to be done. There's actually a lot to be done."

But old attitudes die hard. Dr. Gertz tells the story of being approached by a cardiologist at a recent meeting, who asked him, "Well, does it make a difference if we make the diagnosis or not? There's no treatment." Mayo is considered to be one of the two major amyloidosis centers in the United States—the other is at Boston University—and it treats virtually all its patients, according to Dr. Gertz. He and his colleagues have, in fact, found many

needles, in many haystacks.

So have Muriel Finkel and Elinda Lado. Finkel is president of the Amyloidosis Support Groups, and Lado is secretary of the ASG and a facilitator of the support group in Philadelphia. Both see patients who've been misdiagnosed for years, who've been given inappropriate treatments, who've had unnecessary surgeries, and who've died mere weeks after being given the correct diagnosis. Based on the experiences of the people in their support groups, they say it takes physicians from one to five years to arrive at a correct diagnosis.

Lado's husband, a surgeon, has amyloidosis, and now receives regular care at Mayo. He found it easy to ignore one early sign of trouble—carpal tunnel syndrome—because of his occupation. Later on, his cardiologist suggested trying a heart medication “just to see what would happen,” says his wife, her voice still registering disbelief. It took three biopsies—and a persistent physician insisting that amyloid was present—before the correct diagnosis was made.

Clinicians and patients put pathologists at the frontlines. Lado estimates that among members of her support group, at least 25 percent were given the correct diagnosis only after the pathologist became suspicious for the disease and decided to look for it. “Their cardiologist was scratching his head, or their GI guy was scratching his head. It was the pathologist who was astute enough to take the sample and Congo red stain it,” Lado says.

It might be convenient to dismiss the strong, often angry words of patients as mere anecdotes if they weren't so frequently echoed by leading amyloid specialists. “If the pathologist is waiting for clinical information suspecting amyloid, they'll miss the majority of patients,” says Dr. Gertz.

“It often takes visits to a few different physicians before the biopsy is ultimately done,” says Carl J. O'Hara, MD, chief of surgical pathology, Boston Medical Center Pathology Department, and associate professor of pathology, Boston University School of Medicine. In other cases, he says, diagnoses aren't made until an affected organ begins to fail.

No one is faulting physicians for what they were taught years ago, or for what they may or may not remember from those lessons. But there's plenty new to be learned.

The field has changed immensely in recent decades, says Dr. Gertz. The understanding of the biochemistry of amyloid has improved, and technically it's much easier than in the past to classify the subunit protein that's responsible for the intact amyloid fibril. There are new techniques that allow physicians to classify amyloid and monitor therapy. And with the introduction of new agents, as well as with transplants, physicians have unprecedented ability to treat many types of amyloidosis, extending life and improving organ function tremendously.

All that is for naught if the correct diagnosis isn't made to begin with. And according to many, it often isn't.

“Diagnosis is still made too late,” says Dr. Gertz.

Physicians falter in any number of ways. Making the diagnosis first requires a level of suspicion. But few consider it, given how rare the disease is. "It's still pretty uncommon," Dr. Gertz concedes, before adding, "But just because it's esoteric doesn't mean it lets physicians off the hook."

The simplest solution—keep amyloidosis in the back of your mind—is harder than it sounds. "The symptoms are so vague, it's a wonder that any of us would ever think of it," says Martha Skinner, MD, director of the Amyloid Treatment and Research Program at BU and professor of medicine at Boston University.

Dr. Skinner suggests amyloid is underdiagnosed because its symptoms mimic those of many other diseases. Shortness of breath usually points to heart disease. "You don't think amyloid at that point," she says. "And protein in the urine points to kidney disease, but you don't think of amyloid." The only point-blank symptom is macroglossia; otherwise, she says, no one clinical finding would make a clinician think, This is amyloid.

Finkel points to her late uncle as a classic case. For years, she says, he'd complain of arthritis in his back; the real problem was in his kidneys, which were affected by amyloid deposits. Her uncle also had gastrointestinal problems (constipation and diarrhea are common among patients with amyloidosis) and had his gallbladder removed—unnecessarily, as it turns out. (Finkel says she later posted a question on her association's 600-member listserv asking them about gallbladder-related issues. "You would be amazed at the response I got," she says. "It appears that everyone at one time or another was diagnosed with gallbladder problems" before a diagnosis of amyloid was made.)

If clinicians aren't thinking about amyloidosis, can pathologists raise the index of suspicion?

Dr. O'Hara says, "The clinical presentation is so diverse and so varied, that when I give the lectures to the medical students at BU, I jokingly say to them that anytime anybody asks them for a differential diagnosis, just throw in amyloid, because they probably be right."

Guillermo A. Herrera, MD, chairman of the Department of Pathology and professor of pathology, cellular biology and anatomy, and medicine, LSU Health Services Center, Shreveport, echoes Finkel and Lado when he says that it's not unusual for pathologists to make the call even before the clinician is considering it.

When clinicians ask pathologists to rule out amyloid, he continues, the matter is more straightforward, and such a request usually results in the proper stains being done. "It is when nobody's suspecting it that the pathologist gets into trouble," he says.

Otherwise, says Maria M. Picken, MD, PhD, most pathologists consider amyloid only when they see obvious deposits of homogenous material, which indicates advanced disease. "It's important to think about amyloid not only when you look at the H&E and it looks 'suspicious,' but deposits can be very subtle, and we should be striving to catch it early on," says Dr. Picken, director, renal pathology and electron microscopy laboratory, and professor of pathology,

Loyola University Medical Center, Maywood, Ill.

Dr. Picken says that when she makes a diagnosis of amyloid, she reviews previous biopsies when they're available. "Quite frequently I do detect amyloid in prior biopsies," she says.

In her mind, it's rarely a question of, How did they miss this? "It can be very subtle, and I don't want to throw stones at pathologists," Dr. Picken says. But it happens often enough that she questions whether pathologists consider amyloidosis as often as they should.

Amyloidosis is entangled in several Catch-22s. It's rare, so physicians may not bother to look for it; if they're not looking, they may not see it; if they do look, it may go unseen because they're inexperienced in detection techniques; they lack that experience because the disease is rare.

If there's one thing everyone does know about amyloidosis, it's the Congo red stain. This stain usually suffices in the simplest scenario, when there's plenty of amyloid present, indicated by the telltale apple-green birefringence. Positive and negative controls are important. False-positives often occur from overstaining collagen, which is birefringent on its own. Nor should pathologists make the diagnosis of amyloid based on the presence or absence of salmon-pink color, which is seen in bright light. Instead, the tissue needs to be polarized, which "is not something that many pathologists are immediately familiar with," Dr. Picken says.

Pathologists often use a homemade substitute—two sheets of film—to cross-polarize tissue. "As you can imagine, the sensitivity of this sort of setup is very low," Dr. Picken says. She also makes the point that reading Congo red requires very strong light. "You need to turn the light source to the max, and to see small deposits you should read it in the dark," a step she says is not routinely taken.

Dr. Picken suggests that in some cases, pathologists might want to reconsider how cost fits into the equation. "People don't think twice about upgrading their computers. But yet I still see many pathologists working with outdated equipment, which they get used to using and they don't think much about upgrading," she says.

Merrill Benson, MD, sees a similar penny-wise, pound-foolish reasoning at play. "For some reason or other, they spend a few thousand dollars for a microscope and refuse to pay another hundred dollars for a set of polarizers," says Dr. Benson, professor, Department of Pathology and Laboratory Medicine, Indiana University School of Medicine.

When asked what pathologists do instead of using polarizers, Dr. Benson says, "They miss things." In her department, Dr. Picken has become the de facto amyloid expert and reads all the Congo red stains. Reading Congo red isn't hard—"but there is some how-to involved," as she puts it. "If one is not quite familiar with interpretation, it is quite all right to send it out or to ask somebody to verify it" at an academic or larger hospital laboratory.

Dr. Herrera insists that even small labs should become comfortable using Congo red stains and polarizing tissue. "Remember, in some cases, they are

going to have to be the ones to pick it up. So they're going to have to identify that something is suspicious, and they're going to have to have available to them a good way to rule it in or rule it out. Otherwise, they will be making big mistakes."

Almost any pathologist should be able to identify amyloid in larger deposits, he continues. Small quantities and slight differences in tincture are problematic. "That's when they either ignore it or consider it to be something else."

Few laboratorians are getting the practice they need to become proficient, Dr. Herrera contends. "Histotechs don't do amyloid stains every day, and they may not perform the stain the way they should," he says. In a typical 150- to 200-bed community hospital, amyloid stains may be done 10 times a year, if that. And most of those are rule-out situations.

A generic diagnosis of amyloid needs to be followed by typing. "People say that typing amyloid is difficult, that there are a lot of false-negatives and false-positives," Dr. Picken says. "And I would agree that typing amyloid does require a certain degree of expertise. It's not easy. But it can be done. It should be done. After making a generic diagnosis of amyloid, we need to go further."

Many pathologists still need to be convinced. "As far as amyloid is concerned, people tend to think that this is something you write down in the diagnosis field," says Dr. Picken, "and then you may not see any subsequent specimens from these patients. It just sort of disappears" for the pathologist, she says, who assumes the patient does poorly or dies, "end of story."

Adds Dr. Herrera: "They think, 'Once we call it amyloid, we have done our job, and from there on we don't need to worry about anything else.' And that is not true."

Typing is typically done using immunofluorescence and immunohistochemical techniques. Pathologists need to use a panel of antibodies, which typically would include a stain for amyloid P component, which is present in all types of amyloid regardless of the nature of the fibril protein itself.

With renal biopsies, says Dr. Herrera, typing begins with fluorescent staining for kappa and lambda light chains. If the renal biopsy is neither kappa- nor lambda-positive, pathologists need to "look at your heavy chains very carefully." Heavy chain associated amyloidosis—either IGG or IGM—was first described in 1993. If those are negative, other options include using antibodies to AA and to fibrinogen.

Most amyloid cases turn out to be AL amyloid, and most of those are lambda light chain related. "Just excluding light chain amyloidosis and AA, you're going to diagnose 95-plus percent of cases," says Dr. Herrera. "The hereditary types and the other types are very rare."

It's easier to pick up early cases of amyloid in renal biopsies than in other organs, even when there's no clinical suspicion. "It doesn't take much amyloid in the kidney to result in significant proteinuria," explains Stephen Bonsib, MD, director of surgical pathology, Indiana University. And standard special stains for the kidney, particularly the silver stain, will usually reveal amyloid deposits

even in small amounts. Experienced observers using electron microscopy can easily recognize even small quantities of amyloid.

Silver stain in patients with amyloid shows a distinctive appearance, says Dr. Bonsib. That in itself doesn't prove amyloid, because other diseases can stain in similar fashions. But it raises a red flag, he says, "and amyloid will usually crop in as a possibility."

In some cases, amyloid on the silver stain is completely negative, Dr. Bonsib continues. "There are not very many things that accumulate in the kidney that are silver negative." Sometimes amyloid has a distinctive organizational pattern—rather than being randomly arrayed (the most common pattern), they're parallel arrayed. "Then they have an affinity for silver stain."

The best method of identifying the fibrils may be to extract the amyloid and do amino acid sequencing to identify the responsible protein. The downside, of course, is this approach is extremely labor-intensive and requires instrumentation that most laboratories don't have.

That leaves pathologists with either IHC or a panel of antibodies directed against various fibrils. Immunofluorescence is best done on frozen tissue, so it's often used by renal pathologists. "That has been reasonably successful in terms of identifying the fibril," says Dr. O'Hara. "I had a conversation with my colleague across town, who is a renal pathologist. In his hands, he feels that he can successfully identify the fibril in most of the cases. That said, however, I happened to see a paper that was written last year from a group in Alabama reporting that in 12 of 34 patients (35 percent) with proven AL amyloidosis there was negative immunofluorescence staining for kappa and lambda light chains" (Novak L, et al. *Nephrol Dial Transplant*. 2004; 19:3050-3053).

At Boston, Dr. O'Hara and his colleagues identify fibrils by IHC, which is "not without its nuances," he says. "There is usually a problem with background staining, which is most likely due to the increased sensitivity of the primary antibodies in detecting kappa/lambda light chain immunoglobulins, AA, or transthyretin, etc., using very sensitive detection systems." It makes sense, he says—the antigens of interest are present normally in tissues, and the primary antibodies used in IHC cannot distinguish these normal antigens from those present in the amyloid deposits. Dr. O'Hara and his colleagues use blocking and antigen retrieval methods to counteract this problem, with reasonable success. "The frustrating part is that one can run a case one day and have it work out fine but on another case the amyloid is positive for all the antibodies tested. These latter cases obviously have to be viewed as inconclusive and we have to resort to something else."

That "something else" is a modification called immuno electron microscopy, or immunogold. This procedure uses antisera tagged with gold particles to localize antigens at an ultrastructural level—in this case the extracellular fibrils of amyloid, Dr. O'Hara explains. For reasons perhaps related to the fixation and to the various cleansing steps in between, the background problem is reduced. The downside, however, is that it's labor-intensive. Dr. O'Hara and his colleagues are working with a modified version of the method, adapted to light microscopy. "That's in its very early stages, so I can't really comment much more on it," he says. "But it appears that it might offer us another modality. The downside again is that you need to know about it upfront to fix the tissue

appropriately. Unfortunately for cases that are submitted in consultation or for confirmation of the diagnosis, already fixed and embedded in paraffin, it is not as useful."

If typing seems beyond the reach of most laboratories, the initial steps of identifying amyloid certainly are not.

First, think about amyloidosis as a possible differential. That should be second nature for renal pathologists, says Dr. Picken. But other pathologists should keep it in mind, too. "It may not be the No. 1 differential, but there is room for asking, Could this be amyloid? Especially if something doesn't fit. And I think the fact that the clinician doesn't tell us [the history] doesn't excuse us from asking the clinician."

Dr. Picken and others suggest pathologists should be doing Congo red stains much more readily, starting with kidney and older patients who have otherwise unexplained proteinuria or renal failure. Within the GI tract, Dr. Picken says, "I've seen patients with little erosions and ulcers, and diagnosis of collagenous colitis and ischemic colitis is frequently made in patients with amyloid."

Dr. O'Hara recommends taking a closer look at tissue removed from patients with the carpal tunnel syndrome. "Periodically we'll come across ones where we say, Geez, that looks awfully funny-looking, maybe we should get some stains. And lo and behold, it turns out to be amyloid."

Taking a step back, Dr. O'Hara notes the first clue to amyloid can pop up even earlier. "It's all well and good to talk about the Congo red stain, but ordinarily that's the second line. The first line of identification really is on the H&E or the PAS stain. That's where we become aware of this amorphous deposit and should be highly suspicious is amyloid. That's where it all begins. Obviously if you don't think it's amyloid, then you're not going to order the stains that would help you support the diagnosis."

U.S. physicians typically think about underlying plasma cell dyscrasia/multiple myeloma, because it's the most frequent type of amyloid in this country, says Dr. Picken. But AA amyloid, typically associated with chronic inflammatory states, can be diagnosed in younger patients. "We tend to forget that younger patients may also have amyloid," she says.

Dr. Benson says that as a baseline, physicians need to think about amyloidosis in any patient who develops kidney problem, kidney failure, or proteinuria and who has a history of chronic inflammatory disease. A growing number of patients infected with HIV are developing a Castleman's-type syndrome, he adds, which is a reactive lymphadenitis and which can cause amyloid.

There's also chronic joint disease, rheumatoid arthritis, spondyloarthropathy, and the ever-expanding group of familial periodic fevers and related disorders. Included in this latter group are conditions like Crohn's disease and sarcoidosis. Patients with any of these diseases may develop AA amyloid, says Dr. Picken.

Familial amyloidosis is drawing a fresh look. Some 20 years ago, it was reported that only two percent of amyloid patients were affected by a hereditary form of the disease; more recent data, from Boston, suggest it

affects perhaps 10 percent of patients. Another study, from the U.K., showed that some 10 percent of patients who were suspected of having amyloid derived from the immunoglobulin light chain, that is, AL amyloid, were subsequently found to have familial amyloidosis. "The most striking feature was that these patients did not have any family history. So we need to rethink our approach," says Dr. Picken. "And of course we may be dealing with a de novo mutation."

A clinical history that includes organ system disease in more than one organ should prompt clinicians to think about amyloid, says Dr. Skinner. And if clinical history is available, it could prompt pathologists too—but the history is likely to be lacking. Teresa McHale, MD, is hardly unique when she talks about the dearth of clinical information to accompany biopsy material. "You'd be amazed at the things you find out about patients that the clinicians didn't put on the requisition," says Dr. McHale, assistant professor, University of Pittsburgh Medical Center. "So talking to clinicians about the possibility of amyloid might help to pull the clinical picture together."

For years, says Dr. Herrera, the standard take on amyloid was that it was unimportant. "It was seen as waste material, and nothing happened as a consequence of it," he says. But the last 15 or so years have revolutionized the field.

One new realization is that amyloid is not just one disease, but several—each with its own treatment and prognosis. Some 20-plus different protein types can make amyloid.

Dr. Benson notes that there are also other protein deposition diseases, "which sort of are amyloid *wan na bes*," such as Huntington's and Parkinson's diseases. "So the field has expanded to be called protein-folding diseases. Our journal, which was called *Amyloid*, is now called, *Amyloid—the journal of protein-folding diseases*." All these diseases likely share pathogenic mechanisms, says Dr. Benson, but each is a different disease. "And we now define them by their proteins and not by the old terms of primary, secondary, hereditary, or whatever. So secondary amyloid is now AA, or amyloid A."

There's also been growing discussion about a pre-amyloidotic state, or silent deposits of amyloid. "What it means in the long run is not altogether clear, and even our working definition of amyloid may change as we learn about the condition," says Dr. Picken.

Each new advancement would be reason for physicians to press for a clear, detailed diagnosis, although in Dr. Benson's view, a more basic reason should be compelling enough. "I'm a real believer in making a correct diagnosis and pursuing a diagnosis, and it really bothers me when a doctor says, 'Well, will it make any difference if we make the diagnosis?' To them perhaps not," he says. "To me it would." And for patients, most definitely.

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