The role of serum bilirubin levels in determining venous thromboembolism

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ABSTRACT

Objective: Venous thromboembolism (VTE) is a disease that includes both deep venous thrombosis (DVT) and pulmonary embolism (PE). Bilirubin is an endogenous anti-inflammatory marker associated with atherothrombosis. The purpose of our study was to investigate the association of serum bilirubin levels with the presence of VTE.

Methods: A total of 103 patients with VTE (distal DVT, n = 34; proximal DVT, n = 30; PE, n = 39) and 50 control patients were cross-sectionally enrolled. Peripheral venous duplex ultrasound and computed tomography were used for the diagnosis of VTE. Fasting blood samples were drawn for biochemical analyses.

Results: Baseline characteristics were not different between groups. The VTE group had lower bilirubin level (9.0 ± 2.6 µmol/L vs 7.3 ± 3 µmol/L; $P = .001$) and higher high-sensitivity C-reactive protein (hs-CRP) concentration (0.8 [0.3-2] mg/L vs 1.1 [0.2-3] mg/L; $P = .008$) and white blood cell count (7.4 ± 1.5 × 10^9/L vs 8.2 ± 2.7 × 10^9/L; $P = .02$) compared with control patients. In the analysis of variance, the levels of total direct bilirubin and hs-CRP were clearly different between the control group and VTE subgroups (distal and proximal DVT and PE). The receiver operating characteristic curve analysis showed a cutoff value of 8.9 µmol/L for total bilirubin (sensitivity, 74%; specificity, 55%) and an area under the curve of 0.659 ($P < .001$).

Conclusions: Bilirubin level, hs-CRP concentration, and white blood cell count were independently associated with VTE. (J Vasc Surg: Venous and Lym Dis 2019;7:635-9.)

Keywords: Thromboembolism; Bilirubin; Pulmonary embolism; Antioxidant

Venous thromboembolism (VTE) is a disease that includes deep venous thrombosis (DVT) and pulmonary embolism (PE). VTE is a serious health problem that is associated with PE.[1,2] Predisposing factors for VTE are immobilization, trauma, surgery, malignant disease, age, nutrition, infection, postpartum period, dehydration, and hormone therapy. Inflammation plays a role in arterial and venous thrombosis, which may be a common mechanism that triggers thrombus formation in different vessels.[3,4]

Atherothrombosis is considered a chronic inflammatory disease. VTE may cause inflammatory marker levels to rise.[5] Because endothelial injury is a common mechanism in both arterial and venous thrombosis, inflammation may be a common risk factor.[6] Previous reports suggested that antioxidant capacity increases in mild unconjugated hyperbilirubinemic individuals, and slightly elevated bilirubin may have a protective role in cardiovascular disease.[7] Bilirubin is a possible antithrombotic agent that inhibits platelet activation and, potentially, organ infarction, which could contribute to the reduced mortality rate in mildly hyperbilirubinemic individuals.[8]

In the literature, inflammation markers and antioxidants in venous thrombosis have not been investigated as much as in arterial thrombosis. To our knowledge, there are no reports regarding the role of bilirubin in venous thrombosis. In our study, we planned to investigate the relationship between serum bilirubin level and the presence of VTE.

METHODS

Study population. This study was performed in accordance with the Declaration of Helsinki and was approved by the local ethics committee. All patients were informed and gave consent before the study. The study population included 103 consecutive patients with VTE (distal DVT, n = 34; proximal DVT, n = 30; and PE, n = 39) and 50 control patients. The control group was selected from 75 patients who underwent venous Doppler ultrasound examination and did not have clinical or laboratory findings of VTE.

Exclusion criteria were as follows: pregnancy, known infectious disease, active malignant disease, rheumatic and inflammatory bowel diseases, obesity, antiphospholipid syndrome, and chronic renal or liver disease.

Diagnosis of VTE. Peripheral vascular duplex ultrasound was used for diagnosis of VTE. Acute VTE was defined as either distal or proximal DVT, PE, or both.

DVT was diagnosed if an intraluminal thrombus was present in a deep vein that lacked compressibility or had incomplete compressibility.[9] Proximal DVT was defined if thrombosis occurred at the level of the popliteal veins or above. Distal DVT was defined if thrombosis occurred within the calf veins. The diagnosis of PE was confirmed in all cases by computed tomography. Patients in whom PE was suspected either clinically or because of laboratory results underwent pulmonary computed tomography angiography. Idiopathic VTE was defined if VTE was not associated with known predisposing factors, such as surgery or trauma.

Analysis of blood samples. Fasting peripheral venous blood samples were collected on the day of diagnosis. Routine blood samples were analyzed directly on admission. Samples for hs-CRP were centrifuged and stored maximally for 12 hours in −30°C. Fasting blood glucose, total and direct bilirubin, creatinine, total cholesterol, triglyceride, and high-density lipoprotein cholesterol concentrations were measured routinely using an Abbott Diagnostics C8000i (Abbott, Abbott Park, Ill) autoanalyzer in the biochemistry laboratory of our hospital with commercially available kits. Hemoglobin level, white blood cell (WBC) count, red cell distribution width, and platelet count were measured as part of the automated complete blood count using a Coulter LH 780 Hematology Analyzer (Beckman Coulter Inc, Miami, Fla). Serum hs-CRP levels were measured using the nephelometric method (UniCel DxC 800 System; Beckman Coulter Inc). The conversion factor for direct bilirubin and total bilirubin from SI units (mmol/L to mg/dL) is to divide by 17.1.

Statistics. All statistical analyses were performed with SPSS version 18 software (SPSS Inc, Chicago, Ill). A P value of <.05 was considered significant in all statistical analyses. Continuous variables were given as mean, standard deviation, and median (minimum-maximum). Categorical variables were given as percentage. Student t-test or Mann-Whitney U test was used for the distribution of data for continuous variables, and the χ^2 test was used for categorical variables. Variance analysis (one-way analysis of variance) was used to compare the mean of more independent groups in two quarters. In multiple comparisons, the Tukey honest significant difference test was applied to find the difference between groups. To determine a significant difference between the two groups' average of 1.70 units (for total bilirubin), the minimum number of patients required in each group was 37 with type I error of .05 and test power of 80% and 49 with type I error of .05 and test power of 90%. Multicollinearity occurs when independent variables in a regression model are correlated. To detect multicollinearity between total and direct bilirubin, variance inflation factor and condition index were calculated using linear regression analysis. Variance inflation factor of

total and direct bilirubin was 1.036. Condition index at dimension 5 was 12.591. Because direct and indirect bilirubin levels were moderately correlated, we did not include direct bilirubin in multivariate analysis. Receiver operating characteristic (ROC) curve analysis was performed to determine the cutoff value of total bilirubin and hs-CRP. The predictive validities were quantified as the area under the curve (AUC; C statistics), and the comparisons of C statistics were performed by MedCalc statistical software (MedCalc Software, Ostend, Belgium). Multivariate logistic regression analyses were performed to determine the independent variables associated with VTE.

RESULTS

Baseline characteristics. Baseline characteristics of the patients are presented in . A total of 103 patients and 50 control patients were included in the study. Age, presence of diabetes mellitus, smoking history, estimated glomerular filtration rate, liver enzymes, hemoglobin, and hypertension history were not significantly different between the groups. Sex was not significantly associated with level of bilirubin (VTE: male, 7 ± 2.7 μmol/L; female, 7.5 ± 3.2 μmol/L [P = .387]; controls: male, 9.9 ± 2.7 μmol/L; female, 8.3 ± 2.4 μmol/L [P = .387]). The effect of sex on total bilirubin may be clinically relevant, but this difference is not statistically significant because of low sample power. We included sex in multivariate analyses, but we did not reveal a significant association.

Inflammatory parameters and bilirubin. Total bilirubin concentrations (7.3 ± 3 μmol/L vs 9 ± 2.6 μmol/L; P = .001) were lower whereas hs-CRP levels (1.1 [0.2-3] mg/dL vs 0.8 [0.3-2] mg/dL; P = .008) and WBC counts (8.2 ± 2.7 × 10^9/L vs 7.4 ± 1.5 × 10^9/L; P = .02) were significantly higher in the VTE group compared with the control patients. As we compared VTE subgroups (distal DVT, proximal DVT, PE) and the control group, WBC count was not significantly different between the groups, but bilirubin and hs-CRP levels were significantly different between the VTE and control groups. Post hoc analysis

Table I. General clinical and laboratory features of the study group

Parameters	Control group (n = 50)	VTE group (n = 103)	P value
Age, years	66 ± 15	67 ± 13	.44
Male	47	37	.28
Diabetes	14	19	.50
Hypertension	30	25	.60
Smoking	32	35	.85
OC use history	4	7	.75
History of malignant disease	—	4	—
Heart failure	3	4	.68
Stroke history	3	2	1
Broken/plaster history	—	9	—
Idiopathic VTE	—	50	—
GFR, mL/min/1.73 m^2	72 (48-104)	68 (46-98)	.16
Hemoglobin, g/dL	13.6 ± 1.7	14.0 ± 1.6	.21
Platelet count, ×10^9/L	222 ± 49	233 ± 69	.16
MPV, fL	8.6 ± 1.8	8.8 ± 1.7	.44
WBC count, ×10^9/L	7.4 ± 1.5	8.2 ± 2.7	.02
hs-CRP, mg/L	0.8 (0.3-2)	1.1 (0.2-3)	.008
AST, U/L	27.8 ± 7.7	25.8 ± 6.2	.13
ALT, U/L	28 ± 4.9	27.8 ± 4.4	.86
Total bilirubin, μmol/L	9 ± 2.6	7.3 ± 3	.001
Direct bilirubin, μmol/L	3.6 ± 1.6	3.1 ± 1.5	.03

ALT, Alanine transaminase; *AST*, aspartate transaminase; *GFR*, glomerular filtration rate; *hs-CRP*, high-sensitivity C-reactive protein; *MPV*, mean platelet volume; *OC*, oral contraceptive; *VTE*, venous thromboembolism; *WBC*, white blood cell.
The conversion factor for direct bilirubin and total bilirubin from SI units (mmol/L to mg/dL) is to divide by 17.1.Categorical variables are presented as percentage. Continuous variables are presented as mean ± standard deviation or median (minimum-maximum).

was performed to distinguish between the controls and VTE subgroups. Patients with PE had the lowest bilirubin and highest hs-CRP values. Total bilirubin levels were significantly higher in the control group with respect to the PE group. The hs-CRP value showed a significant difference between all groups (Table II).

Multivariate analysis. Multiple logistic regression analysis identified that total bilirubin (odds ratio [OR], 0.82; 95% confidence interval [CI], 0.73-0.94; *P* = .004), hs-CRP (OR, 2.52; 95% CI, 1.24-5.09; *P* = .01), and WBC count (OR, 1.17; 95% CI, 0.98-1.41; *P* = .082) are independently associated with VTE. Sex (OR, 0.68; 95% CI, 0.32-1.43; *P* = .32) was not independently associated with VTE (Table III).

ROC analysis. ROC analysis showed a cut point of 8.9 μmol/L for total bilirubin (AUC, 0.659; specificity, 53%; sensitivity, 74%; 95% CI, 0.581-0.73; *P* = .002) and 0.8 for hs-CRP (AUC, 0.653; specificity, 60%; sensitivity, 69%; 95% CI, 0.57-0.72; *P* = .003; Fig). However, AUC comparison of these parameters for VTE did not reach statistical significance (*P* > .05).

DISCUSSION

Our study mainly demonstrated that low serum bilirubin levels were significantly associated with the presence of VTE. Subgroup analyses revealed that total bilirubin levels were markedly lower in the PE group than in the control group, and hs-CRP levels were higher in the PE group. The hs-CRP concentrations were also significantly higher in the subgroups of the VTE group than in the control group. ROC analysis showed similar AUC values of hs-CRP and total bilirubin. Last, bilirubin, hs-CRP, and WBC count levels were independently associated with VTE.

Patients with VTE are at increased risk of death, especially within the first year after diagnosis. VTE is a risk factor for death, even during a long follow-up period.[10] PE is a disease that can result in death, requiring rapid diagnosis and treatment.[11] PE is an independent predictor of reduced survival, and for nearly one-quarter of PE patients, the first clinical picture is a sudden death. Furthermore, the number of associated PE deaths is

Table II. The distribution of bilirubin, white blood cell (*WBC*) count, and high-sensitivity C-reactive protein (*hs-CRP*) values between the control group and the subgroups of venous thromboembolism (VTE)

Parameters	Control group (n = 50)	Distal DVT (n = 34)	Proximal DVT (n = 30)	PE (n = 39)	Between-groups P value
Total bilirubin, μmol/L	9 ± 2.6[a]	7.9 ± 2.8	8 ± 3.7	6.4 ± 2.4[a]	.001
Direct bilirubin, μmol/L	3.6 ± 1.6[c]	3.1 ± 1.6	3.6 ± 1.7[b]	2.6 ± 1[b,c]	.01
WBC count, ×10^9/L	7.4 ± 1.4	8.1 ± 2.6	8 ± 3.7	8.6 ± 1.7	.15
hs-CRP, mg/L	0.84 ± 0.5[f]	0.76 ± 0.34[d,e]	1.2 ± 0.71[d,f]	1.3 ± 0.72[e]	<.001

DVT, Deep venous thrombosis; *PE*, pulmonary embolism.
The conversion factor for direct bilirubin and total bilirubin from SI units (mmol/L to mg/dL) is to divide by 17.1. Post hoc analysis was performed to compare study parameters between subgroups.
[a]PE vs control, *P* < .001.
[b]PE vs proximal DVT, *P* = .045.
[c]Control vs PE, *P* = .011.
[d]Proximal DVT vs distal DVT, *P* = .012.
[e]PE vs distal DVT, *P* < .001.
[f]Control vs proximal DVT, *P* = .035.

Table III. Multivariate logistic regression analyses demonstrating association of variables with the presence of venous thromboembolism (VTE)

Variables	Multivariate OR (95% CI)	*P* value
Total bilirubin	0.82 (0.73-0.94)	.004
hs-CRP	2.52 (1.24-5.09)	.010
WBC count	1.17 (0.98-1.41)	.082
Sex	0.68 (0.32-1.43)	.320

CI, Confidence interval; *hs-CRP,* high-sensitivity C-reactive protein; *OR,* odds ratio; *WBC,* white blood cell.

Fig. Comparison of receiver operating characteristic (ROC) curves of total bilirubin and high-sensitivity C-reactive protein (*hs-CRP*) in venous thromboembolism (VTE).

almost the same as myocardial infarction and stroke-related deaths.[12] Therefore, the presence of VTE increases mortality and morbidity significantly.

Inflammation may be part of the event at various stages of hemostasis through the activation of coagulation or by the inhibition of the fibrinolysis and anticoagulant pathways. CRP levels are independently associated with cardiovascular disease and may play a role in the identification and stratification of individuals at risk of cardiovascular diseases.[13] Despite the negative finding of a previous study regarding the role of hs-CRP in VTE, recent reports hold evidence that hs-CRP might be associated with the development of VTE.[14] The study by Kunutsor et al[15] suggested that high hs-CRP concentration is associated with VTE risk with a linear dose-response relationship. In addition, Quist-Paulsen et al[16] reported that CRP levels were 1.6-fold higher in patients with VTE. Similarly, hs-CRP levels were independently associated with extent of VTE. Our basal characteristics findings are parallel to existing publications.[4] A recent study also demonstrated that chronic inflammation plays an important role in venous thrombosis.[6,17] Bakirci et al[18] reported that nonspecific inflammatory markers including WBC count, neutrophil to lymphocyte ratio, and hs-CRP concentration were related to anatomic extent of VTE in a retrospective analysis. We also discovered higher inflammatory markers in VTE and identified that hs-CRP level and WBC count were independently associated with VTE.

Bilirubin has anti-inflammatory properties in addition to antioxidant effects.[7,19] Kubota et al[20] found a relationship between elevated serum uric acid levels and VTE and suggested that this might be a new risk factor for VTE, which is related to the antioxidant role of serum uric acid levels. A retrospective case-control study by Cervellin et al[21] identified a significant relationship between low total bilirubin levels and PE. Mustafa et al[22] showed that presence of heme oxygenase 1 genetic variation increases the risk of recurrence in VTE. Bilirubin inhibits collagen-induced platelet activation, vascular smooth muscle cell migration and proliferation, platelet aggregation, and neointimal formation.[8] Recent data showed a significant relationship between risk factors for VTE and atherosclerosis.[23] Considering the relationship of atherosclerosis
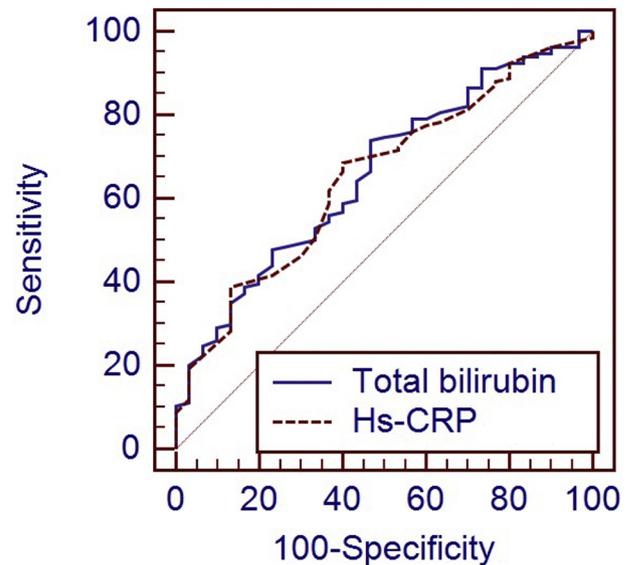
and VTE,[24,25] our study demonstrated association of serum bilirubin levels with the presence of DVT.

Study limitations. Our study has several limitations. Mainly, our study has a small number of patients. Because our study is cross-sectional in design, our results cannot implicate causality. The control group included those in whom DVT was suspected, so they did not represent all non-DVT patients. We had only bilirubin and hs-CRP values at admission and do not have additional data regarding the change during follow-up and previous bilirubin levels. Last, we did not investigate subclinical atherosclerosis and hereditary thrombophilia.

CONCLUSIONS

We investigated the relationship between bilirubin level and VTE. The study showed that bilirubin concentration is independently associated with the presence of VTE. In addition to systemic inflammation markers, WBC count and hs-CRP level were associated with VTE.

AUTHOR CONTRIBUTIONS

Conception and design: HD, SÖ
Analysis and interpretation: HD, SÖ, TE, BK, MD
Data collection: HD, SÖ, TE, BK
Writing the article: HD, SÖ, TE, BK
Critical revision of the article: HD, MD
Final approval of the article: HD, SÖ, TE, BK, MD
Statistical analysis: HD
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Overall responsibility: HD

REFERENCES

1. Croce K, Libby P. Intertwining of thrombosis and inflammation in atherosclerosis. Curr Opin Hematol 2007;14:55-61.

2. Heit J, Silverstein M, David M, Petterson TM, O'Fallon WM, Melton LJ. Risk factors for deep vein thrombosis and pulmonary embolism. Arch Intern Med 2000;160:809-15.

3. Roumen-Klappe EM, den Heijer M, van Uum SH, van der Ven-Jongekrijg J, van der Graaf F, Wollersheim H. Inflammatory response in the acute phase of deep vein thrombosis. J Vasc Surg 2002;35:701-6.

4. Reiter M, Bucek RA, Koca N, Dirisamer A, Minar E. Deep vein thrombosis and systemic inflammatory response: a pilot trial. Wien Klin Wochenschr 2003;115:111-4.

5. Rabinovich A, Cohen JM, Cushman M, Kahn SR, Anderson DR, Chagnon I, et al. Association between inflammation biomarkers, anatomic extent of deep venous thrombosis, and venous symptoms after deep venous thrombosis. J Vasc Surg Venous Lymphat Disord 2015;3:347-53.e1.

6. Poredoš P. Interrelationship between venous and arterial thrombosis. Int Angiol 2017;36:295-8.

7. Bulmer AC, Blanchfield JT, Toth I, Fassett RG, Coombes JS. Improved resistance to serum oxidation in Gilbert's syndrome: a mechanism for cardiovascular protection. Atherosclerosis 2008;199:390-6.

8. Kundur AR, Singh I, Bulmer AC. Bilirubin, platelet activation and heart disease: a missing link to cardiovascular protection in Gilbert' s syndrome? Atherosclerosis 2015;239:73-84.

9. Mazzolai L, Aboyans V, Ageno W, Agnelli G, Alatriat A, Bauersachs R, et al. Diagnosis and management of acute deep vein thrombosis: a joint consensus document from the European Society of Cardiology working groups of aorta and peripheral vascular diseases and pulmonary circulation and right ventricular function. Eur Heart J 2018;39:4208-18.

10. Søgaard KK, Schmidt M, Pedersen L, Horváth-Puhó E, Sørensen HT. Thirty-year mortality after venous thromboembolism: a population-based cohort study. J Vasc Surg 2015;61:281.

11. Xenos ES, Davis G, Green A, He Q, Smyth S. The implementation of a pulmonary embolism response team in the management of pulmonary embolism. J Vasc Surg 2018;67: 13-4.

12. Hooper WC, Holman RC, Heit JA, Cobb N. Venous thromboembolism hospitalizations among American Indians and Alaska Natives. Thromb Res 2002;108:273-8.

13. Pearson TA, Mensah GA, Hong Y, Smith SC Jr; CDC; AHA. CDC/AHA workshop on markers of inflammation and cardiovascular disease. Circulation 2004;110:543-4.

14. Hald EM, Brækkan SK, Mathiesen EB, Njølstad I, Wilsgaard T, Brox J, et al. High-sensitivity C-reactive protein is not a risk factor for venous thromboembolism: the Tromsø study. Haematologica 2011;96:1189-94.

15. Kunutsor SK, Seidu S, Blom AW, Khunti K, Laukkanen JA. Serum C-reactive protein increases the risk of venous thromboembolism: a prospective study and meta-analysis of published prospective evidence. Eur J Epidemiol 2017;32:657-67.

16. Quist-Paulsen P, Naess IA, Cannegieter SC, Romundstad PR, Christiansen SC, Rosendaal FR, et al. Arterial cardiovascular risk factors and venous thrombosis: results from a population-based, prospective study (the HUNT 2). Haematologica 2010;95:119-25.

17. Ogdie A, Yu Y, Haynes K, Love TJ, Maliha S5, Jiang Y, et al. Risk of major cardiovascular events in patients with psoriatic arthritis, psoriasis and rheumatoid arthritis: a population-based cohort study. Ann Rheum Dis 2015;74:326-32.

18. Bakirci EM, Topcu S, Kalkan K, Tanboga IH, Borekci A, Sevimli S, et al. The role of the nonspecific inflammatory markers in determining the anatomic extent of venous thromboembolism. Clin Appl Thromb Hemost 2015;21: 181-5.

19. Bhutani VK, Johnson L, Sivieri EM. Predictive ability of a predischarge hour-specific serum bilirubin for subsequent significant hyperbilirubinemia in healthy term and near-term newborns. Pediatrics 1999;103:6-14.

20. Kubota Y, McAdams-DeMarco M, Folsom AR. Serum uric acid, gout, and venous thromboembolism: the Atherosclerosis Risk in Communities study. Thromb Res 2016;144:144-8.

21. Cervellin G, Buonocore R, Sanchis-Gomar F, Lippi G. Low serum bilirubin values are associated with pulmonary embolism in a case-control study. Clin Chem Lab Med 2016;5: 229-30.

22. Mustafa S, Weltermann A, Fritsche R, Marsik C, Wagner O, Kyrle PA, et al. Genetic variation in heme oxygenase 1 (HMOX1) and the risk of recurrent venous thromboembolism. J Vasc Surg 2008;47:566-70.

23. Mi Y, Yan S, Lu Y, Liang Y, Li C. Venous thromboembolism has the same risk factors as atherosclerosis: a PRISMA-compliant systemic review and meta-analysis. Medicine (Baltimore) 2016;95:e4495.

24. Akboga MK, Canpolat U, Sahinarslan A, Alsancak Y, Nurkoc S, Aras D, et al. Association of serum total bilirubin level with severity of coronary atherosclerosis is linked to systemic inflammation. Atherosclerosis 2015;240:110-4.

25. Canpolat U, Aytemir K, Yorgun H, Hazırolan T, Kaya EB, Şahiner L, et al. Association of serum total bilirubin levels with the severity, extent and subtypes of coronary atherosclerotic plaques detected by coronary CT angiography. Int J Cardiovasc Imaging 2013;29:1371-9.