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# The serum Arginase-1 correlation to child-pugh scores in predicting the severity of cirrhosis



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## ABSTRACT

**Background:** The Child-Pugh score is an internationally accepted system for grading the severity of chronic liver disease such as cirrhosis. It assesses the severity of the liver disease to avoid subjectivity and inconsistency. However, some non-invasive serological markers may also be useful to evaluate the stage of liver fibrosis in determining the severity of cirrhosis. Some of the known markers are Arginase-1 (ARG-1), IL-13, fibronectin (FN), tissue inhibitor of metalloproteinases-1 (TIMP-1), and metalloproteinases-1 (MMP-1).

**Objective:** To evaluate the correlation between the circulating levels of ARG-1, IL-13, FN, TIMP-1, MMP-1 with the score Child-Pugh in cirrhosis patients.

**Materials and Methods:** We conducted a cross-sectional study enrolling fifty-six patients of the chronic liver with Child-Pugh A, B, and C. The score was calculated based on the value of serum bilirubin,

serum albumin, internationalized normal ratio (INR), and the severity of ascites and hepatic encephalopathy. Serum ARG-1, IL-13, FN, TIMP-1, MMP-1 were analyzed using an enzyme-linked immune sorbent assay. The differences were analyzed using Mann-Whitney test, Spearman's correlation (univariate analysis), and multivariate analysis by the multi-regression test.

**Results:** There was a significant correlation of ARG-1 serum with Child-Pugh scores ( $r = -0.589$ ,  $p < 0.05$ ). However, we did not find a significant correlation between the Child-Pugh scores and the IL-13 ( $r = 0.238$ ,  $p = 0.078$ ), FN ( $r = -0.151$ ,  $p = 0.265$ ), TIMP-1 ( $r = -0.158$ ,  $p = 0.244$ ), and MMP-1 ( $r = -0.006$ ,  $p = 0.967$ ) with the Child-Pugh scores. Conclusion: The serum marker of ARG-1 was found correlated with the severity of the chronic liver disease.

**Keywords:** Serum ARG-1, IL-13, FN, TIMP-1, MMP-1, Child-Pugh Scores, Cirrhosis Patients

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## INTRODUCTION

Chronic liver diseases are major global health problems with approximately 800,000 deaths worldwide annually.<sup>1,2</sup> Chronic liver injury, irrespective of the cause, is associated with progressive liver fibrosis.<sup>3</sup> Liver fibrosis is caused by excessive deposition and abnormal distribution of extracellular matrix (ECM) components.<sup>3</sup> Progression of fibrosis eventually leads to end-stage cirrhosis.<sup>3</sup> Some inflammatory mediators have been shown to promote the progression of the chronic liver disease.<sup>4,5</sup> Many of the inflammatory mediators are either targets or activators of nuclear factor- $\kappa$ B (NF- $\kappa$ B). NF- $\kappa$ B is an important transcriptional regulator of the inflammatory response.<sup>6,7</sup> It plays an essential role in the regulation of inflammatory signaling pathways in the liver. Evidence showed that in every chronic liver disease, NF- $\kappa$ B is activated.<sup>8,9</sup> It regulates multiple essential functions in hepatocytes, Kupffer cells and hepatic stellate cells (HSCs). A disturbance in NF $\kappa$ B signaling components may cause liver spontaneous injury, fibrosis and carcinogenesis.<sup>8</sup> It suggests that NF- $\kappa$ B importance in liver homeostasis and wound healing processes.<sup>8,10,11</sup>

Some studies had provided evidence that in chronic liver disease, inflammation actively

promotes the disease progression. A study explained that NF- $\kappa$ B in chronic liver disease determines the wound-healing responses and eventually determine the outcome whether the disease resolves or forming an organ fibrosis and or a hepatocellular carcinoma (HCC).<sup>12</sup> In HSCs and or hepatic myofibroblasts (HMF), the activation of NF- $\kappa$ B seems to promote hepatic fibrosis through various pathways. The increased activation of NF- $\kappa$ B in HSC/HMF may also be instrumental in establishing a tumor-friendly microenvironment and quantitative and qualitative changes of the ECM of a fibrotic liver.<sup>13</sup> However, there had not been any further investigation to establish a role of NF- $\kappa$ B in HSC or HMF in initiating or promoting tumor in a liver fibrosis.<sup>13</sup>

A histology examination of biopsied liver tissue is the gold standard to diagnose liver fibrosis. The liver fibrosis is graded using Knodel score. It categorizes the cirrhosis into 5 degrees: F0 for normal; F1 for portal fibrosis; F2 for a few fibrotic septa; F3 for numerous septa; and F4 for cirrhosis. However, most patients reject the procedure because it is painful, high cost, and may incur extra expenses for handling the complication.<sup>14,15</sup> Furthermore, the weaknesses of liver biopsy are the high liability

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**Table 1** The sample characteristics

Characteristics	f(%)	Mean $\pm$ SD	min-max
Gender			
Male	32 (57,1%)		
Female	24 (42,9%)		
Age (years)		55.2 $\pm$ 14.9	13-85
Bilirubin (mg/dL)		4.92 $\pm$ 6.58	
Albumin (g/L)		2.8461 $\pm$ .73121	
INR		1.27 $\pm$ .40	
ALT* (IU/L)		82.4643 $\pm$ 124.64	
AST** (IU/L)		113.79 $\pm$ 119.81	
Etiology			
HepB chronic infection	26 (45.8%)		
Biliary obstruction	9 (15.4%)		
Alcohol abuse	5 (8.7%)		
Autoimmune	1 (2.8%)		
Cryptogenic	15 (27.3%)		
Child-Pugh Scores			
A (5-6)	14 (25%)		
B (7-9)	26 (46,5%)		
C (10-15)	16 (28,5%)		

\*ALT = alanine aminotransferase, \*\*AST = aspartate aminotransferase.

**Table 2** The circulating markers based on Child-Pugh grades

Markers	Child-Pugh A	Child-Pugh B	Child-Pugh C
ARG-1 (ng/ $\mu$ L)	26.87 $\pm$ 6.95	27.01 $\pm$ 7.57	17.18 $\pm$ 2.91
IL-13 (pg/ $\mu$ L)	46.53 $\pm$ 20.26	71.39 $\pm$ 84.27	73.17 $\pm$ 42.04
FN (pg/ $\mu$ L)	7949.00 $\pm$ 2198.46	7949.00 $\pm$ 2397.60	7007.31 $\pm$ 2916.63
TIMP-1 (pg/ $\mu$ L)	302.77 $\pm$ 105.37	254.74 $\pm$ 84.72	247.79 $\pm$ 123.42
MMP-1 (pg/ $\mu$ L)	1322.22 $\pm$ 3068.30	229.97 $\pm$ 585.97	88.10 $\pm$ 55.15

**Table 3** The Spearman's test comparing the markers to the Child-Pugh grades

Serum Markers	Spearman's Correlation Index	p-value
ARG-1	r = -0.589	0.000
IL-13	r = 0.238	0.078
FN	r = -0.151	0.265
TIMP-1	r = -0.158	0.244
MMP-1	r = -0.006	0.967

to suffer an uncontrolled bleeding, the proneness to sampling errors, and the high inter- and intra-observer histopathological examination disagreement. Additionally, liver biopsy cannot be used to monitor the disease evolution or to evaluate effect of the therapy.<sup>16,17</sup>

The Child-Pugh score is simple and has a good predictive value for assessing the degree and

prognosis of liver injury, especially cirrhosis.<sup>18</sup> Included in the scoring system are serum protein and blood-clotting factors to evaluate a cirrhotic patients' liver reserve function through clinical parameters.<sup>18</sup> Nevertheless, Child-Pugh grades B and C cirrhotic patients with ascites or hemorrhagic tendency may be treated with albumin or blood transfusion.<sup>18</sup> The interventions may affect the real numerical value of the mentioned clinical parameters.<sup>18</sup>

The mechanism contributing to inflammation, liver injuries and fibrosis (cirrhosis) is not entirely known. Some literature support that the role of HSC is significant in the pathogenesis of liver fibrosis. The role of HSC also reciprocal with arginase, cytokine interleukin-13 (IL-13), ECM fibronectin (FN), and tissue inhibitor of metalloproteinases-1 (TIMP-1).<sup>19</sup> Single or combined (test of battery) indirect marker of Arginase-1 (ARG-1), IL-13, and the direct marker of FN, metalloproteinases-1 (MMP-1), TIMP-1, may be able to show the liver fibrosis severity. The markers may demonstrate a persistent necroinflammation, marker arginase, continual mobilization of T lymphocyte, the increase of IL-13, fibroblast activation, FN deposition and the change in the balance of fibrogenesis and fibrinolysis with the increase of TIMP due to the increase of expression of mRNA and gene of TIMP-1.<sup>20,21</sup>

The improvement of circulating marker is necessary to track the severity of the cirrhosis. A valid, non-invasive and accurate, direct and indirect circulating marker should be able to describe the immune process of chronic liver inflammation, liver impairment, the activation of ECM accumulation, and liver metabolism in the patients with progression of liver cirrhosis

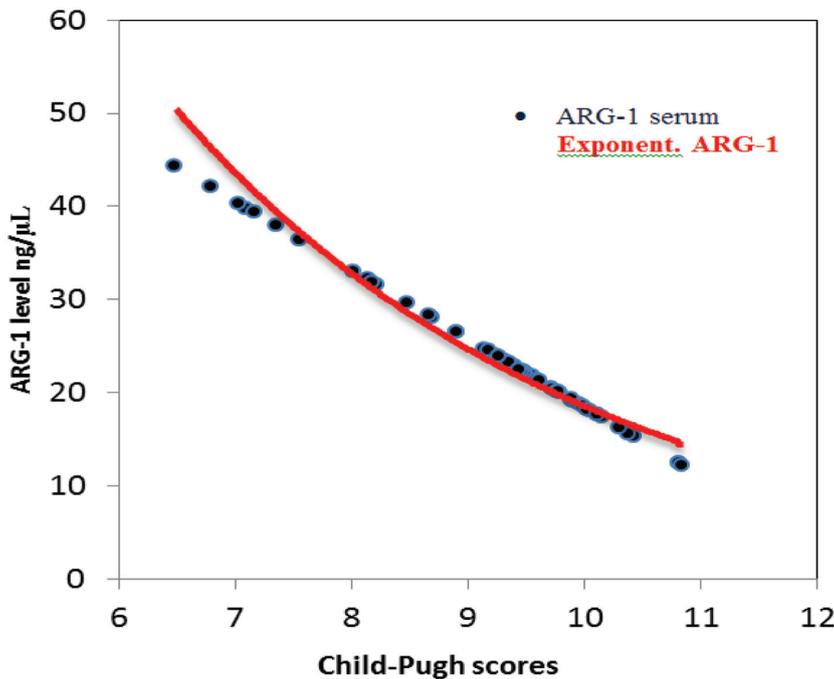
Our study aimed to evaluate whether in cirrhotic patients the ARG-1, IL-13, FN, TIMP-1, MMP-1 circulating markers may assess the dynamic process of liver cirrhosis severity progression. We also evaluate the correlation between the markers with Child-Pugh scores.

## MATERIALS AND METHODS

Our study is a cross-sectional study using a consecutive sampling method. We recruit our sample from the Internal Medicine ward and the Gastro-Hepatology Outpatient Clinic Dr. Sardjito Hospital. The blood samples drawn were examined in the Clinical Laboratory Department of Dr. Sardjito, Hospital Yogyakarta. The ethical clearance was obtained from the Medical and Health Research Ethics Committee of the Faculty of Medicine, Gadjah Mada University.

**Table 4** Circulating markers of cirrhosis severity based on the Child-Pugh score

Variables	Multivariate analysis Beta (standardized coefficients)	p-value
ARG-1	-.477	.000
IL-13	.016	.110
TIMP-1	-.200	.899

**Figure 1** The correlation line shows an inverse relationship between the circulating ARG-1 and the Child-Pugh scores ( $p < 0.05$ ).

The blood samples were collected with minimal venostasis. The serum was obtained by centrifugation within one hour of sampling. The serum was stored in a minus 80°C freezer. The quantitative method of an enzyme-linked immunoassay (Qusabio, USA) was done to obtain the ARG-1 level. An enzyme-linked immunoassay (Booster, USA) was used to obtain the level of FN, IL-13, TIMP-1, and MMP-1. Liver function tests (LFTs) including serum albumin and prothrombin time were executed.

The inclusion criteria were patients diagnosed with liver cirrhosis based on ultrasound result by clinicians who hospitalized in Internal Medicine ward or visited Gastro-Hepatology outpatient clinic of Dr. Sardjito Hospital in January to December 2015, adults, and gave informed consent. The exclusion criteria were patients who had been drinking alcohol in the last two weeks or complicated by other diseases such as diabetes mellitus (DM), chronic kidney disease (CKD), gastrointestinal bleeding or

blood transfusion in the last two weeks before their blood was drawn for the research purposes.

The samples were classified into three groups based on their Child-Pugh grades. Grade A when the score is 5 to 6, B 7 to 9, and C 10 to 15. The Child-Pugh scoring gives 1 point if the total serum bilirubin < 2, serum albumin > 35, internationalized normal ratio (INR) < 1.7, no ascites, no hepatic encephalopathy. It gives 2 points if the serum total bilirubin 2-3, serum albumin 28-35, INR 1.71-2.20, the presence of ascites is suppressed with medication, hepatic encephalopathy grade I-II or suppressed with medication. It gives 3 points if the serum total bilirubin > 3, serum albumin < 28, INR > 2.20, the ascites refractory, hepatic encephalopathy grade III-IV or refractory. The greater the Child-Pugh score showed the more severe condition the liver cirrhosis is.

The statistical analysis was done using the SPSS software version 15.0 for Windows. We provided the descriptive analysis. Mann-Whitney test was used to compare the markers. The Spearman test was applied to examine the correlation of the markers with the Child-Pugh scores. Indicators of cirrhosis severity and the Child-Pugh scores were analyzed using multiple regression models. The tests were two-tailed and  $\alpha = 0.05$ .

## RESULTS

Our sample of 56 patients consisted of 32 males and 24 females. The characteristics are shown in Table 1. There were 14 patients (25%) with Child-Pugh grade A, 26 (46.5%) with grade B, and 16 (28.5%) with grade C.

The cirrhosis patients were grouped into A, B and C based on their Child-Pugh grades, see Table 2. IL-13, FN, TIMP-1, and MMP-1 showed no significant correlation to the Child-Pugh scores, as shown in Table 3. In contrast, Arginase-1 has a significant negative correlation with Child-Pugh scores ( $r = -0.589$ ,  $p < 0.05$ ). It means the greater the Child-Pugh score, the lower the ARG-1 serum level as shown in Figure 1.

For predicting the severity of cirrhosis liver using the regression analysis, ARG-1 was a significant factor (Table 4). In the multivariate analysis, ARG-1 was a predictable prognostic factor for the severity of Child-Pugh group in cirrhosis patients.

## DISCUSSION

ARG-1 is the core enzyme of the urea cycle which occurs in the hepatocyte and periportal hepatocyte. The arginases catalyze the divalent

cation-dependent hydrolysis of L-arginine to form urea and the nonprotein amino acid L-ornithine.<sup>22</sup> In the liver, this reaction constitutes the final step in urea biogenesis.<sup>22</sup> Nitric oxide synthase (NOS) utilizes L-arginine to produce nitric oxide (NO). NO has a major role in the circulation. It decreases vascular smooth muscle's tone, inhibits platelet and leukocyte activation, halts smooth muscle cell proliferation and ECM deposition.<sup>22</sup> Additionally, it reduces endothelial cell death.<sup>22</sup> An increased enzymatic activity of ARG-1 may deplete endothelial L-arginine.<sup>22,23</sup> Therefore, the NO production is hindered which leads to endothelial dysfunction.<sup>22,23</sup>

Some nonhepatic tissues where complete urea cycle was impeded showed arginase activity. ARG-1 participates in the ornithine biosynthesis main compound for proline (collagen), glutamate (glutamine) and polyamine (for regulating the cell proliferation and regeneration). It also competes with NOS for the general substrate, and arginine. Arginase might be the key point in the development of any pathological processes because it is involved in so many essential biochemical pathways. Thus, it is possible that ARG-1 facilitates fibrosis development.<sup>23</sup>

Our study showed a decrease of arginase activity in cirrhosis was associated with the declining cirrhotic liver function. In the clinically declining liver function in cirrhosis, the arginase activity may be related to hepatocytes loss and the simultaneous fibrous tissues development. In our study, the ARG-1 level declined with the increasing severity of the liver fibrosis based on Child-Pugh grades, as supported by Chrzanowska et al.<sup>23</sup> The decrease of ARG-1 may impair the urea cycle in the periportal zone.<sup>23</sup> Detecting the level of serum ARG-1 may be useful to assess the severity of cirrhosis. Indeed, the combination ARG-1 and the Child-Pugh score may provide more accuracy in evaluating the liver reserve function of cirrhosis patients to decide the robust timing for surgery. However, a more elaborate study with a greater number of sample is needed to assess the validity of serum ARG-1 to evaluate the severity of cirrhosis against the Child-Pugh scoring system.

## CONCLUSION

We found a significant negative correlation between the serum level of ARG-1 against the severity of cirrhosis based on Child-Pugh scores.

## DISCLOSURE STATEMENT

The author declares to have no conflict of interest.

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