Prognostic Value of Insulinlike Growth Factor I and Its Binding Protein in Patients With Alcohol-Induced Liver Disease

SØREN MØLLER,[1] ULRIK BECKER,[2] ANDERS JUUL,[3] NIELS E. SKAKKEBÆK,[3] ERIK CHRISTENSEN,[4] AND THE EMALD GROUP*

Insulinlike growth factor I (IGF-I) is a single-polypeptide chain with important anabolic and endocrine activities. The liver is the major source of IGF-I and its binding protein, IGFBP-3. Circulating concentrations of IGF-I and IGFBP-3 are decreased in patients with chronic liver disease and correlate with the severity. The aim of this study was to assess the additional prognostic value of IGF-I and IGFBP-3 in patients entered in a large multicenter study (EMALD). Three hundred thirty-seven patients with alcohol-induced liver disease were studied in a randomized placebo-controlled trial of malotilate with a mean follow-up period of 569 days (range, 7-1,544). A multivariate Cox regression analysis of pertinent clinical and biochemical variables showed a significant independent prognostic value of years of alcohol intake, coagulation factors 2, 7, and 10, alkaline phosphatases, serum creatinine, and immunoglobulin (Ig) M. When IGF-I or IGFBP-3 were added into this model, a Cox regression analysis showed that either had a significant independent prognostic value. Because IGF-I and IGFBP-3 were closely correlated, they contained almost the same prognostic information. Inclusion of IGF-I gave these results: IGF-I ($P < .03$), alcohol intake ($P < .02$), coagulation factors 2, 7, and 10 ($P < .01$), creatinine ($P < .001$), and IgM ($P < .01$) contained independent prognostic information. Inclusion of IGFBP-3 gave these results: IGFBP-3 ($P < .02$), alcohol intake ($P < .05$), coagulation factors 2, 7, 10 ($P < .01$), creatinine ($P < .001$), and IgM ($P < .02$) were independent predictors of survival. In conclusion, IGF-I or IGFBP-3 provide important additional information on survival in patients with alcohol-induced liver disease. (HEPATOLOGY 1996;23:1073-1078.)

Insulinlike growth factor I (IGF-I) is a single-chained polypeptide with important anabolic actions on protein, carbohydrate, and lipid metabolism.[1-3] IGF-I is bound in the circulation to a complex system of at least six binding proteins; IGF-binding protein-3 (IGFBP-3) is the most abundant, playing a major role in determining the total concentration of IGF-I in the circulation.[3,4] Circulating IGF-I is mainly produced in the liver, whereas the production site of IGFBP-3 is uncertain.[4-6] Low concentrations of both IGF-I[7-9] and IGFBP-3[10-12] have been reported in chronic liver disease. IGF-I concentrations in cirrhosis have been shown to correlate significantly with liver function and may be used as a good indicator of functional hepatocellular capacity.[11-15] Clinical and biochemical characteristics in patients with cirrhosis can provide useful, but rather imprecise, information on prognosis, and new markers of survival are needed.[16,17] In a recent study, we found a significant correlation between serum concentrations of IGF-I and survival in a smaller number of patients.[14] The aim of the current study was thus to determine the independent prognostic value of IGF-I and IGFBP-3 in a large number of patients with alcohol-induced liver disease.

MATERIALS AND METHODS

Study Population. The study population represented patients from a large multicenter trial of the effect of malotilate on survival in patients with alcohol-induced liver disease.[18] Briefly, the results of this trial showed a slightly better survival in patients receiving a medium dose of malotilate of

Abbreviations: IGF-I, insulinlike growth factor I; IGFBP-3, insulinlike growth factor–binding protein 3; B/B_0, bound/free ratio; Ig, immunoglobulin.

From the [1]Department of Clinical Physiology and Nuclear Medicine 239 and the [2]Department of Gastroenterology 261, Hvidovre Hospital, University of Copenhagen, Copenhagen, Denmark; the [3]Department of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark; and the [4]Department of Medicine B, Bispebjerg Hospital, University of Copenhagen, Copenhagen, Denmark.

Received March 21, 1995; accepted December 6, 1995.

Supported by King Christian X′ Foundation grant 6973/1991 and The Danish Hospital Foundation for Medical Research, Region of Copenhagen, The Faroe Islands, and Greenland.

Address reprint requests to: Søren Møller, M.D., Department of Clinical Physiology 239, Hvidovre Hospital, DK-2650 Copenhagen, Denmark.

*The EMALD Group: S. Keiding, Transplantation Centre, Aarhus University Hospital, Denmark; J. H. Badsberg, Statistical Research Unit, University of Copenhagen, Denmark; K. D. Bentsen, Odense University Hospital, Odense, Denmark; O. Bonnevie, Frederiksberg Hospital, Copenhagen, Denmark; J. Caballeria, Hospital Clinic i Provincial, Barcelona, Spain; J. Eriksen, Frederiksberg Hospital, Copenhagen, Denmark; F. Hardt, Hvidovre Hospital, Copenhagen, Denmark; N. Keiding, Statistical Research Unit, University of Copenhagen, Denmark; M. Morgan, Royal Free Hospital, London, United Kingdom; H. Poulsen, Hvidovre Hospital, Copenhagen, Denmark; L. Ranek, Rigshospitalet, Copenhagen, Denmark; J. Rodés, Hospital Clinici Provincial, Barcelona, Spain; G. Schou, Rigshospitalet, Copenhagen, Denmark; R. Walker, Walton Hospital, Liverpool, United Kingdom; and N. Tygstrup, Rigshospitalet, Copenhagen, Denmark.

0270-9139/96/2305-0021$3.00/0

TABLE 1. Clinical and Biochemical Characteristics of 354 Patients With Alcohol-Induced Liver Disease

Variable	Median (Range)
Age (yr)	50 (27-85)
Sex (male/female)	266/88
Cirrhosis (present/absent)	204/148
Encephalopathy (present/absent)	30/323
Ascites (present/absent)	110/243
Alanine aminotransferases (U/L, 10-40)	42 (5-460)
Albumin (μmol/L, 540-800)	526 (214-831)
Bilirubin (μmol/L, 2-17)	16 (2-530)
Alkaline phosphatases (U/L, 50-275)	292 (87-1,933)
Coagulation factors 2, 7, and 10 (Arb. units, 0.70-1.30)	0.8 (0.1-1.3)
IgG (μmol/L, 36-89)	14.4 (1.9-44.3)
IgA (μmol/L, 3.8-21.0)	5.1 (1.0-28.9)
IgM (μmol/L, 0.3-3.7)	1.9 (0.3-14.2)
Creatinine (mmol/L, 0.05-0.12)	0.08 (0.03-0.26)
IGF-I (μg/L)	93 (9-440)
IGF-$I_{z\ score}$	−2.19 (−6.03-3.25)
IGFBP-3 (μg/L)	2,003 (399-6,665)
IGFBP-$3_{z\ score}$	−2.87 (−6.27-5.79)

NOTE. Values are shown in numbers, percentages, or medians and ranges. Units and reference intervals in parentheses.

750 mg/day than in patients receiving placebo or patients receiving a malotilate dose of 1,500 mg/d. Of the 407 patients enrolled in the multicenter trial, 354 patients with complete follow-up data and values of IGF-1 and IGFBP-3 were entered. Diagnosis of alcohol-induced liver disease was histologically verified in 352 patients and based on accepted biochemical and clinical criteria in the remaining patients, because liver biopsy was contraindicated. The study population comprised 266 men and 88 women with a median age of 49.0 years (range, 23-85). Clinical and biochemical characteristics of the patients are summarized in Table 1. All patients had a daily alcohol intake of 80 g or more for the preceding 4 years. Patients were randomized to three treatment groups with daily doses of malotilate of 1,500 mg, 750 mg, or placebo. Ascites, encephalopathy, and gastrointestinal bleeding were treated, and disulfiram (Antabuse) was administered in accordance with local practice. The patients mainly receive disulfiram, B vitamin complex, and diuretics, which have not been reported to interact with the circulating concentrations IGF-I and IGFBP-3. The patients participated after giving their informed consent according to the Helsinki II declaration, and the study was approved by the local ethics committees of the hospitals involved.

IGF-I and IGFBP-3 Assays. IGF-I was determined in all subjects with a radioimmunoassay using truncated IGF-I (des[1-3]-IGF-I) as radioligand, as originally described by Bang et al.,[19] modified by the use of a monoiodinated isomer as tracer (Tyr^{31}-des[1-3]-IGF-I).[20] Serum was extracted by acid-ethanol and cryoprecipitated (AEC) before analysis to remove interfering IGFBPs. A monoiodinated IGF-I isomer (^{125}I-Tyr-31-des[1-3]IGF-I) was used as radioligand. The intraassay coefficients of variation were 5% (bound/free ratio [B/B_0] of 0.2), 4% (B/B_0 of 0.4), and 10% (B/B_0 of 0.7), respectively (n = 15; not corrected for recovery). The interassay coefficients of variation for the extraction procedure were 10% (at B/B_0 of 0.2), 9% (at B/B_0 of 0.4) and 14% (at B/B_0 of 0.7), respectively (n = 45). The day-to-day variation of IGF-I measurements was 8%.

Serum concentrations of IGFBP-3 were measured by radioimmunoassay as described by Blum et al.[21] Reagents for the IGFBP-3 radioimmunoassay were obtained from Mediagnost G.m.b.H., Tübingen, Germany. Briefly, 100 μL of unprocessed serum (diluted 1:651) was added to 100 μL of polyclonal rabbit antiserum and 100 μL of ^{123}I-labeled IGFBP-3 tracer obtained from a 30.5-kd stable IGFBP-3 fragment isolated from Cohn fraction IV. The sensitivity of the assay, defined as 3 SD from the mean of the zero standard was 0.291 μg/L, and half maximal displacement occurred at 47 μg/L. In cross-reactivity studies, dilution curves obtained with glycosylated recombinant human IGFBP-3 as well as serum from healthy individuals paralleled the standard curve. Cross-reactivity with IGFBP-1 and IGFBP-2 was undetectable in the range of 0.6 to 80 μg/L. The intraassay coefficient of variation (n = 17) was 2% (at B/B_0 of 0.30), 2% (at B/B_0 of 0.4), and 6% (at B/B_0 of 0.80), respectively. The interassay coefficient of variation (n = 144) was 11% (at B/B_0 of 0.48) and 8% (at B/B_0 of 0.78) over a period of 2.5 years. The normal range for healthy adults has previously been published.[22] Intraassay and interassay variations were less than 6% and 10%, respectively.

Statistical Analysis. Variables are summarized as percentages, medians, and total ranges. Correlation analyses were performed by the Spearman rank correlation test.

Survival Analysis. The prognostic significance of IGF-I and IGFBP-3 was studied using the log rank test to compare the Kaplan-Meier survival curves of the patients stratified into four groups of equal size according to the IGF-I and IGFBP-3 values.[23] The additional prognostic influence of IGF-I and IGFBP-3 to the previously identified prognostic variables was studied by including IGF-I or IGFBP-3 in the Cox regression analysis,[24,25] together with the variables previously found to have independent prognostic information in these patients, i.e., years of high alcohol intake, coagulation factors 2, 7, and 10, alkaline phosphatases, creatinine, and immunoglobulin (Ig) M [$\chi^2 = 77.8$; degrees of freedom: 5; $P < .001$].[18] To adjust for the influence of malotilate therapy in this purely prognostic study, the Cox regression analyses were performed stratified according to therapy (placebo, malotilate 750 mg, or malotilate 1,500 mg daily).[18]

Because IGF-I and IGFBP-3 covary with age and have markedly skewed distributions, we used age-adjusted normalizing z score transformations derived from a study of a normal population.[20] Thus, in the Cox regression analyses, IGF-I was scored as $Z_{IGF\text{-}I} = (\sqrt{}IGF\text{-}I_{measured\ value} - IGF\text{-}I_{estimated\ value\ for\ age})/1.779$, where $IGF\text{-}I_{estimated\ value\ for\ age} = -0.1144 \cdot age + 19.1966$. IGFBP-3 was scored as $Z_{IGFBP\text{-}3} = (IGFBP\text{-}3_{measured\ value} - IGFBP\text{-}3_{estimated\ value\ for\ age})/513.7$, where $IGFBP\text{-}3_{estimated\ value\ for\ age} = -10.1423 \cdot age + 4{,}014$.

After inclusion in the Cox regression analysis, insignificant variables were removed stepwise, until all remaining variables were significant ($P < .05$) (backward elimination). The assumptions of the Cox model were assessed as described elsewhere[24] and were not found to be violated. It turned out that the z scores of IGF-I and of IGFBP-3 fitted better in the models than did the raw and logarithmic values.

A prognostic index can be derived from the prognostic model as the sum of the patient's variable scorings each multiplied by its regression coefficient as previously described.[24,26] Plots of the estimated survival probability corresponding to different values of the prognostic index was obtained as previously described.[24]

TABLE 2. Spearman Rank Correlation Coefficients of Selected Patient Characteristics and IGF-I and IGFBP-3 in 354 Patients

Variable	IGF-I R (S)	IGFBP-3 R (S)
Age	−0.36	−0.33
Albumin	0.62	0.59
Bilirubin	−0.46	−0.47
Alkaline phosphatase	−0.34	−0.26
Coagulation factors 2, 7, & 10	0.57	0.64
IgG	−0.49	−0.48
IgA	−0.57	−0.55
IgM	−0.25	−0.22
Creatinine*	−0.04	−0.07
IGF-I	—	0.87

* $P > .05$. Other $P < .001$.

RESULTS

The patient's characteristics are given in Table 1. The mean follow-up period was 569 days (range, 7-1,544 days); 84 patients died. Table 2 shows the results of correlation analysis of IGF-I and IGFBP-3 and of pertinent variables. The association between IGF-I and survival is shown in Fig. 1. Low IGF-I values were significantly associated with poor prognosis ($P < .001$). The association between IGFBP-3 and survival shown in Fig. 2 was also highly significant ($P < .001$). Almost the same results were obtained from log rank analyses when stratification was performed according to the z scores of IGF-I and IGFBP-3 ($P < .001$, figures not shown).

Both IGF-I and IGFBP-3 showed significant independent prognostic value when they were added separately to the model comprising the significant variables: years of high alcohol intake, coagulation factors 2, 7, and 10, alkaline phosphatases, creatinine and IgM

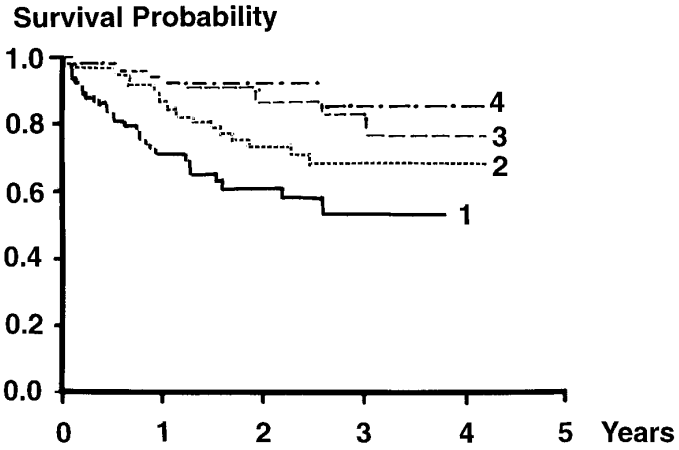

FIG. 1. Survival curves (Kaplan-Meier plots) in groups of patients with alcohol-induced liver disease defined according to IGF-I. (1) IGF-I ≤ 56 μg/L, n = 89. (2) 56 < IGF-I ≤ 93, n = 91. (3) 83 < IGF-I ≤ 145, n = 88. (4) IGF-I > 145 μg/L, n = 86. $P < .001$.

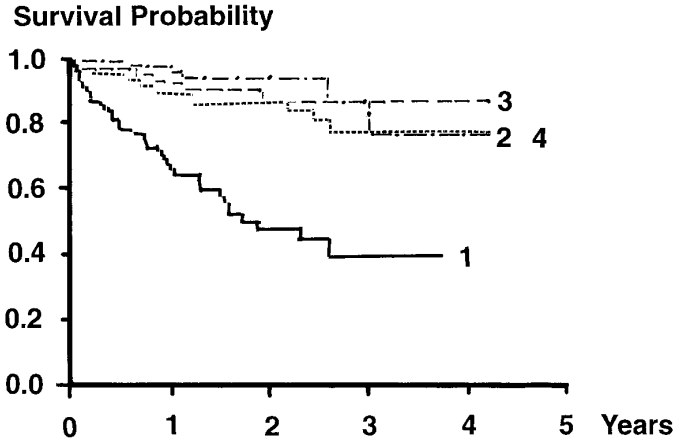

FIG. 2. Survival in patients with alcohol-induced liver disease stratified according to IGFBP-3. (1) IGFBP-3 ≤ 1,355 μg/L, n = 88. (2) 1,355 < IGFBP-3 ≤ 2,003, n = 89. (3) 2,003 < IGFBP-3 ≤ 2,903, n = 90. (4) IGFBP-3 > 2,903 μg/L, n = 87. $P < .001$.

($\chi^2 = 77.8$; degrees of freedom: 5; $P < .001$), but alkaline phosphatases was no longer significant and therefore was eliminated from the model. Because IGF-I and IGFBP-3 were closely correlated, they provided almost the same prognostic information (Tables 3 and 4). The model containing IGF-I ($\chi^2 = 73.1$; degrees of freedom: 5; $P < .0001$) was not significantly different from that containing IGFBP-3 ($\chi^2 = 74.4$; degrees of freedom: 5; $P < .0001$).

Because the presence of alcohol-induced hepatitis in combination with cirrhosis may be associated with the worst prognosis, we studied a possible differential prognostic value of IGF-I and IGFBP-3 in patients having alcohol-induced hepatitis combined with cirrhosis (n = 102), alcohol-induced hepatitis alone (n = 41), or cirrhosis alone (n = 85). Although there was a trend of a stronger prognostic value of IGF-I in patients with cirrhosis and alcohol-induced hepatitis, no statistical significance occurred. This also held true with respect to IGFBP-3.

A prognostic index for a patient with specified patient characteristics can be deduced from the regression models in Tables 3 and 4, and the survival probability can be estimated from Fig. 3. For example, a patient aged 39 years with a 15-year consumption of

TABLE 3. Time-Dependent Cox Regression Model, Including IGF-I

Variable	Scoring	Regression Coefficient	SE	P
Alcohol intake	Years	0.03	0.01	<.02
Coagulation factors 2, 7, 10	Log (value in Arb. units)	−1.11	0.35	<.01
Creatinine	mmol/L	15.26	2.91	<.001
IgM	μmol/L	0.17	0.06	<.01
IGF-I	z score	−0.24	0.10	<.03

TABLE 4. Time-Dependent Cox Regression Model Including IGFBP-3

Variable	Scoring	Regression Coefficient	SE	*P*
Alcohol intake	Years	0.03	0.01	<.05
Coagulation factors 2, 7, 10	Log (value in Arb. units)	−0.99	0.37	<.01
Creatinine	μmol/L	14.19	2.82	<.001
IgM	μmol/L	0.16	0.06	<.02
IGFBP-3	*z* score	−0.26	0.11	<.02

alcohol, coagulation factors 2, 7, and 10 of 1.24 arbitrary units, serum creatinine of 71 μmol/L, serum IgM of 1.80 μmol/L, IGF-I of 373 μg/L, and IGFBP-3 of 2,803 μg/L would have a prognostic index for the two models of 1.00 and 1.34, respectively. Figure 3 shows that the estimated 4-year survival is approximately 95% for prognostic indices at these levels. In another patient aged 37 years with a 5-year consumption of alcohol, coagulation factors 2, 7, and 10 of 0.22 arbitrary units, serum creatinine of 150 μmol/L, serum IgM of 3.10 μmol/L, IGF-I of 119 μg/L, and IGFBP-3 of 1,615 μg/L, the prognostic indices for the two models are 5.18 and 5.25, respectively. For these higher values of the prognostic indices, the 4-year survival of this patient, with markedly lower values of IGF-I and IGFBP-3 in addition to more abnormal values of other variables in the prognostic model, is only approximately 10% to 15% (Fig. 3).

DISCUSSION

The main finding of this study is that circulating plasma concentrations of IGF-I and IGFBP-3 provide additional important information on the mortality of patients with alcohol-induced liver disease.

The prognosis of patients with cirrhosis has remained poor, despite treatment of complications such as ascites and hepatic nephropathy, hepatic encephalopathy, and bleeding from esophageal varices. The ultimate treatment of cirrhosis is liver transplantation,[27] and for this an accurate estimate of the prognosis is essential. Several studies have tried to identify predictors of survival, but until now the prognostic information has been relatively imprecise.[16,17] Thus, there is a need for better prognostic variables. The liver is the major source of circulating IGF-I, whereas the major production site of IGFBP-3 is uncertain.[28,29] Recent animal studies have suggested that IGFBP-3 is mainly synthesized by the Kupffer cells and IGF-I mainly by parenchymal liver cells.[30,31] Low concentrations of both IGF-I and IGFBP-3 have previously been found in patients with chronic liver disease,[7,8,11,12,14,32] and the survival of patients with very low IGF-I levels has been reported to be poor.[14] Apart from hepatic dysfunction, a concomitant state of malnutrition may further suppress the circulating concentrations of IGF-I and IGFBP-3 in patients with alcohol-induced liver disease.[4] In the current study, IGF-I and IGFBP-3 proved to have an independent prognostic value as determined by the two regression models. This may reflect a combined effect of the liver disease and malnutrition, but the design of the current study does not allow one to draw conclusions as to differential effects on prognosis of diverse conditions. Despite different production sites, IGF-I and IGFBP-3 were closely correlated and contained almost the same prognostic information. Both probably reflect the synthetic capacity or the dysfunction of the liver, and measurement of one of the

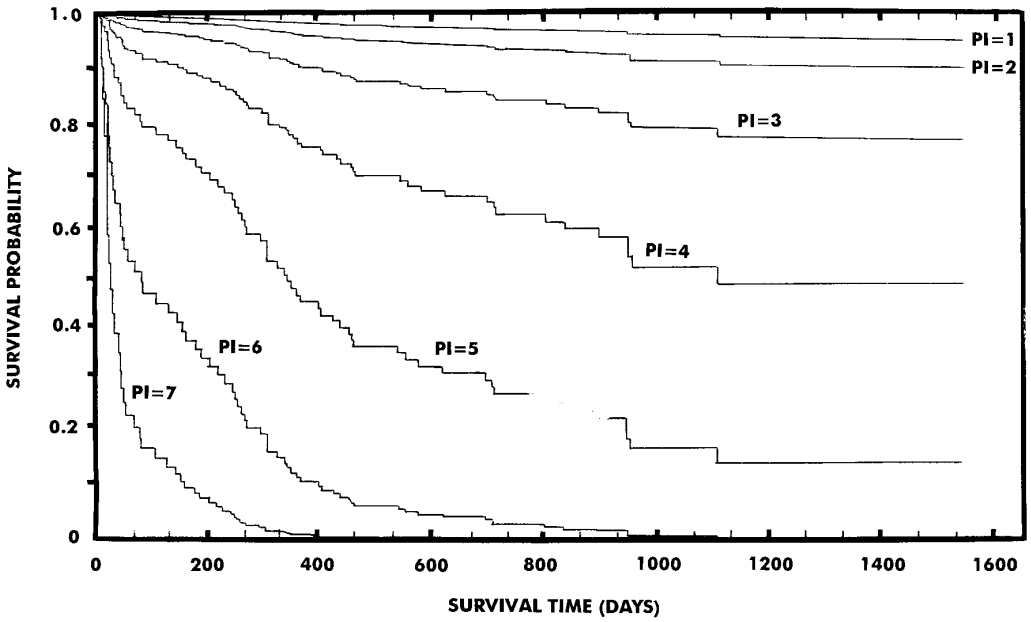

FIG. 3. Estimated survival curves for different values of the prognostic index (PI) for any of the two regression models. Higher values of PI mean a higher hazard or shorter survival; lower values mean lower hazard or longer survival.

peptides will suffice. It was striking that in the regression analyses it turned out that IGF-I and IGFBP-3 had stronger prognostic influence than albumin to which they are correlated (Table 2), and hence, IGF-I and IGFBP-3 may have greater value for the purpose of assessing prognosis. Measurement of IGF-I, in particular, with the new commercial kits is inexpensive and easy to perform, so it may be more widely used as a prognostic variable in the future.

Liver transplantation is the ultimate treatment of cirrhosis, and to ensure its maximum benefit the identification of the optimal time for surgery is essential.[33] Repeated measurements of IGFBP-3 and especially IGF-I may be useful. Because IGF-I in particular seems to be a true marker of hepatic dysfunction, changes occurring in its concentration over time may make a valuable contribution to the assessment of the appropriate time for transplantation. To solve this problem, more studies of the course of IGF-I and its binding proteins before and after liver transplantation are needed in a large population of transplant patients.

In conclusion, our study has shown that concentrations of IGF-I and IGFBP-3 are low in patients with chronic liver disease and that they correlate with the degree of liver dysfunction. Both IGF-I and IGFBP-3 supply significant independent prognostic information, which may be useful to further refine prognostication in patients with alcohol-induced liver disease.

REFERENCES

1. Guler HP, Zapf J, Froesch ER. Short-term metabolic effects of recombinant human insulin-like growth factor 1 in healthy adults. N Engl J Med 1987;317:137-140.
2. Hartmann H, Schmitz F, Christ B, Jungermann K, Creutzfeldt W. Metabolic actions of insulin-like growth factor-1 in cultured hepatocytes from adult rats. HEPATOLOGY 1990;12:1139-1143.
3. Cohick WS, Clemmons DR. The insulin-like growth factors. Annu Rev Physiol 1993;55:131-153.
4. Langford KS, Miell JP. Review: the insulin-like growth factor-I/binding protein axis: physiology, pathophysiology and therapeutic manipulation. Eur J Clin Invest 1993;23:503-516.
5. Blum WF, Ranke MB. Insulin-like growth factor binding proteins (IGFBPs) with special reference to IGFBP-3. Acta Pædiatr Scand 1990;367(suppl):55-62.
6. Baxter RC. Circulating binding proteins for the insulin-like growth factors. Trends Endocrinol Metab 1993;4:91-96.
7. Møller S, Becker U. Insulin-like growth factor-I and growth hormone in chronic liver disease. Dig Dis Sci 1992;10:239-248.
8. Sheppard MS, Minuk GY, Bhaumick B, Bala RM. Insulin-like growth factors (IGF) in liver disease: differential changes of IGF-I and IGF-II. Clin Invest Med 1987;10:49-53.
9. Wu JC, Daughaday WH, Lee SD, Hsiao TS, Chou CK, Lin HD, Tsai YT, et al. Radioimmunoassay of serum IGF-1 and IGF-II in patients with chronic liver diseases and hepatocellular carcinoma with or without hypoglycemia. J Lab Clin Med 1988;112:589-594.
10. Kratzsch J, Blum WF, Schenker E, Keller E. Serum levels of IGF-I, IGF-binding proteins (IGFBP) 1, 2 and 3 and GHBP in liver cirrhosis. Presented at the 31st Annual Meeting of ESPE. Horm Res 1992;37(suppl 4):55.
11. Møller S, Juul A, Becker U, Flyvbjerg A, Skakkebæk NE, Henriksen JH. Concentrations, release, and disposal of insulin-like growth factor (IGF)-binding proteins (IGFBP), IGF-I, and growth hormone in different vascular beds in patients with cirrhosis. J Clin Endocrinol Metab 1995;80:1148-1157.
12. Donaghy A, Ross R, Gimson A, Cwyfan Hughes S, Holly J, Williams R. Growth hormone, insulin-like growth factor-I, and insulin-like growth factor binding proteins 1 and 3 in chronic liver disease. HEPATOLOGY 1995;21:680-688.
13. Caufriez A, Reding P, Urbain D, Golstein J, Copinschi G. Insulin-like growth factor I: a good indicator of functional hepatocellular capacity in alcoholic liver cirrhosis. J Endocrinol Invest 1991;14:317-321.
14. Møller S, Grønbæk M, Main K, Becker U, Skakkebæk NE. Urinary growth hormone (U-GH) excretion and serum insulin-like growth factor-I (IGF-I) in patients with alcoholic cirrhosis. J Hepatol 1993;17:315-320.
15. Kratzsch J, Blum WF, Schenker E, Keller E, Jahreis G, Haustein B, Ventz M, et al. Measurement of insulin-like growth factor-I (IGF-I) in normal adults, patients with liver cirrhosis and acromegaly: experience with a new competitive enzyme immunoassay. Exp Clin Endocrinol 1993;101:144-149.
16. Christensen E, Schlichting P, Fauerholdt L, Juhl E, Poulsen H, Tygstrup N, The Copenhagen Study Group for Liver Diseases. Changes of laboratory variables with time in cirrhosis: prognostic and therapeutic significance. HEPATOLOGY 1985;5:843-853.
17. Christensen E, Schlichting P, Kragh-Andersen P, Fauerholdt L, Schou G, Vestergaard Pedersen B, Juhl E, et al. Updating prognosis and therapeutic effect evaluation in cirrhosis with Cox's multiple regression model for time-dependent variables. Scand J Gastroenterol 1986;21:163-174.
18. Keiding S, Badsberg JH, Becker U, Bentsen KD, Bonnevie O, Caballeria J, Eriksen J, et al. The prognosis of patients with alcoholic liver disease: an international randomized, placebo-controlled trial on the effect of malotilate on survival. J Hepatol 1994;20:454-460.
19. Bang P, Eriksson U, Sara V, Wivall IL, Hall K. Comparison of acid ethanol extraction and acid gel filtration prior to IGF-I and IGF-II radioimmunoassays: improvement of determinations in acid ethanol extracts by the use of truncated IGF-I as radioligand. Acta Endocrinol 1991;124:620-629.
20. Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jørgensen K, Müller J, et al. Serum insulin-like growth factor-I in 1030 healthy children, adolescents and adults; relation to age, sex, stage of puberty, testicular size and body mass index. J Clin Endocrinol Metab 1994;78:744-752.
21. Blum WF, Ranke MB, Kietzmann K, Gauggel E, Zeisel HJ, Bierich JR. A specific radioimmunoassay for growth hormone (GH)-dependent somatomedin-binding protein: its use for diagnosis of GH deficiency. J Clin Endocrinol Metab 1990;70:1292-1298.
22. Juul A, Main K, Blum WF, Lindholm J, Ranke MB, Skakkebæk NE. The ratio between serum levels of insulin-like growth factor (IGF)-I and the IGF binding proteins (IGFBP-1, -2, and -3) decreases with age in healthy adults and is increased in acromegalic patients. Clin Endocrinol 1994;41:85-93.
23. Peto R, Pike MC, Armitage P, Breslow NE, Cox DR, Howard SV, Mantel N, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. Br J Cancer 1977;35:1-39.
24. Christensen E. Multivariate survival analysis using Cox's regression model. HEPATOLOGY 1987;7:1346-1358.
25. Cox DR. Regression models and life tables (with discussion). J R Stat Soc B 1972;34:187-220.
26. Christensen E, Altman DG, Neuberger J, Destavola BL, Tygstrup N, Williams R. Updating prognosis in primary biliary cirrhosis using a time-dependent Cox regression model. Gastroenterology 1993;105:1865-1876.
27. Williams R. Current problems and new advances in liver transplantation. In: Rodés J, Arroyo V, eds. Therapy in liver diseases. Barcelona: Ediciones Doyma, 1992:175-179.
28. Binoux M, Lassare C, Hardouin N. Somatomedin production by rat liver in organ culture. Acta Endocrinol 1982;99:422-430.
29. Phillips ID, Arany E, Strain AJ, Han VKM, Hill DJ. Rapid clearance of insulin-like growth factor (IGF)-binding protein

species from blood and an associated fall in circulating IGF-I following partial hepatectomy in the rat. J Endocrinol 1993; 137:271-280.
30. Villafuerte BC, Koop BL, Pao C-I, Gu L, Birdsong GG, Phillips LS. Coculture of primary rat hepatocytes and non-parenchymal cells permits expression of insulin-like growth factor binding protein-3 in vitro. Endocrinology 1994;134:2044-2050.
31. Chin E, Zhou J, Dai J, Baxter RC, Bondy CA. Cellular localization and regulation of gene expression for components of the insulin-like growth factor ternary binding protein complex. Endocrinology 1994;134:2498-2504.
32. Schimpff RM, Lebrec D, Donnadieu M. Serum somadomedin activity measured as sulphation factor in peripheral, hepatic and renal veins of patients with alcoholic cirrhosis. Acta Endocrinol 1978;88:729-736.
33. Keiding S, Ericzon BG, Eriksson S, Flatmark A, Höckerstedt K, Isoniemi H, Karlberg I, et al. Survival after liver transplantation of patients with primary biliary cirrhosis in the Nordic countries. Scand J Gastroenterol 1990;25:11-18.