

# Prevalence of homocysteinemia and effect of vitamin supplementation in retinal vein occlusion

Maxwell Levi ,<sup>1,2</sup> Amro A Harb,<sup>3</sup> Andrew Trippiedi ,<sup>1,4</sup> Sophia Rodriguez,<sup>1</sup> Nicholas Vianna,<sup>1</sup> Lisa M Higgins,<sup>1</sup> Lauren Kallina,<sup>1</sup> Lee Angioletti,<sup>1</sup> Justin Gutman,<sup>1</sup> Patrick M Higgins<sup>1</sup>

**To cite:** Levi M, Harb AA, Trippiedi A, et al. Prevalence of homocysteinemia and effect of vitamin supplementation in retinal vein occlusion. *BMJ Open Ophthalmology* 2022;7:e001139. doi:10.1136/bmjophth-2022-001139

Received 12 August 2022  
Accepted 11 October 2022

## ABSTRACT

**Objective** The objective of this study was to determine the prevalence of homocysteinemia in patients with retinal vein occlusion (RVO). We investigated the association of B complex vitamin (BCV) and multivitamin (MVI) supplementation on homocysteine levels in RVO patients.

**Methods and analysis** This cross-sectional study occurred at the Retina Center of New Jersey. We investigated 312 patients diagnosed with RVO between 2011 and 2019. Homocysteine levels were measured on diagnosis of RVO and 4–8 weeks after, following recommended daily supplementation with BCV, MVI or combination MVI+BCV.

**Results** The median patient age was 71.00, IQR (61.00, 78.25), with 164 (52.6%) being women. Prevalence of homocysteinemia ( $>13\text{ }\mu\text{mol/L}$ ) was 150 of 312 (48.1%), with a median baseline homocysteine level of 12.80 (10.17, 15.90)  $\mu\text{mol/L}$ . The follow-up cohort, 105 patients, demonstrated significant reduction in median homocysteine levels from 14.50 (12.30, 17.90) to 10.70 (9.30, 13.50)  $\mu\text{mol/L}$  following vitamin supplementation. Multivariate analysis found having baseline homocysteinemia was significantly associated with at least 25% reduction in homocysteine following vitamin supplementation.

**Conclusions** In this study, 48.1% of patients with RVO had elevated homocysteine ( $>13\text{ }\mu\text{mol/L}$ ). Supplementation with BCV or MVI+BCV was associated with a significant reduction in homocysteine from 14.50 (12.30, 17.90) to 10.70 (9.30, 13.50)  $\mu\text{mol/L}$ .

## INTRODUCTION

Retinal vein occlusions (RVOs) are severe causes of visual impairment that occur more frequently in the elderly population.<sup>1,2</sup> RVOs affect vessels of different sizes and can be segmented into six main categories, including ischaemic central retinal vein occlusion (CRVO), non-ischaemic CRVO, ischaemic branch retinal vein occlusion (BRVO), non-ischaemic BRVO, ischaemic Hemi-CRVO and non-ischaemic Hemi-CRVO.<sup>3</sup> The aetiology underlying RVOs is not well understood. Previous studies have demonstrated that risk factors associated with RVOs include age, hypertension, elevated homocysteine levels,

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Elevated homocysteine levels may lead to a hypercoagulable state, with some studies reporting this to be an independent risk factor for arterial and venous thrombotic diseases, such as stroke and coronary artery disease. Data from previous studies investigating the relationship between elevated homocysteine levels and incidence of retinal vein occlusion (RVO) have been mixed.

## WHAT THIS STUDY ADDS

⇒ Comparing our results to the findings from the National Health and Nutrition Examination Survey dataset suggest a higher prevalence of homocysteinemia in patients with RVO than similarly aged adults in the USA general population. B vitamin supplementation was associated with a significant decrease in homocysteinemia in this subpopulation; while the effects of vitamin supplementation on lowering homocysteine levels have been reported in the general population,<sup>25</sup> our study identified a research gap in the present literature, as we could not find any studies demonstrating homocysteine levels in patients with RVO at baseline and following supplementation with B complex vitamin and multivitamin.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Patients who present with RVO may warrant broader medical examination for diseases related to underlying hypercoagulable states including the testing of baseline homocysteine levels, which are not currently covered by Medicare, and further research is needed to determine if homocysteine reduction with vitamin supplementation would be effective in reducing the incidence or improving the disease progression in patients with RVO.

open angle glaucoma, obesity, cardiovascular disease, diabetes mellitus, dyslipidaemia and antiphospholipid antibodies.<sup>4–9</sup> It has been postulated that patients with clotting cascade mutations involving factor V Leiden and prothrombin, in addition to patients with deficiencies in physiological coagulation



© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

<sup>1</sup>Ophthalmology, Retina Center of New Jersey, Bloomfield, New Jersey, USA

<sup>2</sup>Ophthalmology, Eastern Virginia Medical School, Norfolk, Virginia, USA

<sup>3</sup>Columbia University Vagelos College of Physicians and Surgeons, New York City, New York, USA

<sup>4</sup>Hackensack Meridian School of Medicine, Nutley, New Jersey, USA

Correspondence to  
Dr Patrick M Higgins;  
Higginsmds@gmail.com



inhibitors such as plasminogen activator inhibitor, may also be at higher risk for RVO.<sup>7 10 11</sup>

Elevated homocysteine levels may lead to a hypercoagulable state, with some studies reporting this to be an independent risk factor for arterial and venous thrombotic diseases, such as stroke and coronary artery disease.<sup>12–14</sup> Data from previous studies investigating the relationship between elevated homocysteine levels and incidence of RVO have been mixed, with some suggesting a positive association,<sup>10 15–17</sup> or no association.<sup>18</sup> Similarly, mixed findings have also been published regarding the relationship between C677T and A1298C methylenetetrahydrofolate reductase (MTHFR) genotype polymorphisms and RVO.<sup>15–19</sup>

Homocysteine is an amino acid derived from methionine whose metabolism is facilitated by three enzymes: methionine synthetase, MTHFR and cystathione synthetase.<sup>20</sup> Folate, vitamin B12, vitamin B6 and flavin adenine dinucleotide are coenzymes necessary for these reactions.<sup>20</sup> It has been reported that elevated levels of homocysteine may lead to vascular disease via a combination of vascular endothelial dysfunction, vascular smooth muscle proliferation and coagulation abnormalities, leading to venous thrombosis.<sup>21</sup> Predisposing factors of elevated homocysteine levels include diseases such as hypothyroidism, renal failure, proliferative diseases, and type II diabetes mellitus, drugs such as metformin, estrogens, methotrexate, levodopa, and alcohol, vitamin deficiencies including folate, B12, B6 and polymorphisms of the MTHFR gene.<sup>22</sup> It has been reported that a homozygous C677T polymorphism may result in a 70% decrease in MTHFR enzyme function,<sup>23</sup> and a compound heterozygous A1298C and C677T polymorphism may result in a 50%–60% decrease in MTHFR enzyme function.<sup>24</sup>

Supplementation with the coenzymes necessary for homocysteine metabolism, specifically vitamins B12, B6 and B9, may increase the efficiency of the viable homocysteine metabolising pathways enough to reduce homocysteine levels to a normal level. It has been reported that folic acid supplementation (0.5–5 mg/day) can reduce total homocysteine levels by 25%, and B12 supplementation (0.5 mg/day) can reduce total homocysteine levels by an additional 7%, with a combination therapy reducing homocysteine levels up to 33%.<sup>25 26</sup> Hence, the objective of this study was to determine the prevalence of elevated homocysteine levels in patients with RVO and to investigate the association of supplementation with B complex vitamins (BCVs), multivitamins (MVs) or a combination of both (MVI+BCV) on homocysteine levels in these patients.

## MATERIALS AND METHODS

### Study design

This was a cross-sectional study in which patients with RVO were tested for elevated homocysteine levels and followed for 4–8 weeks after recommendation for vitamin supplementation.

### Treatment and vitamin supplementation

On diagnosis of RVO, all patients were treated to the standard of care. Patients presenting with macular oedema were treated with intravitreal injections of bevacizumab, ranibizumab or aflibercept. Patients without macular oedema or decrease in vision were monitored and not treated with intravitreal injection.

Following standard of care treatment, patients were recommended to supplement their diets with a BCV containing B12, B6 and B9 and/or an MVI. The BCV formulations recommended were either FABB, TL Guard and Folplex, all containing 25 mg B6 as pyridoxine HCl, 2.2 mg B9 as folic acid and 1 mg B12 as cyanocobalamin. The MVI formulation recommended was Centrum Silver.

Homocysteine levels were recorded at baseline and at 4–8 weeks after initiation of vitamin supplementation for patients who returned to the clinic and reported following a supplementation regimen. All vitamins were advised to be taken daily for the entirety of this period. These patients were divided into three cohorts depending on which regimen they reported following: MVI only, BCV only or a combination therapy of MVI+BCV.

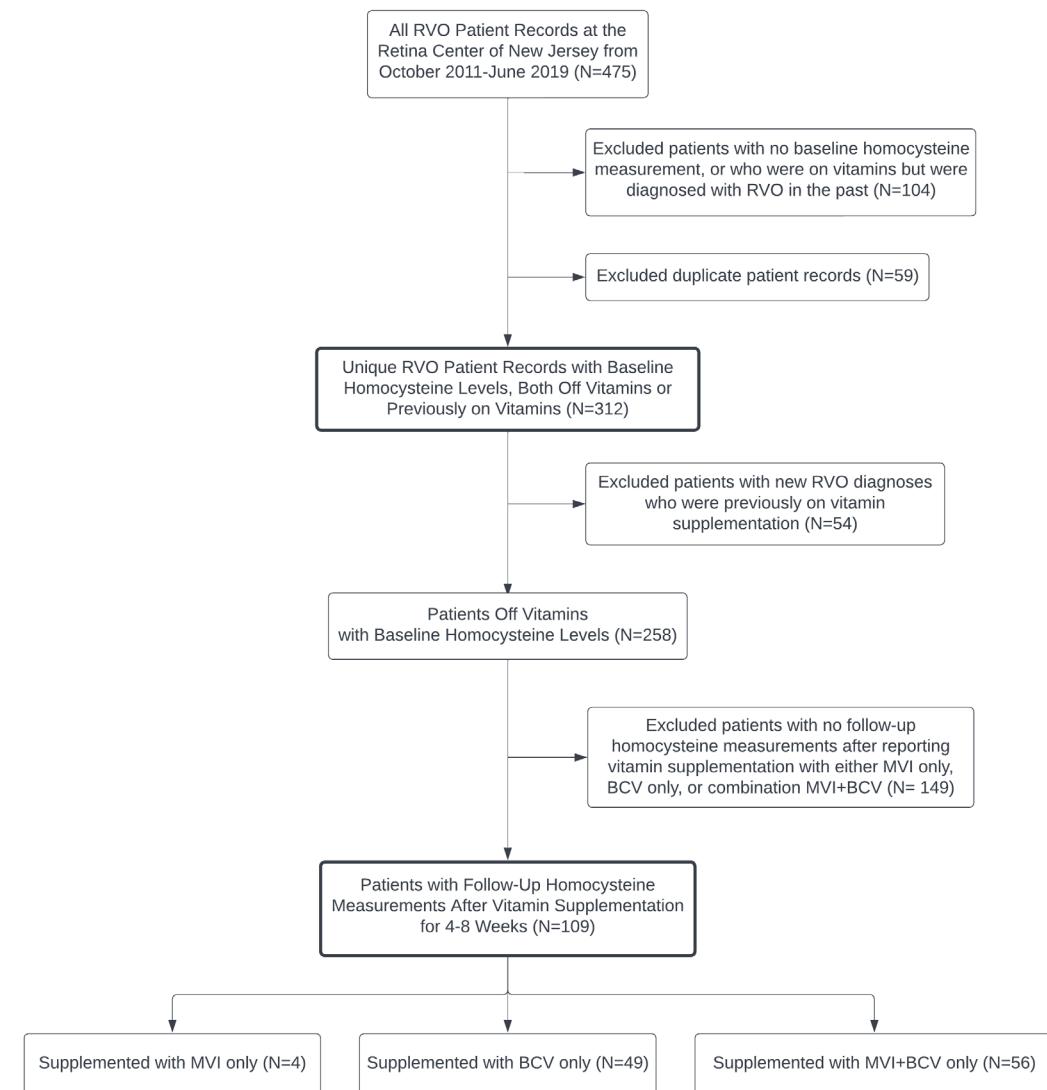
### Diagnostic criteria

RVOs were diagnosed via ophthalmoscopic fundus examination and fluorescein angiography. Ophthalmoscopic fundus examination assessed for dilated or tortuous veins, disc swelling and hyperaemia, cystoid macular oedema, retinal haemorrhages, microaneurysms, cotton-wool spots, and retinal and iris neovascularisation. Fluorescein angiography evaluated increased venous transit time, areas of capillary closure and venous filling defects.

Fasting homocysteine levels were measured, and patients were considered to have elevated homocysteine (homocystinemia) if their total fasting plasma homocysteine level was >13 µmol/L. MTHFR polymorphisms were assessed via PCR and restriction enzyme analysis.

### Study population selection

We identified 475 consecutive patient records with a diagnosis of BRVO, CRVO or Hemi-CRVO who presented to the Retina Center of New Jersey from October 2011 to June 2019. We excluded all patients who did not have baseline data for homocysteine levels and patients who had pre-existing RVO before the defined study period (N=104). We then excluded all duplicate patient records (N=59), yielding a total study population of 312 unique patients for our baseline characteristic analyses. For our follow-up analyses on the association of vitamin supplementation on homocysteine levels in RVO patients, we identified 109 patients who were previously untreated with MVI or BCV who had baseline and follow-up homocysteine levels after 4–8 weeks of reporting either MVI only, BCV only or combination MVI+BCV supplementation. Since there were only four patients who reported vitamin supplementation with MVI only, we excluded these patients from our follow-up cohort, yielding a total



**Figure 1** Study population. Flowchart of study population selection. BCV, B complex vitamin; MVI, multivitamin; RVO, retinal vein occlusion.

of 105 patients. A flowchart of our study population selection process is displayed in figure 1.

### Statistical analysis

We first characterised the demographics, RVO type, comorbidities, MTHFR genotype and complete blood counts (CBCs) for our primary case series with baseline homocysteine levels, stratified by age. Specifically, we characterised patients age $\geq$ 60 in one cohort, and patients age<60 in the other cohort, as well as the total study population. We then characterised the demographics, prevalence of RVO type and reduction in homocysteine levels for our follow-up series with homocysteine levels after vitamin supplementation, stratified by the regimen followed (MVI only, BCV only or combination MVI+BCV). Since there were only four patients who reported supplementation with MVI only, we solely

characterised patients who were on BCV only or combination MVI+BCV therapy.

We created paired boxplots to compare the median homocysteine levels before and after vitamin supplementation, stratified by the regimen followed (BCV only or combination MVI+BCV). We then created boxplots to compare the calculated medians of differences in homocysteine levels after vitamin supplementation. We used multiple logistic regression to analyse if age $\geq$ 60 versus age<60 was associated with the primary outcome of this study, which was at least 25% reduction in homocysteine levels after vitamin supplementation. We chose 25% as a cut-off based on the results of a large meta-analysis finding that folic acid supplementation reduced homocysteine concentration by 25%.<sup>26</sup> We created three models: an unadjusted model, a model adjusted for sex and a final model additionally adjusted for homocysteinemia, BRVO

versus CRVO/Hemi-CRVO and any MTHFR polymorphism. We created a forest plot to visualise the ORs for the final model.

We used R V4.0.1 for our statistical analyses.<sup>27–30</sup> We reported categorical variables as proportions (%) and continuous variables as medians (IQR). We used the  $\chi^2$  test with Yates' continuity correction or Fisher's exact test as appropriate to compare categorical variables. We used the Mann-Whitney U/Wilcoxon rank-sum tests to compare continuous variables and our boxplots of the medians of differences in homocysteine levels by regimen of vitamin supplementation followed. The statistical test used for our paired boxplots comparing median homocysteine levels before and after vitamin supplementation was the Wilcoxon signed-rank test. We considered  $p<0.05$  to be statistically significant.

### Patient and public involvement

Patients were not directly involved with the research process. This study was conducted as a retrospective cross-sectional study. Research questions were developed through physician experience with managing RVO and influenced by the previous literature. Patients, their priorities, experiences and preferences played no role in the development of research questions. Patient's data was deidentified with all analysis blinded. Patients were not involved with the design and conduct of the study, choice of outcome measures or recruitment. Patients were not directly contacted in regard to this study and therefore were not asked about the burden or time involved regarding participation. Patients were not involved in planning for the dissemination of the study results; however, patients will be shown aggregated results of the study. In the interest of increasing patient and public involvement, we will elucidate from our population how to best involve them in the future.

## RESULTS

### Baseline characteristics

**Table 1** displays the baseline characteristics of the entire case series, stratified by two cohorts: age $\geq 60$  and age $<60$ . Of the 312 patients with RVO and baseline homocysteine levels in this study, the median age was 71.00 (61.00, 78.25), with 241 (77.2%) being age $\geq 60$  and 164 (52.6%) being women. There were 141 patients with BRVO, 83 of these being ischaemic; 136 patients with CRVO, 68 of these being ischaemic; and 35 patients with Hemi-CRVO, 21 of these being ischaemic. The median baseline homocysteine level for the entire case series was 12.80 (10.17, 15.90)  $\mu\text{mol/L}$ , with 150 (48.1%) patients found to have homocysteinemia. Of these 312 patients with baseline homocysteine levels, 258 were not taking BCV or MVI supplementation at the time of RVO diagnosis and had a median baseline homocysteine level of 13.50 (11.00, 16.90)  $\mu\text{mol/L}$ . The other 54 patients who were taking BCV or MVI at the time of RVO diagnosis had a median baseline homocysteine level of 9.90 (7.90, 11.80)  $\mu\text{mol/L}$ . A variety of MTHFR genotypes were identified

in this study, with 246 (81.5%) patients having at least one MTHFR polymorphism. All CBCs had median values within normal range.

Notably, homocysteinemia was more prevalent in patients age $\geq 60$  (53.9% vs 28.2%,  $p<0.001$ ), with median baseline homocysteine levels being higher as well (13.40 (10.70, 16.50) vs 11.60 (8.10, 13.35),  $p<0.001$ ). Hypertension was more prevalent in patients age $\geq 60$  (87.0% vs 72.5%,  $p=0.007$ ). Having at least one MTHFR polymorphism was more prevalent in patients age $\geq 60$  (84.0% vs 72.3%,  $p=0.05$ ). There were no significant differences between patients age $\geq 60$  and  $<60$  regarding sex, race/ethnicity, hypercholesterolaemia, diabetes and individual MTHFR polymorphisms. Similarly, we did not identify associations for most CBCs, except for median RBC and platelet levels.

### Association of vitamin supplementation on homocysteine levels

Of the 105 patients in our follow-up cohort, 56 reported using combination MVI+BCV and 49 reported using BCV only. There were no significant differences in demographics between patients who reported using combination MVI+BCV and BCV only (**table 2**). Within each regimen followed, there was a statistically significant decrease in median homocysteine levels following supplementation with BCV only (-3.40 (-5.20, -2.10)  $\mu\text{mol/L}$ ) and combination MVI+BCV (-3.30 (-6.12, -1.80)  $\mu\text{mol/L}$ , **figure 2A**). Supplementation with BCV only reduced median homocysteine levels from 14.40 (12.30, 17.90) to 11.10 (9.30, 13.70)  $\mu\text{mol/L}$  ( $p<0.001$ , **figure 2A**), while combination MVI+BCV supplementation reduced median homocysteine levels from 14.60 (12.23, 18.90) to 10.35 (9.40, 13.20)  $\mu\text{mol/L}$  ( $p<0.001$ , **figure 2A**). When comparing the medians of differences in homocysteine reduction for each regimen followed, there was no statistically significant difference ( $p=0.992$ , **figure 2B**).

Analysis of the entire follow-up cohort revealed the median homocysteine level before vitamin supplementation was 14.50 (12.30, 17.90) and 10.70 (9.30, 13.50)  $\mu\text{mol/L}$  after vitamin supplementation, a significant reduction (**table 2**). Of these patients, 69 (65.7%) met the criteria for homocysteinemia before vitamin supplementation, while 29 (27.6%) met the criteria after vitamin supplementation, and hence vitamin supplementation was associated with a significant decrease in homocysteinemia within this subpopulation ( $p<0.001$ ).

### Multiple logistic regression

In our three models, the ORs for a homocysteine level reduction of at least 25% were not statistically significant when comparing patients age $\geq 60$  vs age $<60$ . In our final adjusted model, this OR was 1.69 (95% CI: 0.57 to 5.26). All covariate ORs in our final adjusted model are displayed in a forest plot in **figure 3**. We did not find any statistically significant ORs for most of the other covariates (age, sex and any MTHFR polymorphism) except

**Table 1** Baseline characteristics

	Total RVO patients (N=312)	Patients age<60 (N=71)	Patients age≥60 (N=241)	P value
Demographics				
Age (median (IQR))	71.00 (61.00, 78.25)	54.00 (48.00, 57.00)	74.00 (69.00, 80.00)	<0.001
BMI (median (IQR))*	27.90 (24.32, 31.30)	30.41 (26.63, 36.72)	27.61 (24.09, 30.70)	<0.001
Sex (%)				
Female	164 (52.6)	38 (53.5)	126 (52.3)	0.961
Race/ethnicity (%)†				
White	163 (52.6)	36 (50.7)	127 (53.1)	0.822
Black	48 (15.5)	12 (16.9)	36 (15.1)	0.850
Hispanic	84 (27.1)	18 (25.4)	66 (27.6)	0.822
Other	15 (4.8)	5 (7.0)	10 (4.2)	0.347
Smoking status (%)‡				
Former or current	87 (38.5)	16 (36.4)	71 (39.0)	0.880
Type of RVO (%)				
CRVO (non-ischaemic)	68 (21.8)	18 (25.4)	50 (20.7)	
CRVO (ischaemic)	68 (21.8)	13 (18.3)	55 (22.8)	
Hemi-CRVO (non-ischaemic)	14 (4.5)	3 (4.2)	11 (4.6)	
Hemi-CRVO (ischaemic)	21 (6.7)	2 (2.8)	19 (7.9)	
BRVO (non-ischaemic)	58 (18.6)	18 (25.4)	40 (16.6)	
BRVO (ischaemic)	83 (22.6)	17 (23.9)	66 (27.4)	
Comorbidities (%)				
Hypertension§	258 (83.8)	50 (72.5)	208 (87.0)	0.007
Hypercholesterolaemia¶	175 (70.0)	34 (68.0)	141 (70.5)	0.863
Diabetes**	102 (39.5)	22 (40.0)	80 (39.4)	1.000
Homocysteine				
Baseline levels (median (IQR))	12.80 (10.17, 15.90)	11.60 (8.10, 13.35)	13.40 (10.70, 16.50)	<0.001
Homocysteinemia	150 (48.1)	20 (28.2)	130 (53.9)	<0.001
MTHFR polymorphisms (%) ††				
Any MTHFR polymorphism	246 (81.5)	47 (72.3)	199 (84.0)	0.050
A1298C (heterozygous)	68 (22.5)	20 (30.8)	48 (20.3)	0.103
C1298C (homozygous)	20 (6.6)	4 (6.2)	16 (6.8)	1.000
C677T (heterozygous)	69 (22.8)	13 (20.0)	56 (23.6)	0.652
T677T (homozygous)	34 (11.3)	3 (4.6)	31 (13.1)	0.091
A1298C/C677T	57 (18.9)	9 (13.8)	48 (20.3)	0.322
CBC‡‡				
WBC (median (IQR))	6.50 (5.30, 7.70)	6.35 (5.15, 7.70)	6.50 (5.40, 7.70)	0.483
RBC (median (IQR))	4.60 (4.27, 4.89)	4.78 (4.50, 4.98)	4.51 (4.23, 4.85)	0.001
Platelets (median (IQR))	232.00 (191.00, 282.00)	251.50 (212.75, 302.50)	228.00 (188.00, 275.00)	0.006
Haemoglobin (median (IQR))	13.50 (12.40, 14.60)	13.95 (12.62, 14.83)	13.30 (12.30, 14.60)	0.118
Haematocrit (median (IQR))	40.30 (37.50, 43.60)	41.15 (37.75, 43.58)	40.00 (37.50, 43.60)	0.169

\*BMI data missing for 78 (22 age&lt;60, 56 age≥60) patients.

†Ethnicity data missing for two patients age≥60.

‡Smoking status missing for 86 (27 age&lt;60, 59 age≥60) patients.

§Hypertension data missing for four (two age&lt;60, two age≥60) patients.

¶Hypercholesterolaemia data missing for 62 (21 age&lt;60, 41 age≥60) patients.

\*\*Diabetes data missing for 54 (16 age&lt;60, 38 age≥60) patients.

††MTHFR genotyping missing for 10 (6 age&lt;60, 4 age≥60) patients.

‡‡CBC lab data missing for 39 (11 age&lt;60, 28 age≥60) patients.

BMI, body mass index; BRVO, branch retinal vein occlusion; CBC, complete blood count; CRVO, central retinal vein occlusion; MTHFR, methylenetetrahydrofolate reductase; RVO, retinal vein occlusion.

**Table 2** Effect of vitamin supplementation in follow-up patients

	Total follow-up patients (N=105)	Reported BCV use only (N=49)	Reported combination MVI+BCV use (N=56)	P value
Demographics				
Age (median (IQR))	72.00 (65.00, 79.00)	72.00 (66.00, 79.00)	71.00 (65.00, 77.25)	0.338
BMI (median (IQR))*	28.44 (24.33, 32.47)	28.34 (25.93, 32.67)	29.30 (24.33, 32.42)	0.835
Sex (%)				
Female	55 (52.4)	26 (53.1)	29 (51.8)	1.000
Race/ethnicity (%)				
White	52 (49.5)	20 (40.8)	32 (57.1)	0.141
Black	17 (16.2)	8 (16.3)	9 (16.1)	1.000
Hispanic	32 (30.5)	19 (38.8)	13 (23.2)	0.130
Other	4 (3.8)	2 (4.1)	2 (3.6)	1.000
Type of RVO (%)				
CRVO (non-ischaemic)	26 (24.8)	15 (30.6)	11 (19.6)	
CRVO (ischaemic)	30 (28.6)	16 (32.7)	14 (25.0)	
Hemi-CRVO (non-ischaemic)	3 (2.9)	0 (0.0)	3 (5.4)	
Hemi-CRVO (ischaemic)	10 (9.5)	0 (0.0)	10 (17.9)	
BRVO (non-ischaemic)	20 (19.0)	7 (14.3)	13 (23.2)	
BRVO (ischaemic)	16 (15.2)	11 (22.4)	5 (8.9)	
Homocysteinemia (%)				
Off vitamins	69 (65.7)	33 (67.3)	36 (64.3)	0.902
On vitamins	29 (27.6)	13 (26.5)	16 (28.6)	0.988
Homocysteine levels				
Off vitamins (median (IQR))	14.50 (12.30, 17.90)	14.40 (12.30, 17.90)	14.60 (12.23, 18.90)	0.893
On vitamins (median (IQR))	10.70 (9.30, 13.50)	11.10 (9.30, 13.70)	10.35 (9.40, 13.20)	0.755
Median of differences (median (IQR))	-3.30 (-5.40, -1.90)	-3.40 (-5.20, -2.10)	-3.30 (-6.12, -1.80)	0.992

\*BMI data missing for 14 BCV use only patients, 3 combination MVI+BCV use patients.

BCV, B complex vitamin; BRVO, branch retinal vein occlusion; CRVO, central retinal vein occlusion; MVI, multivitamin; RVO, retinal vein occlusion.

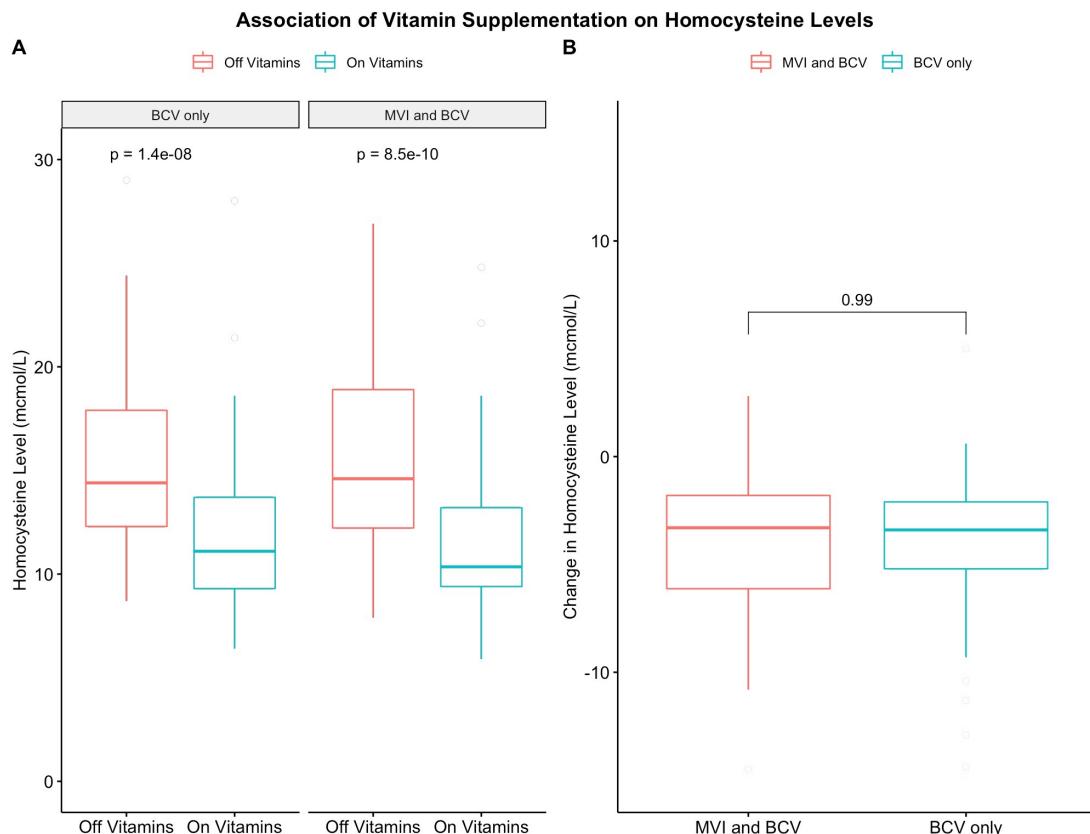
for homocysteinemia, which had an adjusted OR of 4.26 (95% CI: 1.74 to 11.14).

## DISCUSSION

The prevalence of homocysteinemia in this population of 312 patients with RVO was 150 (48.1%), with a median baseline homocysteine level of 12.80 (10.17, 15.90)  $\mu\text{mol/L}$ . In the general US population, data from the National Health and Nutrition Examination Survey (NHANES) found a prevalence of homocysteinemia in 18.5% (95% CI: 16.3% to 21.0%) of adults  $\geq 60$  between 2003 and 2006.<sup>31</sup> In our study, for 241 adults  $\geq 60$ , the prevalence of homocysteinemia was 130 (53.9%). In the NHANES dataset, for adults from ages 40–59, the prevalence of homocysteinemia was 7.0% (95% CI: 5.6% to 8.0%), while in our study the prevalence of homocysteinemia was 20 of 71 (28.2%) in similarly aged adults  $< 60$ .<sup>31</sup> Homocysteine levels increase with age due to a variety of factors, including decreased renal function and nutrition, and so this trend was expected in both our results and in NHANES.<sup>32</sup> Importantly, the NHANES dataset defined

elevated homocysteine as  $> 13 \mu\text{mol/L}$ ,<sup>31</sup> the same cut-off used in our study. Comparing our results to the findings from the NHANES dataset suggest a higher prevalence of homocysteinemia in patients with RVO than similarly aged adults in the US general population. Considering that elevated homocysteine levels are associated with arterial and venous thrombotic diseases, such as stroke and coronary artery disease, patients who present with RVO may warrant broader medical examination for related diseases.<sup>10–12</sup> Additionally, the relatively high prevalence of homocysteinemia in RVO patients provides a strong rationale for testing baseline homocysteine levels in this patient population, a lab not currently covered by Medicare.<sup>33</sup>

Regarding the effect of vitamin supplementation on homocysteine levels in RVO patients, there was a statistically significant decrease in median homocysteine levels following recommendation of BCV only or combination MVI+BCV supplementation (figure 2A). Overall, in the follow-up cohort of 105 patients, 69 patients (65.7%) met the criteria for homocysteinemia before vitamin



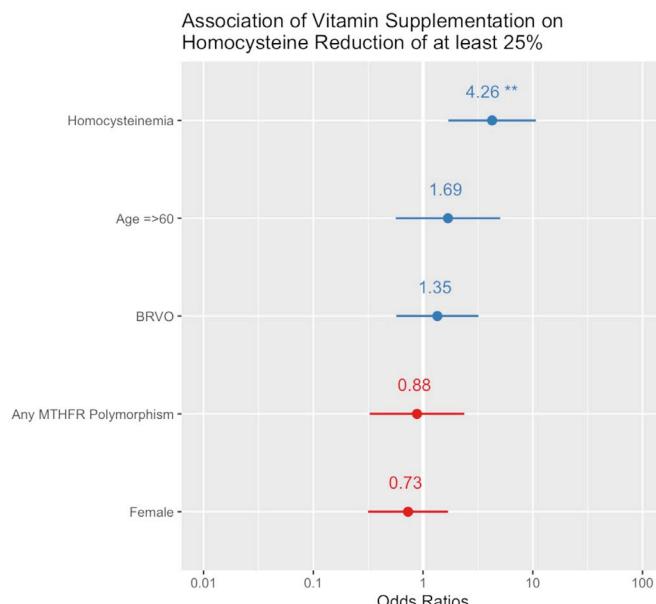
**Figure 2** Boxplots demonstrating association of vitamin supplementation on homocysteine levels. (A) Supplementation with B complex vitamin (BCV) only and combination multivitamin (MVI)+BCV were both associated with significantly reduced homocysteine levels in patients with retinal vein occlusion after 4–8 weeks of follow-up. (B) There was no statistically significant difference in the medians of differences following either supplementation regimen.

supplementation, while 29 (27.6%) met the criteria after vitamin supplementation, a significant reduction. BCV only supplementation was associated with a median homocysteine level reduction of  $-3.40$  ( $-5.20$ ,  $-2.10$ )  $\mu\text{mol/L}$ , decreasing median levels from  $14.40$  ( $12.30$ ,  $17.90$ ) to  $11.10$  ( $9.30$ ,  $13.70$ )  $\mu\text{mol/L}$ . Combination MVI+BCV supplementation was associated with a median homocysteine level reduction of  $-3.30$  ( $-6.12$ ,  $-1.80$ )  $\mu\text{mol/L}$ , decreasing median levels from  $14.60$  ( $12.23$ ,  $18.90$ ) to  $10.35$  ( $9.40$ ,  $13.20$ )  $\mu\text{mol/L}$ . When comparing the medians of differences in homocysteine reduction for each regimen followed, there was no statistically significant difference (figure 2B). Additionally, multiple logistic regression found that having baseline homocysteinemia was associated with an outcome of at least 25% reduction in homocysteine levels following vitamin supplementation (figure 3). As homocysteinemia potentially requires additional enzyme activity to metabolise, supplemental coenzymes in the form of B6, B9 and B12 may have helped catalyse these reactions, and resulted in a significant reduction in homocysteine.<sup>20</sup> In patients with RVO, there is likely benefit in having the baseline B6, B9 and B12 levels determined to assess for obvious deficiencies in the homocysteine metabolism pathway.

Additionally, in our multiple logistic regression analysis, age, sex, type of RVO and having at least one MTHFR polymorphism did not have any statistical effect on the association of vitamin supplementation with lowering homocysteine levels by at least 25%. Taken together, our study suggests that supplementing with either BCV only or combination MVI+BCV is sufficient in lowering homocysteine levels in patients with RVO, regardless of baseline demographics or genotyping. Based on the increased prevalence of homocysteinemia in older patients, screening this population for elevated homocysteine and potentially treating them with vitamin supplementation may be warranted. Importantly, no conclusion can be made from this study regarding whether lowering homocysteine levels would have any effect on RVO incidence, prevention or progression.

### Strengths

While the effects of vitamin supplementation on lowering homocysteine levels have been reported in the general population,<sup>25 26</sup> our study identified a research gap in the present literature, as we could not find any studies demonstrating homocysteine levels in patients with RVO at baseline and following supplementation with BCV and MVI. Despite this study only taking place in New Jersey,



**Figure 3** Association of vitamin supplementation on homocysteine reduction of at least 25%. Multiple logistic regression adjusting for age $\geq$ 60, homocysteinemia, branch retinal vein occlusion (BRVO), any methylenetetrahydrofolate reductase (MTHFR) polymorphism and sex. Baseline homocysteinemia was the only covariate associated with a homocysteine reduction of at least 25% following vitamin supplementation. \*\*This was the only covariate studied associated with a statistically significant homocysteine reduction of at least 25% following vitamin supplementation.

the patient population consisted of a wide demographic that closely mirrored the NHANES dataset; specifically, our patients were 27.1% Hispanic, 15.5% black and 52.6% white, while the NHANES population was 20.2% Mexican Americans, 20.8% non-Hispanic black and 51.9% non-Hispanic white,<sup>31</sup> which helps increase the generalisability of our study to the US population.

## Limitations

Being a case series, our study lacked matched controls, limiting the conclusions we could make regarding our findings and the general population without RVO. As a single-centre study, our patient population was limited to a narrow geographic location, which limited generalisability. We implemented an observational study design due to the logistic constraints of monitoring subjects' daily vitamin intake, which limited our level of evidence. Despite our study finding an association with vitamin supplementation and reduced homocysteine levels in patients with RVO, it does not demonstrate an association between homocysteine reduction therapy and RVO incidence, prevention or progression, and further research is needed in this area.

## CONCLUSION

In this study, 150 of 312 (48.1%) patients with RVO met the criteria for diagnosis of homocysteinemia at baseline. The median homocysteine level of the baseline cohort

of 312 patients was 12.80 (10.17, 15.90)  $\mu\text{mol/L}$ . For the follow-up cohort of 105 patients previously not supplemented with BCV or MVI who had homocysteine levels before and after vitamin supplementation, the median homocysteine level before supplementation was 14.50 (12.30, 17.90) and 10.70 (9.30, 13.50)  $\mu\text{mol/L}$  after. Overall, 69 (65.7%) patients met the criteria for homocysteinemia before vitamin supplementation, while 29 (27.6%) met the criteria after vitamin supplementation, and hence vitamin supplementation was associated with a significant decrease in homocysteinemia in this subpopulation ( $p<0.001$ ). Further research is needed to determine if homocysteine reduction with vitamin supplementation would be effective in reducing the incidence or improving the disease progression in patients with RVO.

**Contributors** The authors listed here have all contributed to the composition of this manuscript in the following ways: ML: conception or design of the work, data collection, drafting the article. AAH: drafting the article, data analysis and interpretation, critical revision of the article. AT: drafting the article, critical revision of the article. SR: data collection, data analysis and interpretation. NV: data collection. LHM: data collection. LK: data collection, critical revision of the article. LA: data collection, critical revision of the article. JG: data collection, critical revision of the article. PMH: (guarantor) conception or design of the work, drafting the article, critical revision of the article, final approval of the version to be published

**Funding** The authors have not declared a specific grant for this research from any funding agency in the public, commercial or not-for-profit sectors.

**Competing interests** None declared.

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

**Patient consent for publication** Not applicable.

**Ethics approval** This study adhered to the guidelines of the Declaration of Helsinki, and WCG IRB (Reverence Number 20180515) reviewed our proposal for a retrospective chart analysis and found to be exempt from IRB approval. Compliance with HIPAA was maintained throughout and after the study.

**Provenance and peer review** Not commissioned; internally peer reviewed.

**Data availability statement** All data relevant to the study are included in the article or uploaded as online supplemental information.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

## ORCID iDs

Maxwell Levi <http://orcid.org/0000-0001-9735-6932>

Andrew Trippiedi <http://orcid.org/0000-0002-1083-029X>

## REFERENCES

- Rogers S, McIntosh RL, Cheung N, et al. The prevalence of retinal vein occlusion: pooled data from population studies from the United States, Europe, Asia, and Australia. *Ophthalmology* 2010;117:313–9.
- Song P, Xu Y, Zha M, et al. Global epidemiology of retinal vein occlusion: a systematic review and meta-analysis of prevalence, incidence, and risk factors. *J Glob Health* 2019;9:010427.
- Li J, Paulus YM, Shuai Y, et al. New developments in the classification, pathogenesis, risk factors, natural history, and treatment of branch retinal vein occlusion. *J Ophthalmol* 2017;2017:1–18.
- Cugati S, Wang JJ, Rochtchina E, et al. Ten-Year incidence of retinal vein occlusion in an older population: the blue Mountains eye study. *Arch Ophthalmol* 2006;124:726–32.

- 5 Rehak M, Rehak J, Müller M, et al. The prevalence of activated protein C (APC) resistance and factor V Leiden is significantly higher in patients with retinal vein occlusion without general risk factors. *Thromb Haemost* 2008;99:925–9.
- 6 O'Mahoney PRA, Wong DT, Ray JG. Retinal vein occlusion and traditional risk factors for atherosclerosis. *Arch Ophthalmol* 2008;126:692–9.
- 7 Janssen MCH, den Heijer M, Cruysberg JRM, et al. Retinal vein occlusion: a form of venous thrombosis or a complication of atherosclerosis? A meta-analysis of thrombophilic factors. *Thromb Haemost* 2005;93:1021–6.
- 8 Hayreh SS, Zimmerman B, McCarthy MJ, et al. Systemic diseases associated with various types of retinal vein occlusion. *Am J Ophthalmol* 2001;131:61–77.
- 9 Newman-Casey PA, Stem M, Talwar N, et al. Risk Factors Associated with Developing Branch Retinal Vein Occlusion Among Enrollees in a United States Managed Care Plan. *Ophthalmology* 2014;121:1939–48.
- 10 Glueck CJ, Bell H, Vadlamani L, et al. Heritable thrombophilia and hypofibrinolysis. Possible causes of retinal vein occlusion. *Arch Ophthalmol* 1999;117:43–9.
- 11 Yioti GG, Panagiotou OA, Vartholomatos GA, et al. Genetic polymorphisms associated with retinal vein occlusion: a Greek case-control study and meta-analysis. *Ophthalmic Genet* 2013;34:130–9.
- 12 Danesh J, Lewington S. Plasma homocysteine and coronary heart disease: systematic review of published epidemiological studies. *J Cardiovasc Risk* 1998;5:229–32.
- 13 Zhao M, Wang X, He M, et al. Homocysteine and stroke risk: modifying effect of methylenetetrahydrofolate reductase C677T polymorphism and folic acid intervention. *Stroke* 2017;48:1183–90.
- 14 He Y, Li Y, Chen Y, et al. Homocysteine level and risk of different stroke types: a meta-analysis of prospective observational studies. *Nutrition, Metabolism and Cardiovascular Diseases* 2014;24:1158–65.
- 15 Li D, Zhou M, Peng X, et al. Homocysteine, methylenetetrahydrofolate reductase C677T polymorphism, and risk of retinal vein occlusion: an updated meta-analysis. *BMC Ophthalmol* 2014;14:147.
- 16 Marcucci R, Giusti B, Betti I, et al. Genetic determinants of fasting and post-methionine hyperhomocysteinemia in patients with retinal vein occlusion. *Thromb Res* 2003;110:7–12.
- 17 Ghaznavi H, Soheili Z, Samiei S, et al. Plasma homocysteine levels, methylene tetrahydrofolate reductase A1298C gene polymorphism and risk of retinal vein thrombosis. *Blood Coagulation Fibrinolysis* 2016;27:679–83.
- 18 McGimpsey SJ, Woodside JV, Bamford L, et al. Retinal vein occlusion, homocysteine, and methylene tetrahydrofolate reductase genotype. *Invest. Ophthalmol. Vis. Sci.* 2005;46:4712–6.
- 19 Gao W, Wang Y-S, Zhang P, et al. MTHFR C677T mutation in central retinal vein occlusion: a case-control study in Chinese population. *Thromb Res* 2008;121:699–703.
- 20 Lenz SR. Homocysteine and vascular dysfunction. *Life Sci* 1997;61:1205–15.
- 21 Boushey CJ, Beresford SA, Omenn GS, et al. A quantitative assessment of plasma homocysteine as a risk factor for vascular disease. probable benefits of increasing folic acid intakes. *JAMA* 1995;274:1049–57.
- 22 Desouza C, Keebler M, McNamara DB, et al. Drugs affecting homocysteine metabolism. *Drugs* 2002;62:605–16.
- 23 Moll S, Varga EA. Homocysteine and MTHFR Mutations. *Circulation* 2015;132:e6–9.
- 24 Weisberg I, Tran P, Christensen B, et al. A second genetic polymorphism in methylenetetrahydrofolate reductase (MTHFR) associated with decreased enzyme activity. *Mol Genet Metab* 1998;64:169–72.
- 25 Clarke R, Armitage J. Vitamin supplements and cardiovascular risk: review of the randomized trials of homocysteine-lowering vitamin supplements. *Semin Thromb Hemost* 2000;26:341–8.
- 26 Collaboration HLT. Lowering blood homocysteine with folic acid based supplements: meta-analysis of randomised trials. *BMJ* 1998;316:894–8.
- 27 GBIF. R: a language and environment for statistical computing, 2015. Available: <https://www.gbif.org/tool/81287/r-a-language-and-environment-for-statistical-computing>
- 28 The Comprehensive R Archive Network, Kassambara A. 'ggplot2' Based Publication Ready Plots [R package ggpqr version 0.4.0], 2020. Available: <https://cran.r-project.org/web/packages/ggpqr/index.html>
- 29 The Comprehensive R Archive Network, Lüdecke D. Data Visualization for Statistics in Social Science [R package sjPlot version 2.8.7], 2021. Available: <https://cran.r-project.org/web/packages/sjPlot/index.html>
- 30 The Comprehensive R Archive Network, Iannone R. Graph/Network Visualization [R package DiagrammeR version 1.0.6.1], 2020. Available: <https://cran.r-project.org/web/packages/DiagrammeR/index.html>
- 31 Centers for Disease Control and Prevention. Second National report on biochemical indicators of diet and nutrition in the US population 2012, 2012. Available: [https://www.cdc.gov/nutritionreport/report\\_2012.html](https://www.cdc.gov/nutritionreport/report_2012.html)
- 32 Ostrakhovitch EA, Tabibzadeh S. Homocysteine and age-associated disorders. *Ageing Res Rev* 2019;49:144–64.
- 33 Centers for Medicare & Medicaid Services. Billing and coding: homocysteine level, serum; Medicare coverage database, 2019. Available: <https://www.cms.gov/medicare-coverage-database/view/article.aspx?articleId=56675>