Imaging technology allows health care professionals to diagnose and monitor liver and biliary diseases in a noninvasive manner (i.e., without surgery).

TOP: A computed tomography (CT) scan of a patient with hepatocellular carcinoma shows a large liver tumor (thick arrow) and a vessel supplying it with blood and nutrients (thin arrow). CT is one of the most widely-used methods to image the liver, as it can show soft tissue and blood vessels. Photo contributed by Dr. Theo Heller, NIDDK.

BOTTOM: Researchers are also developing more advanced techniques to image liver tissue. For example, tumors in the livers of mice injected with cancer cells bioengineered to produce a fluorescent protein can be detected using a special camera, allowing researchers to monitor tumor progression. Adapted with permission from Dr. Shiva Sarraf-Yazdi and Dr. Bryan Clary from Journal of Surgical Research 120: 249-255, 2004, Figure 1. Reprinted with permission from Elsevier.

# CHAPTER 16:
# LIVER IMAGING AND BIOTECHNOLOGY

## INTRODUCTION AND BACKGROUND

In the past century, radiologists have relied mostly on morphologic information obtained with various imaging techniques for disease detection, diagnosis, monitoring of responses, and image-guided therapy. The imaging technologies most commonly used in liver disease include computed tomography (CT), magnetic resonance imaging (MRI), and ultrasound (US), which can be used alone or in combination. US and CT possess the sensitivity necessary to detect dilated intrahepatic or extrahepatic biliary ducts. They are the first methods used to assess patients with suspected obstructive jaundice, in order to differentiate obstructive jaundice that requires surgical or endoscopic intervention from medical jaundice, which requires only medication and monitoring. These imaging techniques can also be used to evaluate changes in liver texture (due to fat or fibrosis), as well as to guide liver biopsy and treatment of focal lesions. Doppler US and MRI are used to detect abnormalities in the hepatic vasculature and blood flow through the liver, as well as for monitoring patency (lack of obstruction) of transjugular intrahepatic portosystemic shunts (TIPS) used to treat portal hypertension. Finally US, CT, and MRI are used to diagnose and assess liver tumors, including their staging.

In addition to these imaging technologies, endoscopic retrograde cholangiopancreatography (ERCP) uses a combination of x-rays and endoscopy to image the biliary tree for diagnostic and therapeutic purposes (e.g., gallstone removal, installation of nasobiliary catheters, and biliary stents). ERCP can be used to diagnose and treat gallstones, primary sclerosing cholangitis, and cancer, as well as to provide tissue samples or brushings from bile ducts to establish the diagnosis of cholangiocarcinoma. A common potential complication of ERCP is pancreatitis, and additional, although less frequent, complications include infection, bleeding, and tearing of the duodenum. Recently magnetic resonance imaging (MRI) and cholangiopancreatography (MRCP) have begun to replace the more invasive technique of ERCP as diagnostic techniques for biliary disease.

With advances in molecular techniques and the successful mapping of the human genome, the era of molecular medicine has clearly arrived, bringing with it important advances in both liver imaging and biotechnology. The integration of techniques from molecular biology, immunology, genetics, chemistry, and tissue engineering with imaging technologies allows for unprecedented capabilities to study system biology in whole organisms, including humans, leading to the development of new diagnostic and therapeutic methods. Many of these novel approaches can be applied to liver diseases.

## RECENT RESEARCH ADVANCES

In the genomic era, there are many new advances in biomedical imaging and bioengineering that are potentially applicable to liver diseases.

**Building on Existing Imaging Technologies:** There are now many target-specific imaging techniques that combine the very high spatial resolution provided by MRI, CT, or US with information about the status of different molecular receptors or pathways. An example is the use of paramagnetic polymerized nanoparticles labeled with different antibodies or ligands to detect upregulation of receptors on endothelial cells through MRI. This approach has been successfully implemented in targeting tumor neovasculature expressing the integrin alpha(v)beta(3) for both detection and treatment. The ability to use the same target-specific drug delivery platform for both imaging and treatment can be used for individualizing treatment. There are many other examples in this line of research.

**Developing New Imaging Technologies:** Positron Emission Tomography (PET) is an important advance in imaging research that uses radiotracers to provide high resolution functional images of biochemical or molecular processes *in vivo*. For these reasons, PET scanning is particularly important as a tool of molecular imaging and noninvasive evaluation of key intracellular biologic pathways. The use of PET scanning is growing exponentially so that many modern medical care centers now use PET technology routinely, principally in the fields of cardiology, neurology, and cancer management. Furthermore, PET scanning has now been combined with CT, enabling the simultaneous collection of anatomic and functional information. PET and CT technologies have been combined with great success in the fields of cardiology and neurology and warrant expanded use in the diagnosis, monitoring, and therapy of liver disease.

**Combining Imaging with Other Clinical Tools:** Many high-throughput techniques for tissue analysis have been developed, including functional genomics, proteomics, and tissue RNA microarrays. Since imaging can provide *in vivo* information about living tissues in a temporally and spatially resolved manner noninvasively, it is important that it be used synergistically with tissue analysis techniques. Theoretically, use of imaging alone or in combination with tissue analysis can facilitate target identification, localize relevant molecular targets *in vivo*, and personalize treatment. Initial experiments demonstrated that image-guided tissue analysis can provide unique information that cannot be obtained by tissue analysis alone.

Traditionally, imaging methods have been used as individual tests with integration of data from different modalities done visually by radiologists. More precise methodologies could be developed for co-registration of data from multiple imaging tests to enable the full characterization of the different voxels of tissues *in vivo* that allow 3-D imaging. This requires both hardware and software co-registration. The newly available PET/CT scanner is a step toward this goal. One potential way of integrating multiple imaging modalities is to develop a common tabletop system that can be moved from one imaging device to another, facilitating co-registration of the imaging data. A flexible, multiparametric display is also required so that the users can display the functional or molecular imaging data from any imaging modality superimposed on any anatomic imaging data. These highly enriched imaging data sets can then be used for guiding biopsies or therapeutic interventions and assessing responses to various therapies.

## RESEARCH GOALS

The major research goals in liver imaging and biotechnology are to develop ways to improve the detection and characterization of liver and biliary disease and to aid in targeting therapies to these sites. Assessment of research goals in imaging and biotechnology cannot be done in isolation. Many other chapters of this *Action Plan for Liver Disease Research* have components related to imaging.

The research goals outlined in this chapter include goals for liver imaging in general, as well as goals for specific liver diseases and their complications.

Standardization of Liver Disease Imaging with Existing Technologies: At present, there are many methods available to image the liver that have varying degrees of sensitivity and specificity for detecting and measuring disease processes or damage. At the same time, there is little agreement as to which technique is optimal or how best to apply these techniques to assess liver disease.

- *Research Goal:* To establish multidisciplinary groups of radiologists, hepatologists, surgeons, oncologists, pathologists, and experts in biotechnology and bioinformatics to work together in developing standard definitions, diagnostic criteria, quantitative methodology, and operating procedures for liver imaging (Matrix Cell A1; see also: Chapter 8, A1; Chapter 9, B2; Chapter 10, A1; Chapter 14, B1).

Once standard definitions and criteria for diagnosis and quantitation are established, they require validation and prospective assessment for sensitivity and specificity in liver disease patients.

- *Research Goal:* To validate standardized definitions, diagnostic criteria, and methodology for imaging the liver in prospectively studied patients with liver disease, including patients from international studies (Matrix Cell B1).

Indeed, the clinical resources for assessing large numbers of patients with liver disease in European and Asian medical centers are often far greater than those available in the United States. These centers could be approached for scientific collaborations.

Too often, different liver disease research communities work separately and without cross-fertilization. For example, a major problem in imaging the liver is the difficulty of detecting hepatocellular carcinoma (HCC) during routine monitoring of patients with chronic liver disease and cirrhosis. A small lesion seen on US, CT, or MRI may represent HCC, but may also constitute a regenerative nodule, with or without dysplasia, or another benign lesion such as hemangioma. For progress to be made in liver imaging, collaborative groups of clinicians, radiologists, and pathologists could be encouraged to work together to develop standardization of diagnostic criteria for small liver masses and of performance and reading of imaging studies. Similar comments can be made regarding the use of imaging studies to detect abnormalities of biliary dilation, hepatic blood flow, portal hypertension, narrowing of TIPS shunts, amount of hepatic fat, and volume of liver lobes. A website providing representative gross, macroscopic images and the corresponding histopathology would be very supportive of this effort, as would a tissue and imaging bank to allow better clinical-radiological correlations and improved distribution of the criteria and standards to the research and clinical practice communities.

- *Research Goal:* To create a liver tissue bank with correlative imaging data to facilitate clinical research (Matrix Cell A2: see also Chapter 14, A1).

Such a tissue and imaging bank must be prepared with care and rigor because of the great methodological difficulties in correlating lesions found on imaging studies with the microscopic findings that represent only a small (and undefined) part of the lesion. Efforts could be directed also toward the integration of different liver imaging techniques (e.g., US, CT, MRI, and PET scanning) for diagnosis and monitoring of different diseases and disease processes.

An important and particularly revealing source of excellent clinical-radiological correlations is the living donor liver transplantation procedure, as imaging studies can be performed immediately before surgery

and liver abnormalities can be carefully defined pathologically in the liver explant. Similarly, subsequent documentation of regeneration of the liver in donors and recipients can be rigorously defined using imaging. Studies of liver transplantation could include optimal use of imaging techniques and validation of methods to assess liver mass, function, and regeneration.

Thus, if methods for detection and quantification of liver fibrosis, fat, or hepatic function are developed and validated, they deserve to be used as primary and secondary endpoints of treatment of liver disease. Imaging techniques should eventually replace liver biopsy as a means of assessing disease activity and fibrosis, as well as amount of liver fat and inflammation. The availability of such noninvasive imaging methods to assess the grade and stage of liver disease would greatly help in clinical evaluation of new treatments for fatty liver disease, hepatic fibrosis, portal hypertension, and prevention of cirrhosis or HCC with medications.

- *Research Goal:* To apply definitions, criteria, and methodology for liver imaging as surrogate endpoints to therapy of liver diseases (Matrix Cell C1).

Development of Novel Technologies for Liver Imaging: In addition to improving existing technologies, another important goal for future research is to introduce new methodologies for imaging the liver. Ultimately, rapid, accurate, and inexpensive imaging for liver disease or liver processes (e.g., fat, fibrosis, inflammation, or quantitative liver function) would be invaluable in population-based studies to document the prevalence and incidence of liver disease and to screen for liver diseases that might be preventable or easily treated if detected early.

- *Research Goal:* To develop practical means of assessing the liver (e.g., fat content, fibrosis, inflam-

mation, functionality) for population-based studies (Matrix Cell C1; see also: Chapter 2, A3; Chapter 6, C2; Chapter 7, B2).

Techniques such as optical imaging using light to activate molecules in contrast material or endogenous compounds deserve evaluation in terms of their applications to liver diseases. Similarly, the promise of PET scanning and other modalities could be more extensively evaluated in assessing diagnosis, activity, and stage of liver disease.

- *Research Goal:* To develop improved techniques for established imaging methods used to detect liver disease, such as optical, MRI, or PET/CT scanning (Matrix Cell A2).

Research on contrast media can also be helpful in advancing the sensitivity and specificity of radiologic diagnosis and improving the ability to define anatomical structures (such as the biliary tree) and physiologic processes (such as bile excretion). These reagents are often expensive to develop and validate, particularly if their ultimate use will be limited to liver and biliary disease. Focused initiatives in developing new contrast media and liver-targeted reactants would facilitate advances in this area.

Molecular imaging techniques, employing antibodies to cell surface molecules, ligands of cell surface receptors, or metabolites that interact with intracellular liver enzymes, promise to provide exciting new means of assessing liver disease and liver processes.

- *Research Goal:* To develop molecular imaging techniques and apply them to animal models that exhibit chronic liver injury, inflammation, fibrosis, and cirrhosis (Matrix Cell A3; see also Chapter 15, A3).

Both academic and private research could be promoted in this area. Once animal models demonstrate

the potential of molecular imaging techniques, these techniques could be applied to humans in clinical trials or clinical investigation.

- *Research Goal:* To apply promising molecular imaging techniques to human liver diseases or processes (Matrix Cell B3).

For example, radiolabeled drugs could be used to study pharmacokinetics and selectivity of targeted therapies *in vivo*. Ultimately, using nanoparticles, antibodies, reporter genes, receptor ligands, or metabolically active compounds, it might be possible to image processes in the liver on the cellular level. Advances in targeted imaging techniques will require particular expertise in chemistry and cell biology to develop innovative agents that target molecular processes, as well as new means of conjugating these agents to safe and reliable probes. Special initiatives promoting the multidisciplinary efforts required to develop molecular imaging methods would be particularly useful. Whole liver imaging for inflammation, fibrosis, or hepatic synthetic function using molecular probes may eventually be possible and would be of enormous benefit to further advances in liver disease research. Furthermore, molecular markers for dysplasia and cancer could be pursued with the objective of developing highly sensitive and specific imaging methods for early diagnosis of liver cancer.

Finally, it is important to make advances in bioinformatics that support molecular and other innovative imaging techniques.

- *Research Goal:* To develop modern bioinformatics tools to support new methodologies of liver imaging (Matrix Cell B2).

Integration of Liver Imaging into Disease Management: Imaging modalities would be most beneficial if they were fully integrated into evaluation and therapy of liver diseases.

- *Research Goal:* To develop imaging techniques that are fully integrated into therapy of liver disease (Matrix Cell C2).

Imaging can also be used to apply specific therapies or as a means for monitoring individual patients. Indeed, image-guided local therapies, such as radiofrequency and thermal ablation of HCC, are already being used, but would benefit from further refinements.

- *Research Goal:* To better define the role, efficacy, and safety of image-guided local therapies for HCC, such as radiofrequency and thermal ablation (Matrix Cell A1; see also: Chapter 12, C1; Chapter 14, B1).

In addition, imaging can be directed to markers of liver cancer and other liver diseases that are specific for underlying etiology, molecular patterns, and possible responsiveness to therapy. These imaging techniques can also integrate information on pharmacokinetics and pharmacodynamics of the specific therapies.

- *Research Goal:* To utilize molecular imaging techniques to provide individualized information for selecting and monitoring therapies of liver diseases (Matrix Cell C3).

Imaging techniques have taken advantage of the many breakthroughs in genetics, molecular medicine, and bioinformatics. These advances should be applied rigorously to develop better methods for diagnosing, monitoring, and treating liver and biliary diseases.

## STEPS TO ACHIEVE RESEARCH GOALS

Considerable progress could be made in improving the accuracy and usefulness of liver imaging if multidisciplinary groups of investigators interested in liver disease would form to develop standardized definitions and diagnostic criteria for liver diseases and liver disease processes, as well as to standardize procedures and means of interpretation of liver images. Such multidisciplinary groups could also help to develop concepts for clinical studies and trials to validate and apply these standardized methodologies to imaging liver disease. A network of investigations developed with the purpose of improving the use of imaging in diagnosis, monitoring, and treatment of liver disease would also serve as a resource to develop tissue and imaging banks, as well as websites for facilitating clinical research on liver disease.

Important advances would also come from the application of innovative biotechnology and bioinformatics to imaging the liver. Investigator-initiated research could be encouraged in the development of new technologies, as well as their application to specific problems in liver disease. All possible funding mechanisms can be used to encourage these advances, including regular research grants, program projects, cooperative agreements, contracts, and small business innovation research grants. Particular focus might be given to training and support of both M.D. and Ph.D. scientists entering the field as new investigators.

## Matrix of Research Goals in Liver Imaging and Biotechnology

|  | Short Term (0-3 years) | Intermediate Term (4-6 years) | Long Term (7-10 years) |
|---|---|---|---|
| **High Risk** | **A3.** Evaluate molecular imaging techniques in animal models of liver disease. | **B3.** Apply promising molecular imaging techniques to human liver diseases or processes using antibody, receptor ligand, metabolically active, or substrate defining probes. | **C3.** Develop molecular imaging methods that provide individualized information for monitoring and therapy of liver disease, including pharmacokinetics and pharmacodynamics of targeted therapies. |
| **Intermediate Risk** | **A2.** Create a liver tissue bank with correlative imaging data to facilitate clinical research.<br><br>Develop improved techniques for established imaging methods for liver disease, such as optical, MRI, or PET/CT scanning. | **B2.** Develop bioinformatics such that computer-aided diagnostics are useful in evaluation of liver disease. | **C2.** Develop imaging techniques that are fully integrated into therapy of liver disease. |
| **Low Risk** | **A1.** Develop standardized definitions, diagnostic criteria, and methodology for liver imaging.<br><br>Better define the role, efficacy and safety of image-guided local therapies for HCC, such as radiofrequency and thermal ablation. | **B1.** Validate standardized definitions, diagnostic criteria, and methodology for liver imaging in prospectively studied patients with liver disease.<br><br>Extend studies on validation to international populations. | **C1.** Apply definitions, criteria, and methodology for liver imaging as surrogate endpoints to therapy of liver diseases.<br><br>Develop practical means of assessing liver (fat content, fibrosis, inflammation, functionality) for population-based studies. |