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Association between homocysteine level and length of stay in patients with lower extremity atherosclerotic disease: a retrospective cohort study

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ABSTRACT

Objectives Homocysteine (Hcy) level has been widely identified as a risk factor associated with adverse outcomes in patients with lower extremity atherosclerotic disease (LEAD). However, there are still some knowledge gaps in research on the association between Hcy level and downstream adverse outcomes, such as length of stay (LOS). This study aims to explore whether and to what extent Hcy level is associated with LOS in patients with LEAD.

Design Retrospective cohort study.

Setting China.

Participants, primary and secondary outcomes We conducted a retrospective cohort study of 748 patients from inpatients with LEAD between January 2014 and November 2021 at the First Hospital of China Medical University in China. We used a slew of generalised linear models to evaluate the association between Hcy level and LOS.

Results The patients' median age was 68 years and 631 (84.36%) were males. A dose–response curve with an inflection point at 22.63 µmol/L was observed between Hcy level and LOS after the adjustment of potential confounders. LOS increased before Hcy level reached the inflection point (β: 0.36; 95% CI: 0.18 to 0.55; p<0.001).

Conclusion

Our results show that an Hcy level <22.63 µmol/L is associated with increased LOS in patients with LEAD, which was independent of some other risk factors. This might shed light on how Hcy can be used as a key marker in the comprehensive management of patients with LEAD during hospitalisation.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study is the first to provide evidence of a non-linear association between homocysteine level and the length of stay in patients with lower extremity atherosclerotic disease.
⇒ Homocysteine level can be easily obtained from the hospital information system.
⇒ Homocysteine level may be used as a key indicator in the clinic.
⇒ This study was conducted at a single centre, which restricts the generalisability of our findings to other centres and nations.
⇒ The results could be susceptible to the impact of unmeasured variables within the data sources.

INTRODUCTION

Lower extremity atherosclerotic disease (LEAD), also known as the main manifestation of peripheral arterial disease (PAD), refers to partial or complete occlusion of the lower extremities due to atherosclerotic occlusion.[1] The latest epidemiological data showed that LEAD affected more than 230 million adults worldwide as of 2021 and is associated with an increased risk of adverse clinical outcomes (eg, necrosis or amputation).[2] Despite its prevalence and adverse clinical outcomes, LEAD remains understudied compared with other atherosclerotic diseases like myocardial infarction and stroke.[3]

Accumulating evidence suggests that high homocysteine (Hcy) levels are a risk factor for LEAD.[4 5] Hcy is a sulfur-containing amino acid that is produced during the metabolism of methionine.[6] Hcy increases the permeability of vascular arteries, damages the integrity of blood vessels and is a factor in the formation of atherosclerosis.[7] Additionally, Hcy levels are associated with vascular disease and can strongly predict clinical outcomes in patients with vascular disease.[8] Ranucci *et al*[9] found that preoperative Hcy level was independently related to increased morbidity and mortality, such as inpatients undergoing coronary artery bypass grafting. A study of 315 Japanese patients undergoing percutaneous coronary intervention reported that preoperative high Hcy levels were significantly associated with a greater risk of long-term mortality.[10] Yan *et al*[11] observed that patients with myocardial infarction diagnosed with both hypertension and hyperhomocysteinaemia tended to suffer major adverse cardiovascular events and that these accordingly prolong their length of stay (LOS) in coronary care units.

LOS is usually regarded as an indicator of the efficiency and effectiveness of hospital services.[12] It is well-established that shortening patients' LOS not only reduces health expenditure and increases patients' satisfaction but also reduces unnecessary bed occupancy and increases hospital productivity.[13] Various studies have shown that LOS is associated with several factors such as age, gender, hospitalisation history, severity of illness, complications, and so on.[14 15] Likewise, several studies have demonstrated that some biochemical indicators are correlated with LOS. A study found that perioperative serum calcium and phosphorus levels could be useful indicators for predicting LOS of patients who underwent major abdominal surgery.[16] Yoshimura *et al*[17] also revealed that a lower baseline haemoglobin level is negatively associated with LOS in hospitalised post-stroke patients with anaemia. In a recent retrospective study, Han *et al*[18] reported that Hcy level at admission affects LOS in patients with community-acquired pneumonia. However, although some studies demonstrated the association between Hcy level and diseases' severity and prognosis, to our knowledge, no previous research has investigated the possible association between Hcy level and LOS in patients with LEAD.

To clarify the association between Hcy level and clinical outcomes, we conducted a retrospective cohort study at the First Hospital of China Medical University. We included all inpatients with LEAD between January 2014 and November 2021 to systematically evaluate the association between Hcy level and LOS.

MATERIALS AND METHODS

Study population

The study included 748 inpatients with LEAD who were admitted to the Department of Vascular Thyroid Surgery between January 2014 and November 2021. Diagnostic criteria refer to the Chinese version of LEAD diagnostic criteria (2007 version).[19]

Data on demographics (gender, age), primary diagnosis, clinical characteristics (eg, medical history, admission/discharge time, symptoms and signs) and laboratory blood measures were recorded in the hospital's electronic information system. The primary diagnosis was coded according to the International Classification of Diseases-10th revision (ICD-10). We extracted hospitalisation data with ICD-10 codes (I70.203) for LEAD. Finally, medical records were reviewed by trained medical record abstractors.

This study was approved by the Ethics Committee of the First Hospital of China Medical University (ethics number: [2021]366). The requirement for informed consent was waived since patient information was anonymised and deidentified prior to our analysis. Our study was conducted in accordance with the WMA Declaration of Helsinki—Ethical Principles for Medical Research Involving Human Subjects.

Patient and public involvement

Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Exposure and outcome

The primary exposure of interest was Hcy level. All patients underwent routine analysis by intravenous sampling in the morning following an 8-hour fast the day of admission. Collected data included Hcy, Urea, C reactive protein (CRP), total protein (TP), low density lipoprotein cholesterol (LDL-C), high density lipoproteins cholesterol (HDL-C), fibrinogen (FIB), activated partial thromboplastin time (APTT), thrombin time (TT) and prothrombin (PT). Blood samples obtained for Hcy, FIB, APTT, TT and PT testing were collected in vacuum blood collection tubes. The blood samples for testing the remaining indicators were collected with citrate anticoagulant tubes. Blood samples were separated within 30 min of collection and immediately frozen at −80°C until the assays were performed. Hcy level was measured using an automated immunoassay (Architect i2000, Abbott, USA). All biochemical measurements were assayed by the Clinical Laboratory at the First Hospital of China Medical University (Shenyang, China).

The primary outcome of interest was LOS, which was treated as a continuous variable. LOS was calculated as the discharge date minus the admission date.

Covariates

Covariates of interest were identified according to the following strategies. An assessment of patients' smoking and drinking history, diseased limb, lower extremity ischaemic symptoms, vascular disease and surgical history was conducted at the time of admission. Our study took these assessments into account as confounders. We also selected confounders based on their associations with LOS (online supplemental table 1). In addition, we included appropriate covariates based on their clinical relevance to LEAD and LOS. The Fontaine classification[20] was used for clinical staging of lower extremity ischaemic symptoms. Additionally, the age-adjusted Charlson comorbidity index (ACCI)[21] was based on the age of the patient and comorbid conditions in their medical records. Therefore, the final covariates included gender, age, number of historical hospitalisations, surgical history, vascular disease history, drinking history, smoking history, insurance type, surgical approach, lesion site, weight loss (loss of ≥2 kg), rest pain, Fontaine classification, ACCI, Urea, CRP, TP, LDL-C, HDL-C, FIB, APTT, TT and PT-INR.

Statistical analysis

First, the Kolmogorov-Smirnov test was used to examine the distribution of the continuous variables. Continuous variables were reported using median and IQR for non-normally distributed data. Categorical variables were expressed as numbers and percentages (%). Hcy values were treated as a continuous variable in statistical tests. Second, univariate

Table 1 Baseline characteristics of the patients (n=748)

Variable	Value
Gender, n (%)	
Male	631 (84.36)
Female	117 (15.64)
Age (years), median (IQR)	68.00 (63.00–76.00)
Number of historical hospitalisations (times), median (IQR)	1.00 (1.00–2.00)
Surgery history, n (%)*	
Yes	379 (51.01)
No	364 (48.99)
Vascular disease history, n (%)*	
Yes	622 (83.72)
No	121 (16.29)
Drinking history, n (%)*	
Yes	236 (31.76)
No	507 (68.24)
Smoking history, n (%)*	
Yes	434 (58.41)
No	309 (41.59)
Insurance type, n (%)	
Out-of-pocket	34 (4.55)
Urban and rural resident medical insurance	123 (16.44)
Social insurance	326 (43.58)
Employee medical insurance	265 (35.43)
Surgical approach, n (%)	
No surgery	135 (18.05)
Open surgery	78 (10.43)
Interventional surgery	535 (71.52)
Diseased limb, n (%)†	
Unilateral lower limb	305 (41.33)
Bilateral lower limbs	433 (58.67)
Weight loss, n (%)‡	
Yes	10 (1.75)
No	560 (98.25)
Rest pain, n (%)§	
Yes	72 (13.38)
No	466 (86.62)
Fontaine classification, n (%)¶	
Class I	32 (4.75)
Class II	563 (83.53)
Class III	23 (3.41)
Class IV	56 (8.31)
ACCI (score), median (IQR)	4.00 (3.00–5.00)
Urea (mmol/L), median (IQR)	6.33 (5.10–7.69)
CRP (mg/L), median (IQR)	6.20 (3.10–20.70)
TP (g/L), median (IQR)	63.40 (59.68–67.00)
LDL-C (mmol/L), median (IQR)	2.81 (2.17–3.46)
HDL-C (mmol/L), median (IQR)	0.98 (0.82–1.19)
FIB (g/L), median (IQR)	3.85 (3.30–4.96)
APTT (s), median (IQR)	38.70 (35.70–42.10)
TT (s), median (IQR)	16.60 (15.90–17.30)
PT-INR, median (IQR)	1.00 (1.00–1.07)
Hcy (μmol/L), median (IQR)	13.32 (10.60–17.91)
Length of stay (day), median (IQR)	9.00 (6.00–13.25)

Data presented as the median (IQR) for continuous variables and frequency (%) for categorical variables. Total percentages within categories may not equal 100% due to rounding.
*Baseline data were missing for 5 patients (0.67%) with baseline covariates of surgery history, vascular disease history, drinking history and smoking history.
†Baseline diseased limb was missing for 10 patients (1.33%).
‡Baseline weight loss was missing for 178 patients (23.80%).
§Baseline rest pain was missing for 210 patients (28.07%).
¶Baseline fontaine classification was missing for 74 patients (9.89%).
ACCI, age-adjusted Charlson comorbidity index; APTT, activated partial thromboplastin time; CRP, C reactive protein; FIB, fibrinogen; Hcy, homocysteine; HDL-C, high density lipoproteins cholesterol; LDL-C, low density lipoprotein cholesterol; PT-INR, prothrombin time international normalised ratio; TP, total protein; TT, thrombin time.

analysis was performed with standard statistical methodology to assess the associations between covariates and LOS. We also calculated the variance inflation factor for each variable in generalised linear models to assess collinearity. The variance inflation coefficients of the variables were all below 2.9. No significant multicollinearity was found among variables. Third, generalised linear models were used to explore the association between Hcy level and LOS. We first conducted a crude model (model 1) without controlling for potential confounders. Next in model 2, we only controlled for age and gender. We further controlled for laboratory indicators in model 3 (Urea, CRP, TP, LDL-C, HDL-C, FIB, APTT, TT and PT-INR). Finally, model 4 adjusted for all confounders. A generalised additive model with a spline smoothing function was applied to explore the association between Hcy level and LOS, with adjustments for all potential confounders. Fourth, we used piecewise linear regression analysis to examine the threshold effect of Hcy level on LOS according to the smoothing curve. To evaluate whether using indicator variables for missing data would bias the results, we imputed missing baseline confounders using multiple imputations. Fifth, interaction and stratified analyses were conducted according to gender, age, surgical history, vascular disease history, drinking history, smoking history, insurance type, surgical approach, diseased limb, weight loss, rest pain and Fontaine classification. Sixth, we examined the possibility of unmeasured confounding between Hcy level and LOS by

calculating the E-value. Finally, to evaluate the effectiveness of Hcy level in predicting a LOS of >9 days (the median LOS), we constructed receiver-operating characteristic curves and compared the areas under the curves (AUC) with and without Hcy level in the model. The bootstrap method with 500 bootstrap samples was used to obtain the 95% CI of the AUC with and without Hcy level in the model. We also assessed model calibration by generating a calibration plot using 500 bootstrap runs. Additional benefit was further assessed based on decision curve analysis.

A two-tailed p<0.05 was considered statistically significant for all analyses. Statistical analyses were conducted using SPSS V.21.0 (IBM Corp, Armonk, New York, USA), Stata V.16.0 (StataCorp, College Station, Texas, USA) and R V.3.6.0 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Patient characteristics

For the 748 patients with LEAD in our study, the median age was 68-years-old (63, 76). There were 622 patients with a vascular disease history and 379 patients with history of surgery. The study population comprised 117 females and 631 males. Baseline characteristics are detailed in table 1.

Association between Hcy level and LOS

In the unadjusted model, Hcy level was associated with LOS (β: 0.10; 95% CI: 0.01 to 0.18; p=0.029). After all covariate adjustments, the association was still statistically significant (β: 0.16; 95% CI: 0.06 to 0.27; p=0.002), and all variables except Hcy level were not significantly associated with LOS (all p>0.05) (online supplemental table 2). We also performed multiple imputations of missing data (missing completely at random) for sensitivity analysis, and the results were robust (see table 2 for details). We then used the E-value to verify possible unadjusted confounding, and the value was 4.33.

The interaction between baseline factors and Hcy level and LOS was not statistically significant based on the subgroup analysis (all p for interaction >0.05) (figure 1).

A non-linear dose–response curve relationship between Hcy level and LOS was observed after all covariate adjustments (see figure 2 for details). In threshold effect analysis, LOS significantly increased with Hcy (β: 0.36; 95% CI: 0.18 to 0.55; p<0.001) in patients with Hcy <22.63 µmol/L. When Hcy level was greater than or equal to 22.63 µmol/L, increased Hcy level was not associated with longer LOS (β: −0.09; 95% CI: −0.30 to 0.13; p=0.436) (see table 3 for details).

Online supplemental figure 1 displays the discrimination performance for LOS of >9 days prediction after adding Hcy level to the reference model. The variables in the reference model included gender, age, number of historical hospitalisations, surgical history, vascular disease history, drinking history, smoking history, insurance type, surgical approach, diseased limb, rest pain, Fontaine classification, ACCI, Urea, CRP, TP, LDL-C, HDL-C, FIB, APTT, TT and PT-INR. The prediction accuracy of the Hcy-added model (AUC: 0.755, p<0.001) improved relative to the reference model (AUC: 0.746, p<0.001) (p values for the comparison >0.05). Results from the bootstrap analysis showed similar results (AUC for the reference model 0.744, p<0.001; AUC for Hcy-added model 0.752, p<0.001). In the calibration plots, observed and predicted probabilities showed good agreement, and adding Hcy level slightly improved the agreement. Moreover, the decision curve analysis demonstrates that the model had potential clinical application value (online supplemental figure 2).

DISCUSSION

We found that an Hcy level <22.63 µmol/L was associated with a prolonged LOS in patients with LEAD. There was a non-linear dose–response relationship with an inflection point at 22.63 µmol/L. To the best of our knowledge, the present study is the first to provide clear evidence of a non-linear association between Hcy level and LOS in patients with LEAD, and to detect the cut-off point of significance in the plot of threshold between them.

Table 2 The association of Hcy level with length of stay before and after performing multiple imputation

	Model 1		Model 2		Model 3		Model 4	
Item	Unadjusted β (95% CI)	P	Adjusted β (95% CI)	P	Adjusted β (95% CI)	P	Adjusted β (95% CI)	P
Before performing multiple imputation								
Hcy (µmol/L)	0.10 (0.01 to 0.18)	0.029	0.09 (0 to 0.18)	0.047	0.19 (0.10 to 0.28)	<0.001	0.16 (0.06 to 0.27)	0.002
After performing multiple imputation								
Hcy (µmol/L)	0.10 (0.01 to 0.18)	0.029	0.09 (0 to 0.18)	0.047	0.11 (0.02 to 0.20)	0.018	0.09 (0 to 0.18)	0.042

Model 2: adjusted for age and gender.
Model 3: adjusted for age, gender, Urea, CRP, TP, LDL-C, HDL-C, FIB, APTT, TT, PT-INR.
Model 4: adjusted for age, gender, number of historical hospitalisations, surgical history, vascular disease history, drinking history, smoking history, insurance type, surgical approach, diseased limb, weight loss, rest pain, Fontaine classification, ACCI, Urea, CRP, TP, LDL-C, HDL-C, FIB, APTT, TT, PT-INR.
ACCI, age-adjusted Charlson comorbidity index; APTT, activated partial thromboplastin time; CRP, C reactive protein; FIB, fibrinogen; Hcy, homocysteine; HDL-C, high density lipoproteins cholesterol; LDL-C, low density lipoprotein cholesterol; PT-INR, prothrombin time international normalised ratio; TP, total protein; TT, thrombin time; β, regression coefficients.

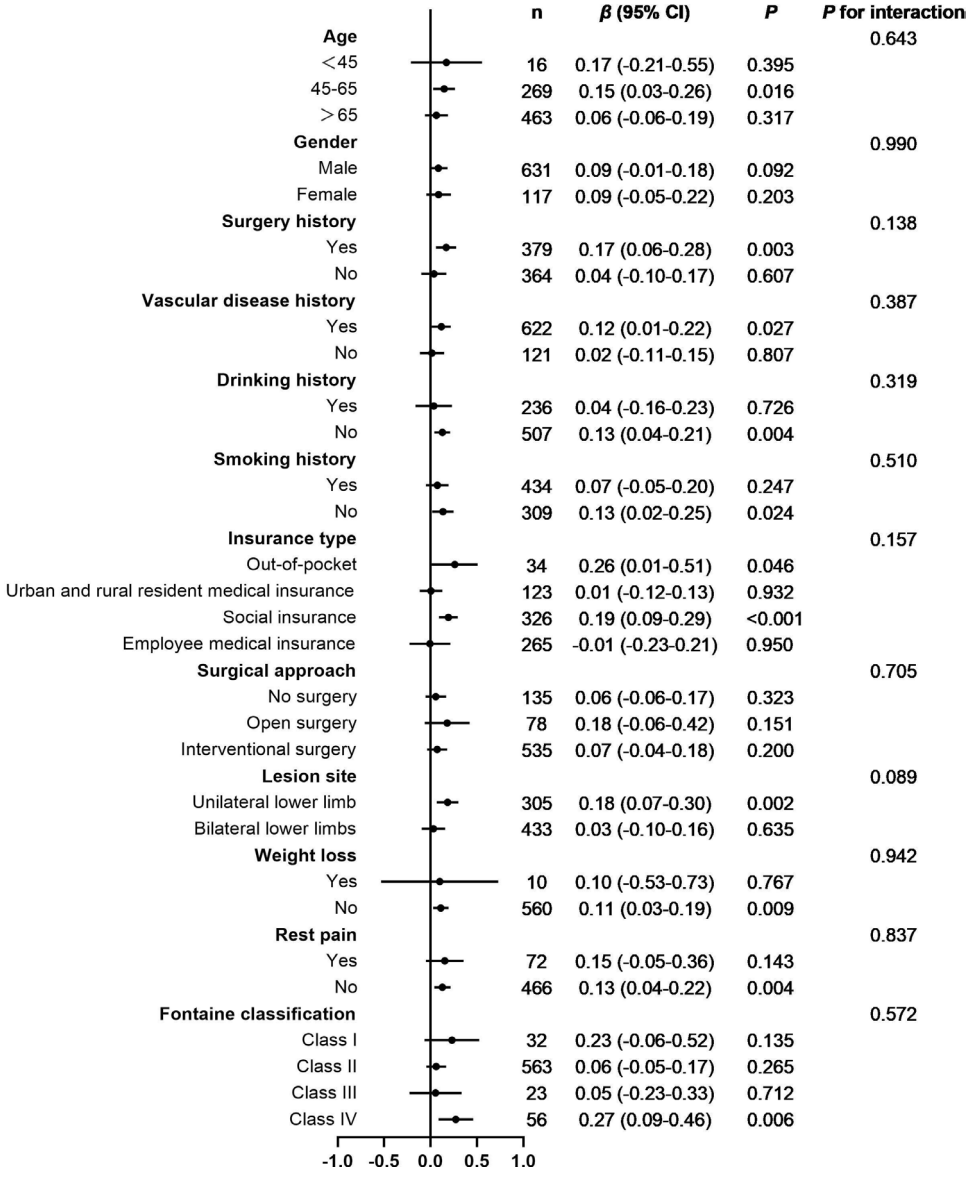

Figure 1 Forest plot for the association between homocysteine level with length of stay in different subgroups.

As for how high Hcy level contributing to atherothrombosis progression, numerous mechanisms have been proposed.[22 23] Atherogenesis may occur when a high Hcy level causes inflammation, oxidative stress and endoplasmic reticulum stress.[24 25] A high Hcy level impairs endothelial function in multiple ways.[26] One of these is the inflammation of endothelium with increased expression of vascular adhesion molecules, increased leucocyte recruitment and increased platelet activation.[27] The mechanisms responsible for exacerbating atherosclerosis by endothelial dysfunction have been extensively studied.[28 29] A hallmark of endothelial dysfunction is the decreased bioavailability of nitric oxide.[30] Decreased bioavailability of nitric oxide is a possible cause of the progression of atherosclerosis.[31] In addition, Hcy can limit the coagulation inhibition effect normally exerted by the thrombomodulin-protein C complex by reducing the activation rate of protein C.[32] The vasodilation of nitric oxide can be reduced by these mechanisms. There may be synergistic effects between decreased bioavailability of nitric oxide, oxidative stress and inflammatory responses.[33] These mechanisms explain the potential role of Hcy level in LOS prediction. We speculate that the non-linear dose–response manner and the height of the threshold between Hcy level and LOS may be due to the highest levels required for the role of Hcy on oxidant stress-mediated vascular inflammation and resulting endothelial dysfunction during the development of atherosclerosis. The degree of endothelial damage, however, can affect the severity of the disease, the course of recovery and the choice of treatment (among other factors). This may affect patients' LOS. Additionally, studies had shown that a high Hcy level is associated with greater functional impairment and a more rapid decline in function (eg, poor baseline physical fitness),[34 35] lower levels of physical activity,[36] more adverse calf muscle characteristics (eg, reduced calf muscle density and poorer calf strength)[37] and increased incidence of macrovascular

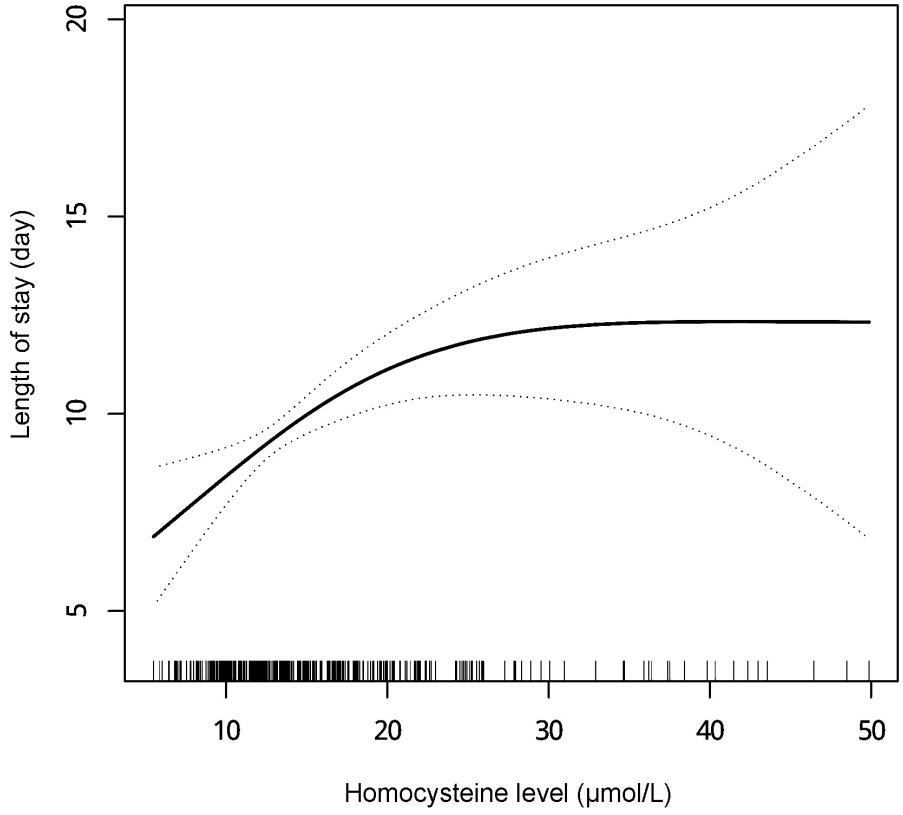

Figure 2 Association between homocysteine level and length of stay. Adjusted for gender, age, number of historical hospitalisations, surgical history, vascular disease history, drinking history, smoking history, insurance type, surgical approach, diseased limb, weight loss, rest pain, Fontaine classification, ACCI, Urea, CRP, TP, LDL-C, HDL-C, FIB, APTT, TT and PT-INR. ACCI, age-adjusted Charlson comorbidity index; APTT, activated partial thromboplastin time; CRP, C reactive protein; FIB, fibrinogen; HDL-C, high density lipoproteins cholesterol; LDL-C, low density lipoprotein cholesterol; PT-INR, prothrombin time international normalised ratio; TP, total protein; TT, thrombin time.

Table 3 Threshold effect analysis of Hcy level on LOS

Items	β (95% CI)	P
Linear regression model	0.16 (0.06 to 0.27)	0.003
Non-linear model, regression coefficients (β)		
Break point of Hcy (K)	22.63	
<K	0.36 (0.18 to 0.55)	<0.001
≥K	−0.09 (−0.30 to 0.13)	0.436
Difference of β value between strata	−0.45 (−0.80 to −0.10)	0.012
Predicted value of LOS at break point	12.51 (10.77 to 14.26)	
P value for likelihood ratio test	0.008	

Adjusted for gender, age, number of historical hospitalisations, surgical history, vascular disease history, drinking history, smoking history, insurance type, surgical approach, diseased limb, rest pain, Fontaine classification, ACCI, Urea, CRP, TP, LDL-C, HDL-C, FIB, APTT, TT and PT-INR.
ACCI, age-adjusted Charlson comorbidity index; APTT, activated partial thromboplastin time; CRP, C reactive protein; FIB, fibrinogen; Hcy, homocysteine; HDL-C, high density lipoproteins cholesterol; LDL-C, low density lipoprotein cholesterol; LOS, length of stay; PT-INR, prothrombin time international normalised ratio; TP, total protein; TT, thrombin time; β, regression coefficients.

complications.[38] These may lead to longer LOS due to prolonged recovery time.

Hcy elevation is pathogenic only on reaching a certain threshold level.[39] Studying the threshold effect can provide valuable information about the occurrence and development of lesions by detecting whether Hcy level reach or exceed the threshold. However, the risk thresholds of Hcy varied for different observed clinical outcomes. Wang *et al*[40] suggested that a cut-off value of 16.56 μmol/L for Hcy may be useful as an index to predict haemorrhagic transformation in patients with acute ischaemic stroke. In a study by Li *et al*,[41] 17.1 μmol/L Hcy value was identified as an effective cut-off point for predicting coronary slow flow syndrome. According to Yao *et al*,[42] a high serum Hcy level may predict poor prognosis after tissue plasminogen activator treatment in patients with acute ischaemic stroke with an optimal cut-off value of 19.95 μmol/L. However, the cut-off points reported by these studies are either focused on specific outcomes or pertain to limited populations. They therefore may only explain trends in Hcy for disease severity change, but do not provide information on the illness's downstream adverse outcomes. This represents an important knowledge gap in research on the association between Hcy level and the downstream adverse outcomes (eg, LOS). Our study complements

this information and provides important insights into the complexity of the possible factors effect on LOS.

Traditional risk factors (smoking, dyslipidaemia)[43 44] and a number of biomarkers, including inflammatory cytokines (CRP),[45] indicators of oxidative stress (Hcy) and coagulation factors (FIB),[46] have been studied widely in relation to LEAD. Our study also analysed the aforementioned factors. Interestingly, however, the results showed that in addition to Hcy, traditional risk factors such as smoking, HDL-C, CRP and FIB were not significantly associated with LOS in patients with LEAD. The present findings suggested that despite the aforementioned factors contribute to the development of an increase in severity of LEAD, they do not result in a prolonged LOS during treatment. Meanwhile, it is important to note that Hcy differs from the aforementioned factors in causing not only increased severity of the disease, but also prolonged LOS. This result has potentially relevant clinical implications, which could benefit patients' care. Hcy in human can be regulated. Previous research has shown that folic acid, vitamin B_6 and B_{12} can regulate Hcy level, which might therefore represent a potential therapeutic target for Hcy.[47] Therefore, clinical attention should be paid to Hcy level at admission, and targeted measures should be taken to control LOS.

The LOS in our study was comparable to that reported in prior studies of PAD.[48–50] The study population consisted of individuals with a median age of 68 years who often have concomitant chronic illnesses, which may prolong hospitalisation time. Furthermore, in China, LOS is typically longer than in Western countries due to differences in social and medical systems. In China, patients are usually discharged directly to their homes after hospitalisation, which incentivizes longer LOS until they achieve significant recovery and target vessel lesion opening with no significant complications. Additionally, medical insurance companies in China only cover medical expenses incurred during hospitalisation, and comprehensive assessments and treatment are often conducted after admission, further prolonging LOS.[51 52] Thus, the prolonged LOS observed in our study is likely attributed to the combination of these aforementioned medical factors.

Several limitations in our study must be acknowledged. First, our study was limited to patients with LEAD in one geographical area in China and therefore the data are not necessarily representative of populations in other locations within China or other countries. The generalisability of our findings requires confirmation in more studies of ethnically diverse adults. And due to the majority of males and claudicants in our study sample, interpretation of the findings is only applicable to a limited population. Second, due to the retrospective nature of the study, other factors related to Hcy and LEAD, such as modifiable lifestyle factors including physical activity, periprocedural complications, and diabetes metrics were not comprehensively evaluated due to paucity of data. It is imperative that future investigations incorporate these factors in the analysis. And future prospective study design would provide more robust evidence and improve the generalisability of the findings. Third, the exclusion of some objective indicators of disease severity, such as ankle-brachial index in the final model is a potential limitation, as it may have an impact on the LOS. Future research is warranted to investigate the potential influence of disease severity on the outcome. Fourth, the inclusion of only 748 patients with valid Hcy test information may limit the representativeness of the study population and affect the external validity of the study findings. Future prospective studies with larger and more diverse patient populations are needed to confirm the observed associations between Hcy levels and adverse outcomes in patients with LEAD. Fifth, this study only employed the Fontaine classification to ascertain the clinical stages. Additional classification systems such as the Rutherford classification could potentially provide a more comprehensive assessment of disease severity and inform appropriate clinical management. Finally, it is known that the occurrence of LEAD is a cumulative process. The present study used only the measured Hcy level at admission once in analysis, thus, whether the observed effect was transient or persistent is unknown, and a longitudinal analysis of Hcy levels should be performed to clarify this uncertainty.

Conclusions

In conclusion, this study extends the understanding of the relationship between Hcy level and LOS in patients with LEAD. A non-linear relationship with an inflection point value of 22.63 µmol/L was found between Hcy level and LOS after the adjustment of potential confounders. Our study helps fill an important gap in understanding about the association between Hcy level and LOS in patients with LEAD, and provides insight into Hcy level optimisation. Current evidence suggests that a Hcy level of 22.63 µmol/L could be regarded as an important and meaningful therapeutic target for the timely adjustment of clinical care plans. Furthermore, the association between Hcy level and LOS in patients with LEAD was independent of some other risk factors and might illustrate that additional studies are necessary to generate therapeutic strategies aimed at lowering Hcy level.

Contributors XW and YY share the first authorship on this work. XW: study design, data analysis, statistical analysis, manuscript drafting and manuscript revision. YY: study design, statistical analysis and manuscript drafting. LX: manuscript revision. LY: manuscript revision. SZ: study design and manuscript revision. XL: manuscript revision. SZ accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish. All authors read and approved the manuscript.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting or dissemination plans of this research.

Patient consent for publication Not applicable.

Ethics approval This study was approved by the Ethics Committee of the First Hospital of China Medical University (ethics number: [2021]366). The requirement

for informed consent was waived since patient information was anonymised and deidentified prior to our analysis.

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement No data are available. No data are available. The datasets analysed during the current study are not publicly available due to privacy policies.

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Supplementary Table 1, online only. Factors associated to length of stay by the univariate analysis (n = 748)

Variable	*β* (95% CI)	*P*
Gender		
Male	Reference	
Female	-3.06 (-5.00- -1.13)	0.002
Age, years	-0.06 (-0.13-0.01)	0.090
Number of historical hospitalizations, times	0.40 (-0.03-0.83)	0.069
Surgery history		
Yes	Reference	
No	-0.33 (-1.75-1.10)	0.652
Vascular disease history		
Yes	Reference	
No	-0.07 (-2.00-1.86)	0.941
Drinking history		
Yes	Reference	
No	-1.28 (-2.81-0.24)	0.100
Smoking history		
Yes	Reference	
No	-1.283 (-2.67-0.21)	0.095
Insurance type		
Out-of-pocket	Reference	
Urban and rural resident medical insurance	-1.09 (-4.84-2.66)	0.570
Social insurance	-0.1 (-3.623-3.36)	0.940
Employee medical insurance	-0.24 (-3.76-3.29)	0.895
Surgical approach		
No surgery	Reference	
Open surgery	8.76 (6.08-11.44)	<0.001
Interventional surgery	2.77 (0.95-4.58)	0.003

Diseased limb		
Unilateral lower limb	Reference	
Bilateral lower limbs	-0.05 (-1.50-1.41)	0.948
Weight loss		
Yes	Reference	
No	-4.21 (-9.12-0.70)	0.094
Rest pain		
Yes	Reference	
No	0.92 (-0.93, 2.76)	0.330
Fontaine classification		
Class I	Reference	
Class II	0.64 (-2.93-4.21)	0.726
Class III	-2.85 (-8.23-2.52)	0.300
Class IV	0.06 (-4.30-4.42)	0.979
ACCI, scores	-0.05 (-0.51-0.41)	0.824
Urea, mmol/L	-0.12 (-0.37-0.14)	0.362
CRP, mg/L	0 (-0.02-0.01)	0.567
TP, g/L	0.10 (-0.04-0.23)	0.166
LDL-C, mmol/L	-0.35 (-1.10-0.41)	0.371
HDL-C, mmol/L	-0.40 (-2.84-2.05)	0.751
FIB, g/L	0.29 (-0.22-0.79)	0.269
APTT, s	0 (-0.08-0.07)	0.994
TT, s	-0.04 (-0.12-0.03)	0.262
PT-INR	0.52 (-4.25-5.30)	0.830

CI, Confidence interval; *β,* regression coefficients; *ACCI,* age-adjusted Charlson comorbidity index; *CRP,* C-reactive protein; *TP*, total protein; *LDL-C*, low density lipoprotein cholesterol; *HDL-C*, high density lipoproteins cholesterol; *FIB*, fibrinogen; *APTT*, activated partial thromboplastin time; *TT*, thrombin time; *PT-INR*, prothrombin time international normalized ratio.

Supplementary Table 2, online only. Estimated coefficients for explanatory variables associated with length of stay by generalized linear model

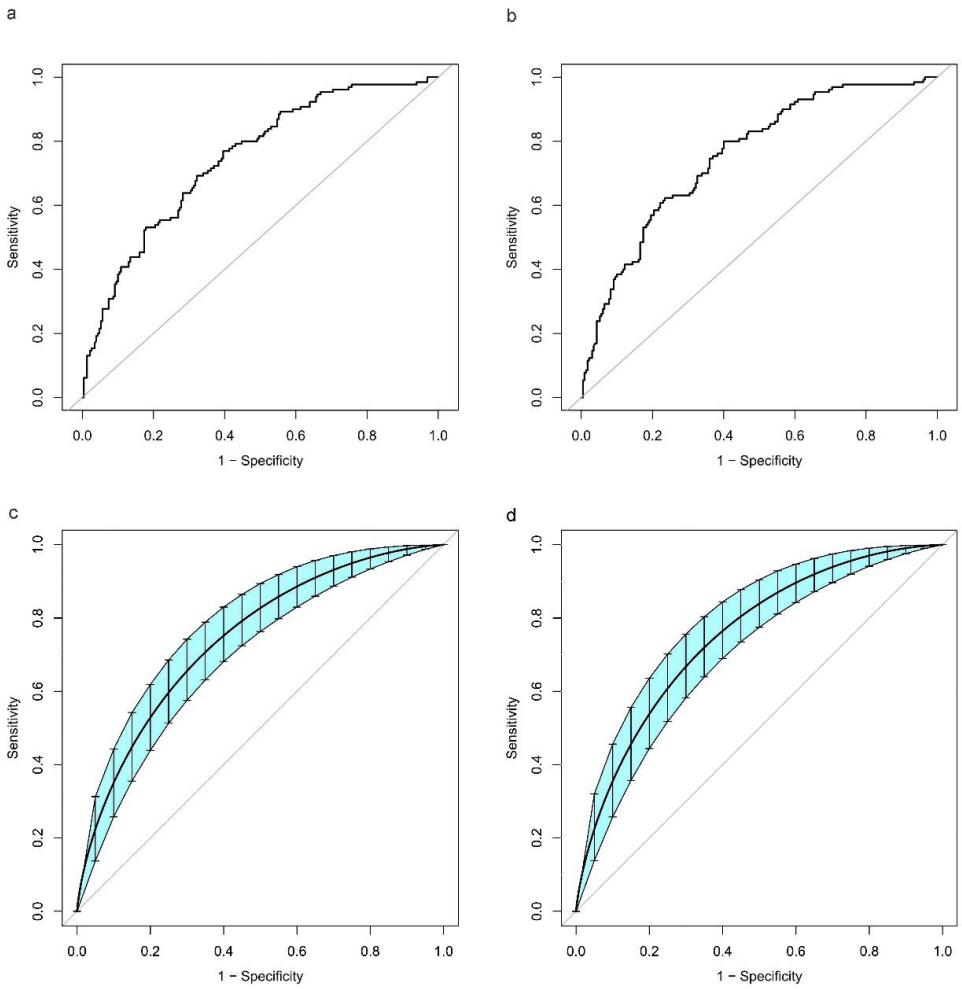
Variable	Estimate	SE	t value	95%CI	*P*
Intercept	11.19	7.96	1.41	-4.40-26.79	0.160
Hcy	0.16	0.05	3.03	0.06-0.26	0.002
Gender					
Male	Reference				
Female	-2.05	1.21	-1.69	-4.42-0.32	0.091
Age, years	-0.07	0.05	-1.41	-0.17-0.03	0.160
Number of historical hospitalizations, times	0.14	0.26	0.55	-0.37-0.66	0.582
Surgery history					
Yes	Reference				
No	-1.02	0.88	-1.15	-2.74-0.71	0.249
Vascular disease history					
Yes	Reference				
No	0.72	1.28	0.56	-1.80-3.23	0.578
Drinking history					
Yes	Reference				
No	0.68	0.94	0.72	-1.16-2.52	0.470
Smoking history					
Yes	Reference				

No	-0.02	0.97	-0.02	-1.92-1.87	0.982
Insurance type					
Out-of-pocket	Reference				
Urban and rural resident medical insurance	-1.25	2.24	-0.56	-5.63-3.13	0.577
Social insurance	1.17	2.07	0.56	-2.89-5.23	0.573
Employee medical insurance	-0.60	2.10	-0.28	-4.71-3.51	0.776
Surgical approach					
No surgery	Reference				
Open surgery	7.88	1.94	4.07	4.08-11.67	<0.001
Interventional surgery	2.21	1.21	1.82	-0.17-4.59	0.070
Diseased limb					
Unilateral lower limb	Reference				
Bilateral lower limbs	0.24	0.81	0.29	-1.35-1.83	0.770
Weight loss					
Yes	Reference				
No	-4.03	3.13	-1.29	-10.17-2.11	0.199
Rest pain					
Yes	Reference				
No	0.30	1.41	0.21	-2.46-3.06	0.830
Fontaine classification					
Class I	Reference				
Class II	-2.35	2.20	-1.07	-6.66-1.97	0.288
Class III	-4.88	3.05	-1.60	-10.86-1.10	0.110

Class IV	-2.73	2.57	-1.06	-7.77-2.31	0.289
ACCI, scores	0.01	0.35	0.03	-0.67-0.69	0.973
Urea, mmol/L	-0.11	0.14	-0.79	-0.38-0.16	0.431
CRP, mg/L	0.01	0.02	0.49	-0.02-0.04	0.621
TP, g/L	0.02	0.07	0.26	-0.13-0.16	0.794
LDL-C, mmol/L	0.02	0.43	0.05	-0.83-0.87	0.961
HDL-C, mmol/L	0.81	1.45	0.56	-2.03-3.65	0.577
FIB, g/L	0.16	0.43	0.36	-0.69-1.00	0.718
APTT, s	-0.03	0.09	-0.40	-0.20-0.13	0.688
TT, s	-0.03	0.04	-0.69	-0.10-0.05	0.491
PT-INR	3.91	3.05	1.28	-2.08-9.89	0.201

Insurance type and surgical approach were coded as binary dummy variables.

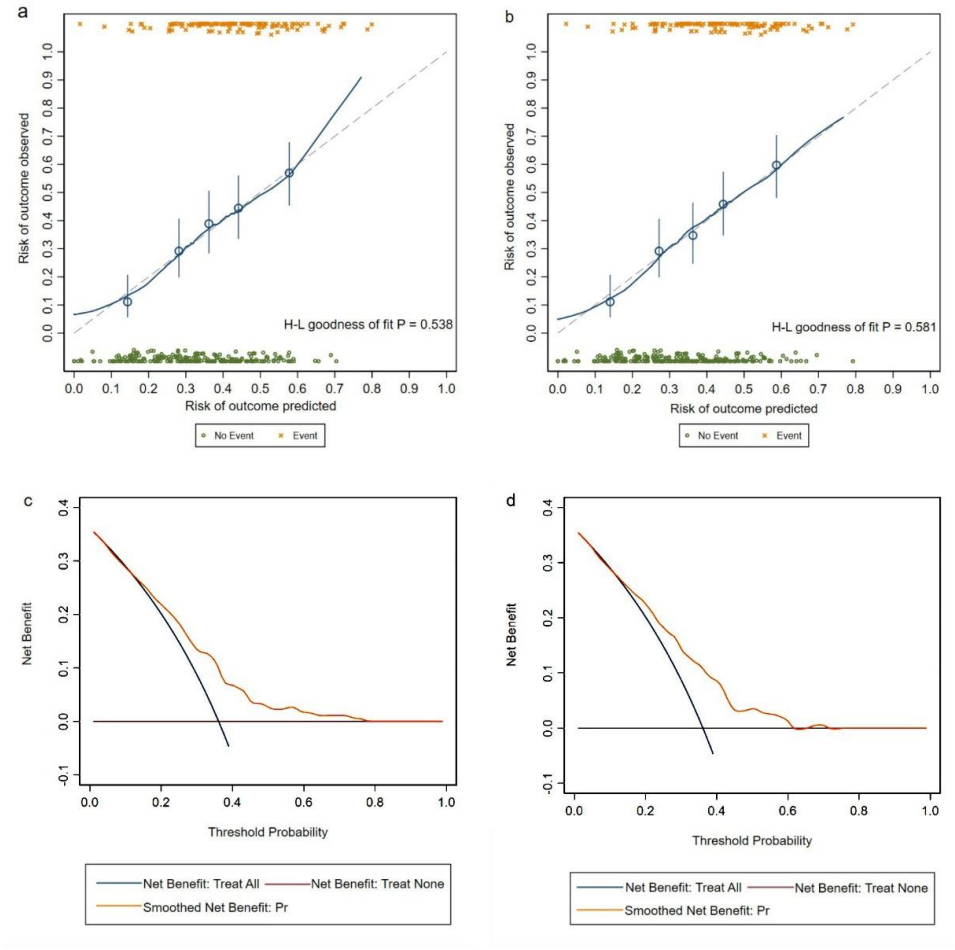
Hcy, homocysteine; *CI*, Confidence interval; *ACCI*, age-adjusted Charlson comorbidity index; *CRP*, C-reactive protein; *TP*, total protein; *LDL-C*, low density lipoprotein cholesterol; *HDL-C*, high density lipoproteins cholesterol; *FIB*, fibrinogen; *APTT*, activated partial thromboplastin time; *TT*, thrombin time; *PT-INR*, prothrombin time international normalized ratio.



Supplementary Figure 1, online only. Receiver operating characteristic curves for predicting a length of stay of > 9 days. (a) Reference model in predicting a LOS of > 9 days in LEAD patients; (b) Reference model adding Hcy level in predicting a LOS of > 9 days in LEAD patients; (c) Reference model in predicting a LOS of > 9 days in LEAD patients by using bootstrap resampling (500 times); (d) Reference model adding Hcy level in predicting a LOS of > 9 days in LEAD patients by using bootstrap resampling (500 times). The variables in the reference model included gender, age, number of historical hospitalizations, surgical history, vascular disease history, drinking history, smoking history, insurance type, surgical approach, diseased limb, rest pain, Fontaine

classification, ACCI, Urea, CRP, TP, LDL-C, HDL-C, FIB, APTT, TT, and PT-INR.

LEAD, Lower extremity arterial disease; *Hcy*, homocysteine; *LOS*, length of stay; *ACCI*, age-adjusted Charlson comorbidity index; *CRP*, C-reactive protein; *TP*, total protein; *LDL-C*, low density lipoprotein cholesterol; *HDL-C*, high density lipoproteins cholesterol; *FIB*, fibrinogen; *APTT*, activated partial thromboplastin time; *TT*, thrombin time; *PT-INR*, prothrombin time international normalized ratio.



Supplementary Figure 2, online only. Calibration plots and decision curve analysis for predicting a length of stay of > 9 days. (a) Calibration plot: reference model in predicting a LOS of > 9 days in LEAD patients; (b) Calibration plot: reference model adding Hcy level in predicting a LOS of > 9 days in LEAD patients. The prediction accuracy increased when the solid line was closer to the dotted line. (c) Decision curve analysis: reference model in predicting a LOS of > 9 days in LEAD patients; (d) Decision curve analysis: reference model adding Hcy level in predicting a LOS of > 9 days in LEAD patients. The orange solid line is from the prediction model, the blue line is for all patients with LOS of > 9 days, and the red horizontal line indicates no patients

with LOS of > 9 days. The graph depicts the expected net benefit per patient relative to the prediction of LOS of > 9 days risk. The net benefit increases as the model curve is extended. The variables in the reference model included gender, age, number of historical hospitalizations, surgical history, vascular disease history, drinking history, smoking history, insurance type, surgical approach, diseased limb, rest pain, Fontaine classification, ACCI, Urea, CRP, TP, LDL-C, HDL-C, FIB, APTT, TT, and PT-INR.

LEAD, Lower extremity arterial disease; *Hcy*, homocysteine; *LOS*, length of stay; *ACCI*, age-adjusted Charlson comorbidity index; *CRP*, C-reactive protein; *TP*, total protein; *LDL-C*, low density lipoprotein cholesterol; *HDL-C*, high density lipoproteins cholesterol; *FIB*, fibrinogen; *APTT*, activated partial thromboplastin time; *TT*, thrombin time; *PT-INR*, prothrombin time international normalized ratio.