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Abstract

Sex, age and 21 routine liver function assays were analyzed by stepwise selection and the best-of-all-possible-combinations method to identify a small group of assays valuable in establishing which liver cirrhosis (LC) patients have a high risk of hepatocellular carcinoma (HCC), when alpha-fetoprotein (AFP) is not elevated. Data was obtained from 115 HCC and 122 LC patients on admission. Tumor size correlated with AFP (0.73), alkaline phosphatase (ALP, 0.47), leucine aminopeptidase (LAP, 0.42), lactic dehydrogenase (LDH, 0.42), and the glutamic oxaloacetic transaminase (GOT)/glutamic pyruvic transaminase (GPT) ratio (GOT/GPT, 0.41). The mean of the correct diagnosis rates (CDR) of HCC and LC utilizing AFP as the sole parameter (89%) was markedly higher than those of the other parameters. The best-of-all-possible-combinations method presented a more powerful combination than stepwise selection. The best combination of 7 parameters (LAP, GOT/GPT, choline esterase, one-hour erythrocyte sedimentation rate, age, albumin/globulin ratio, and total bilirubin) presented a mean CDR of 80%, HCC CDR of 77%, and false positive rate of 18%. LC patients statistically diagnosed as having HCC by these 7 parameters are proposed as high risk patients. Fourteen (78%) of 18 HCC patients who were AFP-negative were statistically diagnosed. This analysis can be applied to LC patients to distinguish those that should be followed closely by imaging diagnostic techniques.

KEYWORDS: hepatocellular carcinoma, liver cirrhosis, high risk hepatocellular carcinoma, liver function tests, differentical diagnosis

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DIAGNOSIS OF HEPATOCELLULAR CARCINOMA IN PATIENTS WITH LIVER CIRRHOSIS USING LIVER FUNCTION ASSAYS

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Abstract. Sex, age and 21 routine liver function assays were analyzed by stepwise selection and the best-of-all-possible-combinations method to identify a small group of assays valuable in establishing which liver cirrhosis (LC) patients have a high risk of hepatocellular carcinoma (HCC), when alpha-fetoprotein (AFP) is not elevated. Data was obtained from 115 HCC and 122 LC patients on admission. Tumor size correlated with AFP (0.73), alkaline phosphatase (ALP, 0.47), leucine aminopeptidase (LAP, 0.42), lactic dehydrogenase (LDH, 0.42), and the glutamic oxaloacetic transaminase (GOT)/glutamic pyruvic transaminase (GPT) ratio (GOT/GPT, 0.41). The mean of the correct diagnosis rates (CDR) of HCC and LC utilizing AFP as the sole parameter (89 %) was markedly higher than those of the other parameters. The best-of-all-possible-combinations method presented a more poweful combination than stepwise selection. The best combination of 7 parameters (LAP, GOT/GPT, choline esterase, one-hour erythrocyte sedimentation rate, age, albumin/globulin ratio, and total bilirubin) presented a mean CDR of 80 %, HCC CDR of 77 %, and false positive rate of 18 %. LC patients statistically diagnosed as having HCC by these 7 parameters are proposed as high risk patients. Fourteen (78 %) of 18 HCC patients who were AFP-negative were statistically diagnosed. This analysis can be applied to LC patients to distinguish those that should be followed closely by imaging diagnostic techniques.

Key words : hepatocellular carcinoma, liver cirrhosis, high risk hepatocellular carcinoma, liver function tests, differential diagnosis.

Hepatocellular carcinoma (HCC) often develops in patients with liver cirrhosis (LC), (1-3). The early detection of malignancy in LC patients requires frequent re-examinations by such procedures as hepatic arteriography, computed tomography, ultrasonography, scintigrams and peritoneoscopy (2). The large number of LC patients, however, prohibits extensive re-examinations. Thus, it is desirable to establish some parameters which differentiate the high risk patients.

Serum alpha-fetoprotein (AFP) is a poweful marker of HCC (4), being positive in 66-84 % of these patients (1, 3, 5-7). However, studies have shown that though AFP levels were frequently assayed and HCC was detected during the rapidly rising phase, the tumor at the time of discovery was already large and

nonresectable (2, 3). Moreover, 16-34 % of HCC produce little or no AFP (7). It is clear that parameters in addition to AFP levels are necessary to determine the high risk patients. In this study, HCC and LC patients were differentially diagnosed by a multivariate analysis of routine liver function tests. A criterion based on the results of these tests for designating which patients have a high risk of HCC is proposed.

MATERIALS AND METHODS

One hundred fifteen (115) HCC patients and 122 LC patients admitted to our department during the 8-year period from Jan. 1973 to Jan. 1981 were included in the study. HCC was diagnosed histologically in 77 of the 115 HCC patients (67 %). The remainder were diagnosed on the basis of hepatic angiography, computed tomography, liver scintigram, ultrasonic echogram or AFP levels. LC was diagnosed by peritoneoscopy and biopsy in 102 patients (84 %) and standard clinical criteria in 20 patients.

Age and sex were determined, and 21 laboratory tests were performed on admission. The assays were : total bilirubin (T-Bil) mg/dl, direct bilirubin (D-Bil) mg/dl, glutamic oxaloacetic transaminase (GOT) IU/l, glutamic pyruvic transaminase (GPT) IU/l, GOT/GPT ratio (GOT/GPT), alkaline phosphatase (ALP) Bessey-Lowry units, leucine aminopeptidase (LAP) IU/l, gamma-glutamyl transpeptidase (γ-GTP) IU/l, choline esterase (CHE) delta pH, lactic dehydrogenase (LDH) IU/l, cholesterol mg/dl, total protein (T-Pro) g/dl, albumin/globulin ratio (A/G), γ-globulin (γ-Gl) %, zinc turbidity test (ZTT) Kunkel units, indocyanine green plasma disappearance rate (KICG), RA test (RA), hepatitis B surface antigen (HBsAg) AFP level, and erythrocyte sedimentation rate at one hour (ESR1) and at 2 h (ESR2) mm, AFP was determined initially by immunodiffusion in 69 patients and later by radioimmunoassay in 143 patients. As the sensitivity of the immunodiffusion was 400 ng/ml, AFP values less than 400 ng/ml as measured by radioimmunoassay were classified as negative, values between 400 and 1,000 as one positive (+), and values over 1,000 two positive (++). The sum of the tumor diameters demonstrated by imaging was measured at the time of diagnosis and categorized into 3 classes : less than 5 cm, up to 10 cm, and over 10 cm.

Distribution of the data was determined, and 11 parameters (T-Bil, D-Bil, GOT, GPT, GOT/GPT, ALP, LAP, r-GTP, LDH, ESR1 and ESR2) were converted to logarithms in order to normalize their distributions. The differences between means of each parameter in HCC and LC patients were analyzed using Student's t test, and the difference in incidence by the chi square test. After converting to the antilogarithms the data of the 11 determinants were expressed linearly as the mean − the standard deviation (SD) and the mean + SD.

Discriminant function analysis was applied to the 23 parameters with the exception of AFP to distinguish HCC from LC. To select the parameters valuable for discriminating HCC from LC, forward selection and backward elimination procedures (8-10), and the best-of-all-possible-combinations method (9, 11) were used. Forward selection represents stepwise addition of single variables in order of descending specificity to the previous best combination. The backward elimination procedure is the opposite : a stepwise removal of the parameter in ascending order of predictive value from the previous combination of parameters. The best-of-all-possible-combinations method selects the most predictive combination of parameters. This selection was analyzed for combinations containing up to 7 determinants. The theoretical probability and the correct diagnosis rate (CDR) were used as the selection standards. When a data-point of a case was absent, the entire case was omitted from calculations. Discriminant

analysis was calculated using a microcomputer (Sord Computer Systems Inc., M223 mark III, Tokyo) ; the program was written in BASIC by one of the authors (TI).

RESULTS

Comparison of means and incidences between HCC and LC. AFP was positive in 90 (80 %) out of 112 HCC patients, but in only 2 % of LC. ALP (Fig. 1), LDH (Fig. 2), LAP, γ-GTP, GOT, GOT/GPT (Fig. 3), and ESR1 were more elevated and CHE was more depressed in HCC than in LC patients ($p<0.001$) (Table 1). D-Bil, cholesterol and ESR2 were higher in HCC than LC patients ($p<0.01$). HCC patients were older than LC patients by 3 years on the average ($p<0.01$). T-Bil was increased and A/G was decreased in HCC patients ($p<0.05$). There were no differences in GPT, T-Pro, γ-Gl, ZTT, KICG, RA and HBsAg between HCC and LC patients. Additionally there was no sex difference between the two groups.

TABLE 1. COMPARISON OF PARAMETERS

	Hepatocellular carcinoma			Liver cirrhosis	
Item	Mean	M−SD M+SD		Mean	M−SD M+SD
Age	54.4	(44.9−63.9)	>>	51.4	(41.4−61.4)
Sex	M(100)	F(15)	=	M(105)	F(17)
T-Bil*	1.76	(0.67−4.65)	>	1.38	(0.70−2.73)
D-Bil*	0.83	(0.24−2.86)	>>	0.58	(0.24−1.37)
GOT*	153	(67−348)	>>>	92	(47−180)
GPT*	79	(36−175)	=	74	(32−170)
GOT/GPT	1.93	(1.02−3.66)	>>>	1.22	(0.72−2.05)
ALP*	4.46	(2.24−8.88)	>>>	2.73	(1.82−4.09)
LAP*	308	(189−504)	>>>	214	(149−306)
γ-GTP*	109	(51−236)	>>>	61	(23−160)
CHE	0.48	(0.31−0.65)	<<<	0.57	(0.39−0.75)
LDH*	483	(301−776)	>>>	361	(280−464)
Chole	177	(113−241)	>>	154	(105−203)
T-Prot	7.1	(6.3−7.9)	=	7.1	(6.3−7.9)
A/G	0.96	(0.61−1.31)	<	1.05	(0.79−1.31)
γ-Gl	27.5	(18.1−36.9)	=	27.9	(19.7−36.1)
ZTT	11.2	(6.5−15.9)	=	12.1	(8.5−15.7)
KICG	0.089	(0.045−0.13)	=	0.093	(0.057−0.13)
RA	+ (40)	− (59)	=	+ (46)	− (65)
HBsAg	+ (34)	− (76)	=	+ (27)	− (93)
AFP	+ (90)	− (22)	>>>	+ (2)	− (97)
ESR1*	25	(9−67)	>>>	16	(6−38)
ESR2*	46	(20−102)	>>	33	(16−69)

>>>, $p<0.001$; >>, $p<0.01$; >, $p<0.05$; = not significant

* ; items analyzed statistically after conversion to logarithmic scale and expressed linearly after reconversion to antilogarithms.

Correlation of the size of liver tumor with liver function tests. The sum of the tumor diameters as determined by imaging was less than 5 cm in 8 patients, up to 10 cm in 26 and over 10 cm in 81. Correlations of liver tumor size category with individual parameters were determined. In descending order, the correlation coefficients obtained were : AFP (0.73), ALP (0.47), LAP (0.42), LDH (0.42), GOT/GPT (0.41), GOT (0.32) and r-GTP (0.31) ($p < 0.01$). These variables were subsequently compared to AFP : GOT/GPT (0.40), GOT (0.35), LAP (0.31) and ALP (0.31) ($p < 0.01$),

Correct diagnosis rates by individual determinants. The 15 varibales whose means demonstrated statistically significant differences between HCC and LC were evaluated. The CDR of HCC and the mean of the CDRs of HCC and LC are shown in Fig. 4. The mean CDR utilizing AFP as the sole variable (89.2 %) was markedly higher than those of the 15 other variables (68.0-55.5 %).

Discriminant function analysis of forward selection and backward elimination procedures. Our findings confirmed the power of AFP as a discriminator of HCC from LC

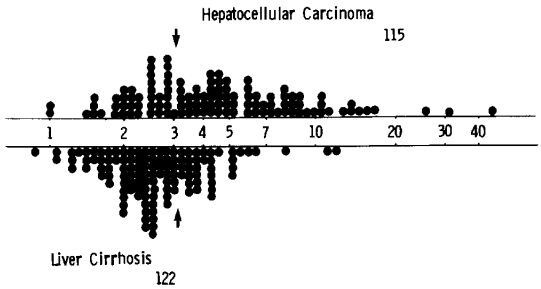

Fig. 1. Dirtribution of alkaline phosphatase (Bessey-Lowry unit) in HCC and LC. Arrows indicate the discriminating point.

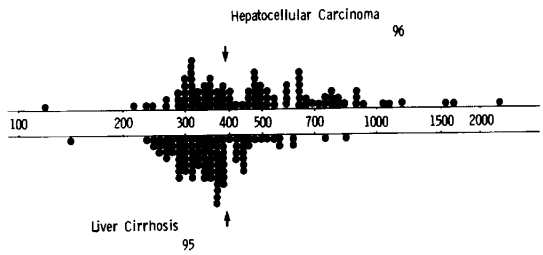

Fig. 2. Distribution of lactic dehydrogenase.

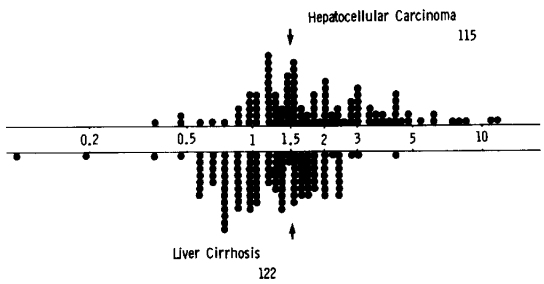

Fig. 3. Distribution of the GOT/GPT ratio.

(Figs. 4, 5). AFP was negative, however, in 20 % of the HCC cases, so we analyzed other 14 parameters in all cases. The CDR improved sharply by adding up to 3 additional variables, thereafter the rate of improvement slackened. The best combination of 3 parameters (ALP, GOT/GPT and LDH ; mean CDR 77.0 % ; HCC CDR, 66.7 %) was selected in both forward selection and backward elimination procedures. The discriminating function (Y(3)) was as follows.

$$Y(3) = 5.12 \times \frac{(\ln(\mathrm{ALP}) - 1.24)}{0.62} + 4.52 \times \frac{(\ln(\mathrm{GOT/GPT}) - 0.42)}{0.62} + 4.26 \times \frac{(\ln(\mathrm{LDH}) - 6.04)}{0.41} - 0.43$$

Determinants are arranged in descending order of discriminant coefficient and each one is standardized, that is, the difference of each value and the mean is divided by its standard deviation. When the discriminating value is equal to or greater than 0, the statistical diagnosis is HCC and when the value was less than 0, the diagnosis is LC (Fig. 6).

The best mean CDR was obtained by 10 variables both in forward selection

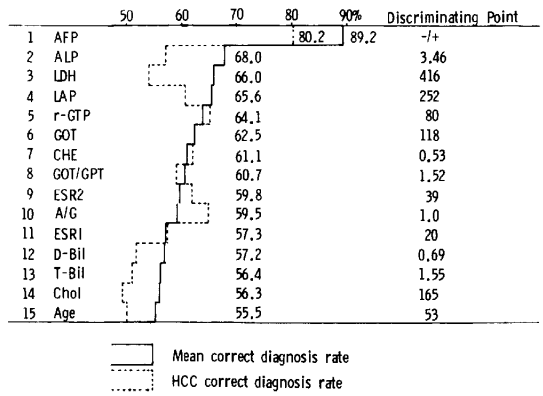

Fig. 4. Correct diagnosis rates and discriminating points of HCC and LC by single parameters, which are arranged in descending order of the mean correct diagnosis rate.

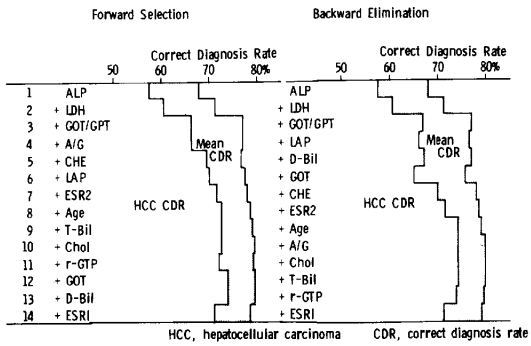

Fig. 5. Order of contribution of parameters to the mean correct diagnosis rate, which was decided by the forward selection and backward elimination method.

(79.7 %) and in backward elimination (79.8 %). The HCC CDR was always lower than the mean CDR. Therefore, HCC is considered to be more difficult to diagnose.

The best-of-all-possible-combinations method. The most valuable combinations of parameters were obtained by analyzing all combinations of up to 7 determinants (Fig. 7). LAP and GOT/GPT represented the most discriminating duet, differing from that designated by the stepwise procedures, ALP and LDH. The most helpful triplet was identified by all 3 analyses as ALP, GOT/GPT, and LDH. ALP and LDH were common to the best combinations of 3 to 5 determinants. In larger series, 6 and 7 members, LAP, GOT/GPT, CHE, ESR1, Age and A/G formed the cores.

The most powerful triplet achieved a mean CDR of 77.0 %, but an HCC CDR of only 66.7 %. The false positive rate utilizing this combination was 12.6 % and the false negative 33.3 %. Employing a 7-parameter grouping, the mean CDR was raised only slightly (79.9 %), but the HCC CDR significantly to 77.4 %.

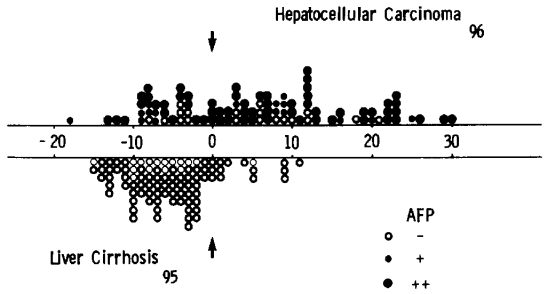

Fig. 6. Differential diagnosis of HCC from LC by discriminant function using the 3 parameters ALP, GOT/GPT and LDH. Arrows indicate the discriminant point. Each case is shown in relation to alpha-fetoprotein (AFP). See text for details.

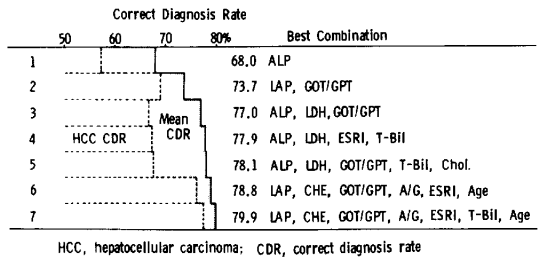

Fig. 7. Best combination of parameters for differential diagnosis of HCC from LC evaluated by the best-of-all-possible-combinations method.

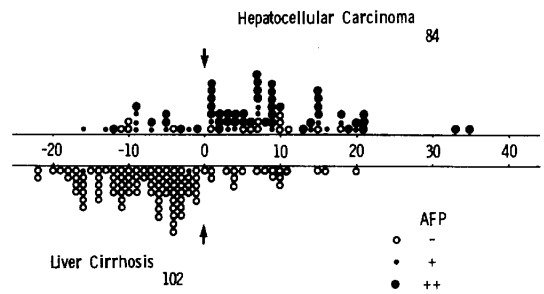

Fig. 8. Differential diagnosis of HCC from LC by discriminant function using 7 parameters : LAP, GOT/GPT, CHE, ESR1, Age, A/G and T-Bil.

The specificity was somewhat decreased (false positive = 17.6 %) but the sensitivity improved (false negative = 22.6 %). The discriminant function of the larger series (Y(7)) was :

$$Y(7) = 7.00 \times \frac{(\ln(\text{LAP}) - 5.33)}{0.46} + 5.19 \times \frac{(\ln(\text{GOT/GPT}) - 0.42)}{0.62} - 3.42 \times \frac{(\text{CHE} - 0.53)}{0.18} + 2.37 \times \frac{(\ln(\text{ESR1}) - 2.98)}{0.96} + 2.35 \times \frac{(\text{Age} - 52.9)}{9.9} + 2.14 \times \frac{(\text{A/G} - 1.00)}{0.31} - 1.90 \times \frac{(\ln(\text{T-Bil}) - 0.44)}{0.84} - 0.36$$

Each case was analyzed by this series (Fig. 8). These derived values did not always correlate with AFP (Fig. 8). Fourteen (78 %) of 18 cases with a negative AFP level were correctly classified as HCC.

DISCUSSION

We have attempted to distinguish a high HCC risk population in LC patients so as to detect HCC in the early, resectable stage. Conventional liver function tests such as ALP (12-15), GOT (13-16), GOT/GPT (6, 13), T-Bil (12, 13), LDH (6, 17), LAP (17, 18), γ-GTP (6, 17), CHE (6, 17) and ESR (14, 15) are usually abnormal in patients with HCC. As abnormalities are also common in LC, these assays have not been considered useful for the diagnosis of HCC (19). However, results of routine liver function tests have not been compared statistically between HCC and LC (12, 17, 21). HBsAg and Anti-HBe are commonly used to distinguish a high risk population (20), however only 31 % of our HCC patients were positive in this respect. Additionally, AFP levels are only marginally useful as the tumors are often too large or disseminated for resection at the time of diagnosis (2, 3). Therefore, we determined the best combination of parameters of routine liver function tests for the differential diagnosis of HCC in LC patients. A patient was considered as having a high risk of developing HCC when he was diagnosed statistically as such.

The diagnosis of HCC by multivariate analysis using discriminant functions was introduced simultaneously in 1966 by Fellingham and Merkel (21) and Shimada and Hirota (22). The former group diagnosed HCC from among 5 liver disease categories by relying upon 8 biochemical tests (T-Bil, D-Bil, ALP, GOT, GPT, LDH, isocitrate dehydrogenase and aldolase). They attained a CDR of 62 %. The latter diagnosed HCC patients with a theoretical CDR of 99 % and a mean CDR of 89 % (23). Their 13 parameters included 6 physical findings and 7 biochemical assays (T-Bil, ALP, GOT, γ-Gl, A/G, BSP and ZTT). However, the physical findings were powerful discriminators only in the late stages of the disease. Fraser and Franklin (24) diagnosed 65 % of HCC patients correctly by

the Bayesian and maximum likelihood methods using 97 parameters to distinguish from among 16 hepatobiliary diseases. Statistical multivariate analysis, however, has not been applied to the differentiation of HCC from LC.

Forward selection, backward elimination (8-10, 25), stepwise (9, 26-29), and interactive stepwise procedures (9, 11) have been utilized to select a valuable combination of parameters for the differential diagnosis of liver diseases. These procedures are relatively quick, and required 5 to 10 h of analysis in the present study. The selected combinations are not always the most powerful ; inclusion or exclusion of a single variable to or from the previously fixed combination does not inherently alter the discriminating power of the group. The best-of-all-possible-combinations method (9, 11) was found to be superior, but several days were required to compute the best combination of 7 out of 14 to 23 parameters of the 237 cases analyzed in this study. The mean CDR as determined by stepwise procedures reached a plateau (80 %) using 10 determinants. In the best-of-all-possible-combinations method, only 7 variables were necessary to achieve the same mean CDR (80 %). Moreover, the HCC CDR of the combinational analysis (77 %) was higher than that (74 %) of the sequential methods.

In determining the parameters valuable to differential diagnosis, several guides may be employed. These include theoretical probability, the CDRs of HCC, LC or total cases, and the mean CDR. Theoretical probability and the mean CDR were used in this study, but there were discrepancies between the results obtained by the two calculations. The discrepancies probably were due to the deviation from the normal of the data distributions. Therefore, the mean CDR was considered the better guiding value with which to select the important determinants.

The best combination of 3 parameters (ALP, GOT/GPT, LDH) presented a false positive rate of 13 % and a false negative rate of 33 %. In a larger group of 7 (LAP, CHE, GOT/GPT, A/G, ESR1, T-Bil and Age), the false positive rate was 18 % and the false negative rate was 23 %. Therefore, when patients are diagnosed statistically as having HCC by 3-parameter analysis, the patients should be further examined by imaging or biopsy, as 84 % of this group will have HCC. As the false negative rate was lower when utilizing 7 parameters, the patients who are statistically diagnosed should be treated as having a high risk of HCC. However, it seems difficult to reduce the false negative rate to less than 18 % since these patients were difficult to distinguish from LC patients using only the laboratory data (Fig. 8). Thus, AFP also should be examined repeatedly. The discriminating scores of the present study did not correlate with AFP levels as 78 % of HCC patients with negative AFP were correctly diagnosed statistically. Statistical analysis of LC patients utilizing the 7 determinants offers the opportunity to detect early stage HCC in AFP-negative patients.

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