In amyloidosis, timely diagnoses lag therapy gains

Karen Titus

July 2014—G-G-G-E♭. Also known as da-da-da-DUM.

Also known as the opening to Beethoven's Fifth Symphony.

It's a simple motif, heard repeatedly in the piece (not to mention across the centuries), yet no less thrilling for that fact.

Maria M. Picken, MD, PhD, finds herself repeating an equally straightforward motif when she speaks about amyloidosis, and it, too, is worth hearing again: The disease is not being diagnosed early enough, and sometimes not at all. That theme has been a steady refrain of hers over the years, and it runs throughout a recent interview with CAP TODAY, so much so that she worries readers will respond with, Oh no, here she goes again.

But she's willing to risk being tuned out. "This is so critical," she says.

The stakes have always been high for patients with the disease. Now they're even higher, given the advent of new treatments and the known long-term survival of patients who've been treated (read: diagnosed) early.

"A lot has changed in the last 10 years, especially with regard to treatment. But the problem of early diagnosis remains," says Dr. Picken, professor of pathology, internal medicine (nephrology), and urology, and director of surgical pathology, Loyola University Chicago Stritch School of Medicine. It came to the fore again at the most recent meeting, this spring, of the International Society of Amyloidosis. "It was shown, again and again, that there is a high level of mortality at the beginning of treatment in a subset of patients—and these are the patients who are diagnosed late." On the other hand, patients who are treated early and who respond to treatment tend to have durable responses, says Dr. Picken, noting that there is a cohort of patients who are alive 10 years post-treatment with bone marrow stem cell transplantation.

And so Dr. Picken persists. At the USCAP meeting in March, she spoke with CAP president Gene Herbek, MD—"very preliminary conversations," she calls them—about how the College might establish recommendations and criteria for early detection and diagnosis of amyloidosis. She's cowritten/coedited a textbook, Amyloid and Related Disorders: Surgical Pathology and Clinical Correlations (Humana Press/Springer). In her own practice as a renal/surgical pathologist, she frequently consults on amyloidosis cases. She also testifies in legal cases related to amyloidosis.

Pathologists, she says, need to claim ownership of the disease. "Nothing can happen unless it's diagnosed in tissue."

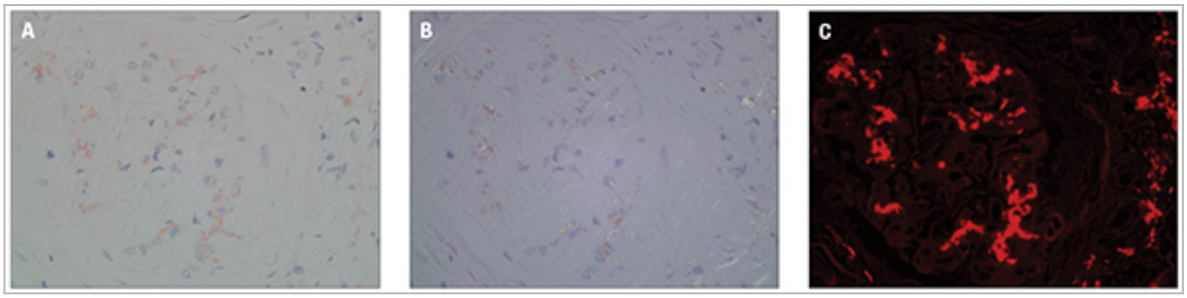
That requires them to look for it, a step that is still too rarely taken, she says. Even at the recent ISA meeting, the lack of pathologist presence was quite evident. "Only a handful of pathologists are active within this society," says Dr. Picken. "The need to educate a broader pathology audience is very apparent."

The first diagnostic step should be familiar to most pathologists, even if they rarely take it: perform a Congo red stain.

Before that can happen, however, physicians need to think about amyloidosis and order the test. Like planning for a summit meeting, the preliminary steps are as critical as the event itself.

While oncologists in particular are getting better at ordering the test—"They see the patient, and they obviously want to treat the patient," says Dr. Picken—it still often falls to pathologists to be the first to think about amyloid

and order the Congo red stain. “There is still not enough awareness among clinicians,” she says.



Three photographs (A–C) demonstrate a Congo red-stained paraffin section with kidney glomerulus. A shows the slide viewed in normal light (bright light), B shows the same field viewed under polarized light, and C shows the same field viewed under fluorescence light, using a TRITC filter. While only deposits detected under polarized light are truly diagnostic of amyloid, examination of Congo red-stained slides under fluorescence dramatically increases sensitivity.

In other cases, pathologists may decide a second opinion is in order, even after initial results are negative. Dr. Picken, who says she’s seen far too many missed diagnoses, calls this “a very good policy. Delayed diagnosis is terrible, and wrong diagnosis is very detrimental,” especially given the significant side effects of most treatments. “We’re not just talking about taking aspirin on a daily basis,” she says.

Treatments have advanced substantially for patients with the most common type of amyloidosis, AL amyloidosis, also known as primary amyloidosis, which is associated with plasma cell dyscrasia. Treatments are individualized and can include risk-adapted strategies (such as stem cell transplant) and combination therapies as well as heart transplantation.

There have been changes in managing hereditary amyloidosis as well, the most common type of which is derived from the transthyretin, or TTR, protein. TTR-related amyloidosis, or ATTR, is associated with a mutation in the transthyretin gene. Since the mid-’90s, when it was introduced, liver transplantation was the only treatment option. Now, however, there are pharmacologic options as well, with several clinical trials available for patients. Others are looking at the possibility of treating carriers of the defective gene prophylactically. “So again, a lot has changed.”

Maria Picken, MD, PhD

‘We are talking about molecular pathology, we are doing molecular testing, and yet people are just using these two pieces

of film to look for polarization.'

But what hasn't—G-G-G-E♭—is the need for early diagnosis. "Somebody, early on, has to make a decision to order Congo red stain."

Skipping the Congo red stain in an amyloidosis workup is unthinkable, akin to skipping the cheese course in France. Even though the stain is such a basic element in the diagnosis, there's still room for education. "There is clearly a need to teach pathologists how to read the stain, because it is not a stain that is easy to read," says Dr. Picken. "It is not pathologist-friendly. It requires certain skills, a certain know-how." That's why, she says, many pathologists would prefer to order a half-dozen other immunostains and skip the Congo red. "And yet we rely so much on this stain, and so much is at stake."

The test can even be a send-out. "Absolutely," says Dr. Picken. "The important thing is to make sure it's done." And while there are ancillary tests that can be done, they are collateral at best. "They can help support the diagnosis, but they will not replace the tissue diagnosis."

Pathologists who perform the stain themselves need good optics. When amyloid areas are very small, she says, it can be difficult to see them under polarization in bright light. For this reason, it is imperative that slides be read in a darkened room.

They also need a good polarizer. Dr. Picken has no patience for practitioners who persist in using two sheets of film to look for polarization, which Dr. Picken says is unacceptable. "We are talking about molecular pathology, we are doing molecular testing, and yet people are just using these two pieces of film to look for polarization. It is like trying to read from an iPad screen in bright sunlight," she says. "It is, frankly, dangerous, and it's high time that the requirements for the evaluation of Congo red stain were defined and implemented. These requirements could be included in the CAP certification."
Even worse than that woeful practice, however, is not ordering the stain at all.

That's starting to change in the clinical sphere, especially among oncologists, she says. "They are getting the message and thinking about it more often." Nevertheless—G-G-G-E♭—"It's still true that the clinician may not think about amyloid, and the pathologist may be the first one to detect it," she says.

Once amyloid has been detected, the second step is to determine whether it's localized or systemic. With localized amyloidosis, relatively little has changed in terms of treatment, Dr. Picken reports. But depending on the organ that's involved, treatments will vary.

If the patient has systemic amyloidosis, treatments are dramatically different and amyloid protein-type dependent, says Dr. Picken.

The first test to determine whether the patient has systemic amyloid is an abdominal fat biopsy. It can be done either by aspiration or surgically. With the first approach, samples frequently are insufficient, Dr. Picken warns. "There is often blood contamination and not enough tissue. So small surgical fat biopsy may be considered," she says.

The gold standard of tissue diagnosis remains: a Congo red stain viewed under polarized light.

Other stains can also be considered, Dr. Picken says; namely, thioflavin S or thioflavin T. Thioflavin can be used on frozen sections and paraffin sections, she notes, and may be easier to read, although it requires the use of a fluorescent microscope.

Interestingly, she says, using a fluorescent microscope may also increase sensitivity of the Congo red stain (which acts as a fluorochrome), at least for prescreening. Dr. Picken, who knows her way around a Congo red slide, says many show a lot of tissue and only minute deposits of amyloid. "So one needs some kind of screening method,"

she explains. Her favored approach is to first examine the Congo red slide under fluorescence, using a TRITC red filter (see photos, above). After identifying the area that shows signal, she switches to polarized light for further examination. “And when I am able to elicit green birefringence [also called apple-green birefringence], then I have a diagnosis of amyloid.” Interestingly, she adds, alternatives to Congo red stain for amyloid diagnosis, which are being explored, also require the use of fluorescence microscopy.

For systemic amyloidosis, in particular, treatments are protein-type dependent; hence, the next critical step involves amyloid typing. Immunohistochemistry has been used for decades, Dr. Picken notes, but it’s not without drawbacks. “Familiarity with the peculiarities of amyloid immunohistochemistry is a must. Amyloid [IHC] is different than other types of immunohistochemistry in surgical pathology,” says Dr. Picken. In fact, she says, it’s best left to laboratories with plenty of experience in doing amyloid IHC. Mass spectrometry-based proteomics is now a highly successful method of amyloid typing in clinical practice, she adds.

The hereditary types are more rare than nonhereditary types. “But collectively they are significant,” Dr. Picken says, accounting for roughly 10 percent of amyloid cases. Another way to think about it, she says, is to remember that for every six cases or so of light chain amyloid—the most common type—“one should anticipate having at least one case of nonlight chain amyloid. So one needs to consider hereditary types.”

In addition, there is amyloid associated with chronic infection, as well as inflammatory processes such as rheumatoid arthritis and Crohn’s disease. Another type of amyloidosis, derived from leukocyte chemotactic factor 2, or ALECT2, has also recently emerged as a newly discovered type. This latter disease primarily targets the kidney; the liver is a secondary target. There is no treatment for this type yet. There is also amyloidosis that can be derived from a nonmutated, “wild type” transthyretin, which primarily affects the heart of older individuals and which is largely undiagnosed. Other, rarer types of amyloidosis can also occur.

When pathologists make a misdiagnosis in amyloidosis cases, says Dr. Picken, it’s usually related to wrong typing of the amyloid protein. It’s not uncommon for physicians to assume, at initial diagnosis, that the disease is systemic and caused by amyloid derived from the immunoglobulin light chain.

Pathologists also need to be on the lookout for other scenarios in which amyloidosis might be mistaken for something else, she says.

With bone marrow biopsies, if there is any clinical evidence that a patient might have underlying plasma cell dyscrasia, then serum protein electrophoresis with immunofixation and serum free light chain assay should be performed. And, of course, the bone marrow biopsy from such patients should also be tested.

Myocardial biopsy should also be tested with Congo red stain to rule out the possibility of amyloid, Dr. Picken says. Especially in older male patients who have shortness of breath, that symptom is often thought to be the result of a more common age-related process. “That may not necessarily be so,” she says. “If the biopsy is not performed, nobody knows for sure what’s behind the cardiac insufficiency.” It’s possible the culprit is wild-type transthyretin-derived amyloidosis.

In kidney biopsy, when done in patients with proteinuria/nephrotic syndrome, amyloidosis is No. 4 in the differential diagnosis, says Dr. Picken. “The list of differentials is rather short, and that’s why renal pathologists do much better in detecting amyloid,” in addition to the fact that kidney biopsies tend to be more comprehensively evaluated using ancillary studies.

Finally, she says, peripheral nerve biopsies “absolutely should be tested for amyloid.”

The matter becomes more difficult with other organ systems, she says. With gastrointestinal biopsies, for example, the list of differentials is long. Nonetheless, says Dr. Picken, pathologists must be alert to mimickers of amyloid. Collagenous colitis is one such mimicker, she says. Likewise, she says, ischemia can mimic amyloid—and vice versa.

If it sounds like Dr. Picken is advocating for many more Congo red stains to be done, that's because she is. "In this day and age, when we do so many stains and testing, I think it should be added to the repertoire of stains that are used regularly in the pathology laboratory." Yes, it has quirks and the need for expertise. The stain itself, however, is available commercially and is quite reproducible.

Nor is it true, she says, that the stain requires thicker sections. "The only true statement is that a thicker section will increase the likelihood of seeing deposits, which are, by the nature of the disease, irregularly distributed." In her own practice, though, she regularly works with precut series of kidney biopsy slides that are routinely cut thinner than other surgical pathology sections. If she decides a Congo red stain is needed, she'll simply stain two slides, which gives her parity with the thicker sections, she says.

Cost isn't an issue, Dr. Picken says. Congo red stain isn't highly expensive; moreover, the human costs (as well as medical costs) of delayed diagnoses are enormous in comparison. When she's invited to speak at meetings of patient support groups, she says, "The most frustrating part is to hear them say—and they do again and again—'I wish I was diagnosed earlier.'"

There's another cost to consider as well. Now that treatment options are available, cases of delayed diagnoses and misdiagnoses are drawing the attention of attorneys. "That's something I'm sorry to have to mention," she says, "but it is a real possibility now. This is something that can no longer be excused by saying, 'Oh, this is rare; I'm not going to see it, or I saw it on an autopsy case.'"

It's time, she says, to change such outdated thinking. "We have patients who are living, now, in dire need of help." [hr]

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