Study of Serum Argininosuccinate Lyase Determination for Diagnosis of Liver Diseases

Jia-fu Feng, Ting-mei Chen, Yang-an Wen, Jian Wang, and Zhi-guang Tu*

Key Laboratory of Laboratory Medical Diagnosis of Education Ministry, Faculty of Laboratory Medicine, Chongqing Medical University, Chongqing, China

The objectives of this research were to establish an automatic analysis method for the determination of serum argininosuccinate lyase (ASL) and to investigate the value of serum ASL test in the diagnosis of various liver disorders. According to the chemical reaction catalyzed by ASL, an enzyme-coupled reaction system was designed, and a methodology evaluation of this method was performed. A total of 291 patients with various liver diseases, 247 patients with nonliver disease and 32 healthy controls, were recruited, their serum levels of ASL and traditional hepatopathy markers, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyltransferase (GGT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), and total bilirubin (TBil), were all determined, and their diagnostic values in liver diseases were analyzed and compared. Liver biopsy and the score of histopathological inflammation grading were performed in 31 patients with hepatopathy to explore the correlation between serum ASL level and hepatic histopathological change. A continuous monitoring assay method of serum ASL activity was established, which could be performed with automatic biochemistry analyzer. Methodological evaluation exhibited that the precision of this method was good indicated by the

4.0% intraassay coefficient of variation (CV), and 5.9% interassay CV. The mean recovery was 100.5%, linear range was from 0 to 167.7 U/L, and the lowest detection limit was approximately 0 U/L. All of the tested hepatopathy markers listed above were significantly increased in the liver disease group. However, levels of traditional markers of hepatopathy were all significantly increased at different degrees (all $P < 0.001$) in patients with nonliver diseases; in contrast, there were no significantly increased ASL levels in all non-hepatopathy groups ($P = 0.335$). The receiver operating characteristic (ROC) curve showed that the sensitivity and specificity of ASL were 100% and 91.1% (cutoff value = 8 U/L), respectively, in the assessment of liver diseases. In contrast, ALT levels were 97.6% and 24.7%, and AST levels were 83.8% and 28.3% (both cutoff values = 40.0 U/L), respectively. A positive correlation ($r = 0.417$, $P = 0.019$) was observed between serum ASL levels (86.9 ± 26.5) and scores of histopathological inflammation grading (SHIG) (9.83 ± 3.36). The sensitivity and specificity of ALS is much higher than that of ALT and AST for the diagnosis of liver diseases. ASL may be a more valuable marker for estimating hepatopathy. *J. Clin. Lab. Anal.* 22:220–227, 2008. © 2008 Wiley-Liss, Inc.

Key words: alanine aminotransferase (ALT); aspartate aminotransferase (AST); argininosuccinate lyase (ASL); liver disease; continuous monitoring method; receiver operating characteristic curve

INTRODUCTION

Abnormal levels of some enzymes in serum may signal liver injury. Among them aminotransferases are extensively used as enzyme markers of liver disease for a long time (1). However, levels of both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) vary according to age (2), sex (3), and strenuous exercise (4). On other side, both ALT and AST are extensively

Present address for Jia-fu Feng: Mianyang Central Hospital, Sichuan Province, China.

*Correspondence to: Zhi-guang Tu, Key Laboratory of Laboratory Medical Diagnosis of Education Ministry, Faculty of Laboratory Medicine, Chongqing Medical University, Chongqing 400016, China. E-mail: tuzhiguang@yahoo.com.cn

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distributed throughout the body, so that they have a poor specificity in diagnosis of liver disease (5–7).

Argininosuccinate lyase (ASL, EC 4.3.2.1) also named argininosuccinase catalyzes the reversible reaction of argininosuccinate to arginine and fumarate (8–11). It is an important enzyme in the urea cycle in liver. ASL mediates the cleavage of argininosuccinate to arginine and fumarate, which is the key limit step in urea formation (12,13). Urea is predominantly produced in liver, so that it is reasonable to investigate serum level of ASL as a candidate of hepatocellular injury marker. Earlier studies have shown ASL was either undetectable or present in minute amounts in the normal individual serum, but it is markedly elevated in liver disease, and no significantly increased in diseases that primarily affect other organs (14–16). Therefore, it is a hopeful index of liver diseases. In the present study, a novel continuous monitoring assay procedure based on the above biochemical reaction catalyzed by ASL was established for automatic determination of serum ASL, and serum ASL levels were compared with traditional liver injury markers in various kinds of hepatopathy and other disorders to explore the diagnostic efficiency of ASL for liver diseases.

MATERIALS AND METHODS

Subjects

Patients With Liver Diseases

A total of 291 patients (163 males, 182 females) diagnosed according to relative clinical criteria were enrolled. The mean age of the patients was 59 years with a range of 17–69 years. There were 31 acute hepatitis patients (23 patients hepatitis B virus-deoxyribonucleic acid [HBV-DNA] positive and eight patients hepatitis C virus-ribonucleic acid [HCV-RNA] positive), 165 chronic hepatitis patients (73 patients with chronic type B hepatitis, 39 patients with chronic type C hepatitis, 25 patients with chronic alcoholic hepatitis, 16 patients with drug-induced hepatitis, and 12 patients with autoimmune hepatitis), 67 cirrhosis patients (43 patients HBV-DNA-positive and 24 patients HCV-RNA-positive), and 28 primary hepatocellular carcinoma patients (23 patients HBV-DNA-positive, and 17 patients HCV-RNA-positive).

Nonliver Disease Patients

A total of 247 nonliver disease patients (139 males, 108 females), excluding liver diseases according to the case history, clinical signs, physical and laboratory examination, and image or ultrasound examination, were investigated. The mean age was 56 years with a range of 15–66 years. There were 26 urologic diseases

(seven kidney stones, three bladder stones, seven hyperplasia of the prostate gland, four nephronic syndrome, two acute glomerulonephritis, two acute pyelonephritis, and one uremia), 17 heart diseases (five coronary artery atherosclerosis, four angina pectoris, four myocardial infarction, two juvenile arrhythmia; one premature, and one acute cardiac insufficiency), 13 cerebropathy (four cephalitis, four subarachnoid hemorrhage, two cerebral infarction, two cerebral abscess, and one agitated paralysis), 32 gastrointestinal diseases (11 gastritis, four gastric ulcer, six duodenal ulcer, five pancreatitis, four acute enteritis, and two intestinal polyp), 23 disease of respiratory system (nine pneumonia, six bronchitis, three aeropleura, two lung embolism, and two pleuritis), 19 connective tissue diseases (10 rheumatoid arthritis, three lupus erythematosus, two dermatomyositis, one Sjogren's syndrome, one chorionitis, and one rheumatic fever), 16 endocrine system diseases (11 hyperthyroidism, three thyroiditis, and two menopausal syndrome), 29 cancer without liver metastasis (seven lung cancer, five gastric cancer, four breast cancer, four carcinoma of bladder, two brain cancer, two colorectal cancer, two ovarian cancer, two nasopharyngeal carcinoma, and one laryngocarcinoma), 30 metabolic disease (16 diabetes mellitus, six obesity, six gout, and two hyperlipoproteinemia), 11 ear-nose-throat diseases (four sudden deafness, three otitis media purulenta, two suppurative tonsillitis, and two rhinitis), and 31 infectious diseases (15 nongonococcal urethritis, 12 influenza, two parotitis, one rubella, and one typhoid fever).

Healthy Controls

There were 32 healthy volunteers (21 males, 11 females), the mean age was 55 years with a range of 21–60 years.

Blood Samples

After overnight fasting, approximate 3 mL of venous blood samples were collected in 5-mL Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ), blood samples were centrifuged at 3,000 rpm for 15 min to separate sera 1 hr later. The sera were stored at -30°C until analysis. At the same time, 1 mL venous blood was drawn into another Vacutainer tube for the determination of HBV-DNA and HCV-RNA.

Sample Analysis

Serum enzymes levels, including ALT, AST, γ -glutamyltransferase (GGT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP), were all determined in each sample with the kinetic method

recommended by the International Federation of Clinical Chemistry (IFCC). Total bilirubin (TBil) was measured by using the vanadate method. ALT, AST, and GGT test kits were from Shanghai Shenergy-DiaSys Diagnostic Technology (Shanghai, China). LDH and ALP test kits were from Shanghai Fosun Long March Medical Science (Shanghai, China). TBil kit was from Beijing Strong Biotechnology (Beijing, China). All assays were performed with a 7600-010 Automatic Analyzer (Hitachi, Ibaraki-ken, Japan).

HBV-DNA and HCV-RNA were quantitatively determined with a iCycler IQ Multicolor Real-Time PCR Detection System (Bio-Rad, Hercules, CA). Both kits were purchased from Shenzhen PG Biotechnology (Shenzhen, China).

Assay of Serum ASL activity

Principles of Assay

A new kinetic method for assay of serum ASL activity was developed based on below sequential reaction:

The change of NADH increased rate was determined by continuous monitoring the NADH absorbance change values per minute ($\delta A/\text{min}$) with automatic biochemical analyzer. ASL activity in the sample was calculated by ϵ_{NADH} and $\delta A/\text{min}$.

Reagents

Reagent 1. 20 mmol/L sodium argininosuccinate. 218 mg sodium argininosuccinate (Sigma Chemical Co., St. Louis, MO) was dissolved in 50 mL purified water.

Reagent 2. 0.2 mmol/L phosphate buffer, pH 7.0. 27 g of $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ (Wuhan Chemical Factory, Wuhan, China) was dissolved in 800 mL of purified water. Adjusted to pH 7.0 using ~ 40 mL of 2.5 mol/L NaOH, and diluted to 1,000 mL with purified water.

Reagent 3. Hydrazine sulfate-glycine buffer, pH 9.5. 10.4 g of anhydrous hydrazine sulfate (Tianjing Kemiao Chemical Development Center, Tainjing, China) and 15 g of glycine (Shanghai Chemical Reagent Co., Shanghai, China) was dissolved in 50 mL of 2 mol/L KOH, and 0.4 g of disodium ethylenediamine tetraacetic acid (Na_2EDTA) was added. Adjusted to pH 6.0 using 2 mol/L KOH. Diluted to 100 mL with purified water. This stock solution is stable at -4°C for several weeks. Before using, 10 mL of the stock buffer was adjusted to pH 9.5 with approximate 8.5 mL of 2 mol/L KOH. This solution is stable at -4°C for 2 weeks.

Reagent 4. Fumarase solution, Fumarase isolated from porcine heart (Sigma Chemical Co.; LOT#041K7435, 368 units/mg protein) was dissolved in 3.2 mol/L $(\text{NH}_4)_2\text{SO}_4$.

Reagent 5. 2 mmol/L NAD^+ . 15.0 mg of NAD^+ (α -nicotinamide adenine dinucleotide) (Sigma Chemical Co.; LOT#031K7033) was dissolved in 10 mL of Reagent 3.

Working Reagent Preparation

Working reagents 1 and 2 were prepared by the above five stock reagents. Reagent 1 (R_1): 4 mL of Reagent 1 and 80 mL of Reagent 2 were mixed; Reagent 2 (R_2): 20 μL of Reagent 4 and 1 mL of Reagent 5 were mixed. Both R_1 and R_2 are stable at -4°C for 7 days.

Test Parameter

Method: rate A; Serum volume: 10 μL ; R_1 volume: 200 μL ; R_2 volume: 50 μL ; reactive time: 10 min; Temperature: 37°C ; Read points: 34; Primary wavelength: 340 nm; Secondary wavelength: 405 nm.

Statistical Analysis

Statistic analysis including the receiver operating characteristic (ROC) curve was completed with SPSS 10.0 for Windows (SPSS Inc., Chicago, IL). The results were expressed as mean $\pm$ SD and/or range (minimum--maximum, median). Statistical analysis was performed using *t*-test or analysis of variance (ANOVA), with $P < 0.05$ considered to be statistically significant. Pearson correlation coefficients (*r*) were used to quantify the correlation between the parameters.

RESULTS

Methodological Evaluation

Imprecision

Within-day and between-day imprecision was determined by performing measurements on three pooled serum samples with various ASL concentrations (7 U/L, 22 U/L, and 36 U/L). Within-day testing was done by 20 repeated tests in a day, and between-day testing was carried out on 20 days over a 3-week period. Calibration was performed each testing day. The within-day and between-day imprecision (coefficient of variation [CV]) was determined by replicate analyses of three serum samples. The results showed that within-day imprecision was 5% for 36 U/L ASL, 7% for 22 U/L ASL, and 8% for 7 U/L ASL. The between-day imprecision was 8% for 36 U/L ASL, 9% for 22 U/L ASL, and 11% for 7 U/L ASL.

Linearity

Neither ASL standard nor control serum was available; we performed the serial two-fold dilutions of a serum sample of 168 U/L ASL with a serum containing undetectable ASL activity, and ASL levels were measured in triplicate. The results showed the linearity was good at least in the range of 0–168 U/L ASL ($y = 264.8446x - 2.354$ U/L; $r^2 = 0.997$).

Detection Limit

Owing to no commercially available purified ASL, we measured physiologic saline in 20 replicates and calculated the mean and SD of the measured values. The detection limit, defined as the mean (0.26)-3 SD (0.18), was 0 U/L (measured range: 0–0.5 U/L), because the mean-3 SD value overlapped with zero.

Recovery

A sample containing 91.8 U/L ASL was added to 10 serum samples (1:9, v/v) and measured in triplicate. The arithmetic mean was calculated. Recoveries are shown in Table 1.

Interference

The effects of several potential interfering substances on present method were evaluated. Various concentrations of bilirubin solutions (up to 3,420 $\mu\text{mol/L}$, dissolved with 0.1 mol/L NaOH) were added to a pooled serum (1:9, v/v) so that a series of test samples was prepared. Each sample was measured in 10 replicates. There was no interference from bilirubin ($<342 \mu\text{mol/L}$). The interference of hemoglobin was also detected. A 10-mL venous blood sample of a healthy volunteer was collected into two Vacutainer tubes. The serum samples were prepared, and ASL activity was tested. At the same

time, a 2.4-mL whole blood sample was drawn into another Vacutainer tube with anticoagulant Na_2EDTA . The erythrocytes were broken as far as possible by vigorously stirring with a glass stick. No broken erythrocytes were removed by centrifugation. The concentration of hemoglobin in the supernatant was measured. A concentrated hemolytic serum sample (36.2 g/L hemoglobin) was obtained. A series of hemolytic serum samples were prepared by adding different volume of concentrated hemolytic sample to serum sample. Each sample ASL activity was measured in 10 replicates. The results showed that our assay was interfered with about +3.5% and +10.9% when hemoglobin in serum was 0.06 g/L and 0.2 g/L.

Changes of ASL and Traditional Liver Injury Markers in Patients With Nonliver Diseases

Results of serum ASL and traditional liver injury markers in patients with nonliver diseases are compared in Table 2. There was no significant change of ASL levels compare with healthy controls ($F = 1.108$, $P = 0.355$). However, traditional liver injury markers tested were all significantly increased (all $P < 0.001$ vs. controls). It suggested that all investigated clinical markers of liver injury, except ASL, would be significantly altered in observed nonliver disease patients.

Changes of ASL and Traditional Liver Injury Aminotransferase Markers in Patients With Various Hepatopathies

The results of serum ASL and traditional aminotransferase markers (ALT and AST) in various liver disease patients showed that either ASL or aminotransferase markers were significantly increased (Table 3). Compared to the healthy controls, the increased degrees of serum ASL in various liver disease patients were primary hepatocellular carcinoma group > acute hepatitis group > liver cirrhosis group > chronic hepatitis group. ALT and AST levels in acute hepatitis patients were significantly increased over those in liver cirrhosis, liver cancer, and chronic hepatitis patients. Both ALT and AST activities of primary hepatocellular carcinoma patients were significantly lower than those of other liver diseases (Table 4).

Diagnostic Efficiency Comparison of ASL and Traditional Aminotransferase Markers on the Diagnosis of Hepatopathy

Figure 1 illustrates the ROC curves of ASL, ALT, AST, and ALT+AST on the diagnosis of hepatopathy. The sensitivity and specificity of ASL at a cutoff value of 8 U/L (the upper limit of healthy control group range) to

TABLE 1. Analytical recovery*

Sample no.	Initial concentration (U/L)	Added concentration (U/L)	Measured concentration (U/L)	Recovery (%)
1	7.7	9.2	17.6	107.2
2	24.8	9.2	34.3	102.8
3	66.4	9.2	75	93.3
4	28.7	9.2	37.4	94.5
5	90.7	9.2	99.8	98.7
6	47.9	9.2	57.6	105.7
7	144.3	9.2	153.9	104.7
8	83.3	9.2	93	106.0
9	11.8	9.2	20.6	95.8
10	108.6	9.2	117.5	96.4

*Initial concentration = sample stock concentration $\times$ 0.9; data was rounded to one decimal place.

TABLE 2. Relationship between traditional markers of liver function and ASL in various nonliver diseases (mean $\pm$ SD)

Disease(n)	ASL (U/L)	ALT (U/L)	AST (U/L)	LDH (U/L)	ALP (U/L)	GGT (U/L)	TBil (μ mol/L)
Urologic diseases (26)	4.5 $\pm$ 2.7	55 $\pm$ 18*	50 $\pm$ 16*	150 $\pm$ 49*	158 $\pm$ 56*	26 $\pm$ 14	13.4 $\pm$ 5.6
Heart diseases (17)	3.9 $\pm$ 2.6	43 $\pm$ 12*	103 $\pm$ 26*	280 $\pm$ 76*	93 $\pm$ 21	36 $\pm$ 24*	11.9 $\pm$ 3.2
Cerebroopathy (13)	3.4 $\pm$ 1.7	77 $\pm$ 34*	66 $\pm$ 24*	747 $\pm$ 173*	88 $\pm$ 21	42 $\pm$ 20*	14.5 $\pm$ 5.5
Gastrointestinal diseases (32)	4.0 $\pm$ 1.9	42 $\pm$ 9*	39 $\pm$ 11*	624 $\pm$ 89*	88 $\pm$ 30	39 $\pm$ 15*	12.9 $\pm$ 4.5
Metabolic disease (30)	5.0 $\pm$ 2.8	46 $\pm$ 14*	43 $\pm$ 12*	278 $\pm$ 50*	144 $\pm$ 55*	39 $\pm$ 20*	15.7 $\pm$ 5.2*
Tumors without liver metastasis (29)	4.7 $\pm$ 3.1	78 $\pm$ 19*	88 $\pm$ 34*	231 $\pm$ 76*	141 $\pm$ 45*	58 $\pm$ 18*	16.5 $\pm$ 4.6*
Disease of respiratory system (23)	3.9 $\pm$ 2.5	87 $\pm$ 39*	83 $\pm$ 39*	630 $\pm$ 163*	99 $\pm$ 42*	45 $\pm$ 15*	11.6 $\pm$ 3.9
Connective tissue diseases (19)	3.8 $\pm$ 2.2	101 $\pm$ 47*	95 $\pm$ 37*	496 $\pm$ 229*	95 $\pm$ 18*	64 $\pm$ 17*	16.1 $\pm$ 4.0*
Ear-nose-throat diseases (11)	2.6 $\pm$ 1.6	47 $\pm$ 18*	37 $\pm$ 16	182 $\pm$ 98	81 $\pm$ 27	38 $\pm$ 17	10.0 $\pm$ 4.1
Endocrine system diseases (16)	4.0 $\pm$ 2.7	48 $\pm$ 19	41 $\pm$ 15*	226 $\pm$ 92	110 $\pm$ 27*	41 $\pm$ 17*	15.2 $\pm$ 4.2*
Infectious diseases (31)	4.3 $\pm$ 2.6	71 $\pm$ 33	65 $\pm$ 28*	424 $\pm$ 155*	147 $\pm$ 31*	58 $\pm$ 11*	11.6 $\pm$ 3.4
Healthy controls (32)	4.4 $\pm$ 2.4	20 $\pm$ 7	22 $\pm$ 8	378 $\pm$ 163	74 $\pm$ 23	28 $\pm$ 16	11.8 $\pm$ 4.1
F	1.108	21.828	27.441	50.921	16.063	12.362	5.012
P	0.355	0.001	<0.001	<0.001	<0.001	<0.001	<0.001

* P < 0.05 or 0.001 vs. healthy controls**TABLE 3. ASL, ALT, and AST levels in various liver diseases (mean $\pm$ SD)***

Group (n)	ASL (U/L)	ALT (U/L)	AST (U/L)
Acute hepatitis (31)	109.4 $\pm$ 34.3 (52.6–161.8, 108.2)	513 $\pm$ 222 (196–916, 493)	407 $\pm$ 160 (139–653, 403)
Chronic hepatitis (165)	81.9 $\pm$ 37.4 (8.3–158.5, 85.8)	83 $\pm$ 74 (26–372, 51)	107 $\pm$ 130 (20–621, 47)
Cirrhosis (67)	82.4 $\pm$ 26.6 (43.6–156.9, 75.1)	81 $\pm$ 7 (69–96, 79)	85 $\pm$ 8 (72–102, 83)
Liver cancer (28)	114.9 $\pm$ 32.2 (60.2–171.6, 114.4)	52 $\pm$ 8 (38–66, 54)	63 $\pm$ 10 (43–81, 61)
Healthy controls (32)	4.4 $\pm$ 2.4 (0.0–8.2, 4.3)	20 $\pm$ 7 (6–31, 20)	22 $\pm$ 7 (7–38, 21)
F	57.328	185.986	67.995
P	0.000	0.000	0.000

*Range and median values (minimum–maximum, median) are in parentheses.

TABLE 4. Statistical differences among ASL, ALT, and AST levels in various liver diseases*

Group	Healthy control	Cirrhosis	Liver cancer	Chronic hepatitis	Acute hepatitis
Healthy control					
ASL		11.11, 0.000	13.08, 0.000	12.27, 0.000	12.75, 0.000
ALT		3.27, 0.001	1.43, 0.154	3.76, 0.000	22.63, 0.000
AST		2.77, 0.006	1.48, 0.140	4.16, 0.000	14.40, 0.000
Cirrhosis					
ASL	11.11, 0.000		4.43, 0.000	0.11, 0.912	3.80, 0.000
ALT	3.27, 0.001	–	1.48, 0.139	0.157, 0.875	23.02, 0.000
AST	2.77, 0.006		0.95, 0.344	1.43, 0.155	13.96, 0.000
Liver cancer					
ASL	13.08, 0.000	4.43, 0.000		4.96, 0.000	0.66, 0.509
ALT	1.43, 0.154	1.48, 0.139	–	1.74, 0.082	20.45, 0.000
AST	1.48, 0.140	0.95, 0.344		2.05, 0.041	12.45, 0.000
Chronic hepatitis					
ASL	12.27, 0.000	0.11, 0.912	4.96, 0.000		4.30, 0.000
ALT	3.76, 0.000	0.157, 0.875	1.74, 0.082	–	25.42, 0.000
AST	4.16, 0.000	1.43, 0.155	2.05, 0.041		14.44, 0.000
Acute hepatitis					
ASL	12.75, 0.000	3.80, 0.000	0.66, 0.509	4.30, 0.000	
ALT	22.63, 0.000	23.02, 0.000	20.45, 0.000	25.42, 0.000	–
AST	14.40, 0.000	13.96, 0.000	12.45, 0.000	14.44, 0.000	

*Values given are q , P , throughout.

JCLA07-073 Figure1 final size: 20 picas x 19.5 picas --76% of original

Fig. 1. ROC curves of ASL, ALT, AST, and ALT+AST on diagnosis of liver diseases. Sensitivity and specificity are displayed at various discrimination levels for serum concentrations of ASL, ALT, AST, and ALT+AST. Data were obtained from a total of 570 subjects, including 291 patients with various liver diseases, 247 patients with nonliver disease, and 32 healthy controls.

diagnose liver diseases were 100% and 91.1%, respectively. However, in contrast, sensitivity of ALT in assessment of liver diseases was 97.6%, with a low specificity 24.7%, and sensitivity of AST was 83.8%, also with a low specificity 28.3% (both at cutoff value of 40 U/L). Combining ALT and AST, although sensitivity was 99.3%, a much lower specificity of 16.6% (cutoff = 40 U/L) was obtained. The total diagnostic efficiency on liver diseases indicated by area under the ROC curve (AUC) was approximately 1.00 for ASL, which was much higher than those of ALT (AUC = 0.723), AST (AUC = 0.648), and ALT+AST (0.693). The other markers, such as GGT, LDH, and ALP, had much poorer diagnostic values than those of ASL, ALT, and AST (data not shown). The above results suggested that serum ASL level was of better specificity, sensitivity, and total diagnostic efficiency than that of ALT, AST, ALT+AST, and other traditional markers for the diagnosis of liver diseases.

Correlation Between Serum ASL Levels and Changes of Liver Histopathology

Liver biopsy were performed in 31 patients with hepatopathy (13 liver cirrhosis, 11 primary hepatocellular carcinoma, five chronic type B hepatitis, and 2 chronic type C hepatitis). Scores of histopathological inflammation grading (SHIG) were calculated by the semiquantitative evaluation criteria of the Hepatic

Fibrosis Study Group of the Chinese Liver Diseases Association in 2002. The SHIG calculation formula was $SHIG = P + L + 2(PN + BN)$, where P is periportal inflammation, L is lobule inflammation, PN is patch necrosis, and BN is bridging necrosis. Each index of these may be recorded as 0, 1, 2, 3, or 4 score by the degree of inflammation. A positive correlation ($r = 0.417$, $P = 0.019$) was observed between serum ASL levels (86.9 ± 26.5) and the SHIG (9.83 ± 3.36).

DISCUSSION

Up to now, ALT and AST have been most extensively used as routine enzymatic markers of hepatocellular injury in clinical practice (1,3). Both markers had a high sensitivity on diagnosis of liver diseases, which was approved in our study again; the sensitivities of ALT and AST were 97.6% and 83.8%, respectively. On the other hand, it has been well known that there is considerable overlap of serum ALT and AST levels between liver diseases except acute hepatitis and healthy controls. In addition, although ALT is mainly found in liver, and AST is mainly found in heart, both aminotransferases were widely distributed in various tissues and/or organs throughout the body. Kidney, skeletal muscle, lung, and pancreas, etc., are rich in ALT and/or AST. Therefore, their specificity on diagnosis of liver disease is unsatisfactory (1–4). In our research, it was revealed that ALT and AST were markedly increased in various nonliver diseases especially cardiac disease, tumor outside liver, connective tissue disease, respiratory disease, etc. ROC curve analysis showed that the specificity of ALT and AST for liver disease diagnosis was quite low, only 24.7% and 28.3%, respectively. Though sensitivity was increased, specificity was only 16.6% when ALT and AST were combined. Moreover, the sensitivity and specificity of other tested enzymatic markers, including GGT, ALP, and LDH, on diagnosis of liver diseases were even much poorer. Therefore, it is necessary to explore a specific and sensitive marker to diagnose liver disease (14).

Both ASL and aminotransferase are involved in the metabolism of protein in the body (17). As mentioned above, transamination reaction is produced in many tissues or organs owing to the extensive distribution of aminotransferases (1,3,6,7). In contrast, the formation of urea occurs primarily in the liver, with few amounts of urea formation occurring in the kidney and brain. For this reason, the synthesis of urea would be profoundly influenced by liver function damage. In theory, low urea level in blood could be used to indicate the injury of liver function (18). However, there are a lot of factors, such as the function of renal glomerular filtration, the reabsorption function of distal renal

tubule, the perfusion of the renal blood, the amount of protein intake, the protein metabolism condition, the dehydration, the administration of glucocorticoid drugs, and so on (19,20), that would affect urea synthesis and excretion and consequently influence the urea level in blood. Therefore, it is unsuitable to use low serum urea concentration as the marker of liver injury in clinical practice. But, as the specific and key enzyme of urea production in the ornithine cycle in the liver, to test serum ASL activity as a liver injury marker is theoretically reasonable.

ASL is present in cytoplasm, which catalyzes the reversible reaction of argininosuccinate to arginine and fumarate. Besides being primarily distributed in liver, ASL also was found in intestine and kidney in small amounts, and some activity in other tissues, such as heart, lung, and spleen (8). However, it is interesting that serum ASL levels were not significantly increased in diseases primarily affecting those organs other than liver in previous reports (9) and present research. One possible explanation is that ASL activity is very low in these tissues or organs, so that it cannot be determined while released from these damaged tissues or organs. Another reason is that ASL existing in these tissues or organs mainly tends to act as a synthesizing enzyme to catalyze condensing arginine with fumaric acid to form argininosuccinic acid. It has been reported that kidney ASL not only plays as a role of condensing arginine with fumaric acid to form argininosuccinic acid, but also catalyzes canavanine and fumaric acid to form canavaninosuccinic acid so that their molecular construction may be different (21,22). Therefore, while we control assay conditions to ensure the reaction catalyzed by ASL to directly cleave argininosuccinate to form arginine and fumarate happened in the hepatocytes, the determined serum ASL activity is mainly from injured hepatic parenchymal cells. Of course, whether there are some ASL isoenzymes in different tissues or organs is worthy of further investigation.

Present research demonstrated that only ASL was not significantly changed in all observed markers in sera of nonliver disease patients. Serum ASL levels were significantly increased in patients with various liver diseases, and the range of ASL levels had completely no overlap as comparing to the healthy control. The ROC curve analysis also displayed that the sensitivity, specificity, and total diagnostic efficiency indicated with AUC of ASL were significantly much better than those of AST, ALT, GGT, ALP, and LDH for the diagnosis of liver diseases. The results of liver biopsy examination also suggested that the serum ASL level was positively related to the severity of liver histopathology.

Some scholars have explored the diagnostic ability of serum ASL activity assay for liver diseases and found it

was a good laboratory diagnostic marker of hepatopathy (8,9). But further research was not done due to the technological complexity of applied methods so that it could not be routinely used in clinical laboratories (9,10,14). Therefore, we have established a continuous monitoring method of serum ASL assay with an automatic biochemical analyzer in the present research.

When interference was defined as a change of more than 3% from the theoretical value (23), no interference could be detected from bilirubin < 342 $\mu\text{mol/L}$ or hemoglobin < 0.06 g/L. It has been reported that ASL level in erythrocytes is 100–200 times higher than in serum (16). Since the red blood cells comprise approximately 40% of blood, complete hemolysis would result in a serum ASL value increased approximately 40–80 times. Hemolysis is visible when serum contains 0.2 g/L hemoglobin. Our interference study revealed that ASL level increased only approximately 10% (i.e., < 1 U/L) at this condition for healthy individuals. Therefore, serum ASL activity could not be affected by erythrocyte ASL until serum showed visible hemolysis.

Methodological evaluation has demonstrated that this method could meet the requirements of clinical sample test. Compared with other published procedures (8,9,15,16), the present ASL assay method is practically available and could be used in the common clinical laboratory. The initial clinical applications have shown that serum ASL activity is a very specific and sensitive marker to diagnose various hepatopathies. Using ROC curve analysis, it has been demonstrated that the specificity, sensitivity, and total diagnostic efficiency of ASL on liver diseases are much superior to the traditional enzyme markers such as ALT, ALP, LDH, and GGT. Through further investigation, serum ASL assay will hopefully become a routine laboratory diagnostic item of liver injury.

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