Low testosterone is a better predictor of mortality than sarcopenia in men with advanced liver disease

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Abbreviations: MELD (Model for End-stage Liver Disease), L4 (4th lumbar vertebra), CT (Computerised Tomography), HU (Houndsfield Unit), SHBG (Sex Hormone Binding Globulin), IQR (interquartile range)

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Introduction: Both sarcopenia and low serum testosterone have been associated with increased mortality in men with cirrhosis. It is not known how these variables interact.

Methods: We conducted a retrospective longitudinal cohort study of 145 men referred for liver transplant evaluation between 2005 and 2012. Baseline demographics included hormone profile and MELD score. Baseline computerised tomography was reformatted to calculate skeletal muscle area at L4 using validated, Tomovision software-based methodology. The primary outcome was time to death or liver transplantation.

Results: Median testosterone was low at 6.2nmol/L (ref 10–27.6nmol/L) as was muscle mass at 48.0cm^2/m^2 (ref >52.4cm^2/m^2). Muscle mass correlated with both serum testosterone (tau=0.132, p=0.019) and MELD score (tau=-0.155, p=0.007). In separate multivariable models, both sarcopenia (HR 1.05, p=0.04) and low testosterone (HR 1.08, p=0.01) were significantly associated with mortality independent of MELD score. When the variables MELD score, muscle area and testosterone were entered into a single model, low testosterone but not sarcopenia remained significantly predictive of mortality (HR 1.07, p=0.02; HR 1.04, p=0.09 respectively).

Conclusion: Low testosterone and sarcopenia are both associated with increased mortality in men with advanced liver disease and may identify patients at high risk of mortality that would be missed by the MELD score alone. Low testosterone appears to be a better predictor of mortality than sarcopenia and is a simpler test to improve the prognostic value of the MELD score. Interventional trials are required to determine whether low testosterone and sarcopenia are markers or mediators of mortality in this population.

Keywords: cirrhosis; sarcopenia; testosterone; muscle mass; mortality

Introduction

In patients with end-stage liver disease, loss of muscle mass (sarcopenia) is very common and clearly affects quality of life. It is only in recent years however that sarcopenia has been identified as an independent risk factor for mortality in advanced liver disease [1]. The increase in mortality that was observed in association with sarcopenia has been attributed to a greater number of sepsis-related deaths, and appears to be most apparent in men as opposed to women [2].

Recent data also demonstrates that low testosterone is a risk factor for mortality in cirrhosis, independent of the MELD score [3]. Low testosterone is widely reported in men with cirrhosis, and falls in parallel with increasing liver disease severity [4, 5]. Testosterone is closely linked with muscle mass in men but also modulates bone mineral density, haemoglobin production, insulin resistance and immunity, all of which are commonly impaired in cirrhosis [6-8].

Given the anabolic actions of testosterone, we hypothesised that testosterone deficiency contributes to sarcopenia in men with cirrhosis. Thus the apparent link between sarcopenia and mortality in this population might be partially due to the direct effects of testosterone on muscle. We also hypothesised that low testosterone may be more strongly associated with mortality in cirrhosis than sarcopenia due to the broad range of beneficial effects of testosterone on androgen receptors elsewhere in the body.

We therefore performed a retrospective longitudinal cohort study in a pre-liver transplant setting to assess the effect of sarcopenia, testosterone deficiency and other variables on outcome.

Methods

A retrospective observational study was conducted at the Victorian Liver Transplant Unit by interrogating the existing database to retrieve records of all men referred for transplant evaluation for advanced liver disease from 2005 to 2012. Patients were excluded if transplant referral was for hepatocellular carcinoma without cirrhosis or for other non-cirrhotic conditions. Routine evaluation of the 145 included men includes full biochemistry which allows calculation of the MELD score, and androgen studies. Quadruple phase Computerised Tomography (CT) is performed to assess vasculature and liver parenchyma.

Tomovision SliceOmatic software (version 5.0, Toronto, Canada) was used to reformat transverse CT scans at the L4 region into fat, muscle and bone planes using Houndsfield unit (HU) thresholds. The threshold of 0 to +100 HUs for identifying skeletal muscle was the most accurate and reproducible using this software version, which allowed for calculation of skeletal muscle area as previously described [9]. Muscle area was adjusted for patient height as previously described in other cohorts, with a cut-off of 52.4cm^2/m^2 used to diagnose sarcopenia [2, 9]. This height-adjusted skeletal muscle area is expressed as cm^2/m^2 and for the purpose of this paper will be referred to as muscle mass. This CT technique can differentiate muscle from water, and thus is a more accurate means of quantifying muscle mass than basic body anthropometry or DEXA body composition scanning which can be confounded by ascites [10].

Muscles included at the L4 plane include the psoas, quadratas lumborum, paraspinals, internal and external obliques, rectus abdominus and transverse abdominus. The L4 level was selected as it has been shown to correlate with total skeletal muscle area in previous analyses [11]. All CTs were analysed by a single trained observer. Intra-observer variation in our cohort was less than 2% on multiple testings.

Pre-transplant biochemistry was measured using standard methodologies at the Biochemistry Department, Austin Health. This included serum creatinine, international normalised ratio (INR) and bilirubin to allow calculation of the MELD score, 25-hydroxyvitamin D3 (vitamin D), serum albumin and serum sodium.

Serum total testosterone was measured using the Access testosterone assay (Beckman Coulter, Inc, Fullerton, CA, USA) with a minimum detection limit of 0.35 nmol/L. The intra- and inter-assay coefficients of variation (CVs) assessed for two different concentrations (4.7 nmol/L and 26.0 nmol/L) were 3.9%, 4.8% and 5.7%, 5.0% respectively. The reference range for this total testosterone assay was 10-27.6 nmol/L, based on gas chromatography/ mass spectrometry (GC/MS) measurements obtained from a reference panel of 124 healthy, reproductively normal young men, with <8 nmol/L set as the unequivocal cut-off for testosterone deficiency [12].

Sex hormone binding globulin (SHBG) levels were determined with the Immulite 2000 analyser (Diagnostics Products Corporation, Los Angeles, CA, USA). The reference range for SHBG was 13-71 nmol/L, and the minimum detection limit was 0.02 nmol/L. Free testosterone were calculated from total testosterone, SHBG and serum albumin based on mass action laws with Vermeulen's formula [13]. The reference range for the Access-Testosterone/Immulite SHBG combination for free testosterone was 230-610 pmol/L (Vermeulen).

Date of death or liver transplantation is automatically recorded in the transplant database employed by the Victorian liver transplant unit and this data was directly retrieved. Patients who remained alive without transplantation had the date of their most recent review in the outpatient transplant service recorded as the end of their follow-up period.

This research was approved by the Human Research Ethics Committee, Research Ethics Unit, Austin Health.

Statistical Analysis

Descriptive statistics included median and interquartile ranges (IQR, 25th and 75th percentiles). Differences between two groups were analysed by means of non-parametric Wilcoxon rank sum test or chi-squared test in case of frequencies. Demographic tables were considered explanatory and not corrected for multiple testing. Correlations were based on Kendall's tau rank correlation.

Survival analysis was used to follow up patients until they had died, received a transplant, or their observation period had ended. Patients still alive were censored at the time their status has last been confirmed. Patients who were transplanted were censored at the time of transplant. Serum testosterone and muscle mass were analysed as categorical variables, for Kaplan Meier estimates, and continuous variables for uni- and multi-variable Cox regression analysis.

Kaplan Meier plots were employed to create survival curves. We show difference in survival up to a restricted time period of two years. This time period was chosen due to the chance of sarcopenia and low testosterone being reversible over a longer time.

Analysis of predictive factors for mortality was conducted on all patients who died or were transplanted within a 2 year follow-up period and relied on Cox proportional hazards model. The assumption of proportional hazards was verified by means of Schoenfeld's test and plot. Statistical differences in frequencies were compared by log rank test. For predictors of interest, such as testosterone and muscle mass, crude and adjusted hazard ratios are reported to ascertain their significance independently of other predictors including age and MELD score. Competing models were compared by analysis of variance (ANOVA). Sensitivity and

specificity of mortality prediction were analysed by receiver operating characteristics curves (ROC) derived by logistic regression including patients who have died in the first year or were still alive without receiving a transplant. Paired ROC curves were statistically compared using Venkatraman's test. $P < 0.05$ conferred significance for all tests. The statistical software packages R 3.1.1 for Mac and Deducer 0.7-7 were used for the analyses [14, 15].

Results

Baseline demographics

Complete liver transplant evaluation was conducted for 145 men with cirrhosis and decompensated liver disease during the study period. Baseline demographics are described in Table 1. The median age was 54 years [interquartile range, 47; 59]. The median MELD score was 17 [14; 23], the majority of patients had Child Pugh C cirrhosis (71.7%) with smaller numbers of Child Pugh B and A (21.4% and 6.9% respectively).

The most common underlying aetiology of liver disease was Hepatitis C virus, responsible for 43% of cases (with co-ingestion of alcohol recorded in one third of these cases), with 18% attributable to alcohol alone, 10% to PSC, 8% to Hepatitis B virus, 7% to non-alcoholic steatohepatitis and 14% to other aetiologies. 33 patients (23%) patients also had hepatocellular carcinoma; all falling within transplant criteria.

Median testosterone was low at 6.2 nmol/L [2.9; 10.4] (reference interval 10-27.6 nmol/L) as was the median free testosterone at 75.5 pmol/L [41; 140] (reference interval 230-610 pmol/L). Using the previously described cut-off for height-adjusted muscle mass of 52.4 cm^2/m^2, the prevalence of sarcopenia was 70.3% [2]. The median muscle mass was 48.0 cm^2/m^2 [43.8; 54.1].

The median follow-up duration was 8.3 months [4.08, 14.16] with median time to transplant of 7.2 months [3.96, 12.84] and time to death of 7.44 months [3.48, 14.16]. Of the 145 men undergoing transplantation evaluation, 56 died, 79 were transplanted and 10 remain alive.

Relationship between serum T, muscle mass and MELD score

Muscle mass was positively associated with serum total testosterone levels (tau 0.13, p = 0.02). Muscle mass also negatively correlated with MELD score (tau -0.16, p=0.007) and positively correlated with serum sodium (tau 0.18, p = 0.002). Serum testosterone was also negatively correlated with MELD score (tau -0.20, p < 0.001). There was no significant correlation between muscle mass and age, vitamin D, albumin or Child Pugh score. Figure 1 demonstrates the correlation between serum testosterone and muscle mass as well as MELD score and muscle mass.

Predictors of Mortality

Comparing patients whose serum testosterone was below the cut-off for testosterone deficiency of 8nmol/L with those with serum testosterone above 8nmol/L, patients with low testosterone had significantly higher mortality over 2 years (p=0.019). Similarly, a marked survival disparity was seen for men who met the diagnostic criteria for sarcopenia (muscle mass < 52.4cm2/m2) as compared to those who did not (p=0.028). 2 year survival curves are demonstrated in Figure 2.

When restricting this analysis to subjects with a low MELD score, at a fixed MELD score of 16 or lower, patients with serum testosterone <8nmol/L were significantly more likely to die than those with normal testosterone (p=0.018). At a MELD score of 16 or lower, patients classified as being sarcopenic had a higher mortality than those who were not (p=0.021). These survival curves are depicted in Figure 3.

On uni-variate analysis both for unrestricted observation time (data not shown) and confined to 2 year follow-up, low total testosterone (HR 1.09 per unit decrease, p = 0.006), low muscle mass (HR 1.06, p = 0.009), SHBG (HR 1.009, p = 0.047) and low serum Na (HR 1.08, p =

0.02) were each associated with a shorter time to death. This means that for each reduction in total testosterone by 1 nmol/L, mortality was increased by 9%. A higher MELD score was also associated with a shorter time to death (HR 1.09, $p < 0.001$). This means that for each increase in MELD score by 1 point, mortality was increased by 9%. Results of uni-variate analyses are depicted in Table 2. Of note, free testosterone was not significantly ($p = 0.1$) associated with mortality.

Multivariable analysis

Several models were created to assess the competing effect of individual variables that demonstrated significant associations on uni-variate analysis. Irrespective of significance, age was incorporated into all multi-variable models due to its established impact on both serum testosterone and muscle mass [16]. Serum sodium, SHBG and Vitamin D did not maintain significance on multi-variable analysis and did not improve the strength of the model and thus these models were discarded. It is for this reason that MELD and not MELD-Na was used in further modelling. Given that free testosterone was not significant, total testosterone was used in modelling.

Low serum testosterone (HR 1.08, $p = 0.01$) remained a significant predictor of mortality independent of the MELD score. Low muscle mass (HR 1.05, $p = 0.04$) was also predictive of mortality independent of the MELD score.

To compare the predictive values of these two variables, testosterone and muscle mass were analysed in a single model also incorporating age and the MELD score. In this model, low serum testosterone remained significantly associated with mortality (HR 1.07, $p = 0.02$) but muscle mass failed to reach statistical significance (HR 1.04, $p = 0.09$).

To further compare testosterone and muscle mass, serum testosterone was added to a model including MELD, age and muscle mass which resulted in a significantly improved model,

compared to the 3 variable model (ANOVA, p = 0.01). This suggests that testosterone contributes additional predictive information to that contained in the MELD score and muscle mass. Conversely, incorporating muscle mass to a model of MELD, age and testosterone did not result in a significantly improved predictive model (ANOVA, p = 0.08). This suggests that in conjunction with MELD, testosterone may be a better predictor of mortality than muscle mass.

The progressive improvement in the sensitivity and specificity of adding additional predictors of mortality to the MELD score is demonstrated in Figure 4. These receiver operating characteristics curves demonstrated an improved area-under-the-curve (AUC) with the subsequent addition of serum testosterone and then muscle mass (AUC 0.64, 0.71 and 0.73 respectively, p=0.05). As previously reported, MELD score was associated with increased mortality. When combined with both testosterone and muscle mass, the hazard ratio for mortality was 1.077 (p = 0.001), which translates to a 7.7% increase in mortality with each increase in point of the MELD score. In a sensitivity analysis, we conducted a 12 month survival analysis. With a smaller number of events (39/125) in this period the strength of the analysis was reduced, but the influence of relevant predictors was confirmed. Including age and MELD in a Cox model, low testosterone (HR 1.11, p = 0.004) was again more influential than low muscle mass (HR 1.04, p = 0.12), although sarcopenia was still significantly associated with 12 month mortality on uni-variate analysis (HR1.05, p = 0.04).

Discussion

We have demonstrated that both low testosterone and sarcopenia are predictors of death in men with advanced liver disease, and each is independent of the MELD score. This is similar to previous work demonstrating that both of these factors are linked to mortality [2, 3]. For the first time, we have shown that low testosterone appears to be a better predictor than sarcopenia, in particular looking at early mortality within 12 months. In addition, there was a link between low testosterone and sarcopenia suggesting that low testosterone contributes in part to the muscle loss seen in cirrhosis.

The clinical value of this study lies in the identification of a new objective biochemical marker than can predict prognosis in patients with advanced liver disease referred for liver transplantation. Not only does serum testosterone appear to be a better predictor of mortality than sarcopenia, it is also a much simpler test to perform, requiring only a blood sample as opposed to a CT scan and subsequent computer software reformatting. Furthermore, adding muscle mass to a multivariable model of MELD, age and testosterone did not improve that model. Low testosterone may be particularly helpful in identifying patients at high risk of mortality who would not have been identified by the MELD score alone, thus improving clinical decision making. This is demonstrated by Figure 3a, which shows significantly higher mortality in patients with a MELD score of 16 or less who had low serum testosterone.

There are two potential explanations for our results. The first is that low testosterone and sarcopenia have no direct causative role and both simply reflect the severity of liver disease. Gonadal axis suppression is common not only in chronic liver disease but also in other chronic disease [17]. Moreover, successful liver transplantation is associated with increased serum testosterone, suggesting that the gonadal axis suppression is functional and driven by poor health [5]. Likewise, sarcopenia is a multifactorial condition that is common in any chronic disease state [18, 19].

The second hypothesis is that low testosterone and sarcopenia actually contribute directly to increased mortality in men with advanced liver disease. If this is the case, our data suggest that the adverse effect of low testosterone is partly mediated by its negative impact on muscle mass but also contributes additional risk. We know that testosterone deficiency can reduce haematopoiesis, increase insulin resistance and inappropriately activate the immune system [6, 8, 20]. Anaemia has been linked with mortality in other populations and is frequently observed in cirrhosis [21]. Insulin resistance is common in cirrhosis and has been associated with progression of fibrosis and hepatocellular carcinoma development [22, 23]. Elevated inflammatory mediators and immune dysregulation in cirrhosis is multifactorial but low testosterone may play a role. The major limitation of our study is its retrospective and observational nature and thus inability to prove causality or discern which hypothesis may be correct. However, the study incorporates data from a large cohort of men with advanced liver disease and has identified important associations that could form the basis for future interventional studies. If hypothesis 2 is indeed correct, it raises the possibility that testosterone therapy in advanced liver disease may have a beneficial effect on both muscle mass and survival. This hypothesis needs to be explored in future randomised controlled trials of interventions to modify testosterone and/or muscle mass.

Previous studies have assessed the effect of testosterone therapy in cirrhosis, but poor design, short duration and suboptimal drug delivery mean that they do not provide an answer as to whether or not testosterone therapy is beneficial. An early trial of intramuscular testosterone therapy in cirrhosis did demonstrate a survival benefit as compared to placebo [24], and subsequent trials demonstrated increased albumin, increased muscle strength and less gynecomastia [25-27]. A Cochrane review of 5 trials concluded testosterone did not confer survival benefit in liver disease, but all included alcoholics with high rates of cessation during the study period, only 2 trials included cirrhotics, and patients were not required to be

hypogonadal at trial entry. No trials have evaluated body composition parameters. Thus further trials in hypogonadal cirrhotics are required.

The high short-term mortality rate of our study makes it difficult to exclude that unmeasured acute illness was responsible for both sarcopenia and reduced testosterone levels. However the reality is that this reflects the poor prognosis of patients with advanced liver disease and thus extended longitudinal studies are not feasible. In addition, regardless of any impact of acute illness on testosterone levels or sarcopenia, both remain significant predictors of mortality and such prognostic information is therefore useful in this clinical context. We recognise that co-linearity between testosterone, MELD score and muscle mass can cause statistical problems when parameters compete against each other in the same model. However it is important to note that although a significant correlation exists, testosterone explains for less than half of the variation in muscle area, and the findings were confirmed by model comparison. The predictive value of testosterone also retained significance independent of the MELD score in each model.

The generalisability of our data to other transplant cohorts is also limited by the fact that we included all patients who were referred for consideration of transplantation, many of whom were later deemed unsuitable and thus were never actually waitlisted for transplant. This may explain why our mortality rate was higher than in previous series, at 37%. Also, our numbers are modest at 145 (though we did have a relatively strong event rate) and analysing a larger cohort would be of benefit. In addition, our results are only applicable to men. We do however note that the association between sarcopenia and mortality in patients with cirrhosis appears to be greatest in men [2]. Interestingly, only total and not free testosterone was a significant predictor of mortality, which differs from previous data in cirrhosis. However formulas to calculate free testosterone have not been validated in patients with abnormal SHBG such as ours (median SHBG 78nmol/L), and the form of testosterone that is

bioactive remains controversial [28, 29]. Thus we do not believe that the absence of a strong relationship with free testosterone detracts from the predictive value of total testosterone in this cohort.

This paper represents the first investigation into the predictive value of both testosterone and muscle mass in men with cirrhosis and is the first to examine the relationship between the two variables. It raises the novel concept that low testosterone may in fact be more useful than sarcopenia in predicting mortality. In this cohort muscle mass contributed minimally to the predictive value of a model incorporating MELD and serum testosterone and this requires validation in future studies. These two variables are potentially modifiable, and improvement in muscle mass in cirrhosis has already been shown to improve survival [30]. Testosterone therapy could theoretically improve survival by modifying other factors in addition to its anabolic properties.

Conclusion

Both low testosterone and sarcopenia add additional predictive value to the MELD score in assessing risk for death in advanced liver disease and thus may identify patients at high risk for mortality that may be missed by using the MELD score alone. Low testosterone appears to be a better short-term predictor of mortality than sarcopenia, which may be due to activation of androgen receptors elsewhere in the body. Serum testosterone is also a much more practical test to perform which increases its potential for clinical applicability. It is as yet unclear whether or not low testosterone and sarcopenia are markers or modulators of disease. Prospective interventional trials are required to investigate the possibility that they mediate disease and directly influence mortality.

Figure 1a. Relationship between muscle mass and serum testosterone

*The regression line is indicative only, correlations were assessed by Kendall's tau rank correlation, as reported in the text.

Figure 1b. Relationship between muscle mass and MELD score

*The regression line is indicative only, correlations were assessed by Kendall's tau rank correlation, as reported in the text.

Figure 2a. Men with low serum testosterone have increased mortality compared with men with normal testosterone levels

Figure 2b. Men with sarcopenia have increased mortality compared with men with normal muscle mass.

Figure 3a. Survival with a MELD score of 16 or less is significantly reduced if serum testosterone is <8nmol/L

Figure 3b. Survival with a MELD score of 16 or less is significantly reduced if muscle mass is in the sarcopenic range

Figure 4. Receiver operating characteristics curves at 1 year for MELD score with the progressive addition of serum testosterone and muscle mass demonstrates improvement in the AUC with each additional factor

* p=0.05

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Table 1. Baseline Demographics (n=145)

Variable	Median [IQR]
Time to OLTx	0.60 [0.33;1.07] years
Time to death	0.62 [0.29;1.16] years
Observation period	0.69 [0.34;1.18] years
Age	54.0 [47.0;59.0] years
BMI	25.7 [23.3;30.4] kg/m^2
Height	1.75 [1.70;1.80] m
Muscle mass	48.0 [43.8;54.1] cm^2/m^2
Child Pugh Score	11.0 [9.00;12.0]
MELD score	17.0 [14.0;23.0]
Na	134 [130;137] mmol/L
T	6.20 [2.90;10.4] nmol/L
SHBG	78.0 [54.0;104] nmol/L
Free T	75.5 [41.0;140] pmol/L
Albumin	28.0 [24.0;33.0] g/L
Creatinine	87.0 [70.0;113] micromol/L
Vitamin D	51.0 [37.5;80.5] nmol/L

Table 2. Uni-variate analysis in a Cox model for the outcome of time to death*

Variable	HR	95% CI	p value
MELD	1.090	1.043; 1.138	<0.001
Total Testosterone	0.921	0.867; 0.978	0.006
Muscle mass (cm^2/m^2)	0.946	0.907; 0.986	0.009
Sodium	0.926	0.8675; 0.988	0.020
Vitamin D	0.990	0.981; 0.999	0.031
Muscle area (cm^2)	0.986	0.973; 0.999	0.037
SHBG	0.991	0.982; 0.999	0.048
Free Testosterone	0.996	0.991; 1.001	0.107
Creatinine	1.003	0.998; 1.008	0.187
Child Pugh Score	1.077	0.951; 1.203	0.238
Body mass Index	0.966	0.911; 1.025	0.258
Albumin	1.025	0.976; 1.077	0.316
Age	1.011	0.979; 1.045	0.509

*In the Cox model, follow-up was confined to a period of 2 years from transplant evaluation as deaths after this point were deemed to be unlikely to be related to baseline variables

Table 3a. Multivariable Cox model: MELD, Age, Testosterone

	HR	95% CI	p value
MELD	1.083	1.04; 1.13	<0.001
Age	1.007	0.98; 1.04	0.659
T	0.925	0.87; 0.98	0.012

Table 3b. Multivariable Cox model: MELD, Age, Muscle mass

	HR	95% CI	p value
MELD	1.078	1.032; 1.127	<0.001
Age	1.002	0.971, 1.033	0.912
Muscle mass	0.956	0.915; 0.999	0.042

Table 3c. Multivariable Cox model: MELD, Age, Testosterone, Muscle mass

	HR	95% CI	p value
MELD	1.077	1.030; 1.126	0.001
Age	1.006	0.975; 1.038	0.714
T	0.930	0.875; 0.989	0.021
Muscle mass	0.964	0.924; 1.006	0.090

Appendix 1. Multivariable Cox model: MELD: SHBG, Na, Vit D, Muscle mass

	HR	95% CI	p value
MELD	1.083	1.036; 1.132	<0.001
SHBG	0.994	0.985; 1.004	0.239
Na	0.947	0.884; 1.015	0.121
Vitamin D	0.994	0.987; 1.001	0.083
Muscle mass	0.957	0.917; 0.999	0.044

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