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RESEARCH ARTICLE
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FREQUENCY OF END OF TREATMENT RESPONSE IN GENOTYPE 3 CIRRHOTIC PATIENTS TAKING SOFOSBUVIR, RIBAVIRIN AND DACLATASVIR

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Abstract

A descriptive (case series) study was conducted to find the frequency of end of treatment response in genotype 3 cirrhotic patients taking Sofosbuvir, Ribavirin and Daclatasvir. Triple therapy in combination with daclatasvir leads to a higher rate of SVR both in previously untreated genotype 3 cirrhotic patients and in those who have failed prior antiviral treatment. Study was conducted in the Department of Gastroenterology, Fatima Memorial Hospital, Shadman, Lahore.

Method: A total of 381 of genotype 3 cirrhotic patients' age (18-80) years of both genders were studied with mean age of 47.57 ± 11.49 years who started on Sofosbuvir (400mg once daily), weight-based ribavirin 1200 or 1000 mg/day if ≥75 or <75 kg body weight, respectively) Ribavirin (and Daclatasvir (60mg once daily). Data was analyzed with the help of SPSS version 21. Categorical variables such as gender and achieved end treatment response were expressed in the form of frequency and percentages.

Analysis: Age, BMI and duration of hep C were calculated as mean and standard deviation. Effect modifiers (age, gender, BMI and duration of HCV) were handled by stratification. Post-stratification chi square test was applied and P value less than or equal to 0.05 was considered significant.

Results: After 6 months 24 weeks treatment, HCV was checked for ETR. Out of 381 patients, 238 (62.57%) male and 143 (27.43%) females with the ratio of 1.6:1; the frequency of end treatment was found in 366 (96.06%) patients which was very high.

Conclusion: Sofosbuvir, Ribavirin and Daclatasvir recommended for use in routine for genotype 3 cirrhotic patients to achieve end treatment response that will in turn reduce the morbidity and mortality of our population.

Keywords: Liver Cirrhosis, Sofosbuvir, Ribavirin and Daclatasvir, Genotype 3.

1. Introduction

Cirrhosis is the end stage of chronic liver damage and is characterized by fibrosis resulting in the distortion and destruction of normal liver architecture. Functional liver tissue is destroyed and replaced by regenerating nodules that do not fully restore lost liver function [1]. Cirrhosis is a slowly progressing disease in which healthy liver tissue is replaced with scar tissue, eventually preventing liver from functioning properly. The scar tissue hampers the flow of blood through the liver and slows the processing of nutrients, hormones, drugs, and naturally produced toxins. This damage is characterized by the replacement of normal liver tissue by scar tissue. Typically, the disease develops slowly over months or years. Early on, there are often no symptoms. As the disease worsens, a person may become tired, weak, itchy, have swelling in the lower legs, develop yellow skin, bruise easily, have fluid buildup in the abdomen, or develop spider-like blood vessels on the skin. The fluid build-up in the abdomen may become spontaneously infected. Other complications

include hepatic encephalopathy, bleeding from dilated veins in the esophagus or dilated stomach veins, and liver cancer. Hepatic encephalopathy results in confusion and may lead to unconsciousness.[1] It affects more than 29 million people in Europe and over 300 million people worldwide [2].

WHO report (2013) indicated that Hepatitis C virus (HCV) is a leading cause of chronic liver disease, cirrhosis, and hepatocellular carcinoma. Chronically infected persons are estimated at 170 million, or 3% of the global population[1,2]. 10 million patients are in Pakistan[3,4]. Approximately 75%-85% of HCV-infected persons will progress to chronic HCV infection, and are at risk for the development of extrahepatic manifestations, compensated and decompensated cirrhosis, and hepatocellular carcinoma (HCC)[5]. Blood transfusion and intravenous drug abuse are the most common routes of transmission of HCV. In other modes of transmission of HCV, unsafe injections by medically unqualified personnel, reuse of glass syringes or needles, vertical transmission, non-sexual contact in households, face or armpit shaving at community barber shops, ear piercing, tattooing and inadequately sterilized surgical or dental instruments[6-10].

To reduce the morbidity and mortality in cirrhotic patients, a standard treatment of sofosbuvir (SOF) is considered effective against HCV genotype 3 infections when it is given with weight-based ribavirin (RBV)[11-12]. Triple therapy in combination with daclatasvir leads to a higher rate of SVR both in previously untreated genotype 3 cirrhotic patients and in those who have failed prior antiviral treatment[13].

The current standard-of-care for treatment of HCV genotype 3 (GT-3) patients is the combination of sofosbuvir (an NS5B protein inhibitor) with weight-based ribavirin and Daclatasvir (an NS5A protein inhibitor).

Prior study has shown that ETR was achieved in 99% of patients[14]. Although the number of such studies is scarce as mostly studies are carried out on SVR12 rather than ETR[15,16] .

In Pakistan the data regarding end of treatment response rates in HCV genotype 3 cirrhotic patients taking sofosbuvir and daclatasvir based antiviral therapy is scarce. Results of this study will be helpful for future research. The advent of introduction of new antiviral drugs (sofosbuvir and daclatasvir), availability of these drugs and rising usage in Pakistan response of these drugs needs to be studied.

Objective:

To find the frequency of end of treatment response in genotype 3 cirrhotic patients taking Sofosbuvir, Ribavirin and Daclatasvir.

Operational Definitions

Chronic Hepatitis C: Positive PCR for HCV RNA Qualitative and genotype 3 on PCR

End of treatment response (ETR): defined as undetectable qualitative PCR for HCV RNA after treatment cessation in patients taking Sofosbuvir, Ribavirin and Daclatasvir.

Cirrhosis of Liver: was defined on the basis of ultrasound findings suggestive of cirrhosis i.e. coarse texture and Fibrosis-4 score i.e.

Fibrosis 4 Score = (Age*AST) / (Platelets*√ (ALT))

Cut-off value of 3.25 was taken. If a patient has a score of > 3.25, considered cirrhotic.

Review of Literature

LIVER CIRRHOSIS

Cirrhosis represents the final common histologic pathway for a wide variety of chronic liver diseases. The term cirrhosis was first introduced by Laennec in 1826. It is derived from the Greek term *scirrhus* and refers to the orange or tawny surface of the liver seen at autopsy.

Cirrhosis is defined histologically as a diffuse hepatic process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules. The progression of liver injury to cirrhosis may occur over weeks to years. Indeed, patients with hepatitis C may have chronic hepatitis for as long as 40 years before progressing to cirrhosis. Many forms of liver injury are marked by fibrosis, which is defined as an excess deposition of the components of the extracellular matrix (i.e. collagens, glycoproteins, proteoglycans) within the liver. This response to liver injury potentially is reversible. In contrast, in most patients, cirrhosis is not a reversible process. In addition to fibrosis, the complications of cirrhosis include, but are not limited to, portal hypertension, ascites, hepatorenal syndrome, and hepatic encephalopathy.

Often a poor correlation exists between histologic findings in cirrhosis and the clinical picture. Some patients with cirrhosis are completely asymptomatic and have a reasonably normal life expectancy. Other individuals

have a multitude of the most severe symptoms of end-stage liver disease and have a limited chance for survival. Common signs and symptoms may stem from decreased hepatic synthetic function (e.g. coagulopathy), decreased detoxification capabilities of the liver (e.g. hepatic encephalopathy), or portal hypertension (e.g. variceal bleeding).[17]

EPIDEMIOLOGY

Chronic liver disease and cirrhosis result in about 35,000 deaths each year in the United States. Cirrhosis is the ninth leading cause of death in the United States and is responsible for 1.2% of all US deaths. Many patients die from the disease in their fifth or sixth decade of life.

Each year, 2000 additional deaths are attributed to fulminant hepatic failure (FHF). FHF may be caused viral hepatitis (e.g. hepatitis A and B), drugs (e.g. acetaminophen), toxins (e.g. *Amanita phalloides*, the yellow death-cap mushroom), autoimmune hepatitis, Wilson disease, or a variety of less common etiologies. Cryptogenic causes are responsible for one third of fulminant cases. Patients with the syndrome of FHF have a 50-80% mortality rate unless they are salvaged by liver transplantation.[18-20]

CAUSES

Cirrhosis has many possible causes; sometimes more than one cause is present in the same patient. Globally, 57% of cirrhosis is attributable to either Hepatitis B (30%) or Hepatitis C (27%).[21] Alcohol consumption is another important cause, accounting for about 20% of the cases.[22]

- **Alcoholic liver disease (ALD):** Alcoholic cirrhosis develops for 10–20% of individuals who drink heavily for a decade or more. Alcohol seems to injure the liver by blocking the normal metabolism of protein, fats, and carbohydrates. This injury happens through the formation of acetaldehyde from alcohol which itself is reactive, but also leads to the accumulation of products in the liver.[23] Patients may also have concurrent alcoholic hepatitis with fever, hepatomegaly, jaundice, and anorexia. AST and ALT are both elevated but less than 300 IU/litre with an AST:ALT ratio > 2.0, a value rarely seen in other liver diseases.[24] In the United States, 2/5 of cirrhosis related deaths are due to alcohol.[23]
- **Non-alcoholic steatohepatitis (NASH):** In NASH, fat builds up in the liver and eventually causes scar tissue. This type of hepatitis appears to be associated with obesity (40% of NASH patients) diabetes, protein malnutrition, coronary artery disease, and treatment with corticosteroid medications. This disorder is similar to that of alcoholic liver disease but patient does not have an alcohol history. Biopsy is needed for diagnosis.[24]
- **Chronic hepatitis C:** Infection with the hepatitis C virus causes inflammation of the liver and a variable grade of damage to the organ. Over several decades this inflammation and grade change can lead to cirrhosis. Among patients with chronic hepatitis C 20-30% will develop cirrhosis.[23] Cirrhosis caused by hepatitis C and alcoholic liver disease are the most common reasons for liver transplant. Can be diagnosed with serologic assays that detect hepatitis C antibody or viral RNA. The enzyme immunoassay, EIA-2, is the most commonly used screening test in the US.[24]
- **Chronic hepatitis B:** The hepatitis B virus causes liver inflammation and injury that over several decades can lead to cirrhosis. Hepatitis D is dependent on the presence of hepatitis B and accelerates cirrhosis in co-infection.[24] Chronic hepatitis B can be diagnosed with detection of HBsAG > 6 months after initial infection. HBeAG and HBV DNA are determined to assess whether patient needs antiviral therapy.[23]
- **Primary biliary cirrhosis:** Damage of the bile ducts leading to secondary liver damage. May be asymptomatic or complain of fatigue, pruritus, and non-jaundice skin hyperpigmentation with hepatomegaly. There is prominent alkaline phosphatase elevation as well as elevations in cholesterol and bilirubin. Gold standard diagnosis is antimitochondrial antibodies (positive in 90% of PBC patients). Liver biopsy if done shows bile duct lesions. It is more common in women.[23]
- **Primary solarizing cholangitis:** PSC is a progressive cholestatic disorder presenting with pruritus, steatorrhea, fat soluble vitamin deficiencies, and metabolic bone disease. There is a strong association with inflammatory bowel disease (IBD), especially ulcerative colitis. Diagnosis is best with contrast cholangiography showing diffuse, multifocal strictures and focal dilation of bile ducts, leading to a beaded appearance. Non-specific serum immunoglobulins may also be elevated.[23]
- **Autoimmune hepatitis:** This disease is caused by the immunologic damage to the liver causing inflammation and eventually scarring and cirrhosis. Findings include elevations in serum globulins, especially gamma globulins. Therapy with prednisone and/or azathioprine is beneficial. Cirrhosis due to autoimmune hepatitis still has 10-year survival of 80+ %.[24]
- **Hereditary hemochromatosis:** Usually presents with family history of cirrhosis, skin hyperpigmentation, diabetes mellitus, pseudogout, and/or cardiomyopathy, all due to signs of iron overload. Labs show fasting

transferrin saturation of > 60% and ferritin > 300 ng/ml.[25] Genetic testing may be used to identify HFE mutations. If these mutations are present, biopsy may not need to be performed. Treatment is with phlebotomy to lower total body iron levels.[23]

- **Wilson's disease:** Autosomal recessive disorder characterized by low serum ceruloplasmin and increased hepatic copper content on liver biopsy, and elevated 24-hour urine copper. May also have Kayser-Fleischer rings in the cornea and altered mental status. This condition affects 1 in 30,000 people.
- **Indian childhood cirrhosis:** is a form of neonatal cholestasis characterized by deposition of copper in the liver.[26]
- **Alpha 1-antitrypsin deficiency (A1AD):** Autosomal recessive disorder of decreased levels of the enzyme alpha 1--antitrypsin. Patients may also have COPD, especially if they have a history of tobacco smoking. Serum AAT levels are low and liver biopsy is positive for Periodic acid-Schiff Recombinant AAT is used to prevent lung disease due to AAT deficiency.[27]
- **Cardiac cirrhosis:** Due to chronic right sided heart failure which leads to liver congestion.[28]
- **Galactosemia**
- **Glycogen storage disease type IV**
- **Cystic fibrosis**[29]
- **Hepatotoxic drugs or toxins**

PATHOPHYSIOLOGY

The central pathogenetic processes in cirrhosis are progressive fibrosis and reorganization of the vascular microarchitecture of the liver.[27] In the normal liver, interstitial collagens (types I and III) are concentrated in portal tracts and around central veins, with occasional bundles in the space of Disse. The collagen (reticulin) coursing alongside hepatocytes is composed of delicate strands of type IV collagen in the space of Disse. In cirrhosis, types I and III collagen are deposited in the lobule, creating delicate or broad septal tracts. New vascular channels in the septae connect the vascular structures in the portal region (hepatic arteries and portal veins) and terminal hepatic veins, shunting blood around the parenchyma. Continued deposition of collagen in the space of Disse within preserved parenchyma is accompanied by the loss of fenestrations in the sinusoidal endothelial cells. In the process, the sinusoidal space comes to resemble a capillary rather than a channel for exchange of solutes between hepatocytes and plasma. In particular, hepatocellular secretion of proteins (e.g., albumin, clotting factors, lipoproteins) is greatly impaired.

The major source of excess collagen in cirrhosis is the perisinusoidal stellate cells, which lie in the space of Disse. Although normally functioning as vitamin A fat-storing cells, during the development of cirrhosis they become activated, a process that includes (1) robust mitotic activity in areas developing new parenchymal fibrosis, (2) a shift from the resting-state lipocyte phenotype to a transitional myofibroblast phenotype, and (3) increased capacity for synthesis and secretion of extracellular matrix. It is predominantly the cytokines secreted by activated Kupffer cells and other inflammatory cells that stimulate perisinusoidal stellate cells to divide and to produce large amounts of extracellular matrix. Moreover, the greatest activation of stellate cells is in areas of severe hepatocellular necrosis and inflammation. As shown in figure III, the stimuli for stellate cell activation may come from several sources.[28]

- Chronic inflammation, with production of inflammatory cytokines such as tumor necrosis factor (TNF), lymphotoxin, and interleukin-1 (IL-1).
- Cytokine production by activated endogenous cells (Kupffer cells, endothelial cells, hepatocytes, and bile duct epithelial cells), including transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and lipid peroxidation products.
- Disruption of the extracellular matrix, stellate cells are extraordinarily responsive to the status of their substrate.
- Direct stimulation of stellate cells by toxins.

Acquisition of myofibers by perisinusoidal stellate cells also increases vascular resistance within the liver parenchyma, since tonic contraction of these "myofibroblasts" constricts the sinusoidal vascular channels.

Throughout the process of liver damage and fibrosis, remaining hepatocytes are stimulated to regenerate and proliferate as spherical nodules within the confines of the fibrous septae. The net outcome is a fibrotic, nodular liver in which delivery of blood to hepatocytes is severely compromised, as is the ability of hepatocytes to secrete substances into plasma. Disruption of the interface between the parenchyma and portal tracts obliterates biliary channels as well. Thus, the cirrhotic patient may develop jaundice and even hepatic failure, despite having a liver of normal mass.

SIGNS & SYMPTOMS

Some of the following signs and symptoms may occur in the presence of cirrhosis or as a result of the complications of cirrhosis. Many are nonspecific and may occur in other diseases and do not necessarily point to cirrhosis. Likewise, the absence of any does not rule out the possibility of cirrhosis.

- Spider angiomata or spider nevi: Vascular lesions consisting of a central arteriole surrounded by many smaller vessels because of an increase in estradiol. These occur in about 1/3 of cases.[29]
- Palmar erythema: Exaggerations of normal speckled mottling of the palm, because of altered sex hormone metabolism.
- Nail changes.
  - Muehrcke's lines: paired horizontal bands separated by normal color resulting from hypoalbuminemia (inadequate production of albumin).
  - Terry's nails: proximal two-thirds of the nail plate appears white with distal one-third red, also due to hypoalbuminemia
  - Clubbing: angle between the nail plate and proximal nail fold > 180 degrees
- Hypertrophic osteoarthropathy: Chronic proliferative periostitis of the long bones that can cause considerable pain.
- Dupuytren's contracture: Thickening and shortening of palmar fascia that leads to flexion deformities of the fingers. Thought to be caused by fibroblastic proliferation and disorderly collagen deposition. It is relatively common (33 % of patients).
- Gynecomastia: Benign proliferation of glandular tissue of male breasts presenting with a rubbery or firm mass extending concentrically from the nipples. This is caused by increased estradiol and can occur in up to 66 % of patients.
- Hypogonadism: Manifested as impotence, infertility, loss of sexual drive, and testicular atrophy because of primary gonadal injury or suppression of hypothalamic or pituitary function.
- Liver size: Can be enlarged, normal, or shrunken.
- Splenomegaly (increase in size of the spleen): Caused by congestion of the red pulp as a result of portal hypertension.
- Ascites: Accumulation of fluid in the peritoneal cavity giving rise to flank dullness (needs about 1500 ml to detect flank dullness).
- Caput medusa: In portal hypertension, periumbilical collateral veins may dilate. Blood from the portal venous system may be shunted through the periumbilical veins and ultimately to the abdominal wall veins, manifesting as caput medusa.
- Cruveilhier-Baumgarten murmur: Venous hum heard in epigastric region (on examination by stethoscope) because of collateral connections between portal system and the periumbilical veins in portal hypertension.
- Fetor hepaticus: Musty odor in breath as a result of increased dimethyl sulfide.
- Jaundice: Yellow discoloring of the skin, eye, and mucus membranes because of increased bilirubin (at least 2–3 mg/dL or 30 mmol/L). Urine may also appear dark.
- Asterixis: Bilateral asynchronous flapping of outstretched, dorsiflexed hands seen in patients with hepatic encephalopathy.
- Other. Weakness, fatigue, anorexia, weight loss.

As the disease progresses, complications may develop. In some people, these may be the first signs of the disease.

- Bruising and bleeding resulting from decreased production of coagulation factors.
- Jaundice as a result of decreased processing of bilirubin.
- Itching (pruritus) because of bile salt products deposited in the skin.
- Hepatic encephalopathy - the liver does not clear ammonia and related nitrogenous substances from the blood, which are carried to the brain, affecting cerebral functioning: neglect of personal appearance, unresponsiveness, forgetfulness, trouble concentrating, or changes in sleep habits.
- Sensitivity to medication caused by decreased metabolism of the active compounds.
- Hepatocellular carcinoma is primary liver cancer, a frequent complication of cirrhosis. It has a high mortality rate.
- Portal hypertension - blood normally carried from the intestines and spleen through the hepatic portal vein flows more slowly and the pressure increases; this leads to the following complications:
  - Ascites - fluid leaks through the vasculature into the abdominal cavity.

o Esophageal varices - collateral portal blood flow through vessels in the stomach and esophagus (Portacaval anastomosis). These blood vessels may become enlarged and are more likely to burst.

- Problems in other organs

o Cirrhosis can cause immune system dysfunction, leading to infection. Signs and symptoms of infection may be aspecific and are more difficult to recognize (e.g., worsening encephalopathy but no fever).
o Fluid in the abdomen (ascites) may become infected with bacteria normally present in the intestines (spontaneous bacterial peritonitis).
o Hepatorenal syndrome - insufficient blood supply to the kidneys, causing acute renal failure. This complication has a very high mortality (over 50 %).
o Hepatopulmonary syndrome - blood bypassing the normal lung circulation (shunting), leading to cyanosis and dyspnea (shortness of breath), characteristically worse on sitting up.[30]
o Portopulmonary hypertension - increased blood pressure over the lungs as a consequence of portal hypertension.[30]
o Portal hypertensive gastropathy which refers to changes in the mucosa of the stomach in patients with portal hypertension, and is associated with cirrhosis severity.[31]

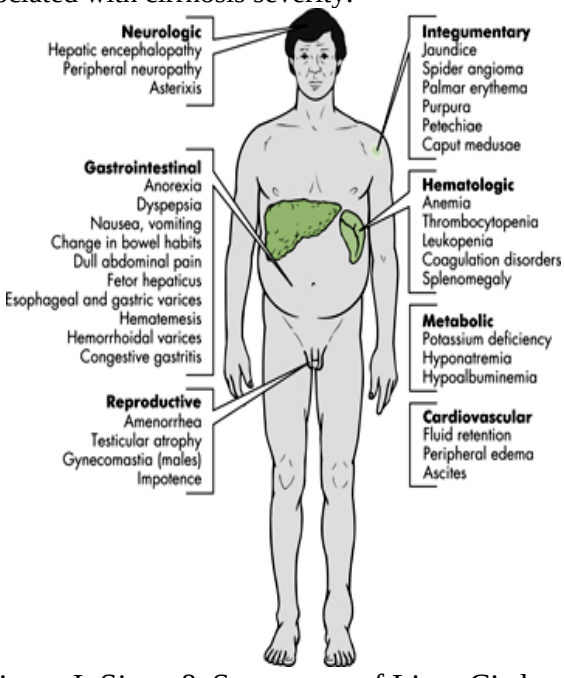

Figure I: Signs & Symptoms of Liver Cirrhosis

MATERIALS AND METHODS

Descriptive, case series study design was used. By using non probability purposive sampling, a sample of 381 was selected at 95% confidence level and 1% margin of error. Inclusion criteria: age of 18 to 80 years male and female patients having positive PCR for HCV Genotype 3Cirrhotic patients (as per-operational definition). The exclusion criteria: HBV HCV co-infection, chronic kidney disease with EGFR of less than 30ML per minute, HCV HIV co-infection, HCC. Percentage of achieved end treatment response was 99%.[11] Study was conducted at the Department of Gastroenterology, Fatima Memorial Hospital, Shadman, Lahore. Data was collected under the supervision of consultant gastroenterologist from the Department of Gastroenterology, Fatima Memorial Hospital, Shadman, Lahore from those patients who had PCR qualitative for HCV RNA genotype 3 as per inclusion criteria. Patients started on Sofosbuvir (400mg once daily), weight-based ribavirin 1200 or 1000 mg/day if ≥75 or <75 kg body weight, respectively) Ribavirin (and Daclatasvir (60mg once daily) by consultant gastroenterologist were followed. The course of this treatment is 6 months i.e. 24 weeks. Once the treatment is complete patients was advised HCV PCR Quantitative analysis at end of treatment to check for ETR. The results were filled on the proforma at the end.

Data was analyzed with the help of SPSS version 21. Categorical variables such as gender and achieved end treatment response were expressed in the form of frequency and percentages. Age, BMI and duration of hep C were calculated as mean and standard deviation. Effect modifiers (age, gender, BMI and duration of HCV)

were handled by stratification. Post-stratification chi square test was applied and P value less than or equal to 0.05 was considered significant.

DIAGNOSIS

The gold standard for diagnosis of cirrhosis is a liver biopsy, through a percutaneous, transjugular, laparoscopic, or fine-needle approach. A biopsy is not necessary if the clinical, laboratory, and radiologic data suggests cirrhosis. Furthermore, there is a small but significant risk to liver biopsy, and cirrhosis itself predisposes for complications caused by liver biopsy.[32] Ascites, low platelet count, and spider nevi are useful physical findings.[33]

Lab Findings

The following findings are typical in cirrhosis:

- Aminotransferases - AST and ALT are moderately elevated, with AST > ALT. However, normal aminotransferases do not preclude cirrhosis.
- Alkaline phosphatase - usually slightly elevated.
- Gamma-glutamyl transferase – correlates with AP levels. Typically much higher in chronic liver disease from alcohol.
- Bilirubin - may elevate as cirrhosis progresses.
- Albumin - levels fall as the synthetic function of the liver declines with worsening cirrhosis since albumin is exclusively synthesized in the liver.
- Prothrombin time - increases since the liver synthesizes clotting factors.
- Globulins - increased due to shunting of bacterial antigens away from the liver to lymphoid tissue.
- Serum sodium - hyponatremia due to inability to excrete free water resulting from high levels of ADH and aldosterone.
- Thrombocytopenia - due to both congestive splenomegaly as well as decreased thrombopoietin from the liver. However, this rarely results in platelet count < 50 000/mL.
- Leukopenia and neutropenia - due to splenomegaly with splenic margination.
- Coagulation defects - the liver produces most of the coagulation factors and thus coagulopathy correlates with worsening liver disease.

There was now a validated and patented combination of 6 of these markers as non-invasive biomarker of fibrosis (and so of cirrhosis): FibroTest.[34] Other laboratory studies performed in newly diagnosed cirrhosis may include:

- Serology for hepatitis viruses, autoantibodies (ANA, anti-smooth muscle, anti-mitochondria, anti-LKM).
- Ferritin and transferrin saturation (markers of iron overload), copper and ceruloplasmin (markers of copper overload).
- Immunoglobulin levels (IgG, IgM, IgA) - these are non-specific but may assist in distinguishing various causes.
- Cholesterol and glucose.
- Alpha 1-antitrypsin

HISTOLOGIC FINDINGS

Lymphocytic infiltration, moderate degrees of inflammation and necrosis, and portal or bridging fibrosis are noted. Regenerative nodules are seen in patients with cirrhosis. Some patients also may have findings indicative of HCC. Most pathologists give separate measurements of disease activity (grade) and fibrosis (stage). Many trials use the Ishak (6-point scale) and Knodell histologic activity index (18-point score); both are useful for assessing improvements in histologic findings in studies but are impractical for clinical use because of interobserver disagreement.[35]

The METAVIR score was developed by the French METAVIR Cooperative Study Group and reported by Bedossa and Poynard in 1996; it is frequently used in European trials. This score consists of a 3-point activity scale and 4-point fibrosis score, with good agreement among pathologists. In the United States, many pathologists use a scale described by Batts and Ludwig in 1995, which consists of an activity grade (0-4) and a fibrosis stage (0-4).[35]

Each method has its advantages and disadvantages, and the system used should be appropriate for the task at hand. In general, more complex systems can provide more information than simple ones, but they are less reproducible.[36]

The numeric scores generated by these systems are very useful in investigational studies that involve large number of patients and require statistical analysis. They are a very good way to show differences in the histologic response between cohorts of patients receiving different forms of therapy, and they have been used successfully in many large clinical trials. However studies have shown fairly poor reproducibility of these scores when applied to individual biopsy specimens, both between different pathologists and for the same pathologist at different times.[36, 37]

KNODELL SCORE

According to Bonis PA,[38] the Knodell score, also known as the histologic activity index (HAI), is composed of the summation of four individual scores representing periportal and/or bridging necrosis, intralobular degeneration and focal necrosis, portal inflammation, and fibrosis; the score ranges from 0 to 22. Several modifications of the HAI have also appeared, which were designed, in part, to address histologic features specific to the disease under study.[39] One modification (referred to as the Ishak score) has six stages of fibrosis, permitting more detailed evaluation of changes in fibrosis compared with the standard Knodell fibrosis score, which has only three stages.[37]

LIMITATIONS OF THE KNODELL SCORE

According to Bonis PA,[38] the changes in the HAI are sometimes interpreted inappropriately: The standard deviation in HAI scoring among six individual observers in the original description of the Knodell score was 2.4. Thus, variation in the HAI score by less than 2.4 does not necessarily represent a true difference in the histologic pattern. This observation is not always adhered to in clinical trials and studies, in which changes of one point or more have sometimes been considered to be clinically or statistically significant.

A difference of one grade has one meaning, but a difference of one stage has vastly great diagnostic and prognostic importance. In the original description of the Knodell score, the inter- and intra-observer reliability was relatively good. However, the Knodell score was originally validated on only five patients (with a total of 14 biopsies), one of whom had hepatitis B and four of whom were presumed to have non-A, non-B hepatitis, which is most likely to have been hepatitis C.[38]

The Knodell score is frequently used in drug trials in chronic hepatitis, particularly hepatitis C, as well as natural history studies. A decrease in the Knodell score is considered to represent histologic amelioration, or less progression to advanced fibrosis. No histologic feature represented in the Knodell score can predict the response to interferon. The reliability of the HAI score has not been well-established in specific forms of liver disease. Reliability refers to the extent to which repeated measurements of a relatively stable phenomenon fall closely to each other.[38]

A subsequent study evaluated the Knodell score among ten pathologists using a cohort of 30 liver biopsy specimens from patients who had documented hepatitis C. Interobserver correlation for three inflammatory components of the Knodell score was relatively poor (with kappa coefficients ranging from 0.25 to 0.46 [a perfect coefficient being 1.00]). The interobserver correlation for the total HAI was also relatively poor ranging from 0.48 to 0.57. Only the fibrosis score had good reliability with a kappa coefficient of approximately 0.80. Similar results were found for intraobserver reliability.

The HAI is weighted toward periportal necrosis and bridging necrosis. However, this weighting may have limitations. First, the Knodell score is relatively insensitive to changes in fibrosis, which is more important because it is fibrosis, and not inflammation per se, which leads to many of the sequelae of chronic liver disease. Furthermore, patients may have the same Knodell score despite having markedly different degrees of fibrosis.[39]

Second, the individual components of the Knodell score have not been well validated in the context of the natural history of viral hepatitis following treatment. Prior to treatment, specific components of liver histology may have different impact on disease prognosis.[40,41] Thus, assessment of improvement following treatment should probably also focus on this inflammatory component of liver histology, and fibrosis (rather than lobular or portal inflammation only). This is particularly relevant in studies assessing the efficacy of long-term interferon therapy in patients with hepatitis C who did not respond to interferon or interferon in combination with ribavirin.[38]

Histologic changes on serial liver biopsies are being used as a surrogate endpoint for determining the efficacy of treatment. Although the Knodell Score can be used as a categorical variable in statistical analysis, it is

unclear what degree of change constitutes a clinically meaningful histologic response. Changes in the mean fibrosis scores in studies are used as end points, but it is not that clearly discernable in individual patients. The Knodell score does not account for features specific to different types of viral hepatitis. As an example, the HAI does not account for lymphoid aggregates, bile duct injury, and macrovesicular fat that are often present in chronic hepatitis C.[38]

Table 1: KNODELL Histological Activity Index

	Score
I.Periportal ± bridging necrosis Score • None • Mild piecemeal necrosis • Moderate piecemeal necrosis (involves less than 50 percent of the circumference of most portal tracts) • Marked piecemeal necrosis (involves more than 50 of most portal tracts) • Moderate piecemeal necrosis plus bridging necrosis • Marked piecemeal necrosis plus bridging necrosis • Multilobular necrosis	 **0** **1** **3** **4** **5** **6** **10**
II.Intralobular degeneration and focal necrosisd • None • Mild (acidophilic bodies, ballooning degeneration and/or scattered foci of hepatocellular necrosis in < 1/3 of lobules or nodules • Moderate (involvement of 1/3 to 2/3 of lobules or nodules) • Marked (involvement of >2/3 of lobules or nodules)	 **0** **1** **3** **4**
III.Portal inflammation • No portal inflammation • Mild (sprinkling of inflammatory cells in <1/3 of portal tracts) • Moderate (increased inflammatory cells in 1/3 to 2/3 of portal tracts • Marked (dense packing of inflammatory cells in >2/3 4 of portal tracts)	 **0** **1** **2** **3**
IV.Fibrosis • No fibrosis • Fibrous portal expansion • Bridging fibrosis (portal-portal or portal- central linkage) • Cirrhosis	 **0** **1** **3** **4**

Table 2: Modified Histological Activity Index- The Ishak Score

Histological Activity Index	**Score**
A. Periportal or periseptal interface hepatitis (piecemeal necrosis) • Absent • Mild (focal, few portal areas) • Mild/moderate (focal, most portal areas) • Moderate (continuous around <50% of tracts or septa) • Severe (continuous around >50% of tracts or 4 septa)	 **0** **1** **2** **3** **4**

B. Confluent necrosis	
• Absent	**0**
• Focal confluent necrosis	**1**
• Zone 3 necrosis in some areas	**2**
• Zone 3 necrosis in most areas	**3**
• Zone 3 necrosis + occasional portal-central (P-C) Bridging	**4**
• Zone 3 necrosis + multiple P-C bridging	**5**
• Panacinar or multiacinar necrosis	**6**
C. Focal (spotty) lytic necrosis, apoptosis and focal inflammation	
• Absent	**0**
• One focus or less per 10 x objective	**1**
• One to four foci per 10 x objective	**2**
• Five to ten foci per 10 x objective	**3**
• More than ten foci per 10 x objective	**4**
D. Portal inflammation	
• None	**0**
• Mild, some or all portal areas	**1**
• Moderate, some or all portal areas	**2**
• Moderate/marked, all portal areas	**3**
• Marked, all portal areas	**4**
Maximum possible score for grading	**18**

Table 3: Modified Histological Activity Index Staging-Architectural Changes, Fibrosis and Cirrhosis Change

Activity Index Staging	**Score**
• No fibrosis	**0**
• Fibrous expansion of some portal areas, with or without short fibrous septa	**1**
• Fibrous expansion of most portal areas, with or without short fibrous septa	**2**
• Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	**3**
• Fibrous expansion of portal areas with marked Bridging, portal-portal (P-P) as well as portal-central (P-C)	**4**
• Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	**5**
• Cirrhosis, probable or definite	**6**
Maximum possible score	**18**

METAVIR Score

According to Bonis PA,[38] the Metavir score was developed in an attempt to address some of the problems with the Knodell score.[42] In contrast to the Knodell score, which was designed as a generic scoring system for chronic hepatitis, the Metavir score was specifically designed and validated for patients with hepatitis C.

The Metavir score is a semiquantitative classifications system consisting of an activity and a fibrosis score: The fibrosis score is assessed on a five point scale (0 = no fibrosis, 1 = portal fibrosis without septa, 2 = few septa, 3 = numerous septa without cirrhosis, 4 = cirrhosis). Compared to the Knodell fibrosis score (which

has only four levels), the Metavir score permits recognition of subtler variation in the degree of fibrosis. The activity score was graded according to the intensity of necroinflammatory lesions (A0 = no activity, A1 = mild activity, A2 = moderate activity, A3 = severe activity).

Table 4: Metavir Score

	Score
1. Focal lobular necrosis	
• Less than one necroinflammatory foci per lobule	**0**
• At least one necroinflammatory foci per lobule	**1**
• Several necroinflammatory foci per lobule or confluent or bridging necrosis	**2**
2. Portal Inflammation	
• Absent	**0**
• Presence of mononuclear aggregates in some portal tracts	**1**
• Mononuclear aggregates in all portal tracts	**2**
• Large and dense mononuclear aggregates in all portal tracts	**3**
3. Piecemeal necrosis	
• Absent	**0**
• Focal alteration of the periportal plate in some portal tracts	**1**
• Diffuse alteration of the periportal plate in some portal tracts or focal lesions around all portal tracts	**2** **3**
• Diffuse alteration of the periportal plate in all portal tracts	
4. Bridging necrosis	
• Absent	**0**
• Present	**1**

The inter-and intra-observer reliability of the activity and fibrosis score of the Metavir system are similar to the Knodell score. In one study, the kappa coefficients of the Metavir activity score and the HAI, and the Metavir fibrosis score and the Knodell fibrosis score were found to be similar (approximately 0.5 and 0.8, respectively).[42] A subsequent study found that the inter-observer agreement of the Metavir score depends highly upon the experience of the hepatopathologist 185. Agreement was influenced more heavily by the interpreter's experience compared with features of the specimen itself such as its length.[38]

On the other hand, a separate study evaluating the fibrosis and activity scores in specimens of various lengths suggested that the length of the biopsy was also important. The kappa coefficients for fibrosis were 0.75, 0.85, and 0.92 comparing specimens of 5, 10, and 15 mm respectively (considering the fibrosis score of a 20 mm specimen as the reference standard). The corresponding figures for the activity scores were 0.73, 0.81, and 0.77, respectively. The authors concluded that a specimen length of at least 10 mm usually reflects the fibrosis and activity scores reliably. Another study, however, suggested that a length of at least 25 mm is necessary to evaluate fibrosis accurately with a semi-quantitative score. Sampling variability becomes a major limitation when using more accurate methods such as automated image analysis.[43]

Thus, the main advantage of the Metavir score for hepatitis C is its relative simplicity, its focus on necroinflammatory lesions, and its increased sensitivity in the fibrosis score due to the addition of one extra fibrosis level. However, many of the limitations of the Knodell score discussed above also apply to the Metavir score. In particular, the fibrosis stages of the Metavir score have not been well-correlated to the natural history of hepatitis C.[38] Thus, earlier on, it was unclear whether individuals progress from early to late stages at a constant, linear rate, although this hypothesis has been proposed. But in subsequent studies it has been shown that the rates of progression of fibrosis were not normally distributed and greatly different estimated rates of progression were found in different patient groups.[44]

IMAGING IN LIVER FIBROSIS

Computed Tomography

CT scanning is useful for demonstrating the morphologic evidence of cirrhosis within the liver and in showing mesenteric and GI tract abnormalities, as well as the development of collateral vessels in portal hypertension. Splenomegaly and the presence of ascites, depicted in the second image above, are readily determined. CT scanning is commonly used to evaluate acutely decompensated patients with suspected subacute bacterial peritonitis, in order to exclude other inflammatory causes. CT scanning is valuable in characterizing lesions shown by US techniques or in evaluating decompensated patients with cirrhosis. In addition, it is increasingly being incorporated into the management of stable patients undergoing screening to identify neoplastic lesions.

Delineating Lesions

With improved technology, which permits rapid dynamic scanning using multi-slice CT scanners, scanning of the liver in multiple phases of contrast enhancement is now routinely recommended as the most sensitive method of detecting space-occupying lesions and evaluating vascular structures. However, substantial limitations remain in delineating small lesions (< 2 cm), particularly in patients with advanced cirrhosis.

Characteristic form of HCCA

The most characteristic form of hepatocellular carcinoma (HCCA) is a hyperattenuating nodule noted on arterial-phase imaging, with hyperattenuation and/or hypoattenuation developing on portal venous–phase imaging, shown below. On CT scanning, hyperattenuation in the arterial phase occurs in a variable proportion of cases, and in many instances, it is characteristic enough to permit confident diagnosis. The characterization of liver nodules is challenging when the findings are not "typical" of HCCA, and the Liver Imaging Reporting and Data (LI-RAD) classification system has recently been introduced to improve consistency and aid in management decisions.[45]

Five major features have been chosen, which, in combination, favor the diagnosis of HCCA: (1) masslike configuration, (2) arterial phase hyperenhancement, (3) portal venous phase or later phase hypoenhancement, (4) increase of 10 mm or more in diameter within 1 year, and (5) tumor within the lumen of a vein. The 5-point categorization scale is based on level of certainty of a benign, indeterminate lesion, or firm diagnosis of HCCA.

Attenuation

Nino-Murcia and colleagues described arterial enhancement with abnormal internal vessels or a variegated appearance.[46] In some instances, a single hyperattenuating focus may be the only evidence of HCCA, with no distinguishing characteristics on precontrast or portal venous-phase images. However, a proportion of lesions are hypoattenuating or isoattenuating on arterial-phase imaging. Dysplastic nodules also may be very similar to HCCA in their enhancement characteristics. However, hypoattenuating nodules depicted on a CT scan have high malignant potential. In the series by Takayasu and coauthors, 36 (60%) of 60 such lesions converted to hyperattenuating lesions, with the cumulative attenuation conversion rates of these 60 lesions reaching 58.7% within 3 years of follow-up.[47] Thirteen of the lesions were biopsied immediately after attenuation conversion was observed to prove that they were HCCA. The presence of hepatitis C viral antibody and lesion size at detection were correlated with the attenuation conversion rate.

Documenting Complications

CT scanning is useful in documenting complications associated with HCCA, such as portal vein thrombosis, and can be used to identify malignant invasion with a high specificity

Degree of Confidence

In terms of degree of confidence, multidetector CT is a robust imaging modality, with a reported sensitivity of 100% for tumors larger than 2 cm, but lower sensitivity (as high as 96%) for lesions in the 1- to 2-cm range.[48] It is incumbent on the radiologist to be vigilant in high-risk patients undergoing screening and pretransplantation evaluation and to suggest confirmation of suspected intrahepatic tumors by additional imaging modalities such as MRI, biopsy, or serial close surveillance if imaging characteristics are atypical, in accordance with American Association for the Study of Liver Diseases (AASLD) guidelines for lesions in the

1- to 2-cm range.[49] Use of the LI-RAD classification system is expected to enhance consistency in reporting and communication of abnormalities.

A major diagnostic dilemma for radiologists is the finding of a small, focal, transiently enhancing lesion (transient hepatic attenuation difference [THAD]) in a patient undergoing CT screening for hepatoma. Even if all other imaging phases are normal, statistically, on hepatic arterial phase images, a high-attenuation focal hepatic lesion of cirrhotic liver is usually HCCA.[50] The likelihood of a hypervascular tumor is of course higher if an area of relative hypoattenuation is present on portal venous–phase images.

THAD may also occur in other tumorous conditions, such as peripheral cholangiocarcinoma, and in nonmalignant neoplasms, such as small hemangiomas. THAD is associated with a change in the blood supply to the liver, which can occur with the development of arterioportal shunts; in perfusion changes related to venous thrombosis, congestion, or fatty infiltration following radiofrequency ablation; and in locations where auxiliary blood supply is present such as segment IV or the gallbladder fossa. If the lesion has a wedge shape, has a straight margin, or if normal vessels can be seen passing through the lesion, the probability of nontumorous THAD becomes greater. The presence of normal signal intensity on T1- and T2-weighted images excludes hypervascular tumor on MRI.[51]The enlargement of the caudate lobe in cirrhosis, with other regions of retraction may be mimicked in patients with breast carcinoma metastatic to the liver who are undergoing chemotherapy. Young and colleagues suggest that the mechanism is through nodular regeneration.[52]

False Positives/Negatives

False negatives may occur as a result of technical limitations or because an advanced degree of liver fibrosis impacts enhancement characteristics. The process of the liver's response to injury results in regenerative nodules and dysplasia, which occur prior to frank transformation into HCCA. Imaging characteristics may not be typical of HCCA and lead to a lower level of confidence and lower LI-RAD classification, which may then result in additional contrast imaging or further surveillance for lesion growth. Dysplastic nodules may mimic HCCA. Nontumorous arterioportal shunting in livers with cirrhosis has been reported by Kim and colleagues as mimicking hypervascular tumor.[53]A higher injection rate may increase the number of small, false-positive, hypervascular lesions. Ichikawa and coauthors studied 60 patients with suspected HCCA[54]; they reported in 2006 that the use of an iodinated contrast injection rate of 5 mL/sec resulted in an 18% reduction in specificity, from 67% to 48%, with no significant change in sensitivity (88% vs 80%) compared with a 3-mL/sec injection rate.[54]

Magnetic Resonance Imaging

MRI offers an alternative noninvasive method of imaging the liver based on tissue-specific characteristics.[55] In addition to demonstrating morphologic changes in cirrhosis, MRI is suited for the evaluation of vascular structures for patency or tumor invasion. T1-weighted images are valuable in providing anatomic detail, and T2-weighted images are more sensitive in detecting mass lesions and characterizing cysts and hemangiomata. MRI technology continues to evolve rapidly, with the development of techniques, such as the use of gradient-echo, fast spin-echo (SE), and diffusion-weighted sequences, that permit the rapid acquisition of images required in association with paramagnetic contrast use. Tumor enhancement patterns following administration of gadolinium-cased contrast agents are incorporated into Liver Imaging Reporting and Data (LI-RAD) criteria. Gadolinium, through its paramagnetic properties, reduces T1 and T2 relaxation times, with an improved signal-to-noise ratio. Gadolinium is chelated to organic compounds in order to form extracellular contrast agents which, having entered the liver, become distributed from the intravascular to interstitial spaces.[50] Agents have now been developed with characteristics of extracellular contrast agents combined with hepatocyte-selective and blood-pool characteristics. The combined agents, such as gadobenate dimeglumine and gadoxetic acid, can be used for dynamic-phase imaging for liver lesion detection and characterization with sensitivity similar to that of extracellular contrast agents. Their uptake into hepatocytes from the blood and excretion into the bile via the organic anion transport protein are analogous to bilirubin uptake. In their hepatocyte-selective phase, these agents provide prolonged opacification of liver parenchyma.[51]

Earlier work evaluating contrast-enhanced images of the liver alone did not appear to be very sensitive; a 2001 study using gadopentetate dimeglumine (Magnevist) in explanted livers for confirmation found an overall sensitivity of only 54% in the detection of hepatocellular carcinoma (HCCA), with sensitivity achieving 80% for lesions larger than 2 cm, 50% for lesions of 1-2 cm, and 33% for lesions smaller than 1 cm. The sensitivity for dysplastic nodules was only 15%.[52] Use of diffusion-weighted sequences appears to be

able to predict development of HCCA in dysplastic nodules. When used with a more recently developed chelate, gadoxetic acid, a hepatobiliary specific agent, nodules that showed hyperintensity on diffusion-weighted images and were hypovascular and hypointense on hepatobiliary-phase imaging were more likely to progress to hypervascular HCCA.[53] The introduction of new, more specific hepatic agents such as gadobenate dimeglumine, ferucarbotran, and gadoxetic acid have also improved accuracy, which is now reported to exceed 95%.[55]

Gadoxetate was recently found superior to multiphase multidetector CT imaging in a population of 58 patients with 87 HCCAs (mean size ± standard deviation, 1.8 cm ±1.5; range, 0.3–7 cm) in a multireader study.[56] Regardless of lesion size, the average diagnostic accuracy and sensitivity were significantly greater with gadoxetate disodium–enhanced MRI (average diagnostic accuracy, 0.88; 95% confidence interval [CI], 0.80-0.97; average sensitivity, 0.85; 95% CI, 0.74-0.96) than with multidetector CT (average diagnostic accuracy, 0.74; 95% CI, 0.65-0.82; average sensitivity, 0.69; 95% CI, 0.59-0.79) ($P < .001$ for each), with inter-reader agreement good to excellent.

The use of newer hepatobiliary-specific agents remains controversial, because they do not provide increased sensitivity with respect to the extracellular agents. Sensitivity may be limited by compromised uptake in patients with advanced cirrhosis and poor liver function. As well-differentiated HCCA may accumulate hepatobiliary-specific agents on delayed hepatocyte imaging, these lesions may be indistinguishable from benign hepatocyte-containing lesions.

MRI has been studied extensively in diffuse liver disease. Tani and coauthors reported that focal and diffuse steatosis are recognized as increased signal intensity on T1-weighted MRIs and as diffuse low signal intensity on opposed-phase, T1-weighted images.[57] Regenerative nodules are seen as small, masslike structures and are hypointense on T2-weighted images.

Degree of Confidence

MRI has an increasing role in screening particularly at specialized transplantation centers. The level of confidence in MRI, particularly when newer contrast agents such as hepatobiliary contrast are used, appears to be equivalent to, or exceed, the level of confidence in dual-phase, spiral CT scanning or triple phase MDCT scanning. In early reporting, overall sensitivity of MRI was reported by Bartolozzi and colleagues to be 86% for a prospective assessment of precontrast and postcontrast images.[58]

Similar sensitivity has been reported by Kondo and coauthors, who retrospectively analyzed images of the liver from 33 patients on a segment-by-segment basis. A total of 261 segments, which included 39 HCCAs and 21 metastases, were independently reviewed by 3 radiologists. Unenhanced and gadolinium-enhanced MRI scans were reviewed first, and then ferumoxides-enhanced MRI scans were added for a combined review. CTAP images and biphasic CT hepatic angiography (CTHA) scans were reviewed together.

It was determined in the study that the sensitivity for the detection of hepatic tumors was equivalent for combined unenhanced, gadolinium-enhanced, and ferumoxides-enhanced MRI scans (86%) and for combined CTAP images and biphasic CTHA scans (87%). Specificity was higher with MRI scans (95%, $P < .01$) than with CT scans (91%), with improved performance achieved by combining ferumoxides-enhanced MRI scans with unenhanced and gadolinium-enhanced MRI scans ($A_z = 0.9$ vs 0.950, $P = .0502$). The radiologists' preoperative ability to detect malignant hepatic tumors using combined unenhanced, gadolinium-enhanced, and ferumoxides-enhanced MRI scans was analogous to that when combined CTAP images and biphasic CTHA images ($A_z = 0.959$) were used.

The introduction of new, more specific hepatic agents such as gadobenate dimeglumine, ferucarbotran, and gadoxetic acid have improved accuracy, now reported to exceed 95%.[48] Therefore, it appears that MRI has a diagnostic accuracy similar to or higher than that of CT scanning for lesions over 1 cm. However, MRI still has significant limitations in the specificity of small-tumor detection, which further development of tissue-specific contrast agents may overcome. MRI does appear to enable the distinction of arterioportal shunts associated with tumor from spontaneous shunts associated with cirrhosis alone. When a superparamagnetic agent (iron oxide) is used, Mori and coauthors noted that tumorous shunts have reduced signal loss, whereas nontumorous shunts resemble normal liver parenchyma in the degree of signal loss, particularly on T2-weighted gradient-echo images.[59]

False Positives/Negatives

Regenerative nodules can resemble hypovascular HCCA; Kim and colleagues recognized that infarcted regenerative nodules can pose particular problems.[60] Liver of patients with nodular regenerative hyperplasia, also known as idiopathic portal hypertension or hepatoportal sclerosis, has morphologic features that are

indistinguishable from cirrhosis. However, the histologic features of these livers demonstrate nodules but not evidence of fibrosis, which is the hallmark of cirrhosis.

Dysplastic nodules occurring in cirrhotic patients, as already noted, may share imaging characteristics of HCCA. Differentiation may be difficult. Hyperintensity on diffusion-weighted images in hypovascular, hypointense nodules on hepatobiliary-phase gadoxetic acid has been found to be strongly associated with progression to hypervascular HCCA,[56] but this finding awaits validation in a population that did not have a history of prior treatment for HCCA.

False negatives may occur in small lesions, related to observer limitations. In a 2013 study tracking 17 patients with elevated alpha-fetoprotein (AFP) and initially negative MRI scans, 10 (59%) of 17 of patients developed HCCA over the next months after a mean of 138 days (range, 41-247 d). Of 10 HCCAs detected at follow-up MRI, 5 were identifiable in retrospect at initial MR studies (mean diameter, 1.4 cm). Fifty-percent of the lesions detected on subsequent MRI were visible in retrospect. Serum AFP levels in patients with HCCAs were significantly higher than those in patients without HCCAs and progressively increased over time ($P = .012$).[61]

Ultrasonography

Real-time US, in combination with color flow Doppler US, is currently the most frequently used diagnostic imaging modality worldwide in the screening and evaluation of patients with cirrhosis.[62] In addition to demonstrating the morphologic characteristics of cirrhosis, including hepatic contour, texture, and the presence of portal collaterals, Doppler US provides useful information on portal hemodynamics.[63]

Portal Blood Flow

Doppler evaluation in a patient with cirrhosis can demonstrate high-velocity blood flow in the enlarged hepatic artery, which becomes tortuous as the underlying degree of fibrosis increases. PI, a measure of hepatic arterial vascular resistance, is elevated in patients with cirrhosis, and Schneider and colleagues have determined that it correlates quite well with the HVPG.[64] The normal direction of portal blood flow is maintained initially, but as the degree of cirrhosis progresses, damping of the usual triphasic signal in the intrahepatic veins and loss of respiratory variation in the portal venous system occur. Flow within the main portal vein gradually diminishes; bidirectional and (subsequently) reversal of flow may be seen, usually with accompanying development of collateral vessels.

Collateral Vessels

These collaterals are most frequently detected in the splenorenal region (21%), or as patent paraumbilical collaterals. Study by Von Herbay et al on 109 patients with cirrhosis, the presence of collaterals correlated significantly with the presence of ascites, esophageal varices, and the inversion of portal flow, but not with splenomegaly.[65]

Doppler US continues to be used in the noninvasive physiologic evaluation of the portal tract in patients who, in an attempt to reduce the risk of GI hemorrhage, undergo pharmacologic modulation of portal pressures. However, Doppler US does not correlate well with intrahepatic pressures or with the portal systemic pressure gradient. For example, the evaluation of systemic flow in the femoral or brachial artery also has been studied, but only a 50% correlation is observed in reduction of femoral blood flow and portal pressure in response to propranolol treatment.

Vascular Impedance

Arterial vascular impedance can be estimated as the RI, which represents the ratio of the difference between the peak systolic and end-diastolic velocities to the peak systolic velocity. This can be measured directly in the superior mesenteric or hepatic artery. In addition to pharmacologic agents, however, numerous factors on the capillary and venous side can affect the RI. These include alteration of blood flow in the portal veins following a meal and the extent of development of collateral vessels, in addition to increased resistance from fibrosis or hepatic congestion because of fatty infiltration or right-sided heart failure.

Screening for Focal Hepatic Masses

US have an established role in screening for focal hepatic masses, despite rather low specificity.

Demonstration of shunt vascularity by Doppler US enables a diagnosis to be made with high specificity, but neovascularization occurring in small lesions may be below the threshold of detection of even sophisticated US systems.
Multifocal lesions occasionally may be obscured, but in general, the lesions can be appreciated as tumor masses that either have vascularity or are avascular, but displacing, vessels.

Harmonic imaging/intravascular contrast agents

The development of intravascular contrast agents (which have little or no toxicity) initiated a re-evaluation of ultrasonographic sensitivity and specificity, which early investigations have suggested are greatly improved. The technical performance of ultrasonographic systems concomitantly has been modified to insonate tissue optimally, as well as to detect and process vascular and parenchymal signals from contrast agents. Techniques that are used include harmonic imaging, which is designed to capture nonlinear resonant frequencies from tissue and microbubbles with enhanced signal compared to background noise.
The microbubbles can be disrupted by insonation at a high mechanical index (MI), which represents the peak negative pressure of the transmitted ultrasonographic pulse, and this produces a strong, very brief echo. The microbubbles can then be visualized at a lower MI intensity (< 0.5) without causing further disruption. The bubbles can be seen within vessels and are detectable within capillaries in which conventional Doppler techniques cannot detect flow.
HCCAs have variable enhancement patterns on contrast-enhanced harmonic US. Homogeneous and heterogeneous enhancement have been described by Kim and colleagues, correlating with CT-scan enhancement patterns.[66] Three of 8 patients in this study also had linear tumor vessels in the lesions, but globular or peripheral enhancement seen in hemangioma and metastases, respectively, were not shown. Wilson and colleagues described perilesional and intralesional vessels. In a pilot study of 3 patients with biopsy-proven HCCA, the authors found variable characteristics, including identification of tumor vessels within the lesion and increased echogenicity within the center of the tumor.
The use of harmonic power Doppler US remains in the investigational phase, as researchers study the impact of technical parameters, such as pulse repetition frequency, wall filter settings, and injection rates on lesion detection. The decreased sensitivity of harmonic power Doppler US, in comparison with conventional power Doppler US on precontrast, is more than compensated for on contrast-enhanced imaging.
Through the evaluation of characteristics related to contrast-enhanced US, including portal-phase enhancement, negative washout (also called negative enhancement), arterial-phase peripheral nodularity and fill-in, and degree of arterial enhancement, algorithms have been developed that allow a logical and accurate differentiation of HCCA from other lesions, such as hemangioma or focal nodular hyperplasia.[67]
In the foreseeable future, the use of contrast in US is expected to reduce the necessity for additional corroborative imaging studies and to increase reliance on this already widely available, reasonably economical and adaptable modality.

Real-Time Elastography

Real-time elastography is a promising technique for the noninvasive evaluation of the severity of hepatic fibrosis. This technique has been commercially developed by Hitachi Medical Systems and was used by Friedrich-Rust and colleagues to assess liver fibrosis in 79 patients with chronic viral hepatitis. Using a stepwise logistic regression analysis in patients and controls to define a tissue elasticity score, diagnostic accuracy was 0.75 for significant fibrosis, 0.73 for severe fibrosis, and 0.69 for cirrhosis, with a highly significant correlation (Spearman's correlation coefficient = 0.48) between the elasticity scores and the histologic fibrosis stage.
Elastography is now widely used as a noninvasive test for staging fibrosis and is now being used in place of liver biopsy to investigate the natural history of chronic liver diseases; however, wide-scale outcome studies are not yet published. Its use is restricted in patients with acute hepatitis, obstructive cholestasis, and passive congestion, which can also alter liver stiffness.[68]

Role of US in Biopsy and Ablative Therapy

The fact that US is readily available and can be used in the guidance of percutaneous biopsy and of ablative ethanol or acetic acid injection of focal lesions, as well as the fact that it can be employed in conjunction with radiofrequency (RF) probes for thermal ablation, means that selected patients to be evaluated, diagnosed, and treated using 1 modality. The use of US contrast agents (SonoVue®) has been helpful in differentiating viable from necrotic tissue, thereby improving diagnostic accuracy, particularly for lesions under 2 cm. Wu and

coauthors achieved a better diagnostic accuracy for lesions that were evaluated by contrast enhancement prior to biopsy than for those that were evaluated with unenhanced US (97.1% vs 78.8%).[69]
In a representative study by Livraghi using percutaneous ethanol ablation, 5-year survival rates for patients with HCCA lesions that were smaller than 5 cm and who suffered from Child C, B, or A cirrhosis were 0%, 29%, and 47%, respectively.[70] Poorer results were obtained for multiple tumors or in the presence of portal thrombosis.

Degree of Confidence

The presence of portal hypertension can be inferred based on the measurement of portal vein diameter; a sensitivity of 75% and a specificity of 100% for a diameter greater than 1.3 cm have been claimed. As previously noted, however, measurements of flow and vessel diameter are only indirectly related to portal pressure, and the degree and level of intrahepatic obstruction (presinusoidal or postsinusoidal), arterial flow to the liver, and capacitance of the collateral flow may affect flow parameters. Other findings, such as loss of respiratory variation in the diameter of the main portal vein or the presence of collaterals, are considered by Zimmerman and coauthors to be approximately 80% sensitive.[71]
Such a wide range of variability exists among patients that measurements of this nature should be considered useful only in research settings. If no other corroborative evidence has been obtained, caution should be used in interpreting these measurements as determinants of the presence of portal hypertension.

Ultrasonographic Characteristics

The ultrasonographic characteristics of HCCAs are variable, reflecting the diversity of neoplastic differentiation. However, certain pathologic characteristics occur with greater frequency and are helpful in characterizing hepatic lesions on ultrasonographic examination. For example, a pseudocapsule may be identified as a halo on ultrasonographic imaging. Neovascularity with arterial-venous shunting, the hallmark of malignant transformation, can be identified by current ultrasonographic systems once a lesion has reached approximately 2 cm. Contrast agents that increase the signal-to-noise ratio enable tumor vascularity to be detected with greater sensitivity.

Ultrasonographic Sensitivity

In patients with cirrhosis attributed to multiple risk factors, Fasani and colleagues report that, compared with CT scanning, US appears to understage patients with multinodular lesions. The sensitivity of US is also reduced in patients with heterogeneous livers. This understaging may be significant when considering patients for transplantation or ablative therapy, indicating that corroborative imaging with MRI or CT scanning may be of benefit in patients with advanced cirrhosis or a multifactorial etiology.

US combined with Intravascular Contrast Agents

US appears to be very promising, particularly when combined with intravascular microbubble contrast agents, in assessing the effectiveness of tumor ablation. Choi studied the tumor characteristics of 40 patients with 45 nodular HCCA lesions 1-3.8 cm in diameter.[72] The patients were undergoing US-guided, percutaneous RF ablation with power Doppler US before and after intravenous injection of a microbubble contrast agent. In 33 of the 45 HCCAs, intratumoral flow was seen at power Doppler US before the administration of a contrast agent. After administration of the contrast agent, an increase in the degree of visualized flow was observed.
After RF ablation, none of the ablated tumors showed intratumoral flow signals at unenhanced power Doppler US, whereas 6 showed marginal intratumoral flow signals at contrast agent–enhanced power Doppler US. This correlated with enhancing foci that were suggestive of viable tumor in corresponding areas, as found at immediate follow-up with contrast-enhanced CT scanning. Thus, these preliminary data suggest that contrast-enhanced power Doppler US can be a promising noninvasive technique for assessing therapeutic response.

False Positives/Negatives

Regenerative nodules, dysplastic nodules, focal fat, and fatty sparing may mimic focal HCCA. Other nonmalignant hepatic neoplasms, such as hemangioma, may appear similar to HCCA, although arteriovenous (AV) shunts are uncommon. Focal nodular hyperplasia and liver cell adenoma may have extensive AV shunting, with this occurring most often in females.

The development of US contrast agents should further increase sensitivity; evidence suggests that the combination of advanced ultrasonographic imaging techniques (harmonic imaging) can increase the conspicuity of liver lesions (hence, the sensitivity of US when combined with microbubble contrast).

Nuclear Imaging

Functional imaging techniques using 99m Tc-labeled sulfur colloid provided some indication of hepatic function. The agent is taken up by reticuloepithelial (RE) cells, and colloid shift to the other RE organs (bone marrow, spleen) provides indirect evidence of portal hypertension. In addition, heterogeneous uptake enables recognition of underlying hepatic dysfunction. Volumetric estimates of the liver can be made but have been superseded by other imaging techniques.

Fluorine-18 fluorodeoxyglucose (18F-FDG) is taken up by tumor cells, but the use of this agent in conjunction with positron emission tomography (PET) scanning appears to be more suited to larger, better-differentiated lesions. Therefore, at present, Trojan and colleagues believe that 18F-FDG PET is unlikely to replace the other techniques.[73] Sensitivity appears in the range of only 55%, compared with the 90% sensitivity of CT scanning, and Khan and coauthors report that better-differentiated tumors tend to have a lower level of uptake.[74] The prognostic implications of this finding have not been elucidated. In an investigation, Kim and colleagues expressed hope that functional imaging techniques may be able to predict tumor response to chemotherapy.[75]

Degree of Confidence

Since the sensitivity of PET is relatively low, this modality is not at present recommended as a clinical screening tool for HCCA, and its use remains investigational. Kurtaran and coauthors report that it may be helpful in discriminating benign hepatic lesions, such as focal nodular hyperplasia (FNH), from malignant lesions, because there is reduced uptake in FNH.

MANAGEMENT

Generally, liver damage from cirrhosis cannot be reversed, but treatment could stop or delay further progression and reduce complications. A healthy diet is encouraged, as cirrhosis may be an energy-consuming process. Close follow-up is often necessary. Antibiotics will be prescribed for infections, and various medications can help with itching. Laxatives, such as lactulose, decrease risk of constipation; their role in preventing encephalopathy is limited.

Alcoholic cirrhosis caused by alcohol abuse is treated by abstaining from alcohol. Treatment for hepatitis-related cirrhosis involves medications used to treat the different types of hepatitis, such as interferon for viral hepatitis and corticosteroids for autoimmune hepatitis. Cirrhosis caused by Wilson's disease, in which copper builds up in organs, is treated with chelation therapy (e.g. penicillamine) to remove the copper.

Pharmacologic Treatment

Specific medical therapies may be applied to many liver diseases in an effort to diminish symptoms and to prevent or forestall the development of cirrhosis. Examples include prednisone and azathioprine for autoimmune hepatitis, interferon and other antiviral agents for hepatitis B and C, phlebotomy for hemochromatosis, ursodeoxycholic acid for primary biliary cirrhosis, and trientine and zinc for Wilson disease.

These therapies become progressively less effective if chronic liver disease evolves into cirrhosis. Once cirrhosis develops, treatment is aimed at the management of complications as they arise. Certainly variceal bleeding, ascites, and hepatic encephalopathy are among the most serious complications experienced by patients with cirrhosis. However, attention also must be paid to patients' chronic constitutional complaints.

Zinc Deficiency

Zinc deficiency commonly is observed in patients with cirrhosis. Treatment with zinc sulfate at 220mg orally twice daily may improve dysgeusia and can stimulate appetite. Furthermore, zinc is effective in the treatment of muscle cramps and is adjunctive therapy for hepatic encephalopathy.

Pruritus

Pruritus is a common complaint in cholestatic liver diseases (e.g. primary biliary cirrhosis) and in noncholestatic chronic liver diseases (e.g. hepatitis C). Although increased serum bile acid levels once were

thought to be the cause of pruritus, endogenous opioids are more likely to be the culprit pruritogen. Mild itching complaints may respond to treatment with antihistamines and topical ammonium lactate.
Cholestyramine is the mainstay of therapy for the pruritus of liver disease. To avoid compromising GI absorption, care should be taken to avoid co-administration of this organic anion binder with any other medication.
Other medications that may provide relief against pruritus include antihistamines (e.g. diphenhydramine, hydroxyzine), ursodeoxycholic acid, ammonium lactate 12% skin cream (Lac-Hydrin, Westwood-Squibb Pharmaceuticals, Inc, Princeton, NJ), doxepin, and rifampin. Naltrexone, an opiate (an opioid antagonist), may be effective but is often poorly tolerated. Gabapentin is an unreliable therapy. Patients with severe pruritus may require institution of ultraviolet light therapy or plasmapheresis.

Hypogonadism

Some male patients suffer from hypogonadism. Patients with severe symptoms may undergo therapy with topical testosterone preparations, although their safety and efficacy is not well studied. Similarly, the utility and safety of growth hormone therapy remains unclear.

Osteoporosis

Patients with cirrhosis may develop osteoporosis. Supplementation with calcium and vitamin D is important in patients at high risk for osteoporosis, especially patients with chronic cholestasis or primary biliary cirrhosis and patients receiving corticosteroids for autoimmune hepatitis. The discovery on bone densitometry studies of decreased bone mineralization may prompt the institution of therapy with an aminobisphosphonate (e.g. alendronate sodium).

Vaccination

Patients with chronic liver disease should receive vaccination to protect them against hepatitis A. Other protective measures include vaccination against influenza and pneumococci.

Drug Hepatotoxicity in the Patient with Cirrhosis

The institution of any new medical therapy warrants the performance of more frequent liver chemistries; patients with liver disease can ill afford to have drug-induced liver disease superimposed on their condition. Medications associated with drug-induced liver disease include the following:

- NSAIDs
- Isoniazid
- Valproic acid
- Erythromycin
- Amoxicillin/clavulanate
- Ketoconazole
- Chlorpromazine
- Ezetimibe

Statins

Hepatic 3-methylglutaryl coenzyme A (HMG CoA) reductase inhibitors are frequently associated with mild elevations of alanine aminotransferase (ALT) levels. However, severe hepatotoxicity is reported infrequently.[76] The literature suggests that statins can be used safely in most patients with chronic liver disease.[77] Certainly, liver chemistries should be followed frequently after the initiation of therapy.
In a study of the effects of statins in 58 patients with primary biliary cirrhosis, Rajab and Kaplan concluded that statin use is safe in patients with this condition.[78] Individuals in the study were on statins for a median period of 41 months, with ALT levels measured every 3 months. The authors found that these levels did not increase, being slightly elevated when statin treatment began and normal by the last follow-up analysis. Patients did not complain of muscle pain or weakness, and serum cholesterol levels fell by 30%.

Analgesics

The use of analgesics in patients with cirrhosis can be problematic. Although high-dose acetaminophen is a well-known hepatotoxin, most hepatologists permit the use of acetaminophen in patients with cirrhosis at doses of up to 2000mg daily.

NSAID use may predispose patients with cirrhosis to develop GI bleeding. Patients with decompensated cirrhosis are at risk for NSAID-induced renal insufficiency, presumably because of prostaglandin inhibition and worsening of renal blood flow. Opiate analgesics are not contraindicated but must be used with caution in patients with preexisting hepatic encephalopathy on account of the drugs' potential to worsen underlying mental function.

Other Drugs

Aminoglycoside antibiotics are considered obligate nephrotoxins in patients with cirrhosis and should be avoided. Low-dose estrogens and progesterone appear to be safe in the setting of liver disease.

A review by Lewis and Stine provided recommendations, including the following, on the safe use of medications in patients with cirrhosis:[79,80]

- Lower medication doses should generally be used, particularly in patients with significant liver dysfunction
- Proton-pump inhibitors and histamine-2 blockers should be used only for valid indications, since they may lead to serious infections in patients with cirrhosis

Transplantation

If complications cannot be controlled or when the liver ceases functioning, liver transplantation is necessary. Survival from liver transplantation has been improving over the 1990s, and the five-year survival rate is now around 80%, depending largely on the severity of disease and other medical problems in the recipient. In the United States, the MELD score is used to prioritize patients for transplantation. Transplantation necessitates the use of immune suppressants (cyclosporine or tacrolimus).

Decompensated cirrhosis

In patients with previously stable cirrhosis, decompensation may occur due to various causes, such as constipation, infection (of any source), increased alcohol intake, medication, and bleeding from esophageal varices or dehydration. It may take the form of any of the complications of cirrhosis.

Patients with decompensated cirrhosis generally require admission to hospital, with close monitoring of the fluid balance, mental status, and emphasis on adequate nutrition and medical treatment - often with diuretics, antibiotics, laxatives and/or enemas, thiamine and occasionally steroids, acetylcysteine and pentoxifylline. Administration of saline is generally avoided as it would add to the already high total body sodium content that typically occurs in cirrhosis Declarations [80-104].

Conclusion

The frequency of treatment response was very high (96.6%) in genotype 3 cirrhotic patients who were taking Sofosbuvir, Ribavirin and Daclatasvir. Majority (63%) of the genotype 3 cirrhotic patients (M-63% and F-27%) from the age group of 18-50 years were the target audience. Patients with HCV HIV co-infection, HBV HCV co-infection and CKD were excluded. Treatment started on Sofosbuvir (400mg once daily), weight-based ribavirin 1200 or 1000 mg/day if ≥75 or <75 kg body weight, respectively) Ribavirin (and Daclatasvir (60mg once daily) by consultant gastroenterologist were followed. The course of this treatment was 6 months i.e. 24 weeks. Patients who completed the treatment were advised for HCV PCR Quantitative analysis at end of treatment to check for ETR. The frequency of end of treatment response in genotype 3 cirrhotic patients taking Sofosbuvir, Ribavirin and Daclatasvir was found in 366 (96.06%) among patients was very high. Triple therapy in combination with daclatasvir leads to a higher rate of SVR both in previously untreated genotype 3 cirrhotic patients and in those who have failed prior antiviral treatment. Treatment found effective against HCV genotype 3 infections. Therefore, Sofosbuvir, Ribavirin and Daclatasvir recommended using in routine for genotype 3 cirrhotic patients to achieve end treatment response that will in turn reduce the morbidity and mortality of our population.

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