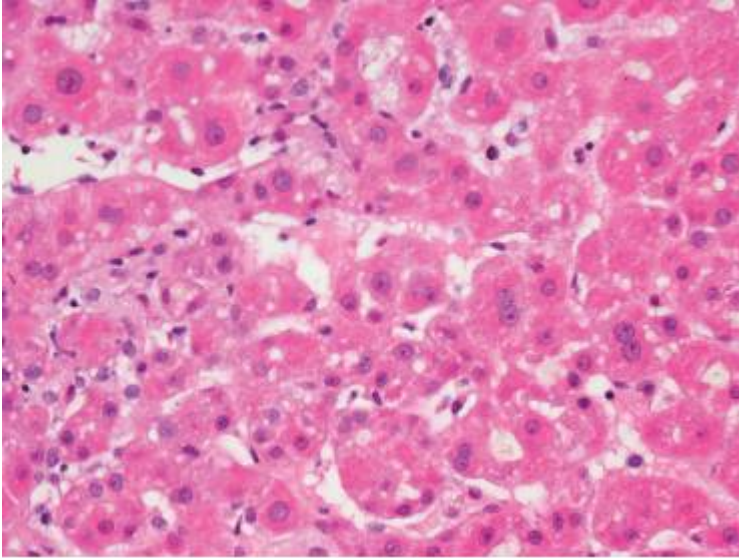


At NCI, on the trail of drug-induced liver injury

Valerie Neff Newitt



Mild parenchymal inflammation and cholestasis from a young man with anabolic-steroid-induced jaundice (H&E, 400×). Images courtesy of David Kleiner, MD, PhD.

When looking at a liver biopsy, always suspect there might be drug-induced liver injury. “But then try hard to prove there isn’t. It’s always a diagnosis of exclusion and pattern evaluation,” says David E. Kleiner, MD, PhD, reference pathologist for the Drug-Induced Liver Injury Network. In that role, he pieces together clues that point to a drug having injured a liver—or not.

Drug-induced liver injury is rare, and few pathologists see it. “The incidence is between one in 100,000 and one in a million, depending on what survey you read,” Dr. Kleiner says.

His advice: “Think of all the other things it could possibly be and rule those out before you say, yes, this is drug-induced liver injury. If it sounds like a big job, well, yes, it is. It’s

probably the most challenging area in liver pathology.”

The Drug-Induced Liver Injury Network, or DILIN, was created in 2003 by the National Institute of Diabetes and Digestive and Kidney Diseases. It was in part “the result of a head-turning 2002 article [Lasser K, et al. *JAMA*. 287(17):2215–2220] that cited liver toxicity as the most common cause of a drug being withdrawn from the market or black boxed,” says Dr. Kleiner, who is a senior research physician in the Laboratory of Pathology at the National Cancer Institute, where he is also chief of the postmortem pathology section. “After that paper, there was a lot of attention by the FDA and Liver Diseases Research Branch of the NIDDK to see if they could explore this issue more.”

DILIN consists of one data coordinating center, at Duke University, and hepatologists working at a half-dozen clinical sites. There are prospective and retrospective studies; Dr. Kleiner works on the prospective side. “Patients suspected of having drug-induced liver injury and meeting certain criteria can be enrolled in the network’s study. They’re followed for up to two years with periodic evaluations. Clinicians collect biosamples, blood, DNA. And then when there is a liver biopsy—and if they can get re-cuts of the biopsy—it is sent, under code, to me.”

All of the drugs the network concerns itself with are considered to cause injuries that are idiosyncratic in nature. “These drugs are generally thought to be one-offs,” Dr. Kleiner says. “Even if patients have susceptibility to these, they may not develop injury. The incidence varies between about one in a thousand

to one in a million patients taking the drugs. Because it's such a low number, usually these drugs are not caught as potential problems during clinical trials."

In contrast, some drugs are considered to be intrinsic dose-related hepatotoxins causing direct injury. "If you give the drug and keep ramping up the dose, you will destroy the liver," Dr. Kleiner says. "Unfortunately, acetaminophen is probably the best example of that." Antibiotics, too, show up in surveys of DILI as causes. "One reason is that antituberculous medications, particularly isoniazid, are problematic." Others, like Augmentin, have an HLA association, he says. "But possibly only one in 600 of those patients who have that HLA allele is at risk for or develops injury. So it's not reasonable for patients to get tested, have HLA determined, and then avoid those drugs." Many are common drugs, though the injury is still uncommon. "Patients could unnecessarily avoid good drugs they really need," he says.

A third group of injury is related to idiosyncratic hepatotoxicity. "This generally refers to hepatotoxicity that requires some processing of the drug to a reactive metabolite, as compared to direct hepatotoxins, which are basically poisons," Dr. Kleiner says.

As new drugs enter the marketplace, other areas of possible hepatotoxicity are emerging. "Checkpoint inhibitors form a distinct group as their hepatotoxicity relates to their mechanism of action," Dr. Kleiner explains. "They work by de-inhibiting the immune system so it can fight the cancer but increase the risk of autoimmune-like injury. The toxicity is therefore frequently referred to as immune-related."

Also to be considered are tyrosine kinase inhibitors. "At least some of them can cause significant injury. Imatinib is a classic example," he says. "The problem we face is that such drugs are being approved very quickly and there is insufficient data on the risk they pose to the liver, yet based on structure or function, some of the new drugs may have significant potential for toxicity."

Since DILIN began, more than 2,000 patients have been enrolled in the prospective study, and about 800 biopsies have been sent to Dr. Kleiner.

"In the beginning, I know nothing about the patients in the study," he says. "I meet them mainly as unstained slides. I stain them, using standard stains, and do an evaluation, looking for different features. I classify the pattern into a number of different patterns seen in drug-induced liver injury—like acute hepatitis, granulomatous hepatitis, cholestatic hepatitis, or nodular regenerative hyperplasia." He has defined about 18 patterns. "I've also created a structured evaluation method to look at the biopsies, grade the various features, and record them in a way that could be put into a database."

When Dr. Kleiner evaluates slides, he does not make a determination about whether there is drug-induced liver injury. Rather, he categorizes the patterns he sees. "A few drugs have very characteristic histologic changes. Amiodarone, for instance, has a distinctive injury pattern, and so even if you don't know anything about the case, sometimes you can recognize that. But for the most part, that's not true. To understand whether a biopsy is showing liver injury usually requires careful correlation of the clinical history with the histology."

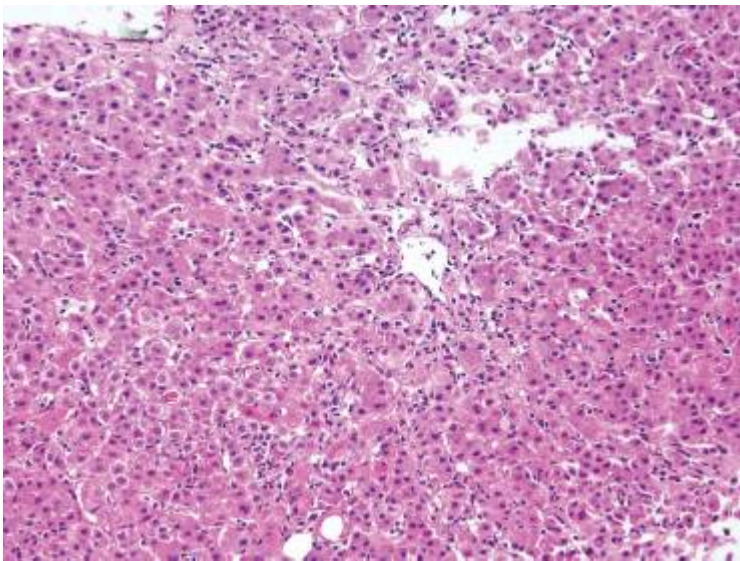
"The hepatologists have a form they follow to look at the details of the case, the history, the laboratory data, et cetera, and then they decide whether they believe there was drug-induced liver injury, and which drug the patient was taking that may have caused it."

Dr. Kleiner's evaluations and the hepatologists' reviews all come together in papers. "A number of projects have come out of this network and the data we've produced. We've written what could be considered classic clinical pathologic correlation papers," Dr. Kleiner says, citing as an example a 2014

article in *Hepatology* (59[2]:661–670). “For instance, on the statins or the cephalosporins, we’ve summarized the clinical presentation, the laboratory data, the histology, and any other information we think might be useful and published those case series.”

In another DILIN project, a subgroup of clinicians is going back and reexamining cases. “We’ve taken out all of the information that came after the liver biopsy was performed. The clinicians might have five days of labs and history to review. We’re asking them to decide, based on that initial information, if they suspect there’s drug-induced injury and whether or not they would do a liver biopsy.”

Later, in a webinar, Dr. Kleiner shows participants the liver biopsies of the cases they reviewed. “I do a separate report, looking at the history and liver biopsy together, and tell what differential diagnosis remains. Then they decide if they would change their minds about how they thought about the case. Sometimes the answer is no, sometimes it is yes.” The purpose is to understand under what circumstances the biopsy is most useful, Dr. Kleiner says. “It’s something that has not been evaluated before. We understand the role of the liver biopsy in other chronic liver diseases, but it’s less well defined how information in the biopsy bears on the determination whether or not the drug caused the injury.”



Moderate acute hepatitis with numerous foci of lobular inflammation and scattered apoptotic hepatocytes in a case of acute liver injury from a green-tea-containing weight-loss product (H&E, 200×).

Clinicians can draw important information from the liver biopsy pertaining to DILI, he says. “You can find out how bad the injury is, and that can help guide therapy or management. If the injury’s not bad, it may be worth the risk to continue a necessary and nonreplaceable medication.” Methotrexate is the obvious example, he says.

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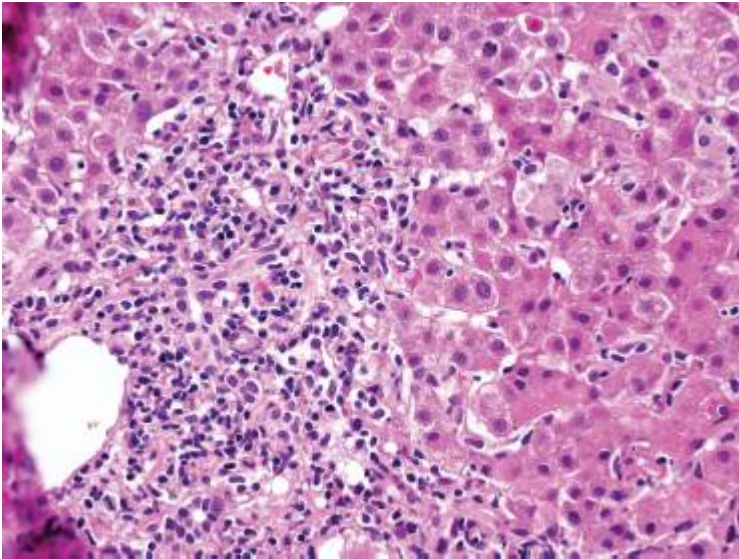
to cause chronic liver injury, and before the development of noninvasive tools, liver biopsy was the only way to assess how much damage had accrued.” Rules were developed that allowed treatment with methotrexate to continue, Dr. Kleiner says, “until the point of bridging fibrosis in the liver, just to a pre-cirrhotic stage. Then you had to stop. You might biopsy the liver if you suspected that the patient was developing more serious liver

disease, but if it was mild, you could keep treating. That sometimes happens with other medications too. If you can’t replace the drug, then maybe it’s worth the risk to keep treating.”

Dr. Kleiner says DILIN has made possible a number of studies of genetic changes. “One of the major points of collecting all of these cases is to have well-characterized cases that can then be used for genetic studies.” The hope, he says, is to find genetic markers that will help clinicians decide if a person is experiencing or at risk for DILI.

Have any such markers been found? “The ambiguous answer is yes and no,” he says, adding that a number of drugs have been associated with particular HLA markers. “Just recently, researchers have identified a signal they think may be a more general risk factor, but they’re still working out the details. It’s not accepted yet universally. Researchers are working through the validation of the genetic change.”

Dr. Kleiner examines liver biopsies for others, separate from DILIN, who may be worried (but uncertain) they are observing drug-induced liver injury. “I evaluate the biopsy and simply see what’s there, and determine if it falls into one of the classic ways that a liver responds to injury. Bile can accumulate, necrosis can occur, there might be inflammation, and then these settle into a couple dozen typical patterns,” he says. The pattern of injury is crucial because it helps determine what the possibilities are.



In the same case, the portal areas showed a dense infiltrate of lymphocytes with scattered plasma cells. There is circumferential interface hepatitis at the edges of the portal area (H&E, 400×).

“Is this acute viral hepatitis? Is it acute autoimmune hepatitis? Is it a drug? Once you’ve gone through that drill and can say, ‘This is what I think is going on, and I’ve looked carefully for all of the things that can happen in drug-induced liver injury,’ you can then go back to the clinical history and ask, ‘What drugs is the patient taking? When did they get them?’” The kind of drug can be correlated with the kind of pattern. “Drugs cause a lot of different patterns as a whole, but every individual drug might only cause two or three. That can help narrow the possibilities. You might be able to say, ‘It wasn’t this drug. It looks like something else,’ or ‘It still could be this drug but first we need to do testing for this virus.’”

Dr. Kleiner sometimes sits with clinicians at the microscope to explain what he sees and ask about the patient. “Then we go through

the possibilities and decide, ‘We still need to rule out obstruction,’ or ‘We need to look for this feature clinically,’ before we can state, ‘This is a drug.’ That’s where the challenge comes in, and that’s why it is a meticulous process. It requires a lot of knowledge of the different ways that ordinary liver disease happens, all of the different causes of liver disease, and how drugs can affect that.”

Online resources are helpful. “I know a lot of my pathology colleagues go to those websites, because remembering the characteristic patterns for a thousand drugs is not something people do. Certainly the more expertise a pathologist has in liver pathology, the better. A general pathologist, however, will find it difficult to walk themselves through this.”

That’s where LiverTox (livertox.nih.gov) comes in; it was created by Jay Hoofnagle, MD, an NIDDK hepatologist. With the help of the National Library of Medicine, it is now incorporated into PubMed as one of the eBooks. “So when you search Liver-Tox for ‘Augmentin hepatotoxicity,’ for example, it will come up with the citations within LiverTox as well as links to the primary literature within the LiverTox articles. It’s all systematically laid out,” Dr. Kleiner says. “There’s a section that describes the clinical presentation of liver injury due to a drug and some of the histologic manifestations.” Many drugs are classified according to how frequently they cause liver injury and how well known they are to cause injury. Many don’t cause injury. “So it’s very easy to search the drug and say, ‘Well, this drug never causes liver injury.’ Thus it’s unlikely to be involved in whatever you’re seeing.”

Asked if the increasing use of herbal and dietary supplements has had an impact on his work within the network, Dr. Kleiner doesn’t hesitate: “You bet it has. It’s a huge problem.”

“There’s no premarketing evaluation, and companies do not have to show up front that their product is safe.” Up to 20 percent of the cases in the network fall into the category of herbals and dietary supplements, and two main groups cause problems. “The first is body-building supplements, which may contain anabolic steroids even when not listed on the label. These compounds can cause severe jaundice but usually do not lead to liver transplants.” The other group is composed of weight-loss supplements, “which can lead to transplants or worse.”

The network is focusing now on supplements that contain green tea. “People are told that green tea is the elixir of life, a cancer fighter, but none of that’s been demonstrated in any clinical study to any great degree. Sure, it might be good for weight loss, but who really knows? I tell people, as long as you drink your green tea in a cup, you’re fine. But don’t take it in pill form.”

As the search for greater understanding into DILI continues, an energetic exploration for biomarkers has begun. “It’s an active area of research in the network and by others studying DILI,” Dr. Kleiner says. “They’re looking at different blood markers mostly, and trying to identify those that will characterize hepatic necrosis separately from just leaking enzymes. The transaminases mostly monitor cell injury, which can indicate an injury that is not lethal or it can mean the cell has died and spilled its contents. Researchers are trying to tease apart the different aspects of the injury to find something that will separate out these bad acute liver injuries from acute viral hepatitis or acute autoimmune hepatitis, et cetera.” So far, he says, nothing is ready for clinical testing. “It’s still all pretty much in the laboratory, looking at patient samples. But it’s certainly a very active area.”

Such biomarkers, once identified, will have an impact in clinical trials, Dr. Kleiner says. “When you’re testing a new drug, you could monitor for the biomarker that is either more sensitive to beginning liver injury or can help you differentiate it from other kinds of liver injury. For clinical trials, there would be a significant interest.”

And in the day-to-day world, “If we actually get biomarkers that are that well characterized and as good as our current transaminases, for example, practitioners could use them to inform treatment when they get someone who is jaundiced or shows other evidence of liver injury. Are they at greater risk for liver failure? Is this something they’ll recover from? Those kinds of questions might be easier to answer,” Dr. Kleiner says. “But we are still a long way from those kinds of applications.”

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