PROGNOSTIC EVALUATION OF PATIENTS WITH PARENCHYMAL CIRRHOSIS : PROPOSAL OF A NEW SIMPLE SCORE. M. ADLER, Ph. THIRY, D. VERSET, H. BOUHDID, N. BOURGEOIS, O. LE MOINE, B. GULBIS, J. VAN DE STADT, M. CREMER. Medicosurgical Dept of Gastroenterology, ULB, Hôpital Erasme, B-1070 Brussels, Belgium.

Due to organ shortage, it is necessary to identify short term prognosis of cirrhotic patients in order to give them higher priority for liver transplantation. We compared 2 exogenous tests (aminopyrine breath test and lidocaine metabolization test), 2 clinical parameters (encephalopathy, ascites), 18 endogenous tests and 5 scores (Pugh, Merkel, Orrego, Adler, Pignon) for predicting mortality within 1 year in patients with parenchymal cirrhosis. Retrospective (n=71 patients) and prospective (n=46 patients) series were analyzed. Univariate (Wilcoxon test), multivariate (discriminant function), ROC (Receiver Operating Characteristic) curves and survival curves (Kaplan-Meier) were realized. Endogenous tests were more discriminant than exogenous tests. The best indicants (encephalopathy, bilirubin, alkaline phosphatase, p cholinesterase and bile acids) were included in a new score with their 25th and 75th percentiles. Area under the curve of this new score was superior to the 5 other scores. Prospectively, sensitivity of our new score compared to the Pugh score is respectively 82% versus 95% (NS) and specificity is 89% versus 56% ($p<0.01$). Our new simple score appears thus very powerful for predicting short term prognosis and shoud be evaluated in other centers.

● **CALPAIN PROTEASES CAN DIRECTLY INDUCE THE MITOCHONDRIAL MEMBRANE PERMEABILITY TRANSITION (MMPT).** H.I. Aguilar, S.F. Bronk, G.J. Gores. Center for Basic Research in Digestive Diseases, Mayo Clinic, Rochester, MN 55905

The MMPT, an abrupt increase in the permeability of the inner mitochondrial membrane to small molecular weight solutes, has been proposed as a mechanism of hepatocyte necrosis. In contrast, we have demonstrated that enhanced activity of calpains (Ca^{++}-dependent, cysteine proteases) contribute to hepatocyte necrosis. To reconcile these data, we developed the **HYPOTHESIS** that stimulation of calpain protease activity induces the MMPT. To test this hypothesis we addressed two questions: Is calpain protease activity present in mitochondria? Does stimulation of mitochondrial calpain protease activity cause the MMPT? **METHODS**: Highly purified rat liver mitochondrial were obtained by differential centrifugation using a percoll-sucrose gradient employing digitonin to separate mitochondria from lysosomes. Calpain-like protease activity was assayed in toluene-permeabilized mitochondria using the fluorogenic substrate, Suc-Leu-Leu-Val-Tyr-AMC; permeabilized mitochondria were used to permit access of the substrate to the matrix. The MMPT was measured in intact mitochondria by a spectrophotometric assay assessing high amplitude mitochondrial swelling. **RESULTS**: Ca^{2+}, 100 µM, was used to stimulate calpain protease activity and induce the MMPT. Calpain-like protease activity was clearly identified in permeabilized mitochondria. Using 50 µM substrate and 1 mg/mL of mitochondria, the velocity of calpain activity was 1.34 ± 0.23 pmol AMC · min^{-1} · mg $protein^{-1}$. Incubation of mitochondria with the calpain inhibitor, Cbz-Leu-Leu-Tyr-CHN_2, decreased calpain-like activity in a concentration-dependent manner with 100 µM of the inhibitor causing a maximum inhibition of 82+2%. Upon addition of Ca^{2+} to intact mitochondria, rapid induction of the MMPT was observed within 10 min. Cbz-Leu-Leu-Tyr-CHN_2 also inhibited the MMPT in a concentration dependent manner similar to its inhibition of calpain-like protease activity. Maximum inhibition of the MMPT was 82 ± 1% with 100 µM of Cbz-Leu-Leu-Tyr-CHN_2. The effect of Cbz-Leu-Leu-Tyr was specific as serine, aspartate and metalloprotease inhibitors did not inhibit either calpain activity or the MMPT. **SUMMARY**: In liver mitochondria, we have directly demonstrated: 1) calpain-like protease activity; and 2) inhibition of the MMPT by a calpain inhibitor. In **CONCLUSION**, these data suggest a unifying hypothesis linking calpain protease activity to the MMPT in cell necrosis. We propose for the first time that activation of mitochondrial calpain activity is the cytolytic trigger initiating the MMPT in hepatocyte necrosis.

MANAGEMENT OF HEPATITIS C VIRUS (HCV) POSITIVE BRITISH BLOOD DONORS MM Ahmed, E Elias, K O'Donnell, J Shaw, RF Harrison, FA Ala*, H Atrah* and DJ Mutimer. Queen Elizabeth Hospital Liver Unit, and Regional Blood Transfusion Service*, Birmingham, UK

Asymptomatic blood donors now comprise the majority of new HCV referrals to hepatology clinics. Since screening began in September 1991, 65 RIBA positive blood donors (48 male, median age 37 years; 17 female median age 37 years) have been investigated at the Birmingham Liver Unit.

Forty donors had an overt parenteral risk factor for HCV exposure- 36 IVDU, 4 prior transfusion. Seventeen patients (26%) had normal transaminases. Median ALT was 48 (range 9-440), AST 48 (range 14-200). Liver biopsy was recommended for all patients, and histology was scored (without reference to clinical details) according to a modified Knodell histological activity index (HAI, maximum possible score 13). Sixty three patients were biopsied - median HAI=1.8, mean HAI=2.8, range 0-8, none cirrhotic. When HAI<6 (54 patients), treatment with interferon was not recommended. For these patients, a 2 year follow-up biopsy was proposed. As of September 1994, 14 biopsies have been repeated in untreated donors - 11 deteriorated, 2 improved, 1 unchanged. When HAI>5 (9 patients), treatment with alpha interferon (3 MU 3 times a week for 6-12 months) was recommended. Long term biochemical response (6 month follow up period) was observed in 2/9 (22%) and histological improvement was seen in 3/6.

Conclusions. Most HCV-infected British donors have a history of IVDU. Initial histological changes tend to be mild. Follow-up is brief, but some patients show histological progression. Results of treatment for patients with higher HAI are disappointing.

QUANTITATION OF HEPATITIS C VIRUS (HCV) IN PATIENTS TREATED WITH INTERFERON. M.M. Ahmed, B.A.B Martin, E. Elias, R.F. Harrison, K. O'Donnell, J.C. Shaw and D.J. Mutimer. Liver Unit, Queen Elizabeth Hospital, Birmingham, UK

We have developed a method for quantitating HCV by co-amplifying a constant amount of viral cDNA with varying amounts of a mutant cDNA fragment (containing the *lac* operator sequence) using multiple nested PCRs. The mutant PCR products were detected colorimetrically following capture on to streptavidin coated microtitre wells. Target cDNA levels were derived from a quantitation curve of optical density versus mutant cDNA concentration.

Patients and Methods. Twenty two patients with chronic HCV (RIBA positive) were treated with alpha interferon (IFN): 3 MU, 3 times a week for up to 12 months. Biochemical response to IFN was divided into three types: long term response (LTR) with normal ALT at >6 months follow up, complete response with relapse after end of treatment (CR/R), and no/partial response (NR). Pre-treatment liver biopsies were graded according to a modified Knodell histological activity index (HAI, range 0-13, 13 is worst). Pre-treatment sera stored at -70°C were quantitated for HCV. In seven patients, serial sera (once before treatment, four times during treatment and once after treatment) were quantitated for HCV to monitor virological response.

Results. Response types were as follows: 3 LTR, 12 CR/R and 7 NR.There was no correlation between pre-treatment HCV cDNA levels and initial ALT (R=-0.095), initial HAI (R=-0.44) or pattern of biochemical response to IFN (p= 0.57, Kruskal Wallis test). Serial HCV cDNA levels measured in 7 patients (3 LTR, 2 CR/R and 2 NR) complemented changes in ALT.

Conclusions. Competitive PCR using a mutant cDNA fragment and colorimetric detection of the PCR products provides a rapid, reproducible and non-radioactive method for HCV cDNA quantitation. HCV cDNA levels may be used to monitor response to IFN especially in patients who have normal pre-treatment ALT. We were unable to demonstrate a relationship between initial viral load and biochemical response.