

# Low Testosterone Levels Predict Increasing Severity and Worse Outcomes of Hepatitis B Virus-related Acute-on-chronic Liver Failure in Males

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## Research

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# Abstract

## Background

Hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) is more prevalent among males than females and has a high rate of short-term mortality, and low serum testosterone has been prevalent in critical illness such as acute respiratory failure and cirrhosis, we investigated the association between testosterone level and severity of hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) in males remains unknown.

## Methods

This single-center observational study involved patients with HBV-ACLF planned to undergo treatment with an artificial liver support system. Morning blood samples were collected and androgen levels analyzed by chemi-bioluminescent immunoassay. Time to death or liver transplantation within 90 days comprised the primary composite outcome. We assessed the relationships between androgen levels, stage (early, middle, or late, categorized according to clinical manifestation), severity scores, and clinical outcomes of HBV-ACLF.

## Results

Among 160 male subjects, 32 had early stage HBV-ACLF, 61 middle stage, and 67 end stage. Serum levels of total testosterone (TT), free testosterone index (FTI), dehydroepiandrosterone sulfate and cortisol were significantly lower among subjects than chronic hepatitis B patients and healthy controls, while androstenedione was higher. Low TT, SHBG, FTI and high androstenedione were associated with increased stage (of HBV-ACLF, ascites, and hepatic encephalopathy) and severity scores (Model for End-stage Liver Disease and Chinese Group on the Study of Severe Hepatitis B-ACLF scores). Low TT (<142.39 ng/dL) was a risk factor for both the composite outcome and for death alone within 90 days. Multivariate analysis revealed TT to be a predictor for the composite outcome independent of age, BMI, SHBG, FTI, cortisol, and androstenedione.

## Conclusion

Low serum testosterone is common among male patients with HBV-ACLF and predictive of increased severity and worse outcome of the disease.

## Background

Acute-on-chronic liver failure (ACLF) is a complex syndrome, characterized by acute and severe liver injury in the context of pre-existing chronic liver disease [1]. It has become a major issue in the field of hepatology due to its rising incidence and ultimate fatality [2]. Among Asian populations, the major underlying disease for ACLF is hepatitis B virus (HBV) infection [3], which is responsible for an estimated 887,000 deaths worldwide in 2015 [4] and ranks seventh in the top causes of mortality worldwide [5].

Hepatitis B virus-related acute-on-chronic liver failure (HBV-ACLF) is associated with a high rate of short-term mortality, ranging from 58% to 74% [6], making this condition a heavy burden to health-care services. However, the 5-year survival rate of patients with HBV-ACLF has been reported to be up to 97.2% for those who survive beyond 90 days after diagnosis [7]. The Global Health Sector Strategy on Viral Hepatitis 2016–2021, approved by the World Health Organization [8], aims to reduce the rate of mortality of viral hepatitis by 65%; therefore, rapid and sensitive identification of critically ill patients with HBV-ACLF is of vital importance in order to facilitate effective multiorgan-supportive treatment and meet this target.

Recent epidemiological investigations and clinical studies [9-10] have revealed that HBV-ACLF affects males more frequently than females. The mechanism underlying this gender disparity may be due to the different sex hormones of males and females. The liver was recently demonstrated to be an androgen-sensitive organ because it expresses androgen receptors, and HBV has been suggested to be a sex-hormone-responsive virus [11]. Researchers have shown that androgen can increase HBV titer through stimulating the production of androgen response elements or via the positive feedback loop of the androgen receptor-androgen complex and HBV X protein [12-13]. Male patients with higher levels of androgen are prone to have higher HBV load than female patients. Furthermore, male patients are more likely to experience chronic conditions because the high viral load may contribute or lead to antigen-specific immune tolerance [14].

Testosterone, an important androgen in males, binds directly to the androgen receptor while androstenedione, dehydroepiandrosterone, and dehydroepiandrosterone sulfate do not bind until they are transformed to testosterone in tissues [15]. Testosterone plays a critical role in the metabolism of proteins, carbohydrates, and fat; therefore, deficiency can contribute to the development of functional disorders throughout the body [16]. Previous studies have reported the high incidence of testosterone deficiency among critically ill patients with condition such as myocardial infarction, acute respiratory failure, and esophageal adenocarcinoma [17-19]. Recent data have demonstrated that, among men with end-stage liver disease, low testosterone is associated with an increased risk of mortality regardless of the etiology of disease [20-22]. Xu et al. [23] found that serum testosterone was decreased in hepatitis B flare compared with in acute liver failure. However, there have been no clinical trials to date exploring the relationship between testosterone levels and the state of HBV-ACLF.

Considering the dynamic transformation between the bound and free forms of testosterone, interactions between steroid hormones and the potential influences of age, body mass index (BMI), and other clinical factors, we compared the circulating serum levels of total testosterone (TT), free testosterone index (FTI), sex hormone binding globulin (SHBG), dehydroepiandrosterone sulfate (DHEAS), cortisol and androstenedione (AND) in men with HBV-ACLF with those of age- and BMI-matched chronic hepatitis B patients or healthy controls without liver disease. Secondly, we evaluated whether testosterone is associated with the severity and outcome of HBV-ACLF in cases. We hypothesized that low testosterone is related to increased severity of disease and risk of death or liver transplantation among men with HBV-ACLF.

# Methods

## Study design and overview

This is a single-center, prospective observational study. The study and all its protocols were approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (approval number 2017[674]). Written informed consent was obtained from patients or their legal representatives prior to enrollment in the study.

## Subjects

We consecutively recruited 229 patients with HBV-ACLF who underwent treatment with an artificial liver support system at the First Affiliated Hospital of Zhejiang University College of Medicine during the 14-month period between December 2018 and January 2020 by invitation. Of these, 69 were excluded from analysis (30 who were female, 28 who had other chronic liver disease, five with hepatocellular carcinoma, four who had undergone previous liver transplant, one who had undergone stem-cell transplantation, and one with pituitary tumor). During the study period, we recruited 151 age- and BMI-matched males with chronic hepatitis B from sample bank of major diseases in Key Laboratory of Clinical In Vitro Diagnostic Techniques of Zhejiang Province as disease controls and 106 matched healthy controls without liver disease from the physical examination center.

## Clinical diagnosis

The diagnostic criteria for HBV-ACLF followed the current guidelines for the diagnosis and treatment of liver failure (2018 edition) recommended by the Liver Failure and Artificial Liver Group, Society of Infectious Diseases, Chinese Medical Association [24]: acute hepatic insult manifesting severe jaundice (total bilirubin [Tbil]  $\geq 171 \mu\text{mol/L}$ ) and coagulopathy (international normalized ratio [INR]  $\geq 1.5$ ) on the basis of chronic hepatitis B. And we categorized subjects according to stage of HBV-ACLF; namely, early stage ( $1.5 \leq \text{INR} < 1.9$  without complications or extrahepatic organ failure), middle stage ( $1.9 \leq \text{INR} < 2.6$  with one complication and/or one extrahepatic organ failure), or end stage ( $\text{INR} \geq 2.6$  with two or more complications and/or extrahepatic organ failures).

## Data collection

Clinical data was obtained from medical records relating to essential information (age, gender, weight, height), comorbidities (diabetes mellitus, hypertension), laboratory indexes (such as serum alanine aminotransferase [ALT], aspartate aminotransferase [AST], albumin, Tbil, INR, creatinine level, complete blood count), and complications (ascites, hepatic encephalopathy, infection, acute kidney injury, gastrointestinal hemorrhage). Severity scores (Model for End-stage Liver Disease [MELD] [25] and Chinese Group on the Study of Severe Hepatitis B-ACLF [COSSH-ACLF] [6] scores) were calculated using the most abnormal value for each organ system during hospitalization. The primary outcome, defined as death or transplantation within 90 days of first treatment of artificial liver support system, was analyzed through evaluation of medical records or by direct contact with the subjects or their families.

## Laboratory analysis

Morning fasting blood samples were drawn from all subjects prior to treatment with the artificial liver support system and from controls at the time of recruitment. We analyzed the androgens using chemioluminescent immunoassay. Serum TT, SHBG and DHEAS were measured on an Architect i4000 analyzer (Abbott Laboratories, Kallang Place, Singapore), cortisol was measured on an ADVIA Centaur XP (Siemens Healthcare Diagnostics Inc., Los Angeles, CA, USA) and androstenedione was measured on an Immulite 2000XPi (Siemens Healthcare Diagnostics Inc.). The FTI was calculated for each participant as  $(TT \times 10)/SHBG$  [26]. The normal ranges for TT, SHBG, FTI, DHEAS, cortisol and androstenedione in males are 142.39–923.14 ng/dL, 17.1–77.6 nmol/L, 20.4–81.2%, 48.6–591.9 µg/dL, 5.27–22.45 µg/dL and 0.6–3.1 ng/mL, respectively.

## Statistical analysis

All continuous variables are expressed as median, interquartile range (IQR; 25<sup>th</sup> and 75<sup>th</sup> percentiles), categorical data are presented as percentage and frequency. TT, FTI and SHBG levels were natural log-transformed and other androgen levels were square root-transformed. In univariate statistical comparisons, the Mann-Whitney non-parametric U test was used for continuous variables and the Kruskal-Wallis test to compare more than two groups. Categorical data were evaluated using a chi-squared test or Fisher's exact test, as appropriate. Generalized linear models were used to predict an increase in stage of HBV-ACLF, ascites, and hepatic encephalopathy with decreasing or increasing androgen level. The relationships between androgen level and severity scores were examined in subjects with HBV-ACLF through multiple linear regression analysis. Kaplan-Meier estimation was used to evaluate the survival rates without transplant of groups with different testosterone levels. The log-rank test was used to compare mortality rates in terms of the composite outcome and in terms of death alone between groups with different testosterone levels. When analyzing mortality rates, subjects who underwent liver transplant were excluded. With regards to HBV-ACLF prognosis, the Cox proportional hazards model was fitted with a forward stepwise selection method (p-in: 0.05 and p-out: 0.01) to identify risk factors associated with the composite outcome of death or transplantation. All statistical analyses were carried out using SPSS version 19.0 (SPSS, Inc., Chicago, IL, USA). A value of  $P < 0.05$  was considered statistically significant.

# Results

## Characteristics of the study population

A total of 160 males aged 45(36–56) years with HBV-ACLF who underwent treatment with an artificial liver support system were enrolled for analysis in the present study. Of these, 32 were categorized as having early stage liver failure, 61 as middle stage, and 67 as end stage. All 160 subjects completed follow-up. The in-hospital 90-day mortality rate was 34.4% (21 subjects underwent liver transplantation and 34 died).

Table 1 compares various characteristics between subjects, chronic hepatitis B patients and healthy controls, as well as in relation to stage of ACLF. Age, BMI, and incidence of basic diseases including diabetes and hypertension were not significantly different between groups. Laboratory data (except ALT and AST) were similar in chronic hepatitis B patients and healthy controls, while TT, FTI, DHEAS and cortisol levels were significantly lower in subjects than chronic hepatitis B patients and healthy controls, and androstenedione was higher. When HBV-ACLF patients with cirrhosis (82 patients) were excluded from subjects, the performance of androgens was consistent. According to the lower limit of the reference range of TT for males, 65% of subjects were TT deficient and 85.6% were FTI deficient. The rate of TT deficiency was highest among end-stage subjects (80.6%) while FTI deficiency was more common among middle-stage subjects (95.1%) compared with other stages. Increased stage of HBV-ACLF was associated with higher rates of ascites (0%, 77.0% and 95.5% in early-stage, middle-stage and end-stage, respectively;  $P < 0.001$ ), hepatic encephalopathy (0%, 8.2% and 44.8%;  $P < 0.001$ ), and infection (0%, 11.5% and 55.2%;  $P < 0.001$ ). The rates of acute kidney injury (5%) and gastrointestinal hemorrhage (1.9%) were very low in our cohort. The MELD and COSSH-ACLF scores and short-term mortality rate increased with increased stage of HBV-ACLF. Based on these findings, we examined the associations between serum testosterone and stage of HBV-ACLF; MELD and COSSH-ACLF scores; and outcomes to identify confounding factors.

### **Association of androgen levels with severity of hepatitis B virus-related acute-on-chronic liver failure**

Lower levels of TT, FTI, and SHBG and higher levels of androstenedione were associated with increased risk of advanced severity stage (Table 2). For every unit change in TT, FTI, SHBG and androstenedione, the risk of advanced HBV-ACLF stage increased by 2- to 8-fold after adjustment for age and BMI. Decreased testosterone level was found to be a predictor of increased severity of ascites and hepatic encephalopathy, but FTI did not predict hepatic encephalopathy. While the prediction of increased severity of ascites with DHEAS, cortisol and androstenedione levels were weak. In addition, linear correlations were identified between androgen levels and severity scores (MELD and COSSH-ACLF scores) after adjustment for age and BMI (Table 3). For instance, each Ln (1 ng/dL) decrease in TT was associated with a higher MELD score (2.794, 95% confidence interval [CI]: 1.740–3.848;  $P < 0.001$ ).

The clinical characteristics and outcomes of subgroups after stratification by TT are presented in Table 4. Low TT was found to be associated with advanced disease stage; MELD and COSSH-ACLF scores; levels of liver-function indicators, prevalence of ascites and hepatic encephalopathy, and in-hospital mortality. Infection exhibited a stepwise increase with increasing stage of HBV-ACLF but was not more frequent among subjects with low TT. There were no significant differences in age, BMI, or incidence of basic diseases (diabetes and hypertension) between subjects with high or low TT.

### **Association of androgen levels with the composite outcome of death or liver transplant**

After dividing patients into survivor and death/liver-transplant groups according to the 90-day outcome, we found TT, SHBG, FTI to be lower and cortisol, androstenedione higher in the death/liver-transplantation group (Figure 1). Cox proportional hazards analysis (Table 5) revealed low TT to be associated with an

approximate quadruple risk of the composite outcome (hazard ratio: 4.49, 95% CI: 2.12–9.53;  $P < 0.001$ ) compared with normal TT. There was no change after adjustment for age (Model 2) and BMI (Model 3). Model 3 was then adjusted for SHBG, free testosterone index, cortisol, and androstenedione (Model 4), which revealed that the significant association between TT and the composite outcome remained. The Model 4 showed that among the six androgens, only TT, SHBG and cortisol levels were independent factors predicting composite outcome rates. As Figure 2 illustrates, the rate of the composite outcome within 90 days was significantly higher among subjects with low TT ( $P < 0.001$  by log-rank test; Figure 2A) and low FTI group ( $FTI < 20.4\%$ ;  $P = 0.018$ ). Analyzing death alone, low TT was associated with a significantly higher risk of 90-day mortality than normal TT ( $P = 0.002$  by the log-rank test; Figure 2B), while no significant differences were seen according to FTI level ( $P = 0.132$ ).

## Discussion

The main findings of the present study are that men with HBV-ACLF have lower levels of TT, FTI, DHEAS and cortisol and higher levels of androstenedione compared with age- and BMI-matched healthy controls and those with chronic hepatitis B. Lower levels of TT, FTI, and SHBG and higher levels of androstenedione are strongly associated with higher stages of HBV-ACLF and increased clinical severity of HBV-ACLF according to complications (ascites and hepatic encephalopathy) and multiple severity scores (MELD and COSSH-ACLF scores). Low TT is associated with an increased risk of death or the need for liver transplantation independent of age; BMI; SHBG; FTI; cortisol and androstenedione, but the association between FTI and this risk is weaker.

This is, to the best of our knowledge, the first prospective study to survey the associations between low serum TT and disease stage, severity, and outcome in men with HBV-ACLF. Our results provide insight into the role of testosterone in end stage-liver disease. Our finding of low serum TT levels in patients with HBV-ACLF is not only in line with previous studies involving men with cirrhosis [20-22], but also true in HBV-ACLF patients without cirrhosis. When FTI is also considered, the prevalence of testosterone deficiency in HBV-ACLF seems to be more widespread. It is unlikely that our results represent an overestimation of this rate, because we collected blood samples in the morning, therefore controlling for circadian variations in testosterone levels, which are highest in the morning and lowest in the late afternoon [27]. Numerous cross-sectional and longitudinal studies have shown TT to decline with age and increasing BMI [28-29]; however, we found the association between TT and disease stage, severity, and outcome to remain significant even after adjustment for age and BMI. Our analysis of DHEAS, cortisol and androstenedione supplements the knowledge of those androgens in liver disease and is in support of the association above. To reduce any influence of treatment, we only recruited patients who were planned to undergo treatment with the artificial liver support system, which may contribute to reducing the mortality rate of ACLF [30]. Indeed, the short-term mortality rate among subjects of our study is lower compared with previous studies [6].

The two severity scores that we evaluated are both based on biochemical and clinical indicators and have been preliminarily validated for severity assessments of HBV-ACLF cohorts. The COSSH-ACLF system

was developed specifically for such cohorts and was found this to be one of the best systems for monitoring the progression of HBV-ACLF [6, 31]. Our results demonstrate that TT is strongly negatively correlated with the two severity scores, meaning that lower TT indicates more advanced HBV-ACLF. Furthermore, we found low TT to be associated with increased risk of advanced severity and poor outcome in patients with HBV-ACLF. High Tbil and low rates of kidney injury were observed among our subjects, meaning that it is difficult to distinguish patients at high risk of mortality from those with favorable prognoses using the MELD score. In contrast, TT is a straightforward and simple indicator which does not involve a narrow score range or complex calculation, enabling quantitative assessment of disease progression and consequent increased potentiality for clinical applicability.

The precise mechanism underlying the association between low TT and increased risk of death or liver transplant among patients with HBV-ACLF is unclear, and there are various biological actions which may be involved. Patients with low testosterone levels have been reported to mount a more efficient, intense, and prolonged immune response [32] which may contribute to the severity of symptoms of HBV-ACLF. In the present study, the pathophysiology of ACLF relating to excessive systemic inflammation [33] presented as an increased rate and severity of ascites and hepatic encephalopathy, which was also observed among patients with low TT and could be predicted by decreased testosterone level instead of other androgens. Furthermore, the low-TT group of the present study exhibited elevated levels of C-reactive protein, a classic inflammatory indicator, in the absence of infection. It may be that the low testosterone levels we observed among subjects could contribute to the severity symptoms by elevating the antiviral immune defense and inflammatory responses, leading to multiple organ injury [34]. In addition, a double-blind placebo-controlled trial [35] demonstrated testosterone deficiency in older men to be associated with decreased hemoglobin levels, which is supported by findings of the present study. The presence of anemia may contribute to the risk of poor outcomes; while testosterone treatment could correct anemia, this may or may not address the cause. Testosterone also has anabolic effects in muscle tissue, and deficiency is often accompanied by lack of exercise and poor nutrition, which is common among patients with HBV-ACLF and may contribute to poor outcomes [36].

Critical illnesses can cause decreased testosterone levels; therefore, HBV-ACLF itself may contribute to lower TT. Testosterone is produced in the testis and the adrenal gland, and the high incidence of adrenal failure in end-stage liver disease [37] may, at least in part, directly result in testosterone deficiency. In the present study, we observed low levels of DHEAS and cortisol, which are the most abundant and important hormone secreted by adrenal cortex and suggested the adrenal failure in HBV-ACLF. In contrast, SHBG—an important determinant of the distribution of circulating testosterone—is secreted by the liver [38-39]. Serious liver injury in the context of HBV-ACLF may cause a reduction in SHBG production with indirect consequences for testosterone levels. We did observe SHBG to decrease as HBV-ACLF progressed, and SHBG was increased among subjects compared with controls and chronic hepatitis B patients, possibly because of SHBG release from the storage pool due to barrier impairment during severe liver injury. Furthermore, new evidence indicates that lipopolysaccharide, which promotes the development of ACLF [40], may initiate inflammation and thus reduce testosterone production in men [41]. Taken together, these

studies clearly demonstrate that low testosterone level is a marker of more advanced disease, although the causality in the case of HBV-ACLF remains unclear.

If low testosterone does contribute to poor outcomes of HBV-ACLF, our findings may support the application of testosterone therapy for men with end-stage liver disease who have low testosterone levels; however, careful selection of patients is required because the risks and benefits of testosterone treatment have not yet been assessed. Immunoregulatory effects make testosterone a double-edged sword; recent studies—both human trials and on animal models—have demonstrated testosterone to have immunosuppressive properties [42] and there is increasing evidence indicating the important role of testosterone in the development of HBV-related hepatocellular carcinoma through down-regulation of the systemic immune response [43-44].

This study has some limitations which should be acknowledged. First, it is a single-center study which analyzed data of 160 subjects, with 55 cases of the composite outcome. Further studies involving larger cohorts recruited from multiple centers would provide information about the generalizability of these results to other populations. Second, we recruited male subjects, but changes in testosterone levels may also occur females and affect female patients with HBV-ACLF. However, due to the rarity of HBV-ACLF in women, studies involving such subjects will require longer-term or multi-center collaborations. Finally, our observational study cannot determine the causality between low testosterone and increased death or need for transplantation among patients with HBV-ACLF. Extended longitudinal studies and prospective interventional trials may help to elucidate the underlying mechanisms of this relationship.

In summary, we demonstrate the high prevalence of decreased serum testosterone levels among male patients with HBV-ACLF and show that low testosterone levels are independently associated with severity and outcomes of HBV-ACLF. Our results indicate low total testosterone level to be a marker of disease progression and poor prognosis in male patients with HBV-ACLF.

## Abbreviations

ACLF, acute-on-chronic liver failure; HBV, hepatitis B virus ; HBV-ACLF, Hepatitis B virus-related acute-on-chronic liver failure; BMI, body mass index; TT, total testosterone; FTI, free testosterone index; SHBG, sex hormone-binding globulin; DHEAS, dehydroepiandrosterone sulfate; AND, androstenedione; Tbil, total bilirubin; INR, International normalized ratio; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; MELD, Model for End-Stage liver disease; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-acute-on-chronic liver failure.

## Declarations

### Ethics approval and consent to participate

The study and all its protocols were approved by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine (approval number 2017[674]). Written

informed consent was obtained from patients or their legal representatives prior to enrollment in the study.

### **Consent for publication**

Not applicable.

### **Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that there are no conflicts of interest.

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### **Authors' contributions**

YC was principal investigators, designed and supervised the study. YH, DY, HZ and BL had roles in recruitment, data collection, and clinical management. YH, DY, RY, YY, MD, DY and FL did clinical laboratory testing and analysis. YH, DY and YC drafted the Article. All authors gave final approval and agree to be accountable for all aspects of the work.

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## Tables

### Table 1. Clinical characteristics of the study population

characteristic	Healthy Controls (n=106)	Chronic Hepatitis B(n=151)	HBV-ACLF (n=160)	HBV-ACLF (n=160)		
				Early stage (n=32)	Middle stage (n=61)	End stage (n=67)
Age (years)	43(35-52)	43(35-51)	45(36-56)	40(32-56)	49(39-57)	45(37-53)
BMI (kg/m <sup>2</sup> )	23.3(21.6-25.2)	23.4(21.8-26.6)	24.0(21.8-26.3)	24.0(21.3-26.5)	23.9(21.8-25.7)	24.2(21.6-26.4)
Diabetes,% (n)	7.5%(8)	7.3%(11)	8.1% (13)	3.1% (1)	11.5% (7)	7.5% (5)
Hypertension, % (n)	17.0% (18)	14.6%(22)	18.1% (29)	12.5% (4)	23.0% (14)	16.4% (11)
ALT (U/L)	15(12-21)	27(19-35) <sup>a</sup>	234(116-442) <sup>b</sup>	305(182-466)	158(101-274) <sup>c</sup>	269(112-587) <sup>d</sup>
AST (U/L)	19(16-22)	24(19-32) <sup>a</sup>	129(84-229) <sup>b</sup>	158(78-350)	110(75-147) <sup>c</sup>	156(92-280) <sup>d</sup>
Albumin (g/dL)	47(45-49)	48(46-51)	31(29-34) <sup>b</sup>	33(31-36)	31(29-33) <sup>c</sup>	31(28-34)
Tbil (μmol/L)	11(9-13)	13(10-18)	350(285-431) <sup>b</sup>	304(264-421)	369(290-420)	370(305-486)
Platelet count (10 <sup>9</sup> /L)	222(192-264)	193(161-230)	106(71-138) <sup>b</sup>	139(108-193)	100(73-127) <sup>c</sup>	98(61-128)
TT (ng/dL)	567(444-714)	573(444-743)	101 (61-202) <sup>b</sup>	291(129-481)	107(59-187) <sup>c</sup>	76(45-130)
SHBG(nmol/L)	33(27-47)	37(28-88)	40(28-55)	55(43-75)	47(31-57)	32(22-43) <sup>d</sup>
FTI(%)	57.3(46.3-68.3)	53(41-64)	9.7(6.1-15.5) <sup>b</sup>	17.6(9.8-25.8)	8.4(6.4-11.9) <sup>c</sup>	8.1(5.4-13.7)
DHEAS(μg/dL)	304(232-380)	286(183-364)	152(86-283) <sup>b</sup>	183(109-394)	106(48-200) <sup>c</sup>	194(97-292) <sup>d</sup>
Cortisol (μg/dL)	13.2(9.3-16.0)	11.7(8.9-15.7)	9.1(6.9-12.5) <sup>b</sup>	9.4(7.5-14.8)	8.8(6.5-11.6)	11.1(5.5-15.0)
Androstenedione (ng/mL)	1.7(1.3-2.3)	1.8(1.4-2.1)	3.5(2.3-4.7) <sup>b</sup>	3.5(2.9-4.8)	2.8(1.6-3.7)	4.2(2.6-5.6) <sup>d</sup>

Data are expressed as median (interquartile range; 25th and 75th percentiles) or percentage (frequency). *P* value < 0.05 for comparisons between chronic hepatitis B and healthy controls<sup>a</sup>, HBV-ACLF and chronic hepatitis B<sup>b</sup>, middle stage and early stage<sup>c</sup>, and between end stage and middle stage<sup>d</sup>.

HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Tbil, total bilirubin; TT, total testosterone; SHBG, sex-hormone-binding globulin; FTI, free testosterone index; DHEAS, dehydroepiandrosterone sulfate; AND, androstenedione.

**Table 2. Prediction of increased stage of HBV-ACLF, ascites, and hepatic encephalopathy with decreased androgen levels**

	HBV-ACLF		Ascites		Hepatic encephalopathy	
	odds ratio (95% CI)	<i>P</i> value	odds ratio (95% CI)	<i>P</i> value	odds ratio (95% CI)	<i>P</i> value
1 - Ln(TT [ng/dL])	3.507(2.285-5.383)	<0.001	2.962(1.957-4.482)	<0.001	3.052(1.690-5.513)	<0.001
1 - Ln(SHBG [nmol/L])	8.757(4.046-18.951)	<0.001	4.293(2.118-8.701)	<0.001	17.010(5.361-53.975)	<0.001
1 - Ln(FTI [%])	2.376(1.469-3.841)	<0.001	2.374(1.472-3.829)	<0.001	1.453(0.783-2.696)	0.236
SQRT(DHEAS [µg/dL])	1.042(0.981-1.106)	0.186	0.971(0.915-1.031)	0.341	1.172(1.079-1.273)	<0.001
SQRT(cortisol [µg/dL])	1.077(0.832-1.393)	0.573	1.028(0.804-1.313)	0.827	1.248(0.924-1.686)	0.148
SQRT(AND [ng/mL])	2.089(1.139-3.830)	0.017	1.390(0.773-2.501)	0.272	3.296(1.473-7.375)	0.004

Model was adjusted for age and body mass index. HBV-ACLF, hepatitis B virus-related acute-on-chronic liver failure; TT, total testosterone; SHBG, sex-hormone-binding globulin; FTI, free testosterone index; SQRT, Square Root; DHEAS, dehydroepiandrosterone sulfate ; AND, androstenedione.

Subjects were categorized according to stage of hepatitis B virus-related acute-on-chronic liver failure (early stage, middle stage, or end stage), ascites (none, mild ascites, or severe ascites), and hepatic encephalopathy (none, grade I/II, or grade III/IV).

**Table 3. Relationships between androgen level and severity scores in hepatitis B virus-related acute-on-chronic liver failure**

	Estimate (95% CI)	<i>P</i> value
<b>MELD</b>		
1 - Ln(total testosterone [ng/dL])	2.794(1.740-3.848)	<0.001
1 - Ln(sex-hormone-binding globulin [nmol/L])	4.621(2.727-6.515)	<0.001
1 - Ln <sub>7</sub> (free testosterone index [%])	2.107(0.703-3.512)	0.004
SQRT(dehydroepiandrosterone sulfate [μg/dL])	0.324(0.145-0.503)	<0.001
SQRT(cortisol [μg/dL])	1.717(0.975-2.459)	<0.001
SQRT(androstenedione [ng/mL])	4.982(3.304-6.660)	<0.001
<b>COSSH-ACLF</b>		
1 - Ln(total testosterone [ng/dL])	0.527(0.346-0.708)	<0.001
1 - Ln(sex-hormone-binding globulin [nmol/L])	1.033(0.720-1.347)	<0.001
1 - Ln <sub>7</sub> (free testosterone index [%])	0.318(0.071-0.565)	0.012
SQRT(dehydroepiandrosterone sulfate [μg/dL])	0.048(0.034-0.062)	<0.001
SQRT(cortisol [μg/dL])	0.234(0.103-0.364)	0.001
SQRT(androstenedione [ng/mL])	0.790(0.492-1.089)	<0.001

Adjusted for age and body mass index. MELD, Model for End-stage Liver Disease; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-acute-on-chronic liver failure; SQRT, Square Root.

**Table 4. Clinical characteristics in relation to total testosterone level**

	TT<142.39 ng/dL (n = 104)	TT≥142.39 ng/dL (n = 56)	P Values
<b>Stage of acute-on-chronic liver failure, % (n)</b>			
Early stage	9.6%(10)	39.3%(22)	<0.001
Middle stage	38.5%(40)	37.5%(21)	<0.001
End stage	51.9%(54)	23.2%(13)	<0.001
<b>Complications, % (n)</b>			
Ascites	80.8%(84)	48.2%(27)	<0.001
Mild Ascites	49%(51)	41.1%(23)	<0.001
Severe Ascites	31.7%(33)	7.1%(4)	<0.001
Hepatic encephalopathy	31.7%(33)	3.6%(2)	<0.001
grade I or II	12.5%(13)	1.8%(1)	<0.001
grade III or IV	19.2%(20)	1.8%(1)	<0.001
Infection	26.9%(28)	28.6%(16)	0.824
Acute kidney injury	7.7%(8)	0%(0)	0.051
Gastrointestinal hemorrhage	2.9%(3)	0%(0)	0.552
<b>Laboratory data</b>			
Albumin (g/dL)	31(28-34)	32(31-34)	0.01
Total bilirubin (µmol/L)	378(301-468)	309(256-398)	<0.001
Total cholesterol(mmol/L)	2.0(1.6-2.3)	2.5(1.9-4.9)	<0.001
Hemoglobin(g/L)	123(111-133)	128(119-135)	0.03
Platelet count (10 <sup>9</sup> /L)	100(67-126)	120(91-180)	<0.001
C-reactive protein(mg/L)	12.3(8.2-20.1)	9.6(6.0-16.2)	<0.001
Total testosterone (ng/dL)	73(47-97)	277(193-375)	<0.001
Sex-hormone-binding globulin (nmol/L)	32(25-46)	54(44-72)	<0.001
Free testosterone index (%)	7.0(5.3-9.7)	18.0(12.5-24.1)	<0.001
Dehydroepiandrosterone sulfate (µg/dL)	160(88-310)	127(70-218)	0.06
Cortisol (µg/dL)	9.2(7.1-13.2)	8.9(5.7-12.2)	0.44

Androstenedione (ng/mL)	3.35(2.23-4.97)	3.60(2.32-4.47)	0.69
<b>Severity score</b>			
MELD	33(29-35)	29(26-31)	<0.001
COSSH-ACLF	6.7(6.2-7.6)	5.8(5.5-6.3)	<0.001
<b>Mortality, % (n)</b>			
90 days	45.2%(47)	14.3%(8)	<0.001

Data are expressed as median (interquartile range; 25th and 75th percentiles) or percentage (frequency).

MELD, Model for End-stage Liver Disease; COSSH-ACLF, Chinese Group on the Study of Severe Hepatitis B-acute-on-chronic liver failure.

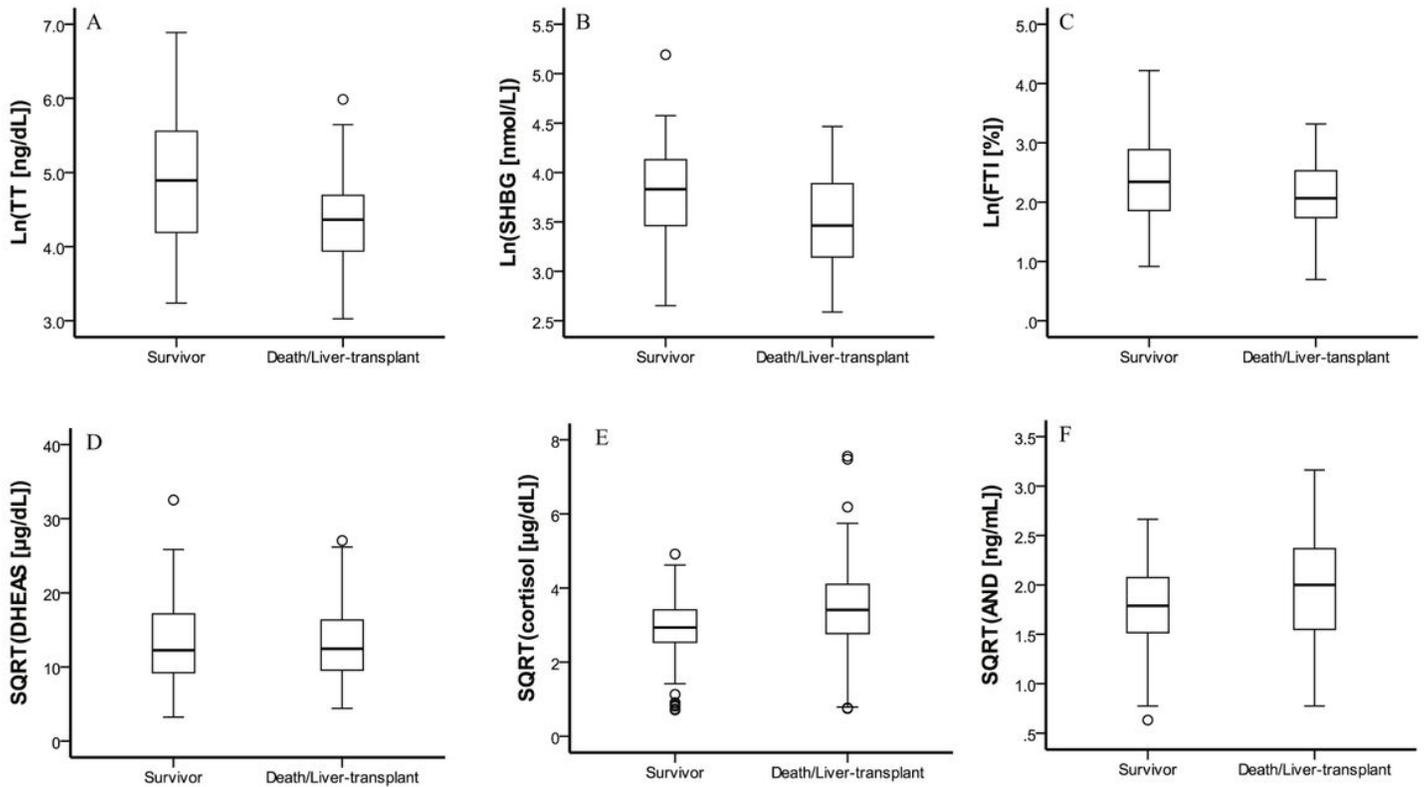
The mortality refers to rate of composite outcome defined as death or transplantation.

**Table 5. Results of Cox proportional hazards estimate of the composite outcome of death or liver transplant**

Variables	Hazard ratio (95% CI)	<i>P</i> value
<b>Model 1</b>		
TT(TT<142.39 ng/dL vs TT $\geq$ 142.39 ng/dL)	4.49(2.12-9.53)	<0.001
<b>Model 2</b>		
TT(TT<142.39 ng/dL vs TT $\geq$ 142.39 ng/dL)	4.49(2.12-9.53)	<0.001
Age (years)		0.218
<b>Model 3</b>		
TT(TT<142.39 ng/dL vs TT $\geq$ 142.39 ng/dL)	4.49(2.12-9.53)	<0.001
Age (years)		0.218
Body mass index (kg/m <sup>2</sup> )		0.896
<b>Model 4</b>		
TT(TT<142.39 ng/dL vs TT $\geq$ 142.39 ng/dL)	3.02(1.33-6.86)	0.008
Age (years)		0.445
Body mass index (kg/m <sup>2</sup> )		0.511
1 - Ln(sex-hormone-binding globulin [nmol/L])	2.04(1.08-3.87)	0.028
1 - Ln(free testosterone index [%])		0.508
SQRT(dehydroepiandrosterone sulfate [ $\mu$ g/dL])		0.570
SQRT(cortisol [ $\mu$ g/dL])	1.75(1.32-2.31)	<0.001
SQRT(androstenedione [ng/mL])		0.353

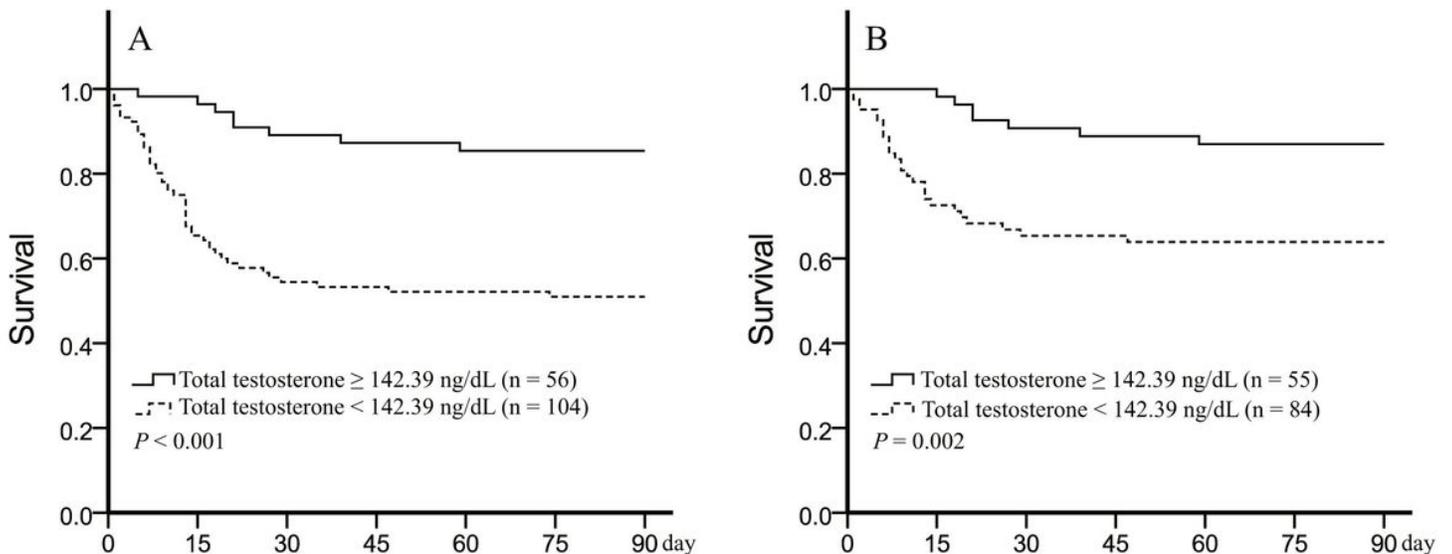
Cox proportional hazards regression models for total testosterone (TT, Model 1) and models additionally adjusted for age; body mass index; sex-hormone-binding globulin; free testosterone index; cortisol; and androstenedione. Hazard ratios are given with 95% confidence intervals within parentheses. The null hypotheses for all proportional hazard assumptions tests were not rejected for total testosterone in any model. Twenty-three patients received liver transplants and 32 died within 90 days.

## Figures



**Figure 1**

Box plot illustrating androgens in survivor and patients who experienced the composite outcome of death or transplant. The composite outcome (n=55) was associated with lower total testosterone ( $P < 0.001$ , A), sex-hormone-binding globulin ( $P < 0.001$ , B), free testosterone index ( $P = 0.032$ , C) and higher cortisol ( $P = 0.007$ , E), androstenedione ( $P = 0.038$ , F) compared with those survived without transplant (n=105). The dehydroepiandrosterone sulfate level was similar among those two groups ( $P = 0.686$ , D). TT, total testosterone; SHBG, sex-hormone-binding globulin; FTI, free testosterone index; DHEAS, dehydroepiandrosterone sulfate; AND, androstenedione; SQRT, Square Root.



## Figure 2

Kaplan-Meier survival curves for cases of hepatitis B virus-related acute-on-chronic liver failure (A) Transplant-free survival without composite endpoint was lower among men with total testosterone levels below the cutoff of 142.39 ng/dL than among those with total testosterone levels above the cutoff value within 90 days. (B) Transplant-free survival without death was lower among men with total testosterone levels below the cutoff of 142.39 ng/dL than among those with total testosterone levels above the cutoff value within 90 days.