A Promising Prognostic Model combining VitaminB12,Mean Corpuscular Volume and Maddrey Discriminant Function for Alcohol Acute-on-Chronic Liver Failure

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Abstract

Background: Acute-on-chronic liver failure (ACLF) which has a high mortality, is frequent during the course of severe alcoholic hepatitis (SAH). Maddrey Discriminant Function (MDF), as a prognostic score special for alcoholic hepatitis, does not show a good prognostic value in ACLF associated with alcoholic liver disease (ALD). It is well-known that the serum levels of vitamin B12 (vitB12) and folic acid, as important substances in the methionine metabolism pathway, were altered in the patients of ALD and may impact on the outcomes of these patients. In this study, the relationship between vitB12 levels, erythrocyte parameters and severity of liver disease in patients with ALD was evaluated to improve the prediction of short-term prognosis in ALD-ACLF by combining with the valuable indexes. Methods: 139 ALD patients were included in the cross-sectional cohort and grouped according to different prognostic scores, the factors that affect the scores were collected and statistically analyzed. In addition, a retrospective cohort of 79 patients was investigated to reveal the correlation between vitB12 and Mean Corpuscular Volume (MCV) with the 28-day mortality of ALD-ACLF. SPSS (20.0) and Medcalc (15.2.2) were used to perform statistical analyses. T-test, chi-square test or Fisher's as well as multivariate Logistic regression analysis were used. The ROC curve was plotted for relevant indicators, and the area under curve (AUC), cut-off value, sensitivity and specificity were calculated. Results: Patients with high scores in MDF, Model for End-stage Liver Disease (MELD) and Age-INR-Bilirubin-Creatine (AIBC), had higher vitB12 levels than those with low scores(1264.78±623.52 VS 634.04±426.57, P<0..001; 1498.47±640.48 VS 750.83±501.95, P<0.001; 1183.71±703.42 VS 813.24±553.32, P<0.05); MCV levels were higher in patients with high MDF scores (105.43±13.02 VS 99.24±13.52, P<0.05). The AUC of a new model of MDF combined with vitB12 and MCV for 28-day mortality was 0.802, which was significantly higher than that of vitB12, MCV, and MDF scores.Conclusions: High levels of vitB12, MCV and the severity of ALD have a statistically significant correlation. MDF combined with vitB12 and MCV have been showed a good specificity to predict 28-day mortality in ACLF-ALD and it may be a new promising prognostic model in ACLF-ALD.

Background

Long-term alcohol consumption can lead to alcoholic fatty liver, alcoholic hepatitis(AH), alcoholic cirrhosis(AC), liver failure, liver cancer and other related diseases, which seriously affect human health[1]. In China, alcohol consumption has been growing at its fastest rate in the past three decades. The number of hospitalized patients with alcoholic liver disease(ALD) and the annual hospitalization rate of ALD gradually increased, among them the annual prevalence of severe AH increased 2.43 times[2]. ALD has become the second most common cause of end-stage liver disease after viral hepatitis in China[3]. Alcohol-related-acute-on-chronic liver failure(ALD-ACLF) is common in patients with severe AH and AC, which is characterized by acute liver decompensation with a 28-days mortality of 50% [4]. It is well-known that several scores including Maddrey Discriminant Function (MDF), Model of end-stage liver disease (MELD) and Age-INR-Bilirubin-Creatine (AIBC) were used to determine the outcome of ALD patients. MDF as a specific score for AH did not show the good performance according to the results from the STOPAH trial[5]. So we need more specific and more sensitive model to predict bad outcome at earlier stage of severe AH, and especially ALD-ACLF.

It is well known that serum vitamin B12(vitB12) levels are significantly higher in alcoholics than in healthy people and elevated vitB12 were associated with increased severity of disease[6], since liver plays a crucial role in storing vitamins. Serum vitB12 levels are an independent predicting factor for the 90d mortality rate in HBV-ACLF patients. Moreover, combining serum vitB12 with MELD score added to prediction power of predicting mortality[7]. Besides that, long-term heavy drinking patients are often associated with anemia and increased Mean Corpuscular Volume (MCV)[8]. So far, most of the studies about vitB12 focused on its role in the pathogenesis in ALD[9, 10], few study was performed to evaluate the prognostic value of vitB12 in ALD-ACLF patients. The purpose of this study was to analyze the correlation between serum vitB12 levels, MCV and the severity of ALD, to identify independent prognostic factors for ALD-ACLF, then find a more specific and promising new prognostic model combining vitB12, MCV and MDF to improve prediction of 28-day mortality in ALD-ACLF.

Methods

Patients

Patients diagnosed with ALD admitted to our hospital from January 2001 to December 2014 were reviewed into a cross-sectional investigation. Among a total of1057 subjects which diagnosed AH, AC or ALD-ACLF, we selected 139 patients who detected serum level of vitB12 and folic acid as well as international normalized ratio (INR), Total Bilirubin (TBil), Creatine (Cre) and Prothrombin Time (PT). Then we collected the clinical data of 39 cases diagnosed ALD-ACLF which admitted to our hospital from 2010 to 2019 to retrospective analyze the prognostic factors, according to liver function index improved significantly(TBil declined more than 50% and INR<1.5) or not at 28-day after admission. This study was approved by the ethics committee of Peking University first hospital.

Inclusion and exclusion criteria

The patient enrolment flow chart is shown in Figure 1. The diagnosis of ACLF was based on the relevant standards of Guideline for diagnosis and treatment of liver failure (2018) updated by the Chinese medical association in 2019. The exclusion criteria were as follows: patients with hepatitis B, hepatitis C virus infection, autoimmune liver disease, drug-induced liver injury and other causes of chronic liver disease, or malignant diseases, as well as other systemic diseases that affect the absorption of folic acid and vitB12,the patients were transfused with red blood cells before serum testing were excluded.

Data collection and Scoring models

All clinical data of the enrolled patients were collected, which including general data(age, gender, duration of drinking, daily amount of alcohol consumption, etc), laboratory data(white blood cell(WBC), hemoglobin(HGB), MCV, platelet(PLT), folic acid, vitB12, albumin(ALB), TBil, alanine aminotransferase(ALT),aspartate aminotransferase(AST), alkaline phosphatase(ALP), glutamine transpeptidase(GGT), prothrombin activity(PTA), INR, etc) and complications(ascites, infection, upper gastrointestinal bleeding(UGIB), acute kidney injury(AKI), hepatic encephalopathy(HE)). The patients' daily alcohol

consumption was calculated with the formula(g=alcohol consumption(mL)*ethanol content(%)*0.8)[11]. The prognosis score systems (MDF, MELD and AIBC) were calculated at admission using the following formula: MDF = [4.6*(PT test - control)] + serum bilirubin (mg/dL), MELD score = 3.8*lg[bilirubin(mg/dL)] + 11.2*lg (INR) + 9.6*lg[creatinine (mg/dL)] + 6.4 (A model to predict survival in patients with end-stage liver disease)[12], and ABIC index = (age in years*0.1) + [bilirubin (mg/dL)*0.08] + [creatinine(mg/dL)*0.3] + (INR*0.8)[13].

Statistical analysis

We used SPSS(20.0) and Medcalc to perform statistical analyses and considered $P \leq 0.05$ as indicative of statistical significance. Independent sample t-test was used to compare the two groups, Continuous data are expressed as means ± SD or medians with interquartile range (P25-P75), the Kruskal–Wallis H test and Mann–Whitney nonparametric U test were used for comparison between groups. Categorical data are expressed as numbers (%), and chi-square test or Fisher's exact probability test was used. A multivariate Logistic regression analysis was used to identify independent prognostic factors for ALD-ACLF. The ROC curve was plotted for relevant indicators, and the area under the curve, cut-off value, sensitivity and specificity were calculated.

Results

MCV and serum vitB12 levels higher significantly in patients with end stage of ALD

Among the 139 patients, 60(43.1%) patients had serum vitB12 levels>894pg/ml. The serum levels of vitB12 and MCV in the patients that with higher scores of MDF(≥32), MELD(≥20) and ABIC(≥9) were significantly higher than that with lower scores of these models. In contrast to vitB12 and MCV, the serum levels of folic acid were much lower in the patients with higher scores. In addition, serum levels of GGT, and HGB were significantly lower in the patients with higher scores of MDF (P<0.05) and HGB also was significantly lower in that with higher scores of MELD (P<0.05). (Shown in Table 1).

We further analyzed the relationship of vitB12 and MCV with parameters of liver function in ALD patients. These results suggested that the serum levels of vitB12 and MCV were significantly correlated with the main indicators of liver function, and there was a positive correlation between the serum levels of vitB12 and ALT, AST, TBil, PT and INR(r=0.181,r=0.237,r=0.626,r=0.685,r=0.615, respectively. P<0.05).

Serum levels of vitB12 and MCV were not associated with the complications in retrospective ALD-ACLF cohort

We selected the patients diagnosed with ALD-ACLF (all the patients were Chinese) to analyzed whether the clinical parameters including the serum levels of vitB12 and MCV have relationship with the complications of end stage liver disease. The ages of all 79 patients (Male/Female: 77/2) range from 24y to 71y, median age 46.66±8.45 years. The most common complication of ALD-ACLF was bacterial infection(49/79,62%), followed by AKI(30/79,38%) and HE(25/79,31.6%)、UGIB(6/79,7.9%). A univariate Logistic regression analysis was performed and there was no statistical significance in Age, gender, BMI, ALB, ALT, AST, GGT, WBC, HGB, PLT, Serum folic acid, vitB12, Fe and Cre (P>0.05) in those patients with various complications of ascites, HE, UGIB and bacterial infection (P>0.05).

Serum levels of vitB12 were associated with liver failure but not liver inflammation or cirrhosis

We collected and analyzed the clinical data of the patients with AH, AC and ALD-ACLF to show whether there were statistically significant differences on the levels of serum vitB12. The results showed there were statistically significant differences on the levels of vitB12 between AH and ALD-ACLF, AC and ALD-ACLF (P<0.001). No statistically significant differences on the levels of vitB12 between AH and AC. There was no statistically significant difference on MCV levels among the three groups (F=2.67, P=0.072). There was a statistically significant difference on vitB12 levels and MCV between non-ACLF and ACLF (F=12.86, P<0.001, F=11.51, P=0.02, respectively). (Table 2)

MCV, vitB12 and MDF were independent prognostic predictors in ALD-ACLF

According to the results of Univariate Logistic regression analysis and clinical significance, we selected duration of alcohol consumption, Amount of alcohol consumption, MCV, vitB12, MDF score and MELD score as independent variables for Multivariate Logistic regression analysis. The analysis revealed that MCV, vitB12and MDF score were independent prognostic predictors in ALD-ACLF patients (P<0.05, Table 3). MCV and MDF score were risk factors for adverse outcomes of ALD-ACLF.

A new model of MDF combined with vitB12 and MCV improved the predictive value of MDF on 28-day outcome in ALD-ACLF patients

According to the results of multi-factor analysis, finally, MDF, vitB12 and MCV were selected to jointly predict the prognosis of patients with ALD-ACLF, and then performed binary logistic regression analysis again to establish a new short-term prognosis model for patients with ALD-ACLF. The calculation formula is as follows: Logit(P)=-9.273 + 0.078 * MCV + 0.036 * MDF +(-1.786*1) +(-0.709*2) +(-0.98*3). [0=vitB12(≥2000), 1=VitB12(<1175), 2=vitB12(1175-1709), 3=vitB12(≥1709-<2000)]. The new model was proved to be valuable and promising to predict 28-day outcome (liver function index improved significantly or not) in the patients diagnosed ALD-ACLF (0.11±1.28 vs. -0.57±1.46, P<0.03; Figure 2 panel A). The ROC curve analysis shows that the area under the ROC curve (AUC) and Youden index of the new model are obviously better than MDF solely (Figure 2 panel B).

Discussion

It is well-known that methionine metabolism imbalance plays an important role in the development of ALD in addition to the toxicity of ethanol and its metabolites, oxidative stress reaction and lipid peroxidation, endotoxin, cytokines and inflammatory mediators, gender and gene polymorphism, etc. [14-16]. In recent years, it has been proposed that ALD can be regarded as a kind of malnutrition results from protein caloric deficiency, nutritional imbalance and the deficiency of various life elements, which includes folic acid, vitamin B6(vitB6) and vitB12[17], that affected the methionine metabolism cycle. Folic acid is the

primary methyl donor for the production of s-adenosine methionine(SAM) and the substrate for DNA and histone methyltransferases, while vitB12 is a cofactor of methionine synthetase(MS),and vitB6 is a cofactor of the cystathionine-β-synthase (CβS) and cystathionase[9]. Chronic alcohol intake leads to vitamin deficiencies, particularly vitB12, vitB6, folic acid, vitamin A and vitamin E, which are the main causes of red cell disease [18]. Previous studies have shown that the serum levels of folic acid and vitB12 is gradually increased during the developing and aggravation of ALD[19][10, 20][21]. In our study, patients with ALD were classified according to MDF, MELD and ABIC scores, serum vitB12 concentration was significantly higher in patients with higher scores than in patients with lower scores ($p<0.05$). Consistent with previous research[18], our results showed that the serum levels of vitB12 was associated with disease adverse outcomes. Furthermore, our data also revealed serum levels of vitB12 were positively correlated with ALT, AST, TBil, PT and INR, which indicated serum vitB12 level were associated with the severity of liver injury in ALD.

Previous study showed that with increasing daily alcohol ingestion, MCV also increased gradually[22], and earlier studies have found that half of chronic alcoholics had higher MCV values(>95fl)[23]. Moreover, it is reported that the increase of MCV can be used as an indicator of long-term heavy drinkers, and the specificity of diagnosis is up to 90%[24, 25]. Among our 139 patients with ALD, MCV was positively correlated with TBil, PT, and INR and there was a significant difference on MCV between 2 groups (MDF≥32 and MDF<32) ($P<0.05$). And in ACLF patients, 82.3% (65/79) patients' MCV were more than 95fl in our study[18, 20, 26]. These results prove that MCV also may be regard as the biochemical indicators to reflect the degree of liver injury and prognosis of ALD patients.

Several prognostic models exist to determine the outcome of ALD patients, which including MDF, MELD, ABIC, MELD-NA, Child-Pugh, and other scoring systems. But even though MDF, as a specific score for alcoholic hepatitis, did not show the satisfactory performance in a recent clinical research for prognosis assessment of AH. In our study, a new model for prognosis of ALD-ACLF which combined MCV and vitB12 with MDF showed better specificity (72.41%) and sensitivity (79.59%), compared with MDF only. The new model may help patients with chronic ALD predict their prognosis more precisely and may be as an early warning to guide early therapy so that the patients gain clinical benefits.

One limitation of this study was the relatively small number of ACLF patients enrolled in this study may impact on the accuracy of the conclusion, so large scale studies need to be carried out to confirm the predictive value of the new model on predicting the prognosis of ALD.

In summary, this study suggests that serum vitB12 is a predictive factor for severity of liver injury and disease outcome in ALD and also an independent risk factor for the 28-day prognosis of patients with ALD-ACLF, which may provide a new idea on a potential and promising supplementary therapy of vitB12 for improving the prognosis in ALD patients, especially in those patients with life-threatening ALD-ACLF. Furthermore, if confirmed by a prospective study with large samples and multiple centers in the future, the new model will be very helpful for the patients with ALD-ACLF to predict the prognosis at earlier stage, suggest start to supplementary therapy and monitor the efficacy of treatment, so that the patients gain survival benefit 28 days.

Conclusions

High levels of vitB12, MCV and the severity of ALD have a statistically significant correlation. MDF combined with vitB12 and MCV have been showed a good specificity to predict 28-day mortality in ACLF-ALD and it may be a new promising prognostic model in ACLF-ALD.

Abbreviations

AC: alcoholic cirrhosis; ACLF: Acute-on-chronic liver failure; AH: alcoholic hepatitis; AIBC: Age-INR-Bilirubin-Creatine; AKI: acute kidney injury; ALD: alcoholic liver disease; ALB: albumin; ALP: alkaline phosphatase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUC: area under curve; Cre: Creatine; CβS: cystathionine-β-synthase; GGT: glutamine transpeptidase; HE: hepatic encephalopathy; HGB: hemoglobin; INR: international normalized ratio; MCV: Mean Corpuscular Volume; MDF: Maddrey Discriminant Function; MELD: Model for End-stage Liver Disease; MS: methionine synthetase; PLT: platelet; PTA: prothrombin activity; PT: Prothrombin Time; SAH: severe alcoholic hepatitis; SAM: s-adenosine methionine; Tbil: Total Bilirubin; UGIB: upper gastrointestinal bleeding; vitB12: vitamin B12; vitB6: vitamin B6; WBC: white blood cell.

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Tables

Variables	MDF≥32 (n =53)	MDF<32 (n =86)	Univariate OR (95% CI) P	MELD≥20 (n =23)	MELD<20 (n = 116)	Univariate OR (95% CI) P	ABIC≥9 (n =23)	ABIC<9 (n =116)	Univariate OR (95% CI) P
Age (y)	47.84±9.59	51.14±10.52	1.034(0.997-1.071) 0.066	48.96±11.14	50.07±10.13	1.011(0.967-1.057) 0.635	58.04±12.12	48.26±9.08	1.034(0.997-1.071) 0.066
Gender (M/F)	51/2	85/1	0.300(0.027-3.392) 0.331	23/0	113/3	328813467.97 0.999	23/0	113/3	328813467.95 0.999
BMI	24.63±4.39	23.01±3.54	0.898(0.819-0.985)[a] 0.019	23.65±3.75	23.64±4.00	0.999(0.892-1.120) 0.992	24.00±3.36	23.57±4.07	0.973(0.870-1.088) 0.628
ALT(IU/L)	29.0(20.0-43.5)	26.5(17.0-46.25)	1.002(0.997-1.006) 0.447	39.0(22.0-48.0)	27.0(17.25-43.0)	1.000(0.996-1.003) 0.897	31.0(22.0-44.0)	28(17.25-45.0)	0.997(0.992-1.001) 0.131
AST (IU/L)	55.0(45.5-101.5)	53.5(32.75-108.5)	0.999(0.995-1.003) 0.606	75.0(49.0-120.0)	52.0(37.0-100.75)	0.996(0.992-1.001) 0.108	83.0(49.0-153.0)	52.0(37.0-99.75)	0.993(0.987-0.999) 0.014
ALP(IU/L)	144.26±73.71	117.37±54.90	0.993(0.987-0.999) 0.015	141.78±79.89	124.82±60.18	0.996(0.990-1.003) 0.250	118.65±34.36	129.41±68.16	1.003(0.995-1.011) 0.460
GGT (IU/L)	58.0(30.5-132.5)	192.0(56.25-468.0)	1.005(1.002-1.008)[a] 0.000	79.0(41.0-154.0)	137.0(35.25-328.5)	1.003(1.000-1.006) 0.060	107.0(58.0-295.0)	109.5(35.25-276.5)	1.000(0.999-1.002) 0.749
WBC	8.90±8.91	6.29±3.40	0.930(0.872-0.992) 0.045	10.63±8.28	6.63±5.53	0.924(0.866-0.984) 0.015	10.58±7.78	6.63±5.67	0.925(0.868-0.985) 0.016
NE%	68.74±11.13	65.16±11.13	0.977(0.950-1.005) 0.110	72.74±11.00	65.29±12.84	0.949(0.912-0.988) 0.011	68.91±16.83	66.05±11.91	0.982(0.947-1.019) 0.328
MCV (fl)	105.43±13.02	99.24±13.52	0.965(0.938-0.992)[a] 0.043	105.65±12.76	100.80±13.70	0.973(0.941-1.007) 0.122	103.21±12.91	101.29±13.79	0.990(0.957-1.023) 0.537
HGB (g/L)	81.57±27.94	99.81±30.63	1.021(1.008-1.034)[a] 0.009	74.82±23.10	96.42±31.00	1.024(1.008-1.041)[b] 0.004	91.61±31.93	93.10±30.76	1.002(0.987-1.016) 0.831
Serum folate (nmol/L)	12.04±11.03	18.62±15.51	1.038(1.009-1.068 0.004	9.43±8.04	17.44±14.90	1.063(1.008-1.122) 0.025	12.63±10.22	16.81±14.91	1.024(0.987-1.063) 0.206
Serum vitB12 (pmol/L)	1264.78±623.52	634.04±426.57	0.998(0.997-0.999)[a] 0.000	1498.47±640.48	750.83±501.95	0.998(0.997-0.999)[b] 0.000	1183.71±703.42	813.24±553.32	0.999(0.998-1.000)[c] 0.008
Length of alcohol consumption (year)	20.62±8.35	24.90±9.97	1.051(1.011-1.092) 0.010	21.09±8.62	23.70±9.74	1.030(0.981-1.082) 0.234	24.74±11.49	22.97±9.19	0.981(0.936-1.028) 0.419
Amount of alcohol consumption (g)	263.98±177.67	249.44±150.04	1.000(0.998-1.003) 0.656	250.65±192.77	243.50±154.40	1.000(0.997-1.002) 0.845	171.61±147.26	259.18±159.78	1.005(1.001-1.008) 0.020

Table 1. MCV and serum VitB12 levels higher significantly in late stage of ALD according to MDF, MELD and ABIC models scores. Multivariate Logistic regression revealed that BMI, serum GGT, serum VitB12, MCV and HGB were significantly different between the two groups (MDF≥32 VS. MDF<32, a P<0.05), among them, the P value of serum VitB12 <0.001; HGB and serum VitB12 were the influence factors of the MELD scores (b P<0.05); Serum VitB12 level was the influence factors of the ABIC scores(c P<0.05).

Total patients(n=177)	Serum VitB12(Mean±SD)	MCV(Mean±SD)
AH（n=13）	508.54±358.85	102.33±13.29
AC（n=86）	577.58±340.13 [a]	100.28±15.27
Non-ALD-ACLF(AH+AC, n=99)	568.52±341.56	100.55±14.98
ALD-ACLF（n=79）	1556.30±461.08 [b,c]	104.96±9.65 [d]

Table 2: Comparison of serum VitB12 and MCV levels between AH, AC, ALD-ACLF groups. Compared with AH, a P<0.05, b P<0.05; compared with Non-ALD-ACLF, c P<0.05, d P<0.05.

Variables	β	SE	Pvalue	Oddsratio	95% CI
MCV (fl)	0.078	0.033	0.019	1.081	1.013-1.154
Serum vitamin B12 (pmol/L)			0.035		
<1175(pmol/L)	-1.790	0.791	0.023	0.167	0.036-0.785
≥1175-<1709(pmol/L)	0.699	0.788	0.373	2.012	0.432-9.378
≥1709-<2000 (pmol/L)	-0.961	0.907	0.287	0.382	0.065-2.244
≥2000 (pmol/L)	0			1.000	
MDF score	0.037	0.017	0.032	1.037	1.003-1.072

Table 3: Multiple Logistic analysis of 79 cases with poor prognosis of ACLF. Multivariate Logistic regression revealed that MCV, vitB12 and MDF score were independent risk factors affecting the short-term prognosis of ACLA-ALD patients.

Figures

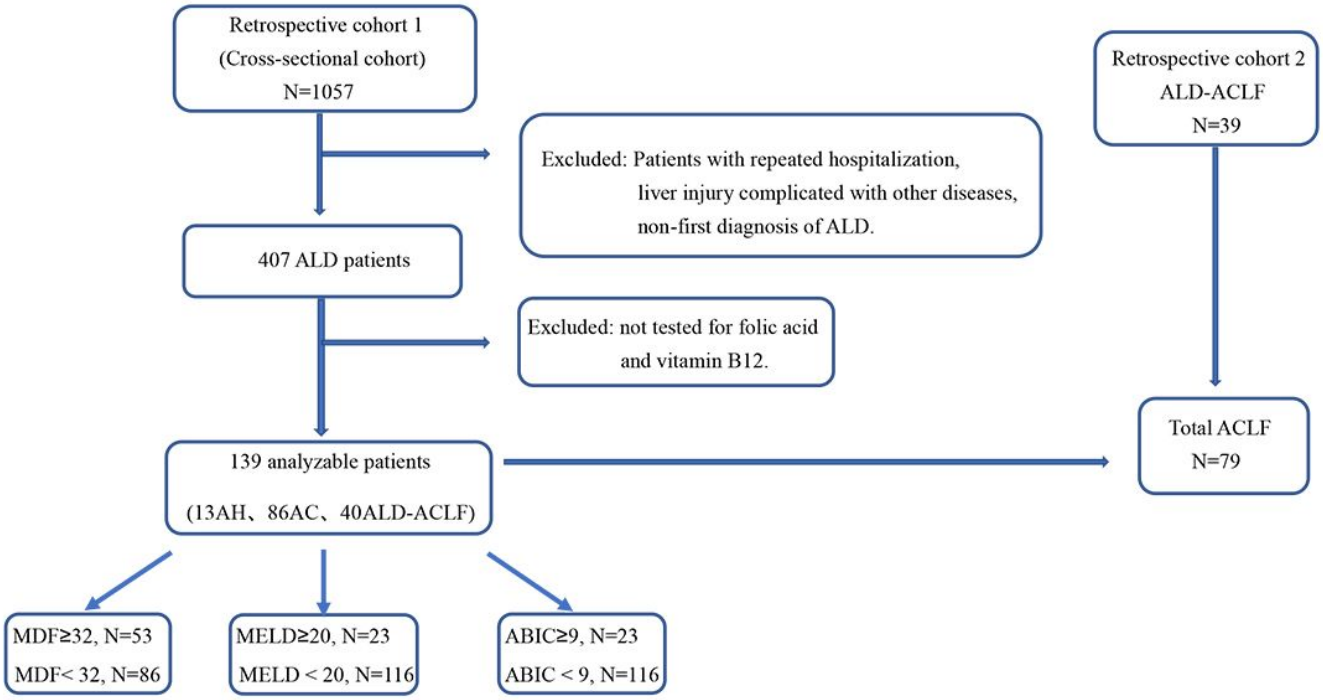

Figure 1

Flow chart.

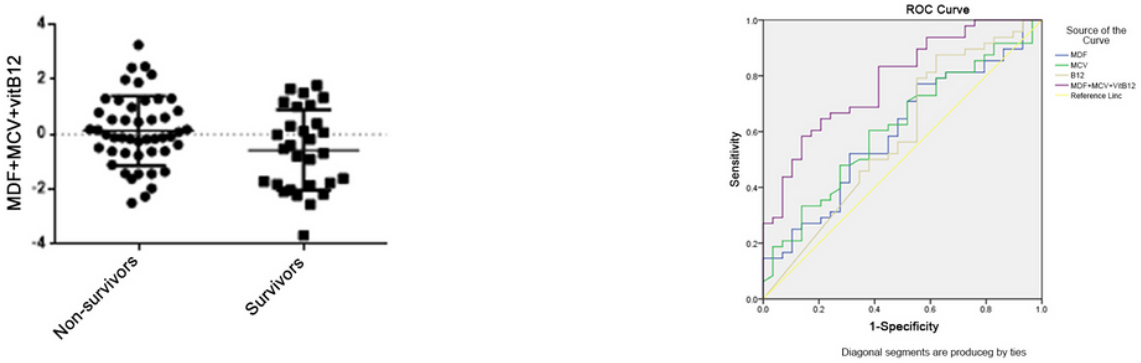

Figure 2

Association between the prognosis of ALD-ACLF patients and the new model score on admission. (A)New score distribution of ALD-ACLF patients in the 79 cases(P=0.03). (B) Comparison of the prognostic accuracy between the new model and prognostic scores in predicting 28d mortality of ALD-ACLF patients. Area under curve (AUC) of the new model and other prognostic scores for predicting 28d mortality in ALD-ACLF patients. The AUC of the “MDF+MCV+VitB12” score model for 28-day mortality was 0.802 (95%CI 0.696-0.883, P<0.0001), with a sensitivity of 79.59% and specificity of 72.41 at a cut-off value of 0.59, which was significantly better than the MDF(AUC=0.598, 95%CI 0.481-0.708, P=0.141), MCV(AUC=0.621, 95%CI 0.504-0.729, P=0.061), VitB12 (AUC=0.582, 95%CI 0.465-0.693, P=0.233).