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Varicose veins are associated with mortality and cardiovascular events: A nationwide cohort study

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Short title: Outcomes of Varicose Veins

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Strengths and limitations of this study

1. The strengths of this study are its population-based design with a large sample size including study and control cohorts.
2. Also, our novel findings indicated that the presence of varicose vein should catch more awareness of potential co-existing risks of mortality and major cardiac adverse events.
3. However, all insurance claims should be reviewed by medical reimbursement specialists. Some risk factors of varicose vein including smoking habits, lack of movement, age, sex, pregnancy history, overweight and glycated hemoglobin levels were not available in this database.

Abstract

Objective: Varicose veins (VV) are common and although considered benign may cause morbidity. Moreover, their potential threat to health is considered to be low. However, the association between VV severity and cardiovascular and mortality risks remains unknown. The aim of this study was to investigate the factors associated with overall mortality in patients with VV.

Setting: Population-based cohort study

Participants: A total of 4807 patients with newly diagnosed VV between 1999 and 2012 were identified from Taiwan's National Health Insurance Database. Moreover, 38,456 age-, sex-, and chronic cardiovascular risk factor–matched controls, as assessed based on propensity score, were included.

Primary and secondary outcome measures: Enrolled patients were analyzed using conditional Cox proportional hazards regression analysis to estimate risk of mortality and major cardiovascular adverse events (MACE) in the VV and control groups. VV severity was classified from grade I to III according to the presentation of ulcers or inflammation. The MACE risk associated with each VV severity grade was calculated using the control group as a reference.

Results: Most patients with VV were free from systemic disease. However, compared with matched controls, patients with VV showed a 1.26-times increased risk of mortality (adjusted hazard ratio [HR]: 1.26; 95% CI 1.10–1.46; p = 0.0012). Compared with matched controls, younger (age <65 years) (HR: 1.65; 95% CI: 1.25–2.17; p = 0.0004) and male patients with VV (HR: 1.42; 95% CI: 1.17–1.72; p = 0.0004) showed increased risk of mortality. Furthermore, MACE risk increased with VV severity. Compared with controls, patients with grades I, II, and III VV showed 26.12-, 48.79-, and 61.79-times increased risks of venous thrombotic events,

respectively, including deep vein thrombosis and pulmonary embolism.

Conclusions: This nationwide cohort study demonstrated that patients with VV are at a risk of cardiovascular events and mortality. Our findings suggest that presence of VV warrants close attention in terms of prognosis and treatment.

Key words: varicose vein, mortality, sex, age, cardiovascular risk

Introduction

Varicose veins (VV) can be considered a common disease with prevalence ranging from 2% to 56% in the adult population.[1] Following clinical examination, VV diagnosis is primarily based on the presence of enlarged and twisted veins in the lower extremities.[1,2] Among people with VV, 1% to 4% of individuals show higher severity grades (Clinical–Etiological–Anatomical–Pathophysiological [CEAP] classification, 5–6).[1,2] Although VV lead to leg swelling, venous eczema, and ulceration in some cases, they are regarded as a benign disease.[3,4] Moreover, the association between the severity of VV and risk of future adverse events remains unknown. In fact, the majority of the previous studies have focused on the importance of superficial venous thrombosis or deep vein thrombosis (DVT).[5] In a 30-year cohort study, mortality risk among patients with DVT and pulmonary embolism (PE) was markedly higher than that in age and sex-matched patients, particularly within the first 30 days.[6] Similarly, another population-based case-control study demonstrated that having VV was a risk factor for venous thromboembolism, although the association of VV severity with survival and cardiovascular events remains unknown.[7] In addition, although age, family history, and female sex are the known risk factors for VV, the effects of underlying diseases or sex on outcomes of VV remain unclear.[1] We hypothesized that presence of VV can be used as a marker for cardiovascular risk. Therefore, the aim of this study was to investigate the association of VV with survival and cardiovascular outcomes.

Methods

Data Source

Taiwan launched a single-payer National Health Insurance (NHI) program on March 1,

1995. This database contains details of almost every Taiwanese resident (coverage rate >98% in 2009), making it one of the world's largest and most complete population-based sources. The data used in this study were retrieved from the Longitudinal Health Insurance Database 2000 (LHID2000)—a subset of the NHI database containing all claims data from 1996 to 2013, covering 1 million beneficiaries randomly selected in 2000. At that time, there were no significant differences in age, sex, and health care costs between patients with VV and matched controls. LHID2000 provided encrypted patient identification numbers; sex; date of birth; admission and discharge dates; International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes of diagnoses and procedures; prescription details; registry data in the Catastrophic Illness Patient Database; and costs covered and paid for by NHI. Details of the National Health Insurance Research Databases (NHIRD) are described in previous studies.[8,9] Moreover, the accuracy of major disease diagnoses in the NHIRD, including stroke and acute coronary syndrome, has been validated.[9] The present study was ethically approved by the Institutional Review Board of Chi-Mei Hospital (CV code: 10406-E01). All procedures followed the principles outlined in the Declaration of Helsinki.

Study Design

This nationwide population-based, retrospective cohort study was conducted to investigate the association between VV and subsequent mortality. Patients with at least 3 claims for outpatient VV diagnosis in 1 year or with 1 claim for inpatient VV diagnosis (ICD-9-CM codes 454, 454.0, 454.1, 454.2, 454.8, and 454.9) were considered as VV cases. Patients with a first-time diagnosis of VV from January 1999 to December 2012 were included in the cohort. Codes for VV were considered reliable for diagnosis based on clinical symptoms. VV was mainly diagnosed based vascular duplex and the judgement of clinical specialists. The date of the first-

time VV diagnosis was considered the index date in this study. To ensure accurate VV diagnosis, and to avoid potentially confounding effects, patients with DVT (ICD-9-CM codes 453.40, 459.1, 671.4, 671.3, 451.83, 459.3, 453.4, and 451.11) or PE (ICD-9-CM codes 415.1, 415.11, 673, 673.2, and 673.8) in an ambulatory setting before the index date were excluded. In addition, VV severity was categorized as grade I uncomplicated (ICD-9-CM code 454.9), grade II with ulcer (ICD-9-CM code 454.0) or inflammation (ICD-9-CM code 454.1), and grade III with both ulcer and inflammation (ICD-9-CM code 454.2).

The control cohort (n = 38,456; 8 control subjects for every enrolled patient with VV) comprised selected patients who were not diagnosed with VV from 1996 to 2013. To eliminate potential selection bias, the controls were selected using propensity score matching for baseline characteristics of age, sex, and chronic cardiovascular risk factors, including hypertension (ICD-9-CM codes 401–405, A260, A269, 4372), diabetes (ICD-9-CM codes 250, A181, A189, A229, A239, 3572, and 3620), hyperlipidemia (ICD-9-CM code 272), and coronary artery disease (CAD; ICD-9-CM codes 410–414). The propensity scores for identified VV cases and controls were estimated using the fitting logistic regression model. Based on greedy algorithm matching, 8 control subjects (the nearest neighbor matching of VV) were selected as matched controls.[10] If a case failed to be assigned to the 8 matched controls, it was dropped from the set of matches. In addition, since the primary VV treatment was covered by insurance, it prevented VV overdiagnosis. The matched controls were assigned the same index date as that of the corresponding VV patient.

Outcomes

The primary outcome was mortality, and the secondary outcome was major cardiovascular adverse events (MACEs), including acute coronary syndrome (ACS, ICD-9-CM codes 410,

410.7, 411.1, 411.81, and 414.8), congestive heart failure (CHF, ICD-9-CM codes 428, 428.0, 428.1, 428.2, and 428.9), ischemic stroke (ICD-9-CM code 436), DVT, and PE. Mortality was identified using the "in-hospital death" or "discharge under critical condition" codes at discharge. Enrollment in the NHI program is mandatory for all people in Taiwan, and registration must be withdrawn within 30 days after death. Patients with the abovementioned mortality-related codes and those withdrawn from the NHI program within 30 days after discharge from the last hospitalization were presumed to have died. All subjects were followed up from the index date to death (lost to follow-up) or until December 31, 2013, whichever was earlier.

Validation of the Accuracy of VV Diagnosis and CEAP Grading

To validate the accuracy of the VV diagnosis, we reviewed the charts of all patients (inpatients and outpatients) using ICD-9-CM diagnosis codes for VV who visited Chi-Mei Medical Center (Tainan, Taiwan) from 2010 to 2015. Our aim was to determine the accuracy of code usage. A vascular specialist reviewed patient discharge and clinical records. In addition to examining the accuracy of VV diagnosis, the reviewer compared CEAP stages with our ICD-9-CM–derived grades in inpatients. Subsequently, we further investigated the sensitivity, specificity, and predictive value of the ICD codes for clinical diagnosis, as well as the applicability of our VV grading system. In particular, as ICD-9-CM coding and VV descriptions are associated with insurance payment, the accuracy of VV diagnosis and the reliability of VV severity grading increased.

Statistical Analyses

Continuous and categorical baseline characteristics between the case and control groups were separately compared using Student's *t*-test and chi-square test or Fisher's exact test, as

appropriate. Comparisons among disease severities were evaluated by one-way ANOVA and chi-square test for continuous and categorical data.

Conditional Cox proportional hazards regression analysis was used to estimate the risk of mortality and MACE in the VV and control groups. Adjusted hazard ratios (HRs) were estimated by adjusting for age, sex, hypertension, diabetes, hyperlipidemia, and CAD. Moreover, the investigation was extended to stratified subgroup analysis. HRs between the VV and control groups were separately estimated in subgroups of population aged <65 years or >65 years; males or females; and subgroups with or without a diagnosis of hypertension, diabetes, hyperlipidemia, or CAD. The Kaplan-Meier method was used to separately estimate the 3-, 6-, and 9-year survival rates in the control and VV groups. Kaplan-Meier curves of mortality and MACE were plotted for controls and patients with 3 grades of VV severity. Differences in survival curves between the control and VV groups were examined using the log-rank test. A two-tailed p value of <0.05 was considered statistically significant. All analyses were performed using the SAS software, version 9.4 (SAS Institute, Cary, NC).

Results

Characteristics of the Study Population

A total of 4807 patients with newly diagnosed VV were identified during January 1999 to December 2012. Moreover, 38,456 age-, sex-, and chronic disease–matched patients without VV were enrolled for comparison. All patients were tracked from the index dates until achieving the primary outcomes or the end of the study. The mean age of patients with VV was 56.27 ± 16.13 years, the majority of the patients were female (60.54%), and most of them did not present with chronic diseases such as hypertension, diabetes, hyperlipidemia, and CAD (Table 1). Patients

with grade III VV showed significantly different distribution of age (aged ≤ years), sex, hypertension, and diabetes (Table 2). Interestingly, more female patients (67.88%) were diagnosed with a lower severity (grade I).

Long-Term Mortality Risk

Compared with matched controls, the outcomes of patients with VV were worse. The estimated survival at 3, 6, and 9 years were 97.7%, 95.6%, and 93.3%, respectively, in patients with VV compared with 98.3%, 96.6%, and 94.6%, respectively, in controls (Figure 1A). A log-rank test revealed a significant difference in survival curves of patients with VV and controls ($p = 0.0008$). The survival curves of controls and patients with different severities of VV are presented in Figure 1B. Lower survival rates over time were observed in patients with higher VV severity grades (II–III) but not in those with grade I. Subsequent post-hoc analysis with Bonferroni correction revealed that the survival curves of patients with higher VV severity grades (II–III) were significantly different (grade II: $p = 0.004$; grade III: $p < 0.0001$) from the survival curves of controls. However, there was no significant difference in the survival curves of patients with grade I VV and controls ($p > 0.9999$).

Overall, HR of all-cause mortality adjusted for age, sex, and cardiovascular risks in patients with VV was 1.26 times higher (adjusted HR: 1.26; 95% CI: 11.10–1.46; $p = 0.0012$) than that in controls (Table 3). Stratified analysis revealed 1.65- and 1.42-times increased risks of mortality in younger (age <65 years; adjusted HR: 1.65; 95% CI: 1.25–2.17; $p = 0.0004$) and male patients with VV (adjusted HR: 1.42; 95% CI: 1.17–1.47; $p = 0.0004$). However, no significant effect of VV was found on the survival of patients with specific concomitant cardiovascular risks.

Long-Term MACE risk

MACE risk significantly increased in patients with VV (adjusted HR: 1.94; 95% CI: 1.79–

2.10; p < 0.0001), particularly in relatively younger (age, <65 years; adjusted HR: 2.10; 95% CI: 1.85–2.38; p < 0.0001) or male (adjusted HR: 2.19; 95% CI: 1.93–2.48; p < 0.0001) patients (Supplement Table 1). In addition, patients with VV showing cardiovascular risk factors, including hypertension, diabetes, hyperlipidemia, and CAD, were at a higher risk of MACE than were matched controls. In patients with VV, 3-, 6-, and 9-year MACE-free rates were 89.6%, 83.3%, and 78.2%, respectively (Figure 2A). These rates dramatically declined further with disease severity (Figure 2B).

In terms of individual cardiovascular outcomes, patients with grade III VV were at a greater risk of CHF (adjusted HR: 2.13; 95% CI: 1.80–2.53; p < 0.001), ACS (adjusted HR: 2.03; 95% CI: 1.60–2.57; p < 0.001), and ischemic stroke (adjusted HR: 1.53; 95% CI: 1.20–1.96; p < 0.001) than were controls (Table 4). In particular, with higher VV severity there was an increasing trend in terms of the risk of venous thrombotic events, including DVT and PE (Grade I: adjusted HR: 26.12; 95% CI, 14.64–46.62; Grade II: adjusted HR: 48.76; 95% CI12.53–189.78; Grade III: adjusted HR: 61.79; 95% CI, 29.13–131.06) (Table 4).

Validation of the Accuracy of VV Diagnosis and ICD-9-CM–Derived VV Grading

During 2010–2015 a total of 2202 outpatients and 347 inpatients were reported to have VV. Among the outpatients, 1188 were coded as uncomplicated VV (ICD-9-CM code 454.9), 775 were coded as VV with inflammation (ICD-9-CM code 454.1), 152 were coded as VV with ulcers (ICD-9-CM code 454.0), and 87 were coded as VV with ulcer and inflammation (ICD-9-CM code 454.2) (Supplement Table 2). Notably, none were coded incorrectly. Compared with CEAP stage, as determined based on chart reviews, only a few inpatients were incorrectly or unclearly diagnosed using ICD-9-CM–derived VV codes (Supplement Table 3). For example, among patients with higher VV grades (CEAP stage 5–6), the positive and negative predictive

values with ICD-9-CM–derived codes were 93% and 98.4%, respectively. Specifically, the sensitivity and specificity of ICD-9-CM–derived grading were up to 95.2% and 97.6%, respectively.

Discussion

The primary findings of this study were that (1) patients with VV were at a significantly higher risk of mortality and MACE when compared with matched controls; (2) having VV had a significant impact on the survival of patients <65 years of age and male patients; and (3) compared with controls, patients with VV were at a higher risk of venous thrombotic events, including DVT and PE. Moreover, the estimated risk increased with the severity of the VV. To the best of our knowledge, this nationwide population-based study is the first to comprehensively describe the association of VV with patients' cardiovascular outcomes.

Although VV are common, their potential threat to health has not been well investigated previously.[1,2] Valve dysfunction-mediated activation of leukocytes, release of enzymes, and remodeling of the vascular wall lead to venous valve destruction and incompetence.[11] VV may cause inflammation, edema, ulcers,[11] endothelial dysfunction,[12] and subsequent DVT.[5] In addition, overexpression of inducible nitric oxide synthase and transforming growth □□ has been documented in patients with VV.[13] In this study, the risk of all-cause mortality and MACE was higher in patients with VV than it was in matched controls, indicating that VV-induced systemic inflammation may be associated with cardiovascular events regardless of the development of venous thromboembolic events. However, only a few studies have compared development of VV with arterial disease and reported inconsistent findings.[2,14] A previous study in Finland has reported a two-fold higher incidence of new arterial disease in individuals with

VV than in those without it, although the incidence of new hypertension was similar.[14,15] Thus, VV and arterial disease may have a common etiology, but VV were not related to hypertension. Furthermore, Chang et al. have reported the association of VV with the incidence of venous thromboembolism and peripheral artery disease.[16] Reportedly, myocardial infarction and heart failure increase the risk of thromboembolism.[17] In contrast, patients with thromboembolic events were at a higher risk of subsequent myocardial infarction and stroke.[18] However, whether this association is causal or represents common risk factors warrants further research. Notably, compared with controls, patients with VV were at a higher risk of mortality independent of age and sex. Specifically, the significant impact of VV was observed in relatively younger or male patients. In previous research, older age and female sex were found to be the most relevant risk factors for VV.[1] VV incidence increases with increasing age. However, Heit et al. have reported that younger patients with VV were at a significantly increased risk of subsequent DVT, whereas the risk was attenuated with increasing age.[19] Earlier onset of VV in the younger population implies a higher risk of concomitant arterial diseases or systemic inflammations. As described previously, female sex, pregnancy, and predominately being in the sitting posture are risk factors for VV.[20] However, despite the valid correlation between use of estrogen supplements and DVT, whether sex hormones contribute to the development of VV remains unclear.

There were several strengths of this study. First, we included an unselected, large, nationwide cohort of patients with VV. By including the data of 4807 patients over a 12-year period, this study provided adequate statistical power for the analysis of long-term outcomes for VV. Second, we compared the VV cohort with a matched, VV-free cohort, which helped distinguish the characteristics of the VV population in terms of survival and outcomes. In addition, among patients with VV, the effects of sex and age on mortality and MACE were

emphasized because VV may have been ignored in these specific populations. Finally, we included patients presenting with VV of various severity grades, which allowed for a comprehensive investigation of overall effects of severity.

However, this study had several limitations. According to previous meta-analysis and research, smoking habits, lack of movement, age, sex, pregnancy history, overweight, rheumatoid factor positivity, cholesterol levels, and glycated hemoglobin levels are considered VV risk factors, with some of these being related to increased mortality risk. Although NHIRD provides a complete clinical medical history over decades for 1 million people, currently the NHIRD lacks information regarding people's lifestyle and clinical laboratory test results. Therefore, the selected confounders in this study were limited to age, sex, and four chronic cardiovascular risk factors. The small corresponding area under the receiver operating characteristic curve indicated that the relevant confounders were not appropriately identified. To explore the effects of VV on mortality and MACE with minimum confounding bias, a future study including more comprehensive VV-related risk factors is imperative. Second, the miscoding of VV severity may have led to the exclusion of uncomplicated cases. Nevertheless, to overcome the inherent limitations, we verified the accuracy of VV diagnosis using chart review by a specialist. Overall, both the validation methods indicated a satisfactory accuracy of VV coding in the NHI database. Third, owing to difficulties in completing CEAP staging according to ICD-9, we established our own grading system. However, even though this novel ICD-9-CM–derived grading system clearly differentiated patients with various severities, it remained different from the generally applied CEAP staging system and disease progression could hardly be represented. Similarly, to validate the reliability of the ICD-9-CM–derived grading system, we reviewed medical records of inpatients with VV and observed satisfactory

sensitivity and specificity. Finally, increased mortality with higher ICD-9-CM–derived grades indicated that our grading system specifically reflected the severity of VV.

Collectively, VV are a common condition typically believed to be benign; however, our results suggest that they warrant close attention. Compared with matched controls, patients with VV were at a higher risk of mortality and cardiovascular events. Therefore, these findings should alert clinicians regarding the importance of detecting VV at an early stage.

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Patient consent: Detail has been removed from this case description/these case descriptions to

ensure anonymity. The editors and reviewers have seen the detailed information available and are satisfied that the information backs up the case the authors are making.

Patient and public involvement: This study is based on database while no patient is actually involved.

Ethics approval: The present study was ethically approved by the Institutional Review Board of Chi-Mei Hospital (CV code: 10406-E01).

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Table 1. Baseline characteristics and comorbid medical disorders for the control cohort and patients with varicose vein (VV).

Characteristic	Varicose vein	Controls	
n (%)	n = 4807	n = 38456	P-value[b]
Age (years)			0.9608
< 65	3255 (67.71)	26058 (67.76)	
≧65	1552 (32.29)	12398 (32.24)	
Age(mean±SD)	56.27 ± 16.13	56.32 ± 15.60	0.8392
Gender			0.3920
Male	1897 (39.46)	14926 (38.81)	
Female	2910 (60.54)	23530 (61.19)	
Hypertension			0.4642
No	3837 (79.82)	30872 (80.28)	
Yes	970 (20.18)	7584 (19.72)	
Diabetes			0.7580
No	4337 (90.22)	34754 (90.37)	
Yes	470 (9.78)	3702 (9.63)	
Hyperlipidemia			0.8111
No	4502 (93.66)	35977 (93.55)	
Yes	305 (6.34)	2479 (6.45)	
Coronary artery disease			0.6037
No	4589 (95.46)	36779 (95.64)	
Yes	218 (4.54)	1677 (4.36)	

P-value was calculated based on the two sample t test and Pearson's chi-square test.

Table 2. Baseline characteristics and comorbid medical disorders for patients with VV categorized by the disease severity.

Characteristic	Grade I	Grade II	Grade III	
n (%)	**2525 (52.53%)**	**704 (14.65%)**	**1578 (32.83%)**	**P-value**
Age (years)				<0.0001
< 65	1789 (70.85)	444 (63.07)	1022 (64.77)	
≧65	736 (29.15)	260 (36.93)	556 (35.23)	
Age (mean±SD)	55.69 ± 14.83	57.68 ± 17.52	56.57 ± 17.39	0.0540
Gender				0.0271
Male	811 (32.12)	297 (42.19)	789 (50.00)	
Female	1714 (67.88)	407 (57.81)	789 (50.00)	
Hypertension				0.0235
No	2056 (81.43)	553 (78.55)	1228 (77.82)	
Yes	469 (18.57)	151 (21.45)	350 (22.18)	
Diabetes				<0.0001
No	2331 (92.32)	634 (90.06)	1372 (86.95)	
Yes	194 (7.68)	70 (9.94)	206 (13.05)	
Hyperlipidemia				0.3894
No	2376 (94.10)	650 (92.33)	1476 (93.54)	
Yes	149 (5.90)	54 (7.67)	102 (6.46)	
Coronary artery disease				0.4968
No	2409 (95.41)	668 (94.89)	1512 (95.82)	
Yes	116 (4.59)	36 (5.11)	66 (4.18)	

P-value was calculated based on the one way ANOVA and Pearson's chi-square test.

Table 3. Crude and adjusted hazard ratios of all-cause mortality in patients with VV compared with the matched control cohort during the follow-up period.

Cohort All (n =)	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)*	p-value
Overall analysis				
VV	1.26 (1.11, 1.44)	0.0005	1.26 (1.10, 1.46)	0.0012
Controls	1[reference]		1[reference]	
Stratified analysis				
Age (years)				
< 65 (years)				
VV	1.67 (1.27, 2.18)	0.0002	1.65 (1.25, 2.17)	0.0004
Controls	1[reference]		1[reference]	
≧65 (years)				
VV	1.15 (0.96, 1.37)	0.1347	1.12 (0.94, 1.34)	0.2148
Controls	1[reference]		1[reference]	
Gender				
Male				
VV	1.36 (1.14, 1.64)	0.0008	1.42 (1.17, 1.72)	0.0004
Controls	1[reference]		1[reference]	
Female				
VV	1.24 (0.98, 1.55)	0.0682	1.16 (0.91, 1.47)	0.2399
Controls	1[reference]		1[reference]	

Hypertension				
VV	1.01 (0.75, 1.35)	0.9643	1.03 (0.76, 1.41)	0.8506
Controls	1[reference]		1[reference]	
Diabetes				
VV	0.87 (0.53, 1.43)	0.5868	0.87 (0.53, 1.43)	0.5785
Controls	1[reference]		1[reference]	
Hyperlipidemia				
VV	0.69 (0.21, 2.27)	0.5461	0.65 (0.16, 2.68)	0.5509
Controls	1[reference]		1[reference]	
Coronary artery disease				
VV	1.31 (0.72, 2.37)	0.3823	1.32 (0.72, 2.43)	0.3677
Controls	1[reference]		1[reference]	

HR = Hazard Ratio; CI = Confidence Interval

* Adjusted age, gender, hypertension, diabetes, hyperlipidemia, coronary artery disease

Table 4. The adjusted hazard ratios of major cardiovascular adverse events (MACE) in patients with VV compared with the matched control cohort during the follow-up period.

	Controls	**Grade 1**	**Grade 2**	**Grade 3**
No. of CHF, N (%)	2553 (6.64)	249 (9.86)	87 (12.36)	204 (12.93)
Adjusted HR for CHF (95% CI)*	Referent	1.80 (1.55, 2.10) †	1.66 (1.28, 2.14) †	2.13 (1.80, 2.53) †
No. of ACS, N (%)	1276 (3.32)	132 (5.23)	29 (4.12)	104 (6.59)
Adjusted HR for ACS (95% CI)*	Referent	1.72 (1.40, 2.11) †	1.14 (0.75, 1.74)	2.03 (1.60, 2.57) †
No. of ischemic stroke, N (%)	1385 (3.60)	106 (4.20)	33 (4.69)	94 (5.96)
Adjusted HR for ischemic stroke (95% CI)*	Referent	1.32 (1.05, 1.64) †	1.28 (0.85, 1.92)	1.53 (1.20, 1.96) †
No. of DVT +PE, N (%)	46 (0.12)	58 (2.30)	14 (1.99)	64 (4.06)
Adjusted HR for DVT +PE (95% CI)*	Referent	26.12 (14.64, 46.62) †	48.76 (12.53, 189.78) †	61.79 (29.13, 131.06) †

†p<0.05 * Adjusted age, gender, hypertension, diabetes, hyperlipidemia, coronary artery disease

HR = Hazard Ratio; CI = Confidence Interval; CHF=congestive heart failure; ACS= acute coronary syndrome; DVT = deep vein thrombosis; PE= pulmonary embolism

Figure Legends

Figure 1. (A) Kaplan-Meier estimates of 12-year survival between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year survival between patients with VV categorized by the disease severities and the matched control cohort.

Figure 2. (A) Kaplan-Meier estimates of 12-year free from MACE between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year free from MACE between patients with VV categorized by the disease severities and the matched control cohort.

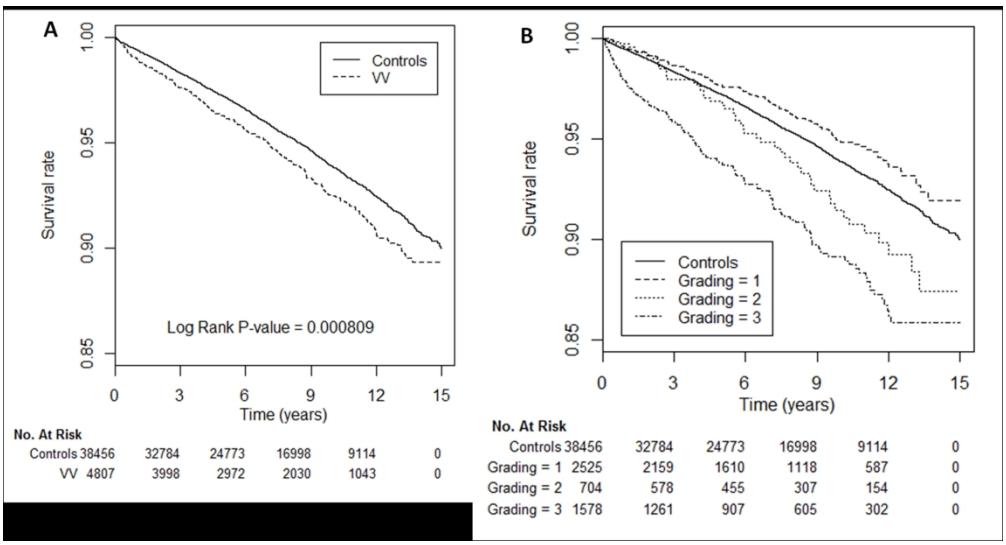

Figure 1. (A) Kaplan-Meier estimates of 12-year survival between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year survival between patients with VV categorized by the disease severities and the matched control cohort.

264x141mm (300 x 300 DPI)

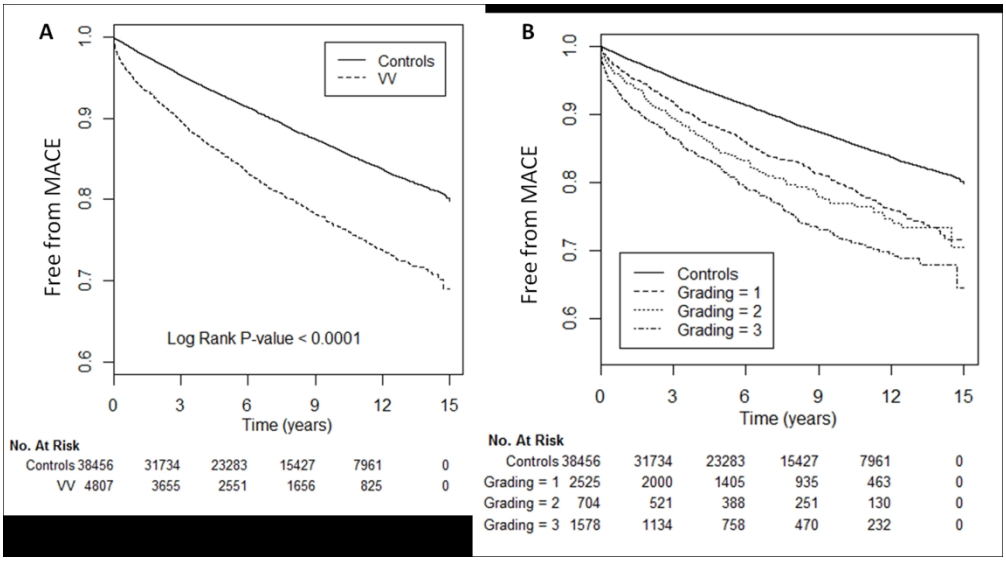

Figure 2. (A) Kaplan-Meier estimates of 12-year free from MACE between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year free from MACE between patients with VV categorized by the disease severities and the matched control cohort.

264x146mm (300 x 300 DPI)

Supplement Table 1. Crude and adjusted hazard ratios of major cardiovascular events in patients with VV compared with the matched control cohort during the follow-up period

Cohort All (n =)	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)*	p-value
Overall analysis				
VV	1.91 (1.77, 2.06)	<.0001	1.94 (1.79, 2.10)	<.0001
Controls	1[reference]		1[reference]	
Stratified analysis				
Age (years)				
< 65 (years)				
VV	2.02 (1.78, 2.28)	<.0001	2.10 (1.85, 2.38)	<.0001
Controls	1[reference]		1[reference]	
≧65 (years)				
VV	1.94 (1.73, 2.18)	<.0001	1.93 (1.72, 2.16)	<.0001
Controls	1[reference]		1[reference]	
Gender				
Male				
VV	2.09 (1.85, 2.35)	<.0001	2.19 (1.93, 2.48)	<.0001
Controls	1[reference]		1[reference]	
Female				
VV	1.76 (1.57, 1.97)	<.0001	1.75 (1.56, 1.97)	<.0001
Controls	1[reference]		1[reference]	
Hypertension				
VV	1.85 (1.57, 2.18)	<.0001	1.87 (1.58, 2.21)	<.0001
Controls	1[reference]		1[reference]	
Diabetes				
VV	1.46 (1.09, 1.96)	0.0104	1.52 (1.13, 2.04)	0.0057
Controls	1[reference]		1[reference]	
Hyperlipidemia				
VV	2.42 (1.48, 3.94)	0.0004	2.34 (1.39, 3.94)	0.0015
Controls	1[reference]		1[reference]	
Coronary artery disease				
VV	2.44 (1.72, 3.46)	<.0001	2.44 (1.72, 3.48)	<.0001
Controls	1[reference]		1[reference]	

HR = Hazard Ratio; CI = Confidence Interval

* Adjusted Grade, age, gender, hypertension, diabetes, hyperlipidemia, coronary artery disease

Supplement Table 2. ICD-9 codes versus chart review diagnosis among outpatients with VV

Code	No.	Clinical diagnosis	No.
454.9 Asymptomatic varicose veins	1188	VV	1188
		Others	0
454.1 With inflammation	775	VV	775
		Others	0
454.0 Varicose veins of lower extremities With ulcer	152	VV	152
		Others	0
454.2 With ulcer and inflammation	87	VV	87
		Others	0

Supplement Table 3. ICD-9 codes-derived severity grading versus Clinical–Etiological–Anatomical–Pathophysiological (CEAP) stages of inpatients with VV

Code	No.	CEAP stage	No.
454.9 Asymptomatic varicose veins	193	C0-2	183
		C3-4	6
		C5-6	4
454.1 With inflammation	56	C0-2	0
		C3-4	56
		C5-6	0
454.0 Varicose veins of lower extremities With ulcer	86	C0-2	6
		C3-4	0
		C5-6	80
454.2 With ulcer and inflammation	12		

	CEAP 0-2	Not CEAP	
Test +	183	10	183/193 (94.8)
Test -	6	136	136/142 (95.7)
	183/189 (96.8)	136/146 (93.1)	

Statistic	**Estimate**	**95% CI**
Sensitivity	96.83%	93.22% to 98.83%
Specificity	93.15 %	87.76% to 96.67%
Positive Predictive Value	94.82%	90.95% to 97.08%
Negative Predictive Value	95.77 %	91.15% to 98.03%

	CEAP 3-4	Not CEAP	
Test +	56	0	56/56 (100)
Test -	6	273	273/279 (97.8)
	56/62 (90.3)	273/273 (100)	

Statistic	**Estimate**	**95% CI**
Sensitivity	90.32%	80.12% to 96.37%
Specificity	100.00 %	98.66% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	97.85 %	95.51% to 98.98%

	CEAP 5-6	Not CEAP	
Test +	80	6	80/86 (93)
Test -	4	245	245/249 (98.4)
	80/84 (95.2)	245/251 (97.6)	

Statistic	Estimate	95% CI
Sensitivity	95.24%	88.25% to 98.69%
Specificity	97.61 %	94.87% to 99.12%
Positive Predictive Value	93.02%	85.79% to 96.71%
Negative Predictive Value	98.39 %	95.92% to 99.38%

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	9
		(*d*) If applicable, explain how loss to follow-up was addressed	9
		(*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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The severe varicose veins and the risk of mortality: A nationwide population-based cohort study

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The severe varicose veins and the risk of mortality: A nationwide population-based cohort study

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Short title: Outcomes of Varicose Veins

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Abstract

Objective: Varicose veins (VV) are common and although considered benign may cause morbidity. However, the association between VV severity and cardiovascular and mortality risks remains unknown. The aim of this study was to investigate the factors associated with overall mortality in patients with VV.

Methods: A total of 4644 patients with newly diagnosed VV between 1999 and 2013 were identified from Taiwan's National Health Insurance Database. VV severity was classified from grade 1 to 3 according to the presentation of ulcers or inflammation. Moreover, 9497, 2541 and 5722 age-, sex-, and chronic cardiovascular risk factor–matched controls, as assessed based on propensity score, were separately selected for 3 grading VV groups. Enrolled patients were analyzed using conditional Cox proportional hazards regression analysis to estimate risk of mortality and major cardiovascular adverse events (MACE) in the VV and control groups.

Results: Most patients with VV were free from systemic disease. However, compared with matched controls, patients with VV showed a 1.37-times increased risk of mortality (95% CI 1.19–1.57; $p < 0.0001$). Compared with matched controls, older (age≧65 years) (adjusted HR: 1.38; 95% CI: 1.17–1.62; $p = 0.0001$) and male patients with VV (adjusted HR: 1.41; 95% CI: 1.18–1.68; $p = 0.0001$) showed increased risk of mortality. Furthermore, compared with controls, patients with VV showed 2.05-times greater risk of MACE. Compared with matched controls, population at grade 3 increased 1.83 times risk of mortality and 2.04 to 38.42 times risk of heart failure, acute coronary syndrome, ischemic stroke and venous thromboembolism.

Conclusions: This nationwide cohort study demonstrated that patients with VV are at a risk of cardiovascular events and mortality. Our findings suggest that presence of VV warrants close

attention in terms of prognosis and treatment.

Key words: varicose vein, mortality, sex, age, cardiovascular risk

Strengths and limitations of this study

1. The strengths of this study are its population-based design with a large sample size including study and control cohorts.
2. All insurance claims should be reviewed by medical reimbursement specialists.
3. However, some risk factors of varicose vein including smoking habits, lack of movement, overweight and glycated hemoglobin levels were not available in this database.
4. Our novel findings indicated that patients at severe grades of varicose vein had higher risks of mortality and major adverse cardiovascular events.
5. The presence of varicose vein should catch more awareness of potential co-existing risks of mortality and cardiovascular events.

Introduction

Varicose veins (VV) can be considered a common disease with prevalence ranging from 2% to 56% in the adult population.[1] Following clinical examination, VV diagnosis is primarily based on the presence of enlarged and twisted veins in the lower extremities.[1,2] Among people with VV, 1% to 4% of individuals show higher severity grades (Clinical–Etiological–Anatomical–Pathophysiological [CEAP] classification, 5–6).[1,2] Although VV lead to leg swelling, venous eczema, and ulceration in some cases, they are regarded as a benign disease.[3,4] Moreover, the association between the severity of VV and risk of future adverse events remains unknown. In fact, the majority of the previous studies have focused on the importance of superficial venous thrombosis or deep vein thrombosis (DVT).[5] In a 30-year cohort study, mortality risk among patients with DVT and pulmonary embolism (PE) was markedly higher than that in age and sex-matched patients, particularly within the first 30 days.[6] Similarly, another population-based case-control study demonstrated that having VV was a risk factor for venous thromboembolism, although the association of VV severity with survival and cardiovascular events remains unknown.[7] In addition, although age, family history, and female sex are the known risk factors for VV, the effects of underlying diseases or sex on outcomes of VV remain unclear.[1] We hypothesized that presence of VV can be used as a marker for cardiovascular risk. Therefore, the aim of this study was to investigate the association of VV with survival and cardiovascular outcomes.

Methods

Data Source

Taiwan launched a single-payer National Health Insurance (NHI) program on March 1, 1995. This database contains details of almost every Taiwanese resident (coverage rate >98% in 2009), making it one of the world's largest and most complete population-based sources. The data used in this study were retrieved from the Longitudinal Health Insurance Database 2000 (LHID2000)—a subset of the NHI database containing all claims data from 1996 to 2013, covering 1 million beneficiaries randomly selected in 2000. At that time, there were no significant differences in age, sex, and health care costs between patients with VV and matched controls. LHID2000 provided encrypted patient identification numbers; sex; date of birth; admission and discharge dates; International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes of diagnoses and procedures; prescription details; registry data in the Catastrophic Illness Patient Database; and costs covered and paid for by NHI. Details of the National Health Insurance Research Databases (NHIRD) are described in previous studies.[8,9] Moreover, the accuracy of major disease diagnoses in the NHIRD, including stroke and acute coronary syndrome, has been validated.[9] The present study was ethically approved by the Institutional Review Board of Chi-Mei Hospital (CV code: 10406-E01). All procedures followed the principles outlined in the Declaration of Helsinki.

Patient and Public Involvement

No patient involved.

Study Design

This nationwide population-based, retrospective cohort study was conducted to investigate the association between VV and subsequent mortality. Patients with at least 3 claims for outpatient VV diagnosis in 1 year or with 1 claim for inpatient VV diagnosis (ICD-9-CM codes

454, 454.0, 454.1, 454.2, 454.8, and 454.9) were considered as VV cases. Patients with a first-time diagnosis of VV from January 1999 to December 2012 were included in the cohort. Codes for VV were considered reliable for diagnosis based on clinical symptoms. The date of the first-time VV diagnosis was considered the index date in this study. To ensure accurate VV diagnosis, and to avoid potentially confounding effects, patients with DVT (ICD-9-CM codes 453.40, 459.1, 671.4, 671.3, 451.83, 459.3, 453.4, and 451.11) or PE (ICD-9-CM codes 415.1, 415.11, 673, 673.2, and 673.8) in an ambulatory setting before the index date were excluded. In addition, VV severity was categorized as grade 1 uncomplicated (ICD-9-CM code 454.9), grade 2 with ulcer (ICD-9-CM code 454.0) or inflammation (ICD-9-CM code 454.1), and grade 3 with both ulcer and inflammation (ICD-9-CM code 454.2).

Three control cohorts (n1=9497, n2=2541 and n3=5722; 4 control subjects for every enrolled patient with VV), not diagnosed with VV from 1996 to 2013, were selected for three VV grade groups separately. To eliminate potential selection bias, the controls were matched using propensity score method at a 4:1 ratio for baseline characteristics of age, sex, and chronic cardiovascular risk factors, including hypertension (ICD-9-CM codes 401–405, A260, A269, 4372), diabetes (ICD-9-CM codes 250, A181, A189, A229, A239, 3572, and 3620), hyperlipidemia (ICD-9-CM code 272), and coronary artery disease (CAD; ICD-9-CM codes 410–414). The propensity scores (PS) for identified VV cases and controls were estimated using the fitting logistic regression model. Based on greedy algorithm matching, 8 control subjects (the nearest neighbor matching of VV) were selected as matched controls.[10] If a case failed to be assigned to the 4 matched controls, it was dropped from the set of matches. In addition, since the primary VV treatment was covered by insurance, it prevented VV from over-diagnosis. The matched controls were assigned the same index date as that of the corresponding VV patient.

Outcomes

The primary outcome was mortality, and the secondary outcome was major cardiovascular adverse events (MACEs), including acute coronary syndrome (ACS, ICD-9-CM codes 410, 410.7, 411.1, 411.81, and 414.8), congestive heart failure (CHF, ICD-9-CM codes 428, 428.0, 428.1, 428.2, and 428.9), ischemic stroke (ICD-9-CM code 436), DVT, and PE. Mortality was identified using the "in-hospital death" or "discharge under critical condition" codes at discharge. Enrollment in the NHI program is mandatory for all people in Taiwan, and registration must be withdrawn within 30 days after death. Patients with the abovementioned mortality-related codes and those withdrawn from the NHI program within 30 days after discharge from the last hospitalization were presumed to have died. All subjects were followed up from the index date to death (lost to follow-up) or until December 31, 2013, whichever was earlier.

Validation of the Accuracy of VV Diagnosis and CEAP Grading

To validate the accuracy of the VV diagnosis, we reviewed the charts of all patients (inpatients and outpatients) using ICD-9-CM diagnosis codes for VV who visited Chi-Mei Medical Center (Tainan, Taiwan) from 2010 to 2015. Our aim was to determine the accuracy and consistency of code usage. A vascular specialist reviewed patient discharge and clinical records. In addition to examining the accuracy of VV diagnosis, the reviewer compared CEAP stages with our ICD-9-CM–derived grades in inpatients. Subsequently, we further investigated the sensitivity, specificity, and predictive value of the ICD codes for clinical diagnosis, as well as the applicability of our VV grading system. In particular, as ICD-9-CM coding and VV descriptions are associated with insurance payment, the accuracy of VV diagnosis and the reliability of VV

severity grading increased. The consistency between CEAP and grading stages were evaluated by kappa score, whose value between 0.8 and 1.0 was considered as an almost perfect agreement.

Statistical Analyses

Continuous and categorical baseline characteristics between the case and control groups were separately compared by standardized mean difference (SMD), an assessment approach for evaluating the balance between variables after PS matching. SMD greater than 0.1 is considered to denote a meaningful imbalance in variables.

Conditional Cox proportional hazards regression analysis was used to estimate the risk of mortality and MACE in the VV and control groups. Adjusted hazard ratios (HRs) were estimated by adjusting for chronic obstructive pulmonary disease (ICD-9-CM codes 490-496) , cancer (ICD-9-CM codes 140-208), atrial fibrillation (ICD-9-CM codes 427.31), heart failure (ICD-9-CM codes 428), ischemic heart disease (ICD-9-CM codes 410-414), chronic renal insufficiency (ICD-9-CM codes 403, 404, 582, 585-588). Moreover, the investigation was extended to stratified subgroup analysis. HRs between the VV and control groups were separately estimated in subgroups of population aged <65 years or ≧65 years; males or females; and subgroups with or without a diagnosis of hypertension, diabetes, hyperlipidemia, or CAD. The Kaplan-Meier method was used to separately estimate the 3-, 6-, and 9-year survival rates in the control and VV groups. Kaplan-Meier curves of mortality and MACE were plotted for controls and patients with 3 grades of VV severity. Differences in survival curves between the control and VV groups were examined using the log-rank test.

With respect to mortality, CHF, ACS, ischemic stroke and DVT +PE endpoints, the risks

for VV with 3 separate severity grades were further estimated by comparison against each matched controls.

Finally, sensitivity analyses were conducted to determine the influence from subjects with pregnancy history (ICD-9-CM codes V22, V23.2, 761.5), peripheral artery disease (PAD, ICD-9-CM codes 440.0, 440.2, 440.3, 440.8, 440.9, 443, 444.0, 444.22, 444.8, 447.8, and 447.9) medical history and patients treated with operations (ICD-9-CM procedure code 3859, 3889 and NHIRD order code 69013, 69014, 69015, 69016, 69017, 69019, 69020, 69021) including ligation and stripping procedures after VV diagnosis.

A two-tailed p value of <0.05 was considered statistically significant. All analyses were performed using the SAS software, version 9.4 (SAS Institute, Cary, NC) and Stata software 15.0 (StataCorp, College Station, TX.)

Results

Characteristics of the Study Population

A total of 4644 patients with newly diagnosed VV were identified during January 1999 to December 2012. Among them, 2467, 668 and 1509 VVs were separately classified into 1, 2 and 3 severity grade. For each VV group, age-, sex-, and chronic disease–matched patients without VV were separately included for comparison. The covariates between VV and matched groups are well balanced after propensity score matching. All patients were tracked from the index dates until achieving the primary outcomes or the end of the study. The mean age of patients with VV was 55.70 ± 16.03 years, the majority of the patients were female (61.33%), and most of them did not present with chronic diseases such as hypertension, diabetes, hyperlipidemia, and CAD (Table 1). Significantly different distribution of age, sex and diabetes among three severity VV

groups were displayed (p-value < 0.05) (Supplementary Table 1). Interestingly, more female patients (68.67%) were diagnosed with a lower severity (grade 1). Also, the baseline characteristics and comorbid medical disorders for 3 grading VV groups and 3 separately matched controls were listed in Supplement Table 2.

Long-Term Mortality Risk

Compared with matched controls, the outcomes of patients with VV were worse. The estimated survival at 3, 6, and 9 years were 97.6%, 95.6%, and 93.5%, respectively, in patients with VV compared with 98.5%, 97.1%, and 95.6%, respectively, in controls (Figure 1A). A log-rank test revealed a significant difference in survival curves of patients with VV and controls (p <0.0001). The survival curves of controls and patients with different severities of VV are presented in Figure 1B. Lower survival rates over time were observed in patients with highest VV severity (grades 3) but not in those with grade 1-2. Significant difference between survival curves between VV grading 3 and corresponding controls were revealed by log rank test (p < 0.0001). However, there is no significant difference were found between survival curves of patients with VV severity grades (1-2) and corresponding controls (grade 1: p = 0.3191; grade 2: p=0.3599).

Overall, HR of all-cause mortality adjusted for chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency in patients with VV was 1.34 times higher (adjusted HR: 1.37; 95% CI: 1.19–1.57; p < 0.0001) than that in controls (Table 2). Stratified analysis revealed 1.38- and 1.41-times increased risks of mortality in older (age ≧65 years; adjusted HR: 1.38; 95% CI: 1.17–1.62; p = 0.0001) and male

patients with VV (adjusted HR: 1.41; 95% CI: 1.18–1.68; p = 0.0001). Notably, despite no significant effect of VV on the survival of patients with hypertension, hyperlipidemia or coronary artery disease, patients with both VV and diabetes presented 1.50 times higher risk of mortality compared with those without VV (adjusted HR: 1.50; 95% CI: 1.05–2.15; p = 0.0254). Furthermore, VV at grade 3 show 1.83 (95% CI : 1.48, 2.27 ; p < 0.0001) greater risk of mortality under the control of chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency.

Long-Term MACE risk

MACE risk significantly increased in patients with VV (HR: 2.05; 95% CI: 1.89–2.23; p < 0.0001), particularly in relatively younger (age, <65 years; adjusted HR: 2.17; 95% CI: 1.92–2.46; p < 0.0001) or male (adjusted HR: 2.32; 95% CI: 2.06–2.62; p < 0.0001) patients (Table 3). In addition, patients with VV showing cardiovascular risk factors, including hypertension, diabetes, hyperlipidemia, and CAD, were at a higher risk of MACE than were matched controls. In patients with VV, 3-, 6-, and 9-year MACE-free rates were 91.17%, 84.99%, and 79.27%, respectively (Figure 2A). These rates dramatically declined further with disease severity (Figure 2B). In terms of individual cardiovascular outcomes, patients with grade 3 VV were at a greater risk of CHF (adjusted HR: 2.05; 95% CI: 1.71–2.46; p < 0.0001), ACS (adjusted HR: 2.04; 95% CI: 1.58–2.63; p < 0.0001), and ischemic stroke (adjusted HR: 2.06; 95% CI: 1.58–2.69; p < 0.0001) than were controls (Table 4). In particular, with the highest VV severity there was an increasing risk of venous thrombotic events, including DVT and PE (Grade 3: adjusted HR: 38.42; 95% CI, 16.38–90.13; p < 0.0001) (Table 4).

Validation of the Accuracy of VV Diagnosis and ICD-9-CM–Derived VV Grading

During 2010–2015 a total of 2202 outpatients and 347 inpatients were reported to have VV in Chi-Mei Medical Center. Among the outpatients, 1188 were coded as uncomplicated VV (ICD-9-CM code 454.9), 775 were coded as VV with inflammation (ICD-9-CM code 454.1), 152 were coded as VV with ulcers (ICD-9-CM code 454.0), and 87 were coded as VV with ulcer and inflammation (ICD-9-CM code 454.2) (Supplement Table 3). Notably, none were coded incorrectly. Compared with CEAP stage, as determined based on chart reviews, only a few inpatients were incorrectly or unclearly diagnosed using ICD-9-CM–derived VV codes (Supplement Table 4). For example, among patients with higher VV grades (CEAP stage 5–6), the positive and negative predictive values with ICD-9-CM–derived codes were 93% and 98.4%, respectively. Specifically, the sensitivity and specificity of ICD-9-CM–derived grading were up to 95.2% and 97.6%, respectively. The calculated kappa score between CEAP stages and grading severity is 0.918 (95%CI = [0.878, 0.957]).

Sensitivity analyses

VV and controls with pregnancy history were identified and exam the influence in sensitivity analysis (Supplement Table 5). After additionally adjustment for history of pregnancy, the results remain showing great impacts on mortality and MACE (adjusted HR for death (95%CI) = 1.37 (1.19, 1.57), p-value<0.0001; adjusted HR for MACE (95%CI) = 2.01 (1.89, 2.23), p-value<0.0001).

After excluding 472 subjects with Myocardial infarction, stroke, coronary angioplasty or

CABG, remaining VV and corresponding controls were included for sensitivity analysis. Comparing with corresponding matched controls, those conservatively treated VV patients were found 1.36 times risks of mortality (adjusted HR (95%CI) = 1.362 (1.18, 1.57), p-value<0.0001) and 1.95 times risks of MACE (adjusted HR (95%CI) = 1.95 (1.80, 2.12), p-value<0.0001).

Discussion

The primary findings of this study were that (1) patients with VV were at increasing risks of mortality and cardiovascular events, especially those with VV at grade 3 compared with matched controls; (2) having VV had a significant impact on the survival of male patients. To the best of our knowledge, this nationwide population-based study is the first to comprehensively describe the association of VV with patients' cardiovascular outcomes.

Although VV are common, their potential threat to health has not been well investigated previously.[1,2] Valve dysfunction-mediated activation of leukocytes, release of enzymes, and remodeling of the vascular wall lead to venous valve destruction and incompetence.[11] VV may cause inflammation, edema, ulcers,[11] endothelial dysfunction,[12] and subsequent DVT.[5] In addition, overexpression of inducible nitric oxide synthase and transforming growth factor-β1 has been documented in patients with VV.[13] In this study, the risk of all-cause mortality and MACE was higher in patients with VV than it was in matched controls, indicating that VV-induced systemic inflammation may be associated with cardiovascular events regardless of the development of venous thromboembolic events. Notably, the lower survival rates were observed in patients with highest VV severity but not in those with grade 1-2. This also reflects that the chronic inflammation induced by a higher grade of VV may be associated with increasing

mortality and MACEs. However, only a few studies have compared development of VV with arterial disease and reported inconsistent findings.[2,14] A previous study in Finland has reported a two-fold higher incidence of new arterial disease in individuals with VV than in those without it, although the incidence of new hypertension was similar.[14,15] Thus, VV and arterial disease may have a common etiology, but VV were not related to hypertension. Furthermore, Chang et al. have reported the association of VV with the incidence of venous thromboembolism and peripheral artery disease.[16] Reportedly, myocardial infarction and heart failure increase the risk of thromboembolism.[17] In contrast, patients with thromboembolic events were at a higher risk of subsequent myocardial infarction and stroke.[18] However, whether this association is causal or represents common risk factors warrants further research. Notably, compared with controls, patients with VV were at a higher risk of mortality independent of age and sex. Specifically, the significant impact of VV was observed in male patients. In previous research, older age and female sex were found to be the most relevant risk factors for VV.[1] VV incidence increases with increasing age. However, Heit et al. have reported that younger patients with VV were at a significantly increased risk of subsequent DVT, whereas the risk was attenuated with increasing age.[19] Similarly, Lohr et al also reviewed that Although a lower grade of VV (CEAP 2-3) has been observed in 50.5% of females and in 30.1% of males, a higher grade of VV with trophic skin changes (CEAP 4-6) were found in 2.8% of females and 5.4% of males.[20] Also, DVT was more common in males compared with females (11.3% vs 7.8%).[20] Earlier onset of VV in the younger population implies a higher risk of concomitant arterial diseases or systemic inflammations. As described previously, female sex, pregnancy, and predominately being in the sitting posture are risk factors for VV.[21] However, despite the valid correlation between use of estrogen supplements and DVT, whether sex hormones contribute to the development of VV

remains unclear.

There were several strengths of this study. First, we included an unselected, large, nationwide cohort of patients with VV. By including the data of 4644 patients over a 12-year period, this study provided adequate statistical power for the analysis of long-term outcomes for VV. Second, we compared the VV cohort with a matched, VV-free cohort, which helped distinguish the characteristics of the VV population in terms of survival and outcomes. Third, among patients with VV, the effects of sex on mortality and MACE were emphasized because VV may have been ignored in these specific populations. Forth, we included patients presenting with VV of various severity grades, which allowed for a comprehensive investigation of overall effects of severity. Finally, a recently published article evaluated and supported the accuracy of several major outcomes, including MI, hypertension, diabetes, stroke, CHF and VV, in NHIRD.[22]

However, this study had several limitations. According to previous meta-analysis and research, smoking habits, quality of life, lack of movement, pregnancy history, overweight and glycated hemoglobin levels are considered VV risk factors, with some of these being related to increased mortality risk. Although NHIRD provides a complete clinical medical history over decades for 1 million people, currently the NHIRD lacks information regarding people's lifestyle and clinical laboratory test results. Therefore, the selected confounders in this study were limited to age, sex, and four chronic cardiovascular risk factors. The small corresponding area under the receiver operating characteristic curve indicated that the relevant confounders were not appropriately identified. To explore the effects of VV on mortality and MACE with minimum confounding bias, a future study including more comprehensive VV-related risk factors is

imperative. Second, the miscoding of VV severity may have led to the exclusion of cases. This might explain why 47% of the included patients are with advanced venous disease (Grade 2 or 3), different from the general distribution of disease severity. Nevertheless, to overcome the inherent limitations, we verified the accuracy of VV diagnosis using chart review by a specialist. Overall, both the validation methods indicated a satisfactory accuracy of VV coding in the NHI database. Third, owing to difficulties in completing CEAP staging according to ICD-9, we established our own grading system. However, even though this novel ICD-9-CM–derived grading system clearly differentiated patients with various severities, it remained different from the generally applied CEAP staging system and disease progression could hardly be represented. Similarly, to validate the reliability of the ICD-9-CM–derived grading system, we reviewed medical records of inpatients with VV and observed satisfactory sensitivity and specificity. Forth, while ligation and stripping surgeries may affect the outcomes, through excluding patients receiving surgical treatment for VV we performed sensitivity test. It also revealed significant increases of risks of mortality and MACE in patients with VV compared with risks in the matched controls. Likewise, after excluding the potential influences of peripheral artery disease, we also found great impacts of mortality and MACE in the population with VV. Finally, increased mortality with higher ICD-9-CM–derived grades indicated that our grading system specifically reflected the severity of VV but the cause of mortality was not available in this database. .

Conclusions

VV are a common condition typically believed to be benign; however, our results suggest

that they warrant close attention. Compared with matched controls, patients with VV were at increasing risks of mortality and cardiovascular events, especially those with VV at grade 3. Therefore, these findings should alert clinicians regarding the importance of detecting VV at an early stage.

Author Contributions

All authors contributed to the revision of the manuscript and approved the final version. All agreed to be accountable. NC Wu contributed to concept and design, critical revision. ZC Chen contributed to data collection and critical revision. IJ Feng contributed to critical revision, data collection, statistical analysis and interpretation. CH Ho contributed to critical revision, data collection, statistical analysis and interpretation. CY Chiang contributed to data collection and critical revision. JJ Wang contributed to data collection and critical revision. WT Chang contributed to concept and design, data collection, interpretation, manuscript writing and critical revision.

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Data availability: All the data is available in National Health Insurance Research Database (NHIRD) in Taiwan

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Figure legends

Figure 1. (A) Kaplan-Meier estimates of 12-year survival between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year survival between patients with VV categorized by the disease severities and the matched control cohort.

Figure 2. (A) Kaplan-Meier estimates of 12-year free from MACE between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year free from MACE between patients with VV categorized by the disease severities and the matched control cohort.

Table 1. Baseline characteristics and comorbid medical disorders for the control cohort and patients with varicose vein (VV).

Characteristic, n (%)	Varicose vein, n = 4644	Matched controls, n = 17742	Standardized difference
Age (years)			0.02275
< 65	3164 (68.13)	12275 (69.19)	
≧65	1480 (31.87)	5467 (30.81)	
Age(mean±SD)	55.70 ± 16.03	56.10 ± 16.04	0.02514
Gender			0.00944
Male	1796 (38.67)	6780 (38.21)	
Female	2848 (61.33)	10962 (61.79)	
Hypertension			0.04519
No	3750 (80.75)	14637 (82.50)	
Yes	894 (19.25)	3105 (17.50)	
Diabetes			0.05807
No	4247 (91.45)	16501 (93.01)	
Yes	397 (8.55)	1241 (6.99)	
Hyperlipidemia			0.08429
No	4413 (95.03)	17157 (96.70)	
Yes	231 (4.97)	585 (3.30)	
Coronary artery disease			0.07832
No	4489 (96.66)	17375 (97.93)	
Yes	155 (3.34)	367 (2.07)	

P-value was calculated based on the two sample t test and Pearson's chi-square test.

Table 2. Crude and adjusted hazard ratios of all-cause mortality in patients with VV compared with the matched control cohort during the follow-up period.

Cohort All (n =22386)	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)*	p-value
Overall analysis				
VV	1.431 (1.247, 1.643)	<0.0001†	1.367 (1.189, 1.572)	<0.0001†
Controls	1[reference]		1[reference]	
Stratified analysis				
Age (years)				
< 65 (years)				
VV	1.487 (1.144, 1.932)	0.0030†	1.202 (0.918, 1.574)	0.1803
Controls	1[reference]		1[reference]	
≧65 (years)				
VV	1.411 (1.199, 1.660)	< 0.0001†	1.377 (1.169, 1.623)	0.0001†
Controls	1[reference]		1[reference]	
Gender				
Male				
VV	1.462 (1.229, 1.738)	< 0.0001†	1.408 (1.182, 1.677)	0.0001†
Controls	1[reference]		1[reference]	
Female				
VV	1.381 (1.098, 1.736)	0.0058†	1.308 (1.038, 1.648)	0.0227†
Controls	1[reference]		1[reference]	
Hypertension				
VV	1.138 (0.869, 1.492)	0.3476	1.157 (0.880, 1.522)	0.2957
Controls	1[reference]		1[reference]	
Diabetes				
VV	1.504 (1.059, 2.137)	0.0226†	1.503 (1.051, 2.148)	0.0254†
Controls	1[reference]		1[reference]	
Hyperlipidemia				
VV	0.992 (0.387, 2.542)	0.9865	1.005 (0.387, 2.610)	0.9914
Controls	1[reference]		1[reference]	
Coronary artery disease				
VV	1.140 (0.640, 2.029)	0.6565	1.051 (0.575, 1.922)	0.8716
Controls	1[reference]		1[reference]	

HR = Hazard Ratio; CI = Confidence Interval

†p<0.05

*Adjusted for chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency

Table 3. Crude and adjusted hazard ratios of major cardiovascular events (MACE) in patients with VV compared with the matched control cohort during the follow-up period

Cohort All (n =22386)	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)*	p-value
Overall analysis				
VV	2.075 (1.912, 2.251)	< 0.0001†	2.053 (1.891, 2.228)	< 0.0001†
Controls	1[reference]		1[reference]	
Stratified analysis				
Age (years)				
< 65 (years)				
VV	2.207 (1.949, 2.499)	< 0.0001†	2.171 (1.916, 2.461)	< 0.0001†
Controls	1[reference]		1[reference]	
≧65 (years)				
VV	1.981 (1.778, 2.208)	< 0.0001†	1.958 (1.756, 2.184)	< 0.0001†
Controls	1[reference]		1[reference]	
Gender				
Male				
VV	2.352 (2.087, 2.651)	< 0.0001†	2.322 (2.059, 2.618)	< 0.0001†
Controls	1[reference]		1[reference]	
Female				
VV	1.866 (1.668, 2.087)	< 0.0001†	1.853 (1.656, 2.074)	< 0.0001†
Controls	1[reference]		1[reference]	
Hypertension				
VV	1.649 (1.418, 1.917)	< 0.0001†	1.621 (1.394, 1.885)	< 0.0001†
Controls	1[reference]		1[reference]	
Diabetes				
VV	1.397 (1.111, 1.757)	0.0042†	1.366 (1.084, 1.721)	0.0081
Controls	1[reference]		1[reference]	
Hyperlipidemia				
VV	1.495 (1.028, 2.175)	0.0353†	1.561 (1.065, 2.289)	0.0224
Controls	1[reference]		1[reference]	
Coronary artery disease				
VV	1.932 (1.380, 2.704)	0.0001†	1.991 (1.407, 2.818)	0.0001†
Controls	1[reference]		1[reference]	

HR = Hazard Ratio; CI = Confidence Interval

†p<0.05

*Adjusted for chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure,

ischemic heart disease, chronic renal insufficiency

Table 4. The adjusted hazard ratios of mortality and major cardiovascular adverse events (MACE) in patients with VV compared with the matched control cohort during the follow-up period.

	Grade 1 Control	Grade 1	Grade 2 Control	Grade 2	Grade 3 Control	Grade 3
No. of mortality, N (%)	343 (3.62)	99 (4.01)	147 (5.79)	44 (6.59)	266 (4.65)	136 (9.01)
Adjusted HR for mortality (95% CI)*	Referent	1.083 (0.864, 1.358)	Referent	1.133 (0.800, 1.603)	Referent	1.833 (1.481, 2.269) †
No. of CHF, N (%)	552 (5.82)	238 (9.65)	181 (7.12)	80 (11.98)	358 (6.26)	190 (12.59)
Adjusted HR for CHF (95% CI)*	Referent	1.680 (1.439, 1.961)†	Referent	1.792 (1.369, 2.345)†	Referent	2.050 (1.711, 2.456) †
No. of ACS, N (%)	291 (3.07)	125 (5.07)	72 (2.83)	24 (3.59)	174 (3.04)	95 (6.30)
Adjusted HR for ACS (95% CI) *	Referent	1.702 (1.376, 2.106)†	Referent	1.247 (0.780, 1.992)	Referent	2.038 (1.576, 2.634) †
No. of ischemic stroke, N (%)	236 (2.49)	99 (4.01)	90 (3.54)	31 (4.64)	162 (2.83)	89 (5.90)
Adjusted HR for ischemic stroke (95% CI) *	Referent	1.586 (1.250, 2.011)†	Referent	1.400 (0.925, 2.118)	Referent	2.063 (1.583, 2.687) †
No. of DVT +PE, N (%)	14 (0.15)	56 (2.27)	7 (0.28)	13 (1.95)	6 (0.10)	63 (4.17)
Adjusted HR for DVT+PE (95% CI) *	Referent	14.896 (8.260, 26.863) †	Referent	6.269 (2.462, 15.959) †	Referent	38.419 (16.376, 90.133) †

†p<0.05; HR = Hazard Ratio; CI = Confidence Interval; CHF=congestive heart failure; ACS= acute coronary syndrome; DVT = deep vein thrombosis; PE= pulmonary embolism

*Adjusted for chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency

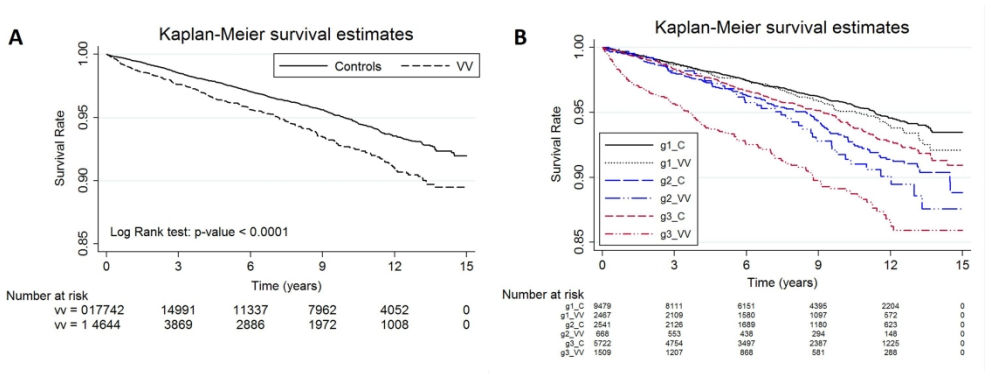

Figure 1. (A) Kaplan-Meier estimates of 12-year survival between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year survival between patients with VV categorized by the disease severities and the matched control cohort.

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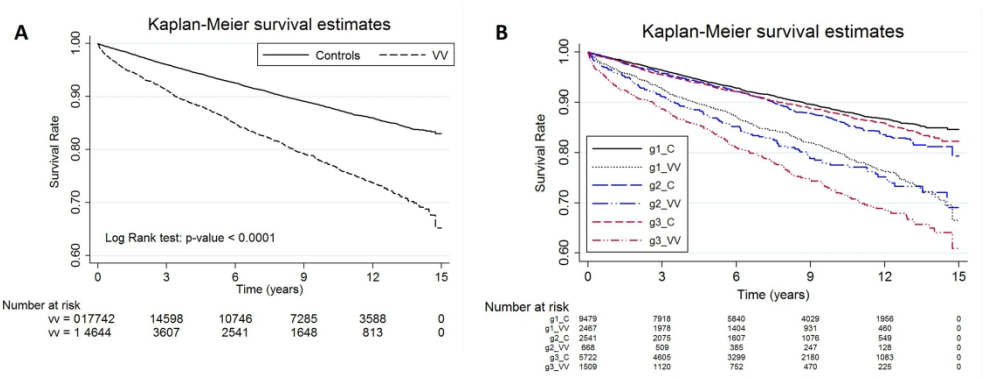

Figure 2. (A) Kaplan-Meier estimates of 12-year free from MACE between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year free from MACE between patients with VV categorized by the disease severities and the matched control cohort.

545x208mm (300 x 300 DPI)

Supplementary Table 1. Clinical characteristics and comorbid medical disorders for patients with VV categorized by the disease severity.

Characteristic	Grade 1, n=2467	Grade 2, n=668	Grade 3, n=1509	
n (%)				**P-value**
Age (years)				< 0.0001
≧65	1756 (71.18)	428 (64.07)	529 (35.06)	
< 65	711 (28.82)	240 (35.93)	980 (64.94)	
Age (mean±SD)	55.57 ± 14.76	57.20 ± 17.45	56.50 ± 17.31	
Gender				0.0332
Male	773 (31.33)	277 (41.47)	746 (49.44)	
Female	1694 (68.67)	391 (58.53)	763 (50.56)	
Hypertension				0.0721
No	2020 (81.88)	539 (80.69)	1191 (78.93)	
Yes	447 (18.12)	129 (19.31)	318 (21.07)	
Diabetes				< 0.0001
No	2296 (93.07)	617 (92.37)	1334 (88.40)	
Yes	171 (6.93)	51 (7.63)	175 (11.60)	
Hyperlipidemia				0.8288
No	2342 (94.93)	633 (94.76)	1468 (95.29)	
Yes	125 (5.07)	35 (5.24)	71 (4.71)	
Coronary artery disease				0.6442
No	2379 (96.43)	648 (97.01)	1462 (96.89)	
Yes	88 (3.57)	20 (2.99)	47 (3.11)	

P-value was calculated based on the two sample t test and Pearson's chi-square test.

Supplement Table 2. Baseline characteristics and comorbid medical disorders for the control cohort and patients with varicose vein (VV).

Characteristic	Varicose vein Grade1	Varicose vein Grade1 control	Standardized difference	Varicose vein Grade2	Varicose vein Grade2 control	Standardized difference	Varicose vein Grade3	Varicose vein Grade3 control	Standardized difference
n (%)	**n = 2467**	**n = 9497**		**n = 668**	**n = 2541**		**n = 1509**	**n = 5722**	
Age (years)			0.02882			0.01557			0.01539
≧65	1756 (71.18)	6870 (72.48)		428 (64.07)	1647 (64.82)		529 (35.06)	1964 (34.32)	
< 65	711 (28.82)	2609 (27.52)		240 (35.93)	894 (35.18)		980 (64.94)	3758 (65.68)	
Age (mean±SD)	55.57 ± 14.76	55.14 ± 14.69	0.02891	57.20 ± 17.45	56.91 ± 17.52	0.01664	56.50 ± 17.31	56.09 ± 17.38	0.02336
Gender			0.00891			0.01014			0.00726
Male	773 (31.33)	2931 (30.92)		277 (41.47)	1041 (40.97)		746 (49.44)	2808 (49.07)	
Female	1694 (68.67)	6548 (69.08)		391 (58.53)	1500 (59.03)		763 (50.56)	2914 (50.93)	
Hypertension			0.03885			0.04022			0.05681
No	2020 (81.88)	7901 (83.35)		539 (80.69)	2090 (82.25)		1191 (78.93)	4646 (81.20)	
Yes	447 (18.12)	1578 (16.65)		129 (19.31)	451 (17.75)		318 (21.07)	1076 (18.80)	
Diabetes			0.04942			0.06897			0.06569
No	2296 (93.07)	8936 (94.27)		617 (92.37)	2391 (94.10)		1334 (88.40)	5174 (90.42)	
Yes	171 (6.93)	543 (5.73)		51 (7.63)	150 (5.90)		175 (11.60)	548 (9.58)	
Hyperlipidemia			0.08073			0.09142			0.08733
No	2342 (94.93)	9153 (96.56)		633 (94.76)	2455 (96.62)		1468 (95.29)	5549 (96.98)	
Yes	125 (5.07)	326 (3.44)		35 (5.24)	86 (3.38)		71 (4.71)	173 (3.02)	
Coronary artery disease			0.07654			0.07737			0.08244
No	2379 (96.43)	9263 (97.72)		648 (97.01)	2495 (98.19)		1462 (96.89)	5617 (98.16)	
Yes	88 (3.57)	216 (2.28)		20 (2.99)	46 (1.81)		47 (3.11)	105 (1.84)	

Supplement Table 3. ICD-9 codes versus chart review diagnosis among outpatients with VV

Code	No.	Clinical diagnosis	No.
454.9 Asymptomatic varicose veins	1188	VV	1188
		Others	0
454.1 With inflammation	775	VV	775
		Others	0
454.0 Varicose veins of lower extremities With ulcer	152	VV	152
		Others	0
454.2 With ulcer and inflammation	87	VV	87
		Others	0

Supplement Table 4. ICD-9 codes-derived severity grading versus Clinical–Etiological–Anatomical–Pathophysiological (CEAP) stages of inpatients with VV

Code	No.	CEAP stage	No.
454.9 Asymptomatic varicose veins	193	C0-2	183
		C3-4	6
		C5-6	4
454.1 With inflammation	56	C0-2	0
		C3-4	56
		C5-6	0
454.0 Varicose veins of lower extremities With ulcer	86	C0-2	6
		C3-4	0
		C5-6	80
454.2 With ulcer and inflammation	12		

	CEAP 0-2	Not CEAP	
Test +	183	10	183/193 (94.8)
Test -	6	136	136/142 (95.7)
	183/189 (96.8)	136/146 (93.1)	

Statistic	**Estimate**	**95% CI**
Sensitivity	96.83%	93.22% to 98.83%
Specificity	93.15 %	87.76% to 96.67%
Positive Predictive Value	94.82%	90.95% to 97.08%
Negative Predictive Value	95.77 %	91.15% to 98.03%

	CEAP 3-4	Not CEAP	
Test +	56	0	56/56 (100)
Test -	6	273	273/279 (97.8)
	56/62 (90.3)	273/273 (100)	

Statistic	**Estimate**	**95% CI**
Sensitivity	90.32%	80.12% to 96.37%
Specificity	100.00 %	98.66% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	97.85 %	95.51% to 98.98%

	CEAP 5-6	Not CEAP	
Test +	80	6	80/86 (93)
Test -	4	245	245/249 (98.4)
	80/84 (95.2)	245/251 (97.6)	

Statistic	**Estimate**	**95% CI**
Sensitivity	95.24%	88.25% to 98.69%
Specificity	97.61 %	94.87% to 99.12%
Positive Predictive Value	93.02%	85.79% to 96.71%
Negative Predictive Value	98.39 %	95.92% to 99.38%

Supplement Table 5. Sensitivity analysis of hazard ratio in patients with VV compared with the matched control cohort

	VV	**Control**
History of pregnancy (ICD-9-CM : V22, V23.2, 761.5)**, N(%)**	169 (3.64)	598 (3.37)
Adjusted HR (95% CI); p-value[2]		
all-cause mortality as endpoint	1.369 (1.191, 1.573); < 0.0001	Reference
MACE as endpoint	2.055 (1.893, 2.231); < 0.0001	Reference
For Grade 3 and matched controls		
all-cause mortality as endpoint	1.838 (1.485, 2.276); < 0.0001	Reference
MACE as endpoint	2.456 (2.138, 2.821); < 0.0001	Reference
Myocardial infarction, stroke, coronary angioplasty or CABG, N(%)	19 (0.41)	457 (2.58)
Adjusted HR (95% CI); p-value[3]		
all-cause mortality as endpoint	1.360 (1.181, 1.567); < 0.0001	Reference
MACE as endpoint	1.952 (1.799, 2.119); < 0.0001	Reference
For Grade 3 and matched controls		
all-cause mortality as endpoint	1.842 (1.487, 2.283) ; < 0.0001	Reference
MACE as endpoint	2.316 (2.018, 2.658); < 0.0001	Reference

HR = Hazard Ratio; CI = Confidence Interval

2 Adjusted for history of pregnancy, age, gender, hypertension, diabetes, hyperlipidemia, coronary artery disease, chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency

3 HR is calculated for population without history of Myocardial infarction, stroke, coronary angioplasty or CABG and the value is adjusted for age, gender, hypertension, diabetes, hyperlipidemia, coronary artery disease, chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	9
		(*d*) If applicable, explain how loss to follow-up was addressed	9
		(*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.

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Severe varicose veins and the risk of mortality: A nationwide population-based cohort study

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Severe varicose veins and the risk of mortality: A nationwide population-based cohort study

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Short title: Outcomes of Varicose Veins

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Abstract

Objective: Varicose veins (VV) are common and although considered benign may cause morbidity. However, the association between VV severity and cardiovascular and mortality risks remains unknown. The aim of this study was to investigate the factors associated with overall mortality in patients with VV.

Methods: A total of 4644 patients with newly diagnosed VV between 1999 and 2013 were identified from Taiwan's National Health Insurance Database. VV severity was classified from grade 1 to 3 according to the presentation of ulcers or inflammation. Moreover, 9497, 2541 and 5722 age-, sex-, and chronic cardiovascular risk factor–matched controls, as assessed based on propensity score, were separately selected for 3 grading VV groups. Enrolled patients were analyzed using conditional Cox proportional hazards regression analysis to estimate risk of mortality and major cardiovascular adverse events (MACE) in the VV and control groups.

Results: Most patients with VV were free from systemic disease. However, compared with matched controls, patients with VV showed a 1.37-times increased risk of mortality (95% CI 1.19–1.57; $p < 0.0001$). Compared with matched controls, older (age≧65 years) (adjusted HR: 1.38; 95% CI: 1.17–1.62; $p = 0.0001$) and male patients with VV (adjusted HR: 1.41; 95% CI: 1.18–1.68; $p = 0.0001$) showed increased risk of mortality. Furthermore, compared with controls, patients with VV showed 2.05-times greater risk of MACE. Compared with matched controls, population at grade 3 increased 1.83 times risk of mortality and 2.04 to 38.42 times risk of heart failure, acute coronary syndrome, ischemic stroke and venous thromboembolism.

Conclusions: This nationwide cohort study demonstrated that patients with VV are at a risk of cardiovascular events and mortality. Our findings suggest that presence of VV warrants close

attention in terms of prognosis and treatment.

Key words: varicose vein, mortality, sex, age, cardiovascular risk

Strengths and limitations of this study

1. The strengths of this study are its population-based design with a large sample size including study and control cohorts.
2. All insurance claims were reviewed by medical reimbursement specialists.
3. However, some risk factors of varicose vein including smoking habits, lack of movement, overweight and glycated hemoglobin levels were not available in this database.
4. Our novel findings indicated that patients at severe grades of varicose vein had higher risks of mortality and major adverse cardiovascular events.
5. The presence of varicose vein should catch more awareness of potential co-existing risks of mortality and cardiovascular events.

Introduction

Varicose veins (VV) can be considered a common disease with prevalence ranging from 2% to 56% in the adult population.[1] Following clinical examination, VV diagnosis is primarily based on the presence of enlarged and twisted veins in the lower extremities.[1,2] Among people with VV, 1% to 4% of individuals show higher severity grades (Clinical–Etiological–Anatomical–Pathophysiological [CEAP] classification, 5–6).[1,2] Although VV lead to leg swelling, venous eczema, and ulceration in some cases, they are regarded as a benign disease.[3,4] Moreover, the association between the severity of VV and risk of future adverse events remains unknown. In fact, the majority of the previous studies have focused on the importance of superficial venous thrombosis or deep vein thrombosis (DVT).[5] In a 30-year cohort study, mortality risk among patients with DVT and pulmonary embolism (PE) was markedly higher than that in age and sex-matched patients, particularly within the first 30 days.[6] Similarly, another population-based case-control study demonstrated that having VV was a risk factor for venous thromboembolism, although the association of VV severity with survival and cardiovascular events remains unknown.[7] In addition, although age, family history, and female sex are the known risk factors for VV, the effects of underlying diseases or sex on outcomes of VV remain unclear.[1] We hypothesized that presence of VV can be used as a marker for cardiovascular risk. Therefore, the aim of this study was to investigate the association of VV with survival and cardiovascular outcomes.

Methods

Data Source

Taiwan launched a single-payer National Health Insurance (NHI) program on March 1, 1995. This database contains details of almost every Taiwanese resident (coverage rate >98% in 2009), making it one of the world's largest and most complete population-based sources. The data used in this study were retrieved from the Longitudinal Health Insurance Database 2000 (LHID2000)—a subset of the NHI database containing all claims data from 1996 to 2013, covering 1 million beneficiaries randomly selected in 2000. At that time, there were no significant differences in age, sex, and health care costs between patients with VV and matched controls. LHID2000 provided encrypted patient identification numbers; sex; date of birth; admission and discharge dates; International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes of diagnoses and procedures; prescription details; registry data in the Catastrophic Illness Patient Database; and costs covered and paid for by NHI. Details of the National Health Insurance Research Databases (NHIRD) are described in previous studies.[8,9] Moreover, the accuracy of major disease diagnoses in the NHIRD, including stroke and acute coronary syndrome, has been validated.[9] The present study was ethically approved by the Institutional Review Board of Chi-Mei Hospital (CV code: 10406-E01). All procedures followed the principles outlined in the Declaration of Helsinki.

Patient and Public Involvement

No patient involved.

Study Design

This nationwide population-based, retrospective cohort study was conducted to investigate the association between VV and subsequent mortality. Patients with at least 3 claims for outpatient VV diagnosis in 1 year or with 1 claim for inpatient VV diagnosis (ICD-9-CM codes

454, 454.0, 454.1, 454.2, 454.8, and 454.9) were considered as VV cases. Patients with a first-time diagnosis of VV from January 1999 to December 2012 were included in the cohort. Codes for VV were considered reliable for diagnosis based on clinical symptoms. The date of the first-time VV diagnosis was considered the index date in this study. To ensure accurate VV diagnosis, and to avoid potentially confounding effects, patients with DVT (ICD-9-CM codes 453.40, 459.1, 671.4, 671.3, 451.83, 459.3, 453.4, and 451.11) or PE (ICD-9-CM codes 415.1, 415.11, 673, 673.2, and 673.8) in an ambulatory setting before the index date were excluded. In addition, VV severity was categorized as grade 1 uncomplicated (ICD-9-CM code 454.9), grade 2 with ulcer (ICD-9-CM code 454.0) or inflammation (ICD-9-CM code 454.1), and grade 3 with both ulcer and inflammation (ICD-9-CM code 454.2).

Three control cohorts (n1=9497, n2=2541 and n3=5722; 4 control subjects for every enrolled patient with VV), not diagnosed with VV from 1996 to 2013, were selected for three VV grade groups separately. To eliminate potential selection bias, the controls were matched using propensity score method at a 4:1 ratio for baseline characteristics of age, sex, and chronic cardiovascular risk factors, including hypertension (ICD-9-CM codes 401–405, A260, A269, 4372), diabetes (ICD-9-CM codes 250, A181, A189, A229, A239, 3572, and 3620), hyperlipidemia (ICD-9-CM code 272), and coronary artery disease (CAD; ICD-9-CM codes 410–414). The propensity scores (PS) for identified VV cases and controls were estimated using the fitting logistic regression model. Based on greedy algorithm matching, 8 control subjects (the nearest neighbor matching of VV) were selected as matched controls.[10] If a case failed to be assigned to the 4 matched controls, it was dropped from the set of matches. In addition, since the primary VV treatment was covered by insurance, it prevented VV from over-diagnosis. The matched controls were assigned the same index date as that of the corresponding VV patient.

Outcomes

The primary outcome was mortality, and the secondary outcome was major cardiovascular adverse events (MACEs), including acute coronary syndrome (ACS, ICD-9-CM codes 410, 410.7, 411.1, 411.81, and 414.8), congestive heart failure (CHF, ICD-9-CM codes 428, 428.0, 428.1, 428.2, and 428.9), ischemic stroke (ICD-9-CM code 436), DVT, and PE. Mortality was identified using the "in-hospital death" or "discharge under critical condition" codes at discharge. Enrollment in the NHI program is mandatory for all people in Taiwan, and registration must be withdrawn within 30 days after death. Patients with the abovementioned mortality-related codes and those withdrawn from the NHI program within 30 days after discharge from the last hospitalization were presumed to have died. All subjects were followed up from the index date to death (lost to follow-up) or until December 31, 2013, whichever was earlier.

Validation of the Accuracy of VV Diagnosis and CEAP Grading

To validate the accuracy of the VV diagnosis, we reviewed the charts of all patients (inpatients and outpatients) using ICD-9-CM diagnosis codes for VV who visited Chi-Mei Medical Center (Tainan, Taiwan) from 2010 to 2015. Our aim was to determine the accuracy and consistency of code usage. A vascular specialist reviewed patient discharge and clinical records. In addition to examining the accuracy of VV diagnosis, the reviewer compared CEAP stages with our ICD-9-CM–derived grades in inpatients. Subsequently, we further investigated the sensitivity, specificity, and predictive value of the ICD codes for clinical diagnosis, as well as the applicability of our VV grading system. In particular, as ICD-9-CM coding and VV descriptions are associated with insurance payment, the accuracy of VV diagnosis and the reliability of VV

severity grading increased. The consistency between CEAP and grading stages were evaluated by kappa score, whose value between 0.8 and 1.0 was considered as an almost perfect agreement.

Statistical Analyses

Continuous and categorical baseline characteristics between the case and control groups were separately compared by standardized mean difference (SMD), an assessment approach for evaluating the balance between variables after PS matching. SMD greater than 0.1 is considered to denote a meaningful imbalance in variables.

Conditional Cox proportional hazards regression analysis was used to estimate the risk of mortality and MACE in the VV and control groups. Adjusted hazard ratios (HRs) were estimated by adjusting for chronic obstructive pulmonary disease (ICD-9-CM codes 490-496) , cancer (ICD-9-CM codes 140-208), atrial fibrillation (ICD-9-CM codes 427.31), heart failure (ICD-9-CM codes 428), ischemic heart disease (ICD-9-CM codes 410-414), chronic renal insufficiency (ICD-9-CM codes 403, 404, 582, 585-588). Moreover, the investigation was extended to stratified subgroup analysis. HRs between the VV and control groups were separately estimated in subgroups of population aged <65 years or ≧65 years; males or females; and subgroups with or without a diagnosis of hypertension, diabetes, hyperlipidemia, or CAD. The Kaplan-Meier method was used to separately estimate the 3-, 6-, and 9-year survival rates in the control and VV groups. Kaplan-Meier curves of mortality and MACE were plotted for controls and patients with 3 grades of VV severity. Differences in survival curves between the control and VV groups were examined using the log-rank test.

With respect to mortality, CHF, ACS, ischemic stroke and DVT +PE endpoints, the risks

for VV with 3 separate severity grades were further estimated by comparison against each matched controls.

Finally, sensitivity analyses were conducted to determine the influence from subjects with pregnancy history (ICD-9-CM codes V22, V23.2, 761.5), peripheral artery disease (PAD, ICD-9-CM codes 440.0, 440.2, 440.3, 440.8, 440.9, 443, 444.0, 444.22, 444.8, 447.8, and 447.9) medical history and patients treated with operations (ICD-9-CM procedure code 3859, 3889 and NHIRD order code 69013, 69014, 69015, 69016, 69017, 69019, 69020, 69021) including ligation and stripping procedures after VV diagnosis.

A two-tailed p value of <0.05 was considered statistically significant. All analyses were performed using the SAS software, version 9.4 (SAS Institute, Cary, NC) and Stata software 15.0 (StataCorp, College Station, TX.)

Results

Characteristics of the Study Population

A total of 4644 patients with newly diagnosed VV were identified during January 1999 to December 2012. Among them, 2467, 668 and 1509 VVs were separately classified into 1, 2 and 3 severity grade. For each VV group, age-, sex-, and chronic disease–matched patients without VV were separately included for comparison. The covariates between VV and matched groups are well balanced after propensity score matching. All patients were tracked from the index dates until achieving the primary outcomes or the end of the study. The mean age of patients with VV was 55.70 ± 16.03 years, the majority of the patients were female (61.33%), and most of them did not present with chronic diseases such as hypertension, diabetes, hyperlipidemia, and CAD (Table 1). Significantly different distribution of age, sex and diabetes among three severity VV

groups were displayed (p-value < 0.05) (Supplementary Table 1). Interestingly, more female patients (68.67%) were diagnosed with a lower severity (grade 1). Also, the baseline characteristics and comorbid medical disorders for 3 grading VV groups and 3 separately matched controls were listed in Supplement Table 2.

Long-Term Mortality Risk

Compared with matched controls, the outcomes of patients with VV were worse. The estimated survival at 3, 6, and 9 years were 97.6%, 95.6%, and 93.5%, respectively, in patients with VV compared with 98.5%, 97.1%, and 95.6%, respectively, in controls (Figure 1A). A log-rank test revealed a significant difference in survival curves of patients with VV and controls (p <0.0001). The survival curves of controls and patients with different severities of VV are presented in Figure 1B. Lower survival rates over time were observed in patients with highest VV severity (grades 3) but not in those with grade 1-2. Significant difference between survival curves between VV grading 3 and corresponding controls were revealed by log rank test (p < 0.0001). However, no significant differences were found between survival curves of patients with VV severity grades (1-2) and corresponding controls (grade 1: p = 0.3191; grade 2: p=0.3599).

Overall, HR of all-cause mortality adjusted for chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency in patients with VV was 1.34 times higher (adjusted HR: 1.37; 95% CI: 1.19–1.57; p < 0.0001) than that in controls (Table 2). Stratified analysis revealed 1.38- and 1.41-times increased risks of mortality in older (age ≧65 years; adjusted HR: 1.38; 95% CI: 1.17–1.62; p = 0.0001) and male

patients with VV (adjusted HR: 1.41; 95% CI: 1.18–1.68; p = 0.0001). Notably, despite no significant effect of VV on the survival of patients with hypertension, hyperlipidemia or coronary artery disease was observed, patients with both VV and diabetes presented 1.50 times higher risk of mortality compared with those without VV (adjusted HR: 1.50; 95% CI: 1.05–2.15; p = 0.0254). Furthermore, VV at grade 3 show 1.83 (95% CI : 1.48, 2.27 ; p < 0.0001) greater risk of mortality adjusted for chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency.

Long-Term MACE risk

MACE risk significantly increased in patients with VV (HR: 2.05; 95% CI: 1.89–2.23; p < 0.0001), particularly in relatively younger (age, <65 years; adjusted HR: 2.17; 95% CI: 1.92–2.46; p < 0.0001) or male (adjusted HR: 2.32; 95% CI: 2.06–2.62; p < 0.0001) patients (Table 3). In addition, patients with VV showing cardiovascular risk factors, including hypertension, diabetes, hyperlipidemia, and CAD, were at a higher risk of MACE than were matched controls. In patients with VV, 3-, 6-, and 9-year MACE-free rates were 91.17%, 84.99%, and 79.27%(Figure 2A). These rates dramatically declined further with disease severity (Figure 2B). In terms of individual cardiovascular outcomes, patients with grade 3 VV were at a greater risk of CHF (adjusted HR: 2.05; 95% CI: 1.71–2.46; p < 0.0001), ACS (adjusted HR: 2.04; 95% CI: 1.58–2.63; p < 0.0001), and ischemic stroke (adjusted HR: 2.06; 95% CI: 1.58–2.69; p < 0.0001) than were controls (Table 4). In particular, with the highest VV severity there was an increasing risk of venous thrombotic events, including DVT and PE (Grade 3: adjusted HR: 38.4; 95% CI, 16.4–90.1; p < 0.0001) (Table 4).

Validation of the Accuracy of VV Diagnosis and ICD-9-CM–Derived VV Grading

During 2010–2015 a total of 2202 outpatients and 347 inpatients were reported to have VV in Chi-Mei Medical Center. Among the outpatients, 1188 were coded as uncomplicated VV (ICD-9-CM code 454.9), 775 were coded as VV with inflammation (ICD-9-CM code 454.1), 152 were coded as VV with ulcers (ICD-9-CM code 454.0), and 87 were coded as VV with ulcer and inflammation (ICD-9-CM code 454.2) (Supplement Table 3). Notably, none were coded incorrectly. Compared with CEAP stage, as determined based on chart reviews, only a few inpatients were incorrectly or unclearly diagnosed using ICD-9-CM–derived VV codes (Supplement Table 4). For example, among patients with higher VV grades (CEAP stage 5–6), the positive and negative predictive values with ICD-9-CM–derived codes were 93% and 98.4%, respectively. Specifically, the sensitivity and specificity of ICD-9-CM–derived grading were up to 95.2% and 97.6%, respectively. The calculated kappa score between CEAP stages and grading severity is 0.92 (95%CI = [0.88, 0.96]).

Sensitivity analyses

VV and controls with pregnancy history were identified and exam the influence in sensitivity analysis (Supplement Table 5). After additionally adjustment for history of pregnancy, the results remain showing great impacts on mortality and MACE (adjusted HR for death (95%CI) = 1.37 (1.19, 1.57), p-value<0.0001; adjusted HR for MACE (95%CI) = 2.01 (1.89, 2.23), p-value<0.0001).

After excluding 472 subjects with Myocardial infarction, stroke, coronary angioplasty or

CABG, remaining VV and corresponding controls were included for sensitivity analysis. Comparing with corresponding matched controls, those conservatively treated VV patients were found 1.36 times risks of mortality (adjusted HR (95%CI) = 1.36 (1.18, 1.57), p-value<0.0001) and 1.95 times risks of MACE (adjusted HR (95%CI) = 1.95 (1.80, 2.12), p-value<0.0001).

Discussion

The primary findings of this study were that (1) patients with VV were at increasing risks of mortality and cardiovascular events, especially those with VV at grade 3 compared with matched controls; (2) having VV had a significant impact on the survival of male patients. To the best of our knowledge, this nationwide population-based study is the first to comprehensively describe the association of VV with patients' cardiovascular outcomes.

Although VV are common, their potential threat to health has not been well investigated previously.[1,2] Valve dysfunction-mediated activation of leukocytes, release of enzymes, and remodeling of the vascular wall lead to venous valve destruction and incompetence.[11] VV may cause inflammation, edema, ulcers,[11] endothelial dysfunction,[12] and subsequent DVT.[5] In addition, overexpression of inducible nitric oxide synthase and transforming growth factor-β1 has been documented in patients with VV.[13] In this study, the risk of all-cause mortality and MACE was higher in patients with VV than it was in matched controls, indicating that VV-induced systemic inflammation may be associated with cardiovascular events regardless of the development of venous thromboembolic events. Notably, the lower survival rates were observed in patients with highest VV severity but not in those with grade 1-2. This also reflects that the chronic inflammation induced by a higher grade of VV may be associated with increasing

mortality and MACEs. However, only a few studies have compared development of VV with arterial disease and reported inconsistent findings.[2,14] A previous study in Finland has reported a two-fold higher incidence of new arterial disease in individuals with VV than in those without it, although the incidence of new hypertension was similar.[14,15] Thus, VV and arterial disease may have a common etiology, but VV were not related to hypertension. Furthermore, Chang et al. have reported the association of VV with the incidence of venous thromboembolism and peripheral artery disease.[16] Reportedly, myocardial infarction and heart failure increase the risk of thromboembolism.[17] In contrast, patients with thromboembolic events were at a higher risk of subsequent myocardial infarction and stroke.[18] However, whether this association is causal or represents common risk factors warrants further research. Notably, compared with controls, patients with VV were at a higher risk of mortality independent of age and sex. Specifically, the significant impact of VV was observed in male patients. In previous research, older age and female sex were found to be the most relevant risk factors for VV.[1] VV incidence increases with increasing age. However, Heit et al. have reported that younger patients with VV were at a significantly increased risk of subsequent DVT, whereas the risk was attenuated with increasing age.[19] Similarly, Lohr et al also reported that although female presented with a higher prevalence of lower grade VV (CEAP 2-3) compared with male (50.5% v.s. 30.1%), there were more higher grade VVs with trophic skin changes (CEAP 4-6) found in male than in female (5.4% v.s. 2.8%).[20] Also, DVT was more common in males compared with females (11.3% vs 7.8%).[20] Earlier onset of VV in the younger population implies a higher risk of concomitant arterial diseases or systemic inflammations. As described previously, female sex, pregnancy, and predominately being in the sitting posture are risk factors for VV.[21] However, despite the valid correlation between use of estrogen supplements and DVT, whether sex hormones contribute to

the development of VV remains unclear.

There were several strengths of this study. First, we included an unselected, large, nationwide cohort of patients with VV. By including the data of 4644 patients over a 12-year period, this study provided adequate statistical power for the analysis of long-term outcomes for VV. Second, we compared the VV cohort with a matched, VV-free cohort, which helped distinguish the characteristics of the VV population in terms of survival and outcomes. Third, among patients with VV, the effects of sex on mortality and MACE were emphasized because VV may have been ignored in these specific populations. Forth, we included patients presenting with VV of various severity grades, which allowed for a comprehensive investigation of overall effects of severity. Finally, a recently published article evaluated and supported the accuracy of several major outcomes, including MI, hypertension, diabetes, stroke, CHF and VV, in NHIRD.[22]

However, this study had several limitations. According to previous meta-analysis and research, smoking habits, quality of life, lack of movement, pregnancy history, overweight and glycated hemoglobin levels are considered VV risk factors, with some of these being related to increased mortality risk. Although NHIRD provides a complete clinical medical history over decades for 1 million people, currently the NHIRD lacks information regarding people's lifestyle and clinical laboratory test results. Therefore, the selected confounders in this study were limited to age, sex, and four chronic cardiovascular risk factors. The small corresponding area under the receiver operating characteristic curve indicated that the relevant confounders were not appropriately identified. To explore the effects of VV on mortality and MACE with minimum confounding bias, a future study including more comprehensive VV-related risk factors is

imperative. Second, the miscoding of VV severity may have led to the exclusion of cases. This might explain why 47% of the included patients are with advanced venous disease (Grade 2 or 3), different from the general distribution of disease severity. Nevertheless, to overcome the inherent limitations, we verified the accuracy of VV diagnosis using chart review by a specialist. Overall, both the validation methods indicated a satisfactory accuracy of VV coding in the NHI database. Third, owing to difficulties in completing CEAP staging according to ICD-9, we established our own grading system. However, even though this novel ICD-9-CM–derived grading system clearly differentiated patients with various severities, it remained different from the generally applied CEAP staging system and disease progression could hardly be represented. Similarly, to validate the reliability of the ICD-9-CM–derived grading system, we reviewed medical records of inpatients with VV and observed satisfactory sensitivity and specificity. Forth, while ligation and stripping surgeries may affect the outcomes, through excluding patients receiving surgical treatment for VV we performed sensitivity test. It also revealed significant increases of risks of mortality and MACE in patients with VV compared with risks in the matched controls. Likewise, after excluding the potential influences of peripheral artery disease, we also found great impacts of mortality and MACE in the population with VV. Finally, increased mortality with higher ICD-9-CM–derived grades indicated that our grading system specifically reflected the severity of VV but the cause of mortality was not available in this database. .

Conclusions

VV are a common condition typically believed to be benign; however, our results suggest

that they warrant close attention. Compared with matched controls, patients with VV were at increasing risks of mortality and cardiovascular events, especially those with VV at grade 3. Therefore, these findings should alert clinicians regarding the importance of detecting VV at an early stage.

Author Contributions

All authors contributed to the revision of the manuscript and approved the final version. All agreed to be accountable. NC Wu contributed to concept and design, critical revision. ZC Chen contributed to data collection and critical revision. IJ Feng contributed to critical revision, data collection, statistical analysis and interpretation. CH Ho contributed to critical revision, data collection, statistical analysis and interpretation. CY Chiang contributed to data collection and critical revision. JJ Wang contributed to data collection and critical revision. WT Chang contributed to concept and design, data collection, interpretation, manuscript writing and critical revision.

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Data availability: All the data is available in National Health Insurance Research Database (NHIRD) in Taiwan

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Figure legends

Figure 1. (A) Kaplan-Meier estimates of 12-year survival between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year survival between patients with VV categorized by the disease severities and the matched control cohort.

Figure 2. (A) Kaplan-Meier estimates of 12-year free from MACE between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year free from MACE between patients with VV categorized by the disease severities and the matched control cohort.

Table 1. Baseline characteristics and comorbid medical disorders for the control cohort and patients with varicose vein (VV).

Characteristic, n (%)	Varicose vein, n = 4644	Matched controls, n = 17742	Standardized difference
Age (years)			0.02275
< 65	3164 (68.13)	12275 (69.19)	
≧65	1480 (31.87)	5467 (30.81)	
Age(mean±SD)	55.70 ± 16.03	56.10 ± 16.04	0.02514
Gender			0.00944
Male	1796 (38.67)	6780 (38.21)	
Female	2848 (61.33)	10962 (61.79)	
Hypertension			0.04519
No	3750 (80.75)	14637 (82.50)	
Yes	894 (19.25)	3105 (17.50)	
Diabetes			0.05807
No	4247 (91.45)	16501 (93.01)	
Yes	397 (8.55)	1241 (6.99)	
Hyperlipidemia			0.08429
No	4413 (95.03)	17157 (96.70)	
Yes	231 (4.97)	585 (3.30)	
Coronary artery disease			0.07832
No	4489 (96.66)	17375 (97.93)	
Yes	155 (3.34)	367 (2.07)	

P-value was calculated based on the two sample t test and Pearson's chi-square test.

Table 2. Crude and adjusted hazard ratios of all-cause mortality in patients with VV compared with the matched control cohort during the follow-up period.

Cohort All (n =22386)	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)*	p-value
Overall analysis				
VV	1.43 (1.25, 1.64)	<0.0001†	1.37 (1.19, 1.57)	<0.0001†
Controls	1[reference]		1[reference]	
Stratified analysis				
Age (years)				
< 65 (years)				
VV	1.49 (1.144, 1.93)	0.0030†	1.2 (0.92, 1.57)	0.1803
Controls	1[reference]		1[reference]	
≧65 (years)				
VV	1.41 (1.2, 1.66)	< 0.0001†	1.38 (1.17, 1.62)	0.0001†
Controls	1[reference]		1[reference]	
Gender				
Male				
VV	1.46 (1.23, 1.74)	< 0.0001†	1.41 (1.181, 1.68)	0.0001†
Controls	1[reference]		1[reference]	
Female				
VV	1.38 (1.1, 1.74)	0.0058†	1.31 (1.04, 1.65)	0.0227†
Controls	1[reference]		1[reference]	
Hypertension				
VV	1.14 (0.87, 1.49)	0.3476	1.16 (0.88, 1.52)	0.2957
Controls	1[reference]		1[reference]	
Diabetes				
VV	1.5 (1.06, 2.14)	0.0226†	1.5 (1.05, 2.15)	0.0254†
Controls	1[reference]		1[reference]	
Hyperlipidemia				
VV	0.99 (0.39, 2.54)	0.9865	1.01 (0.39, 2.61)	0.9914
Controls	1[reference]		1[reference]	
Coronary artery disease				
VV	1.14 (0.64, 2.03)	0.6565	1.05 (0.58, 1.92)	0.8716
Controls	1[reference]		1[reference]	

HR = Hazard Ratio; CI = Confidence Interval

†p<0.05

*Adjusted for chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency

Table 3. Crude and adjusted hazard ratios of major cardiovascular events (MACE) in patients with VV compared with the matched control cohort during the follow-up period

Cohort All (n =22386)	Crude HR (95% CI)	p-value	Adjusted HR (95% CI)*	p-value
Overall analysis				
VV	2.08 (1.91, 2.25)	< 0.0001†	2.05 (1.89, 2.23)	< 0.0001†
Controls	1[reference]		1[reference]	
Stratified analysis				
Age (years)				
< 65 (years)				
VV	2.21 (1.95, 2.5)	< 0.0001†	2.17 (1.92, 2.46)	< 0.0001†
Controls	1[reference]		1[reference]	
≧65 (years)				
VV	1.98 (1.78, 2.21)	< 0.0001†	1.96 (1.76, 2.18)	< 0.0001†
Controls	1[reference]		1[reference]	
Gender				
Male				
VV	2.35 (2.09, 2.65)	< 0.0001†	2.32 (2.06, 2.62)	< 0.0001†
Controls	1[reference]		1[reference]	
Female				
VV	1.87 (1.67, 2.09)	< 0.0001†	1.85 (1.66, 2.07)	< 0.0001†
Controls	1[reference]		1[reference]	
Hypertension				
VV	1.65 (1.42, 1.92)	< 0.0001†	1.62 (1.39, 1.89)	< 0.0001†
Controls	1[reference]		1[reference]	
Diabetes				
VV	1.4 (1.11, 1.76)	0.0042†	1.37 (1.08, 1.72)	0.0081
Controls	1[reference]		1[reference]	
Hyperlipidemia				
VV	1.5 (1.03, 2.17)	0.0353†	1.56 (1.07, 2.29)	0.0224
Controls	1[reference]		1[reference]	
Coronary artery disease				
VV	1.93 (1.38, 2.7)	0.0001†	1.99 (1.41, 2.82)	0.0001†
Controls	1[reference]		1[reference]	

HR = Hazard Ratio; CI = Confidence Interval

†p<0.05

*Adjusted for chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure,

ischemic heart disease, chronic renal insufficiency

Table 4. The adjusted hazard ratios of mortality and major cardiovascular adverse events (MACE) in patients with VV compared with the matched control cohort during the follow-up period.

	Grade 1 Control	Grade 1	Grade 2 Control	Grade 2	Grade 3 Control	Grade 3
No. of mortality, N (%)	343 (3.62)	99 (4.01)	147 (5.79)	44 (6.59)	266 (4.65)	136 (9.01)
Adjusted HR for mortality (95% CI)*	Referent	1.08 (0.86, 1.36)	Referent	1.13 (0.8, 1.6)	Referent	1.83 (1.48, 2.27) †
No. of CHF, N (%)	552 (5.82)	238 (9.65)	181 (7.12)	80 (11.98)	358 (6.26)	190 (12.59)
Adjusted HR for CHF (95% CI)*	Referent	1.68 (1.44, 1.96)†	Referent	1.79 (1.37, 2.34)†	Referent	2.05 (1.71, 2.46) †
No. of ACS, N (%)	291 (3.07)	125 (5.07)	72 (2.83)	24 (3.59)	174 (3.04)	95 (6.30)
Adjusted HR for ACS (95% CI) *	Referent	1.7 (1.37, 2.11)†	Referent	1.25 (0.78, 1.99)	Referent	2.04 (1.58, 2.63) †
No. of ischemic stroke, N (%)	236 (2.49)	99 (4.01)	90 (3.54)	31 (4.64)	162 (2.83)	89 (5.90)
Adjusted HR for ischemic stroke (95% CI) *	Referent	1.59 (1.25, 2.01)†	Referent	1.4 (0.92, 2.12)	Referent	2.06 (1.58, 2.69) †
No. of DVT +PE, N (%)	14 (0.15)	56 (2.27)	7 (0.28)	13 (1.95)	6 (0.10)	63 (4.17)
Adjusted HR for DVT+PE (95% CI) *	Referent	14.9 (8.26, 26.86) †	Referent	6.27 (2.46, 15.96) †	Referent	38.42 (16.38, 90.13) †

†p<0.05; HR = Hazard Ratio; CI = Confidence Interval; CHF=congestive heart failure; ACS= acute coronary syndrome; DVT = deep vein thrombosis; PE= pulmonary embolism

*Adjusted for chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency

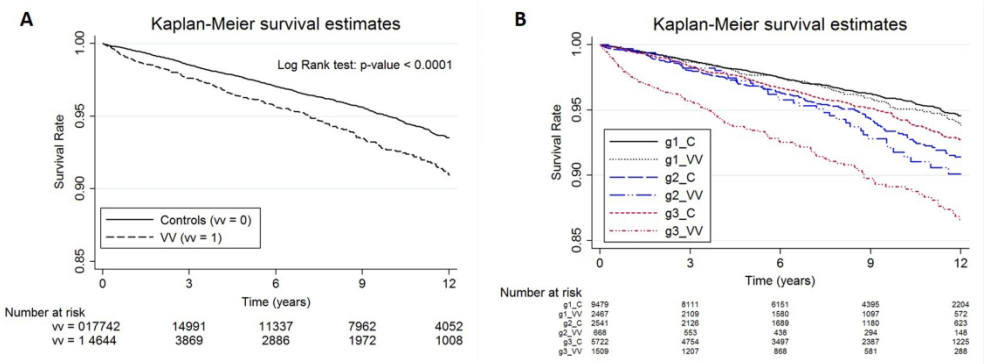

Figure 1. (A) Kaplan-Meier estimates of 12-year survival between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year survival between patients with VV categorized by the disease severities and the matched control cohort.

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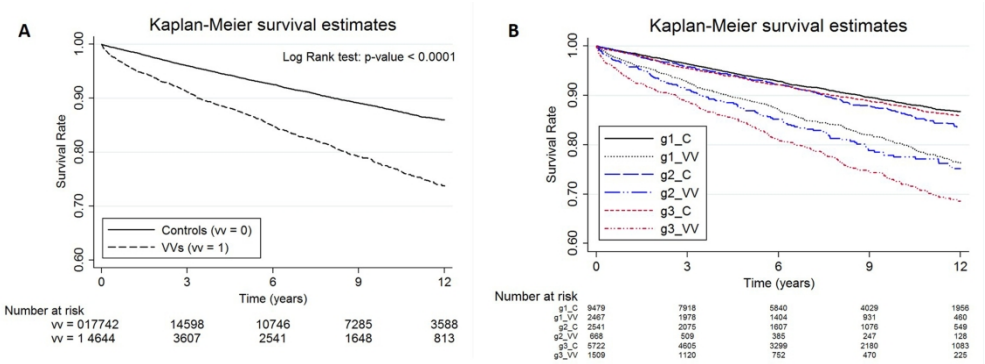

Figure 2. (A) Kaplan-Meier estimates of 12-year free from MACE between patients with varicose veins (VV) and the matched control cohort. (B) Kaplan-Meier estimates of 12-year free from MACE between patients with VV categorized by the disease severities and the matched control cohort.

314x116mm (300 x 300 DPI)

Supplementary Table 1. Clinical characteristics and comorbid medical disorders for patients with VV categorized by the disease severity.

Characteristic	Grade 1, n=2467	Grade 2, n=668	Grade 3, n=1509	
n (%)				**P-value**
Age (years)				< 0.0001
≧65	1756 (71.18)	428 (64.07)	529 (35.06)	
< 65	711 (28.82)	240 (35.93)	980 (64.94)	
Age (mean±SD)	55.57 ± 14.76	57.20 ± 17.45	56.50 ± 17.31	
Gender				0.0332
Male	773 (31.33)	277 (41.47)	746 (49.44)	
Female	1694 (68.67)	391 (58.53)	763 (50.56)	
Hypertension				0.0721
No	2020 (81.88)	539 (80.69)	1191 (78.93)	
Yes	447 (18.12)	129 (19.31)	318 (21.07)	
Diabetes				< 0.0001
No	2296 (93.07)	617 (92.37)	1334 (88.40)	
Yes	171 (6.93)	51 (7.63)	175 (11.60)	
Hyperlipidemia				0.8288
No	2342 (94.93)	633 (94.76)	1468 (95.29)	
Yes	125 (5.07)	35 (5.24)	71 (4.71)	
Coronary artery disease				0.6442
No	2379 (96.43)	648 (97.01)	1462 (96.89)	
Yes	88 (3.57)	20 (2.99)	47 (3.11)	

P-value was calculated based on the two sample t test and Pearson's chi-square test.

Supplement Table 2. Baseline characteristics and comorbid medical disorders for the control cohort and patients with varicose vein (VV).

Characteristic	Varicose vein Grade1	Varicose vein Grade1 control	Standardized difference	Varicose vein Grade2	Varicose vein Grade2 control	Standardized difference	Varicose vein Grade3	Varicose vein Grade3 control	Standardized difference
n (%)	**n = 2467**	**n = 9497**		**n = 668**	**n = 2541**		**n = 1509**	**n = 5722**	
Age (years)			0.02882			0.01557			0.01539
≧65	1756 (71.18)	6870 (72.48)		428 (64.07)	1647 (64.82)		529 (35.06)	1964 (34.32)	
< 65	711 (28.82)	2609 (27.52)		240 (35.93)	894 (35.18)		980 (64.94)	3758 (65.68)	
Age (mean±SD)	55.57 ± 14.76	55.14 ± 14.69	0.02891	57.20 ± 17.45	56.91 ± 17.52	0.01664	56.50 ± 17.31	56.09 ± 17.38	0.02336
Gender			0.00891			0.01014			0.00726
Male	773 (31.33)	2931 (30.92)		277 (41.47)	1041 (40.97)		746 (49.44)	2808 (49.07)	
Female	1694 (68.67)	6548 (69.08)		391 (58.53)	1500 (59.03)		763 (50.56)	2914 (50.93)	
Hypertension			0.03885			0.04022			0.05681
No	2020 (81.88)	7901 (83.35)		539 (80.69)	2090 (82.25)		1191 (78.93)	4646 (81.20)	
Yes	447 (18.12)	1578 (16.65)		129 (19.31)	451 (17.75)		318 (21.07)	1076 (18.80)	
Diabetes			0.04942			0.06897			0.06569
No	2296 (93.07)	8936 (94.27)		617 (92.37)	2391 (94.10)		1334 (88.40)	5174 (90.42)	
Yes	171 (6.93)	543 (5.73)		51 (7.63)	150 (5.90)		175 (11.60)	548 (9.58)	
Hyperlipidemia			0.08073			0.09142			0.08733
No	2342 (94.93)	9153 (96.56)		633 (94.76)	2455 (96.62)		1468 (95.29)	5549 (96.98)	
Yes	125 (5.07)	326 (3.44)		35 (5.24)	86 (3.38)		71 (4.71)	173 (3.02)	
Coronary artery disease			0.07654			0.07737			0.08244
No	2379 (96.43)	9263 (97.72)		648 (97.01)	2495 (98.19)		1462 (96.89)	5617 (98.16)	
Yes	88 (3.57)	216 (2.28)		20 (2.99)	46 (1.81)		47 (3.11)	105 (1.84)	

Supplement Table 3. ICD-9 codes versus chart review diagnosis among outpatients with VV

Code	No.	Clinical diagnosis	No.
454.9 Asymptomatic varicose veins	1188	VV	1188
		Others	0
454.1 With inflammation	775	VV	775
		Others	0
454.0 Varicose veins of lower extremities With ulcer	152	VV	152
		Others	0
454.2 With ulcer and inflammation	87	VV	87
		Others	0

Supplement Table 4. ICD-9 codes-derived severity grading versus Clinical–Etiological–Anatomical–Pathophysiological (CEAP) stages of inpatients with VV

Code	No.	CEAP stage	No.
454.9 Asymptomatic varicose veins	193	C0-2	183
		C3-4	6
		C5-6	4
454.1 With inflammation	56	C0-2	0
		C3-4	56
		C5-6	0
454.0 Varicose veins of lower extremities With ulcer	86	C0-2	6
		C3-4	0
		C5-6	80
454.2 With ulcer and inflammation	12		

	CEAP 0-2	Not CEAP	
Test +	183	10	183/193 (94.8)
Test -	6	136	136/142 (95.7)
	183/189 (96.8)	136/146 (93.1)	

Statistic	**Estimate**	**95% CI**
Sensitivity	96.83%	93.22% to 98.83%
Specificity	93.15 %	87.76% to 96.67%
Positive Predictive Value	94.82%	90.95% to 97.08%
Negative Predictive Value	95.77 %	91.15% to 98.03%

	CEAP 3-4	Not CEAP	
Test +	56	0	56/56 (100)
Test -	6	273	273/279 (97.8)
	56/62 (90.3)	273/273 (100)	

Statistic	**Estimate**	**95% CI**
Sensitivity	90.32%	80.12% to 96.37%
Specificity	100.00 %	98.66% to 100.00%
Positive Predictive Value	100.00%	
Negative Predictive Value	97.85 %	95.51% to 98.98%

	CEAP 5-6	Not CEAP	
Test +	80	6	80/86 (93)
Test -	4	245	245/249 (98.4)
	80/84 (95.2)	245/251 (97.6)	

Statistic	Estimate	95% CI
Sensitivity	95.24%	88.25% to 98.69%
Specificity	97.61 %	94.87% to 99.12%
Positive Predictive Value	93.02%	85.79% to 96.71%
Negative Predictive Value	98.39 %	95.92% to 99.38%

Supplement Table 5. Sensitivity analysis of hazard ratio in patients with VV compared with the matched control cohort

	VV	**Control**
History of pregnancy (ICD-9-CM : V22, V23.2, 761.5)**, N(%)**	169 (3.64)	598 (3.37)
Adjusted HR (95% CI); p-value[2]		
all-cause mortality as endpoint	1.37 (1.19, 1.57); < 0.0001	Reference
MACE as endpoint	2.06 (1.89, 2.23); < 0.0001	Reference
For Grade 3 and matched controls		
all-cause mortality as endpoint	1.84 (1.48, 2.28); < 0.0001	Reference
MACE as endpoint	2.46 (2.14, 2.82); < 0.0001	Reference
Myocardial infarction, stroke, coronary angioplasty or CABG, N(%)	19 (0.41)	457 (2.58)
Adjusted HR (95% CI); p-value[3]		
all-cause mortality as endpoint	1.36 (1.18, 1.57); < 0.0001	Reference
MACE as endpoint	1.95 (1.8, 2.12); < 0.0001	Reference
For Grade 3 and matched controls		
all-cause mortality as endpoint	1.84 (1.49, 2.28) ; < 0.0001	Reference
MACE as endpoint	2.32 (2.02, 2.66); < 0.0001	Reference

HR = Hazard Ratio; CI = Confidence Interval

2 Adjusted for history of pregnancy, age, gender, hypertension, diabetes, hyperlipidemia, coronary artery disease, chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency

3 HR is calculated for population without history of Myocardial infarction, stroke, coronary angioplasty or CABG and the value is adjusted for age, gender, hypertension, diabetes, hyperlipidemia, coronary artery disease, chronic obstructive pulmonary disease, cancer, atrial fibrillation, heart failure, ischemic heart disease, chronic renal insufficiency

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies***

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	5
Objectives	3	State specific objectives, including any prespecified hypotheses	5
Methods			
Study design	4	Present key elements of study design early in the paper	6
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	6
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	6
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	6
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6
Bias	9	Describe any efforts to address potential sources of bias	6
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	7
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	8
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	9
		(*d*) If applicable, explain how loss to follow-up was addressed	9
		(*e*) Describe any sensitivity analyses	9
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	10
		(b) Give reasons for non-participation at each stage	10
		(c) Consider use of a flow diagram	10
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	10
		(b) Indicate number of participants with missing data for each variable of interest	10
		(c) Summarise follow-up time (eg, average and total amount)	10
Outcome data	15*	Report numbers of outcome events or summary measures over time	10

Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	11
		(*b*) Report category boundaries when continuous variables were categorized	11
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	11
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	11
Discussion			
Key results	18	Summarise key results with reference to study objectives	12
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	13
Generalisability	21	Discuss the generalisability (external validity) of the study results	14
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.