Acute leukemia workups, from top to bottom

Karen Titus

May 2017—Plenty can happen in five years. Just ask Cubs fans who watched their team leap from a 101-loss season in 2012 to a 103-win season in 2016 and a World Series title as the cherry on top.

Dr. Daniel Arber (left) and Dr. James Vardiman at the University of Chicago. The acute leukemia guideline recommends consolidated reporting of test results. “There’s not a computer system out there that makes this easy to do,” Dr. Vardiman says.

Or ask Daniel Arber, MD, who co-chaired a hefty new guideline—a half decade in the making—on diagnostic workup of acute leukemia. At the start of the project, “I think everyone going into it realized it was going to be a time-consuming, long process. But I don’t think anyone realized how long,” says Dr. Arber, professor and chair of pathology, University of Chicago, and the CAP co-chair for the guideline group.

But the wait appears worth it. The guideline tackles a Gordian topic on behalf of pathologists and clinicians, laying out a thoughtful approach to tests (including molecular studies), specimens and samples, results reporting, and targeted therapies. The guideline is heavily based on evidence, which is one of the reasons it took so long to produce the final document (Arber DA, et al. *Arch Pathol Lab Med.* Epub ahead of print Feb. 22, 2017. doi: 10.5858/arpa.2016-0504-CP). Every time the group thought it had at least some answers securely in hand, the literature would erupt with new, potentially significant studies, says James Vardiman, MD, professor emeritus of pathology, University of Chicago, and the co-chair on behalf of the American Society of Hematology.

Acute leukemia is complicated, Dr. Arber says. “There are a lot of paths to take, and you can’t really leave

anything out. I'm not sure there was a better way to do this, unless you broke it into pieces and did several guidelines." But that approach would have its own weaknesses. Why write a book about Henry VIII's marital adventures and neglect to mention three of his wives?

In the end, it made no sense to isolate one aspect from another, Dr. Arber says, or to ignore compelling new data. That's why it took so long—and why the guideline delivers 27 statements regarding the initial workup for diagnosis, prognostic factors, and possible future monitoring of AL, including acute lymphoblastic leukemia, acute myeloid leukemia, and acute leukemias of ambiguous lineage, in children and adults.

Adds Dr. Vardiman, "Right now, the field is exploding with information regarding the molecular basis for acute leukemia, and it's important to give pathologists and clinicians guidance on what to do." Given the scope of the subject, those 27 statements may seem like a model of Shaker-like minimalism.

The guideline also presents a compact list of six objectives. Because it's certain the guideline will need to be revised ("It's not out of date yet," says Dr. Vardiman with a laugh, "but it may be in a year or two"), the questions provide a solid foundation not only for the current document but also for future incarnations.

The questions address: 1) what clinical and laboratory information should be available during the initial diagnostic evaluation; 2) what specimens and sample types should be used; 3) what tests are required for all patients at diagnosis; 4) what tests should be done on only subsets of patients; 5) where lab testing should be done; and 6) how to report and correlate test results and the diagnosis.

The six questions function almost like sutras, aimed at the lab instead of yogis. In other words, they touch on enduring concepts, worth revisiting and pondering again and again, given the changing nature of all things, including AL diagnostic workups. For the current guideline, the questions allowed the authors to frame a consuming topic. "As so many tests become available, there's a lot of confusion about which to do, what's really necessary, and what is prognostically significant versus what's just interesting or worthy of further study," says Dr. Arber.

The majority of the guideline's authors are pathologists, but they were chosen by both the CAP and ASH, while the advisory panel was more heavily weighted toward hematologists, Dr. Arber says. "We felt hematologists really needed to be engaged in the process."

As the writers discussed the various topics, a few especially puzzling areas emerged. Most perplexing, says Dr. Arber, is how to use molecular studies. This idea of genetic susceptibility for leukemia "is not well appreciated," he says. Though there was dim awareness previously, only in the past few years has the literature revealed what genes and what germline mutations are associated with specific diseases.

"It's confusing," Dr. Arber concedes. "Even the guideline is a little confusing." A handful of statements run through a host of mutations that can be tested depending on the scenario, including *ETV6-RUNX1, BCR-ABL1, KMT2A (MLL)* translocation, *PAX5, JAK1, JAK2, IKZF1, NOTCH1, FBXW7, FLT3-IDT, IDH1, IDH2, TET2, WT1, DNMT3A, TP53, RUNX1-RUNX1T1,* and *CBFB-MYH11.* But this list is hardly complete.

Clinicians also have questions about molecular testing, he adds. "They tend to want a large number of mutations up front." Pathologists are free to create their own algorithms. "But each center has to decide for itself the best approach, working with the hematologists," Dr. Arber says.

Among pathologists, the role of flow cytometry in evaluating cerebrospinal fluid was another vibrant area of discussion—should it be standard, or is it merely an option? "There were vastly different opinions, based on what people were doing in their labs," Dr. Arber recalls. "Some people did it on every sample and thought it was essential, and other people didn't."

When the writers turned to the literature, they found evidence that flow cytometry on cerebrospinal fluid had prognostic significance. "But there weren't a lot of studies," Dr. Arber says, "so it was ultimately decided that you

couldn't require it." The guideline simply states that pathologists may use flow cytometry.

Given that the evidence didn't lean heavily in either direction, the guideline is unlikely to change what labs are doing with regard to flow, says Dr. Arber.

Molecular studies may be a different story, since the guideline defines what tests truly support a diagnosis or have prognostic significance. Still, the impact may be less than the authors initially thought. As work on the guideline began, Dr. Arber recalls, many molecular tests were being done as single PCR assays. "You had to do a lot of them, and it was a hassle." The guideline aimed to sort matters out.

In the ensuing years, however, most institutions moved to next-generation sequencing panels of myeloid genes. At the University of Chicago, says Dr. Arber, "It's not going to make a dramatic change in how we practice," but, like a Haydn symphony, the guideline, if lacking the power of Beethoven, is engaging and useful nevertheless. "It should help people feel comfortable that their panel is the right one and how to use it."

Molecular studies and flow cytometry could easily steal the spotlight, but the authors speak with equal energy about other aspects of the guideline, including tissue management and results reporting. In acute leukemia, these issues become worthy of their own space-time continuum. Pathologists have to manage testing that usually occurs at more than one institution and is often repeated, plus consider clinicians' future as well as current needs.

Holding back tissue for targeted therapies should be near the top of pathologists' to-do lists. In acute leukemia right now, Dr. Vardiman says, "targeted therapy is extremely important," having moved through the categories of medical progress, from wished-for to working-on-it to maybe-as-soon-as-next-year. It's here. "Pathologists need to be aware that targeted therapy is now something clinicians have in their hand."

But while managing specimens for current use, pathologists also must determine what will be needed for prognosis, to identify minimal residual disease, and to learn the molecular underpinnings of the leukemia that could be targeted later.

"I think in the past pathologists have not thought so much about minimal residual disease," Dr. Vardi-man says, despite its importance as a marker for pediatric acute lymphocytic leukemia and as a predictor of eventual relapse in patients with acute myeloid leukemia. The challenges of doing this are not technical, he says; rather, pathologists simply need to be more aware of the need to hold back tissue.

Pathologists also need to have a good handle on clinical information. What do they need to know before they can make a correct diagnosis? When a bone marrow biopsy is ordered, for example, pathologists should have access to the relevant clinical information, perhaps in checklist form. Such an approach would also be helpful to remind pathologists to obtain information from clinicians if they're not getting it. Likewise, "If you don't know that a patient had prior chemotherapy," says

Dr. Arber, "that would completely change the diagnosis and prognosis of the patient."

Dr. Vardiman agrees. "Talk to clinicians about any therapy that might interfere with any of the tests you're going to do." Some newer drugs produce specific changes in the cells, and some of them, particularly those that are antibody or antigen directed, will mask those antigens and hinder interpretations, he says.

And since some leukemias are inherited, some associated genetic changes might suggest the need for screening the patient and family members. Just knowing there might be family members who may have had a hematologic neoplasm, for example, can be useful information for pathologists, who may then be spurred to look for congenital causes in a case, Dr. Arber says.

Just as important is the issue of results reporting. Like hot running water, a test result, in and of itself, is generally not remarkable—until you don't have it.

The guideline expends good effort to make clear where tests should be performed and where they should be reported. The goal is consolidated reporting. It's neither effective nor fair to ask clinicians to pull together multiple reports. "The pathologist should take a bigger role in doing that," Dr. Arber says. "Because it's complicated information, and each isolated data point may point in a different direction," including an incorrect diagnosis.

Pathologists have their work cut out for them, Dr. Arber says, "because the computer systems [we] use are pretty bad. They're just not good at pulling data out of different sources," leaving physicians to manage an overflow of addendums and amendments.

Thus the guideline is also a cry for help to laboratory information system vendors. Says Dr. Vardiman: "There's not a computer system out there that makes this easy to do. So we want to wake up the computer companies as well as individual pathologists." Nor is it unusual for results to go directly to the electronic medical record, bypassing the LIS, he says. "Or it gets scanned, or a paper copy goes to the ordering physician's office—and again, it's never shared with the pathologist."

Even should these problems be solved, the piecemeal approach will never disappear entirely. Clinicians need information early on and expect—and want—preliminary reports. If it's an acute leukemia, they need to know if it's myeloid, for example. But in Dr. Arber's experience, they also appreciate getting a final report that pulls everything together.

It doesn't help that the typical AL case generally gets worked up at more than one institution. The guideline has plenty to say about coordinating results as well as producing them, just like D-Day was as much about logistics as beach landings.

The guideline recommends conducting as much testing as possible at the treating institution. During the public comment period, the matter of deferring testing was "a little controversial," Dr. Arber says. In some cases, of course, the patient will require treatment before transfer. "That's certainly understood. And in some cases it's a medical emergency; you need to know there's a specific type of leukemia. And that's also fine." But if it's merely a matter of looking at a peripheral blood smear to identify an acute leukemia, "then why not just wait to do all the tests where they're going to be treated?" Dr. Arber asks.

Particularly maddening, says Dr. Vardiman, is when results fail to make it beyond the initial institution. "So the tests get done again, instead of [the pathologist] spending time trying to find them." The guideline strongly recommends that information be transferred with the patient, almost like a passport.

At the University of Chicago, says Dr. Vardiman, it's not uncommon to receive referrals where only a partial workup is done. "We find ourselves repeating the bone marrow," which is not only costly but also distressing for the patient.

Even when testing is done, and done well, he and his colleagues can still find themselves in the dark. If flow cytometry is done at the initial institution, for example, "we would like to see the real-time data, the histograms, which can be saved on a disk or sent over digitally. But we rarely get that. We may get a report about what markers showed, but it doesn't show us the distribution of the cells." In ALL, this information is an important way of following minimal residual disease.

So when the results are missing or appear incomplete, "we order everything all over again," Dr. Vardiman says. Whether a test was done or not, it's all the same if the second institution doesn't see it.

There's another reason to defer testing when possible. If the treating institution seeks to enroll a patient in a clinical trial, additional tests may be needed. "Oftentimes clinicians will then just repeat everything, because to their mind, it wasn't complete," says Dr. Arber. "So why not just hold off?"

Another complication arises when pathologists don't look at results that are available. Dr. Vardiman notes that pathologists frequently neglect to look at the peripheral blood smear. Clinicians know it's de rigueur to look at the

peripheral blood, but as far as Dr. Vardiman is concerned, “There’s *no* reason for the pathologist not to look at the smear.”

His preference is to look at it, and he’ll often make the call to track it down when it’s not included with referral cases. “Many times you don’t absolutely have to have it. But it’s really the starting point,” he says. Are the cells in the peripheral blood? How anemic is the patient? What’s the platelet count? Moreover, if the peripheral blood contains a generous number of blasts, many of the subsequent tests can be done from the peripheral blood sample—especially useful, he says, if the patient is elderly or quite ill and thus can be spared a bone marrow procedure.

During the public comment period, Dr. Vardiman says he was struck by the back-and-forth discussion over whose job was whose. Some said clinicians needed to track down test results, for example; others said that was up to pathologists. “It doesn’t make any difference who does it, just so it gets done,” he says.

Based on the referral cases he sees, there’s room for improvement in many areas, Dr. Vardiman says. “We see patients come in who don’t have the right tests ordered or they haven’t thought about a specific, certain type of leukemia. Or—we’ve seen lots of this—they may order FISH without even getting routine cytogenetics.”

The guideline recommends some FISH analysis. But doing large FISH panels hasn’t been shown to be more effective or to add more value than doing a karyotype. While the guideline doesn’t come out strongly against them, Dr. Arber says, “I think it’s a pretty obvious omission—we’re not recommending broad FISH panels.” Some FISH tests are being done inappropriately, he continues—such as testing for pediatric ALL in adults—and his hope is that the guideline will change such practices.

Dr. Vardiman makes it clear that he’s not expecting pathologists to commit all 27 statements to memory. “But you can get the general thrust of what needs to be done. I think the guideline will give them an approach as to what is absolutely needed in terms of the morphology, looking at the blood, looking at the bone marrow, and getting the tissue,” he says. “And also telling them: We do need flow cytometry, we do need cytogenetics, and we do need to put tissue back for things other than just making the diagnosis.”
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Karen Titus is CAP TODAY contributing editor and co-managing editor. The guideline’s other coauthors are Michael J. Borowitz, MD, PhD; Melissa Cessna, MD; Joan Etzell, MD; Kathryn Foucar, MD; Robert P. Hasserjian, MD; J. Douglas Rizzo, MD; Karl Theil, MD, PhD; Sa A. Wang, MD; Anthony T. Smith, MLS; R. Bryan Rumble, MSc; and Nicole E. Thomas, MPH, CT(ASCP).