after randomization for patients not actively bleeding. Patients whose bleeding after treatment was estimated to exceed 5 units in 24 hr were counted as treatment failures. All of these patients who failed treatment were removed from the protocol and then treated with either ES, THS or both. Despite the break in protocol, the results for the second major outcome of the study (survival) were attributed to the originally assigned treatment groups.

The major findings reported by this study are (i) that the frequency of rebleeding 15 months after randomization (but not 27 months afterward) is significantly reduced by combined therapy with propranolol plus ES compared with propranolol alone, and (ii) that survival is significantly improved at 2 years after randomization (but not earlier) by combined therapy with propranolol plus ES, compared with propranolol alone.

The first question to ask before accepting these findings is whether there are other plausible interpretations of the results. In this regard, problems did occur in the proficiency with which the treatments were administered. Were the assigned treatments received by the subjects? Did they comply with therapy? Were additional treatments including abstinence equally applied in the treatment groups?

With respect to the first issue, all 31 subjects in the ES plus propranolol group received at least one session of sclerotherapy. Although only 29 of the intended 35 subjects in the THS group received sclerosis, the investigators were able to show that this did not influence the results of the study. In the propranolol group, however, eight of the 31 subjects never did receive the drug. Of the 97 subjects, 25 had not become stable enough to receive the drug, and eight did not receive it because they were intolerant or had a contraindication. Thus, 34% of subjects in the study did not receive the drug. In addition, for those subjects who did receive propranolol, compliance was assessed only for the period until the first episode of rebleeding, and the data so collected revealed variable compliance. Because it appears that so few subjects received propranolol alone, what was in essence compared in the study is supportive therapy vs. ES vs. THS. Regarding the second issue of alcohol abstinence, there is no documentation provided in the report to answer this question. The possibility exists that those in the propranolol plus ES group, who had a lower frequency of major rebleeding, did better because there the number of abstainers was greater and hence the rate of progression of the underlying liver disease was lower.

The second question to ask is how should the results for death be interpreted, when there were so many modifications of the assigned therapy once the first major outcome (major rebleeding) had been reached. What was really being compared in the analysis for this outcome is whether a policy of treatment with propranolol alone is better than either a policy of treatment with propranolol plus ES or a policy of propranolol plus THS. Unfortunately, the efficacies of the three treatments were not compared themselves, so that the question of the effect of the three treatments on survival remains unclear.

In summary, the results of this study, which essentially compared supportive medical therapy vs. ES vs. THS in alcoholic men with severe liver disease, concur with the results of previous studies in demonstrating that ES is better than supportive medical therapy alone in decreasing the frequency of rebleeding. Furthermore, the benefits of ES occur in the first 15 months after the index bleed, but are not sustained. Based on this study, it remains unclear whether propranolol plus ES is better than propranolol alone in preventing rebleeding and it remains unclear whether survival is improved by adding ES to propranolol in patients with EVB.

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THE DIFFERENTIATION OF CHOLESTEROL AND PIGMENT GALLSTONES

van Erpecum KJ, van Berge Henegouwen GP, Stoelwinder B, Stolk MFJ, Eggink WF, Govaert WHA. Cholesterol and pigment gallstone disease: comparison of the reliability of three bile tests for differentiation between the two stone types. Scand J Gastroenterol 1988; 23:948–954.

ABSTRACT

Gallbladder biles and stones were obtained at 116 cholecystectomies for symptomatic gallstone disease. All 33 patients younger than 50 years had cholesterol stones, whereas 40% of the older patients had pigment stones. We compared the reliability of three different bile tests for the differentiation between cholesterol and pigment stone patients. Whereas both the presence of cholesterol monohydrate crystals in fresh gallbladder bile and a nucleation time ≤20 days in ultrafiltered gallbladder bile had a specificity of 100% for cholesterol gallstone disease, biliary supersaturation with cholesterol (cholesterol saturation index

>1.0) had a low specificity. The sensitivity of nucleation time ≤20 days for cholesterol gallstone disease was 78% in concentrated gallbladder biles (biliary total lipid concentration ≥5 g/dl) but only 21% in dilute biles (biliary total lipid concentration <5 g/dl). In contrast, examination for the presence of cholesterol crystals in fresh bile was reasonably sensitive both in concentrated and dilute gallbladder biles (sensitivity, 84% and 72%, respectively). In addition, duodenal bile obtained from 16 patients (10 cholesterol, 6 pigment) before cholecystectomy showed cholesterol crystals in 7 of the cholesterol but in none of the pigment stone patients. We conclude that examination of fresh bile for cholesterol crystals is a specific and reasonably sensitive test for cholesterol gallstone disease.

COMMENTS

Gallbladder stones are traditionally classified as pigment or cholesterol stones. Pigment stones are a mixture of insoluble calcium salts of bilirubinate, carbonate, phosphate and long-chain fatty acids, arrayed on a glycoprotein matrix. Pigment stones probably form only if bile is supersaturated with calcium bilirubinate, often accompanied by supersaturation with calcium carbonate. The development of cholesterol gallstones involves a triple defect with supersaturation and abnormal rapid nucleation of cholesterol as well as altered gallbladder motor function (1–3). In 100 cholecystectomized American patients, Trotman et al. (4) found 23 with pigment and 77 with cholesterol cholecystolithiasis. A similar distribution has been reported from several other Western countries and in the present report of van Erpecum et al. There is an increasing need to discriminate pigment stone carriers from cholesterol stone carriers, since the latter patients may be candidates for dissolution therapy and/or extracorporeal shock wave lithotripsy (5, 6). Due to the different mechanisms of stone development in patients with pigment and patients with cholesterol stones, there have been many attempts to differentiate these by analysis of the surrounding bile.

All of these studies have been hampered by the fact that the physicochemical properties of gallbladder bile obtained at cholecystectomy may not necessarily reflect the same properties of gallbladder bile at the time of gallstone formation. Despite this difficulty, three bile tests have been proposed to be useful predictors of cholesterol stones.

In an extension of prior investigations, Carey and Small (1) determined the *cholesterol saturation* in bile regarding the individual total lipid concentration. All patients with cholesterol stones showed supersaturated biles, but so did about 50% of the patients with pigment stones. However, in the same study, cholesterol monohydrate crystals were found in the majority of gallbladder (83%) and hepatic (58%) bile from cholesterol gallstone patients but were not observed in pigment stone patients or controls. Although it seemed possible that cholesterol crystals, detectable by *light microscopy* in the gallbladder bile of patients with gallstones, are shed from the surface of the stone and do not represent *de novo* nucleation, Holan et al. (2) have indicated that the rate of *crystal formation* and growth is much faster in bile samples from cholesterol gallstone patients after removal of crystals by ultracentrifugation, in comparison to bile samples without crystals from patients with pigment stones.

van Erpecum et al. have compared the reliability of these three bile tests for the differentiation between patients with cholesterol or pigment gallstones. They found that the microscopical identification of cholesterol crystals in bile is a highly specific and a rather sensitive method for the diagnosis of cholesterol cholecystolithiasis in concentrated as well as dilute gallbladder bile. This is in agreement with several other reports (1). The sensitivity of microscopical detection of cholesterol crystals for cholesterol gallstone disease was slightly lower when duodenal bile from cerulein-stimulated gallbladders was analyzed. In contrast, the results of the so-called cholesterol nucleation time were less useful for the differentiation of patients with pigment from patients with cholesterol gallstones. Although, a nucleation time <20 days in ultrafiltered gallbladder bile had a specificity of 100% and a sensitivity of 78% for cholesterol gallstone disease in concentrated gallbladder biles, sensitivity decreased to 21% in dilute biles.

There is a discrepancy between the presence of cholesterol crystals in the dilute bile of 72% of the patients with cholesterol stones and the normal nucleation time of cholesterol >21 days found in most of these patients. Methodological problems may be one explanation for this difference. In variance with prior studies (2), van Erpecum et al. performed ultrafiltration instead of ultracentrifugation to remove crystals prior to the nucleation assay. For ultrafiltration, 0.22-μm Millex filters were used, which may retain not only crystals or larger cellular debris but, additionally, cholesterol-phospholipid vesicles, particularly if these are fused and aggregated to larger complexes. Since aggregated vesicles play a key role in the formation of cholesterol crystals (7, 8), removal of part of those vesicles by ultrafiltration could prolong cholesterol nucleation in a subsequent assay. This effect may be less evident in concentrated bile. However, in dilute bile and with decreasing concentrations of bile acids, the vesicular fraction in bile increases, and larger aggregates are more likely to occur. It is therefore conceivable that the results obtained in the cholesterol nucleation studies of van Erpecum may be affected by the use of ultrafiltration. It would be interesting to compare van Erpecum's results to data obtained with the conventional nucleation assay as originally described by Holan et al. (2).

Finally, van Erpecum et al. have proven the reliability of the cholesterol saturation index in the differentiation of cholesterol and pigment stones. Their results confirm many prior investigations (1) that an abnormal cholesterol saturation index (>1.0) is a fairly sensitive but nonspecific parameter for cholesterol gallstone disease.

Taken together, van Erpecum's study shows that the microscopical detection of cholesterol crystals in bile is a rather sensitive and highly specific method for the

diagnosis of cholesterol gallstones. However, a negative test result does not exclude the presence of cholesterol gallstones, especially if duodenal bile is used.

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APLASTIC ANEMIA AFTER LIVER TRANSPLANTATION FOR FULMINANT VIRAL HEPATITIS: BLACK BOX OR BAG OF WORMS?

Tzakis AG, Arditi M, Whitington PF, Yanaga K, Esquivel C, Andrews WA, Makowka L, Malatak J, Freese DK, Stock PG, Ascher NL, Johnson FL, Broelsch CE, Starzl TE. Aplastic anemia complicating orthotopic liver transplantation for non-A, non-B hepatitis. N Engl J Med 1988; 319:393–396.

ABSTRACT

Aplastic anemia developed in 9 of 32 patients (28 percent) undergoing orthotopic liver transplantation for acute non-A, non-B hepatitis, at one to seven weeks after the procedure. No patient previously had evidence of hematologic dysfunction or conditions known to be associated with aplastic anemia. No other cases of aplastic anemia were identified among 1463 patients undergoing liver transplantation for all other indications at the four centers participating in the study (chi-square = 415, P < 0.001; 95 percent confidence interval for the incidence of aplastic anemia after transplantation for non-A, non-B hepatitis, 13 to 44 percent, vs. 0.00 to 0.13 percent for all other indications).

The operative and postoperative treatment of these patients was not otherwise different, indicating that the aplastic anemia was a complication of the hepatitis, not of the transplantation procedure. Four of the nine patients died of complications due to infections. Three of the surviving patients have been followed for less than six months, one for one year, and one for two years. The two patients followed the longest have recovered marrow function to an appreciable degree, and two of the others have evidence of early recovery.

We concluded that patients undergoing orthotopic liver transplantation for non-A, non-B hepatitis are at a high risk for the development of aplastic anemia.

COMMENTS

The discovery of a cohort of patients who develop aplastic anemia after liver transplantation only accentuates an already described phenomenon. Every form of viral hepatitis is associated with both transient and severe bone marrow depression. In the United States, approximately 5 to 7% of all patients with aplastic anemia have had an antecedent episode of clinical hepatitis. Up to 40% of patients being referred to bone marrow transplant centers for aplastic anemia have elevated aminotransferases. The true incidence of hepatitis and viral-associated aplastic anemia is not clear, since most patients do not have an exhaustive evaluation for the dozen or so viruses that have been implicated in causing severe bone marrow depression. Cyclosporin and steroid therapy in and of themselves do not appear to induce aplastic anemia. Most of the 1,495 patients who received liver transplants presumably were treated with cyclosporin and corticosteroids, but only nine patients developed aplastic anemia. Furthermore, aplastic anemia in renal transplant recipients is extraordinarily rare.

Hepatitis A, B and non-A, non-B can directly depress the *in vitro* differentiation and proliferation of normal human bone marrow (1–4). Hepatitis A and B virus depress bone marrow only if there is at least one virion per mononuclear cell. This effect can be prevented by antibodies. Bone marrow is routinely infected with hepatitis B virus during acute and chronic hepatitis B. By *in situ* hybridization and Southern blot analysis, we and others have found hepatitis B virus DNA in human bone marrow and circulating mononuclear cells, including T cells, B cells, and monocytes (5, 6). The percentage of nucleated bone marrow cells that contain viral DNA, HBsAg or HBcAg is 3 to 5%. Despite these *in vitro* findings, we cannot entirely explain bone marrow depression as a direct effect of the virus on stem cell function. Since transient aplasia is usually concurrent with acute hepatitis, bone marrow depression is likely a consequence of immunological attack on infected bone marrow stem cells.

Hepatitis and other types of viral-associated aplastic anemia may be nothing more than manifestations of severe immunological injury of bone marrow that results in destruction of either all progenitor cells or the microenvironment necessary to allow continued growth of bone marrow. Evidence for this hypothesis is exemplified with Epstein-Barr virus (EBV)-associated aplastic anemia and with cytomegalovirus (CMV)-infected bone