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Vitamin B_{12} deficiency and hyperhomocysteinaemia in outpatients with stroke or transient ischaemic attack: a cohort study at an academic medical centre

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ABSTRACT

Objective We sought to assess the current magnitude of the opportunity for secondary stroke prevention with B vitamins.

Design A cohort study.

Setting The Urgent TIA (Transient Ischaemic Attack) Clinic at an academic medical centre.

Main outcome measures We assessed the prevalence of biochemical vitamin B_{12} deficiency (B_{12}Def, serum B_{12} <156 pmol/L), hyperhomocysteinaemia (HHcy; plasma total homocysteine [tHcy] >14 µmol/L) and metabolic B_{12} deficiency (MetB_{12}Def, serum B_{12} <258 pmol/L and HHcy) between 2002 and 2017, by age group and by stroke subtype.

Results Data were available in 4055 patients. B_{12}Def was present in 8.2% of patients overall; it declined from 10.9% of patients referred before 2009 to 5.4% thereafter (p=0.0001). MetB_{12}Def was present in 10.6% of patients, and HHcy was present in 19.1% of patients. Among the patients aged ≥80 years, MetB_{12}Def was present in 18.1% and HHcy in 35%. Among the 3410 patients whose stroke subtype was determined, HHcy was present in 18.4% of patients: 23.3% of large artery atherosclerosis, 18.1% of cardioembolic, 16.3% of small vessel disease, 10.8% of other unusual aetiologies and 13.6% of undetermined subtypes (p=0.0001).

Conclusions Despite a decline in our referral area since 2009, B_{12}Def, MetB_{12}Def and HHcy remain common in patients with stroke/TIA. Because these conditions are easily treated and have serious consequences, all patients with stroke/TIA should have their serum B_{12} and tHcy measured.

Strengths and limitations of this study

- We have data on a large number of patients (4055) referred to the Urgent TIA (Transient Ischaemic attack) Clinic at an academic medical centre, over 15 years, with data on age, sex, serum B_{12}, and plasma total homocysteine and creatinine.
- As we could not rigorously exclude patients who were taking B_{12} supplements at the time of referral, our findings probably represent an underestimate of the prevalence of hyperhomocysteinaemia and metabolic B_{12} deficiency among patients with TIA/stroke, absent supplementation.
- Furthermore, we analysed all patients referred to the Urgent TIA Clinic, so we could not exclude patients with stroke mimics; including them would probably have exaggerated the underestimate.
- However, we have in place a triaging system, whereby the clinic nurse practitioner redirects referrals that seem inappropriate, and we track appropriateness of referrals; our percentage of stroke mimics is <10% of cases.
- Another limitation is that the biochemical methods changed over time, as described in the methods, and different assays may give different results; however, the changes in the reference ranges were small, and the changes in methods did not coincide with the probable increase in B_{12} supplementation after 2009.
- Stroke subtype classification was not available in all patients; however, it was available in a substantial number (3410; 84% of cases).

INTRODUCTION

There is a commonly held belief that "Homocysteine is dead." This apparently has its origins in a widely quoted statement made by Dr Kaare Harald Bønaa in September 2005 at the European Society of Cardiology Congress in Stockholm.[1] Indeed, this very phrase was cited by an official at the National Institute for Health and Care Excellence (NICE) in the UK as the reason that NICE recommends that measurement of plasma total homocysteine (tHcy) not be paid for with public funds (Professor David Smith, personal communication, 15 June 2018).

However, B vitamin therapy for lowering homocysteine to prevent stroke was

resurrected by the China Stroke Primary Prevention Trial (CSPPT), in which folic acid reduced ischaemic stroke by 24% in primary prevention. In higher risk groups, the reduction of stroke was greater: among patients with low-density lipoprotein cholesterol >2 mmol/L, the reduction in ischaemic stroke was 36%,[2] and in patients with tHcy >15 and low platelet counts it was 70%.[3] Folic acid did not reduce the risk of haemorrhagic stroke. A recent meta-analysis indicated that B vitamin combinations and folic acid reduced the risk of stroke; the authors recommended folic acid for this purpose in countries where folate fortification does not exist.[4]

Restricting the recommendation to folic acid is problematic, as in countries where folate fortification is in place, the main nutritional determinant of tHcy is deficiency of vitamin B_{12}.[5 6] It has become apparent in the light of the CSPPT trial that in the early trials of B vitamins for stroke prevention, harm from cyanocobalamin among study participants with renal failure obscured the benefit among participants with good renal function.[7] It is likely that instead of cyanocobalamin, we should be using methylcobalamin or oxocobalamin for stroke prevention.[7]

An important opportunity for prevention of stroke and dementia is metabolic B_{12} deficiency, which is often missed because most physicians do not realise that a serum total B_{12} in the 'normal' range (~180–670 pmol/L) does not define adequacy of functional vitamin B_{12}.[8] In order to assess B_{12} function, it is necessary to measure holotranscobalamin, or measure one of the metabolites that become elevated in B_{12} deficiency: methylmalonic acid (MMA) or tHcy. Although MMA is more specific, homocysteine can substitute for MMA in folate-replete persons (which in countries with folate fortification essentially applies to nearly all patients).[9] The inflection point for serum B_{12} at which both MMA and tHcy become elevated is 400 pmol/L,[10 11] so within the normal range of serum B_{12} there are many patients with metabolic B_{12} deficiency.

Spence reported in 2009 that at age ≥80, 40% of patients referred for stroke prevention had tHcy ≥14,[12] and in 2006 he reported that metabolic B_{12} deficiency was present in 10% of patients with stroke/transient ischaemic attack (TIA) aged <50, 13% aged 50–70 and 30% above the age of 70.[13] He routinely requests of referring doctors that serum B_{12} and plasma lipids be measured before new and follow-up visits, and had noticed that more patients recently had been started on B_{12} supplements before coming to clinic, after the family physician saw the serum B_{12} level. It seemed that as a result of reporting such findings to the hundreds of family physicians of some 40 000 patients with stroke/TIA in our region over 40 years, detection and treatment of B_{12} deficiency in our referral area may have increased.

In this study, we sought to assess the current magnitude of the opportunity for stroke prevention with B vitamins by determining the prevalence of hyperhomocysteinaemia (HHcy) and metabolic B_{12} deficiency in patients with TIAs or minor strokes.

METHODS

Patient population

Data were obtained from outpatients with TIA or minor stroke referred to the Urgent TIA Clinic at University Hospital in London, Ontario, Canada. Data for 3410 patients referred between 2000 and 2012 were obtained from the database of a study on secular trends in stroke subtypes[14]; data on an additional 1776 patients referred between 2012 and 2017 were obtained from the electronic medical records of the hospital during a 3-month visit by SA, undertaken within a Flexible and Enhanced Learning programme of the University of British Columbia Medical School. Because of the limited time available, data collected on patients referred since 2012 were restricted to age, sex and variables pertinent to the diagnosis of metabolic B_{12} deficiency and HHcy: serum B_{12}, plasma tHcy and creatinine. Among the 3410 patients referred between 2000 and 2012, stroke subtypes had been assigned using the Subtypes of IschaemicStroke Classification System (SPARKLE) classification,[15] which incorporates high carotid plaque burden in the large artery atherosclerosis subtype.

Blood tests and analyses

Serum B_{12}, EDTA tHcy and lithium heparin plasma creatinine were measured at the Department of Pathology and Laboratory Medicine of London Health Sciences Centre, London, Ontario, Canada.

Between 2000 and May 2004, serum B_{12} levels were measured by immunoassay on a Siemens Centaur analyser (reference intervals [RIs]: normal 181–672 pmol/L, indeterminate 155–181 pmol/L, deficient <155 pmol/L). The analysis on this platform continued until January 2014, but in May 2004 the RIs changed to 200–672 pmol/L for normal, 156–200 pmol/L for indeterminate and <156 pmol/L for deficient. Serum B_{12} was then measured by immunoassay on a Roche e602 with generation I of reagent (RIs: 156–698 pmol/L) until November 2015, and then on Roche e602 and E170 analysers with generation II of reagent (RIs: 145–569 pmol/L) beyond 2017.

Plasma tHcy was measured by high-pressure liquid chromatography (RI: ≤13.5 µmol/L) from 2000 until December 2001. It was then measured by immunoassay on a Siemens Immulite or Immulite 2000 (RI: 5.0–12.0 µmol/L) until May 2011. From May 2011 until February 2014, tHcy was measured by immunoassay on a Siemens Centaur (RI: 3.7–13.9 µmol/L), then using an ELISA from Immuno-Biological Laboratories (RIs: male 4.8–11.8 µmol/L, female 4.3–10.9 µmol/L) until December 2015, and then by immunoassay on an Abbott Architect (RIs: male 5.1–15.4 µmol/L, female 4.4–13.6 µmol/L) beyond 2017.

Plasma creatinine was measured by the Jaffe method on Beckman CX4, CX7 and LX20 analysers from 2000 until November 2008, and then by an enzymatic method on Roche P-Modules beyond 2017. The RIs throughout this entire time were 62–120 µmol/L for male 12 years to adult and 55–100 µmol/L for female 12 years to

adult. Biochemical B_{12} deficiency was defined as a serum B_{12} <156 pmol/L. Metabolic B_{12} deficiency was defined, as recommended by Stabler *et al*, by a serum B_{12} <258 pmol/L and tHcy >14 µmol/L.[16] Because tHcy is high in patients with renal failure, a second estimate of metabolic B_{12} deficiency excluded patients with an estimated glomerular filtration rate (eGFR) <60 mL/min per 1.73 m^2, computed by the Modification of Diet in Renal Disease (MDRD) equations.[17]

Involvement of patients and the public

There was no involvement of patients other than their data. The public were also not involved.

Statistical methods

Continuous variables were summarised by mean±SD and categorical variables by %. Differences in categorical variables were compared using X^2; post-hoc differences were assessed by computing standardised residual scores for each cell. Continuous variables were assessed by analysis of variance. All tests were performed using SPSS V.25.

RESULTS

Data were available in 4282 patients with values for serum B_{12} and tHcy. There were 218 patients who had unphysiologically high serum B_{12} levels from 700 to 2661 pmol/L, indicating that they were probably taking B_{12} supplements (other possibilities would include laboratory error or errors in data entry): 5.2% of patients before 2009 and 8.1% thereafter (p=0.0001). There were also nine patients whose serum B_{12} was implausibly low (between 1 and 31 pmol/L, possibly due to laboratory error or errors in data entry). After excluding these patients there remained 4055 patients whose results are presented here. A Consolidated Standards of Reporting Trials diagram is shown in online supplementary material. Analyses including the patients with serum B_{12} >700 pmol/L are presented in online supplementary eTable 1 and eTable 2; online supplementary eFigure 1 shows the distribution of serum B_{12} and tHcy from 2002 to 2017. It is certain from the medication lists in the clinic notes that there were many patients with a serum B_{12} <700 pmol/L who were also taking B_{12} supplements, but data on B_{12} supplementation were not collected, so those patients were included in the analyses. Folate supplementation of the grain supply was implemented in Canada in 1989, and we know from observation in the clinic that very few patients took folate supplements; a substantial proportion (~20%) do take a multivitamin tablet daily.

Biochemical B_{12} deficiency was present in 8.2% of patients overall; it declined from 10.9% of patients referred before 2009 to 5.4% thereafter (p=0.0001). Metabolic B_{12} deficiency was present in 10.6% of patients, and HHcy was present in 19.1% of patients. Among patients aged ≥80 years, metabolic B_{12} deficiency was present in 18.1% and HHcy in 35%. Among the 3410 patients whose stroke subtype was determined, HHcy was present in 18.4% of patients: 23.3% of large artery atherosclerosis, 18.1% of cardioembolic, 16.3% of small vessel disease, 10.8% of other unusual aetiologies and 13.6% of undetermined subtypes (p=0.0001).

Figure 1 shows the distribution of serum B_{12} and tHcy from 2002 to 2008 versus 2009–2017. The distributions were significantly different by a Kruskal-Wallis test (p=0.0001). Online supplementary data eFigure 1 shows the distribution of serum B_{12} and tHcy in all the cases, including those with serum B_{12} >700 pmol/L.

Table 1 shows the characteristics of the patients by metabolic B_{12} status. Patients with metabolic B_{12} deficiency were significantly older, male and had smoked more tobacco.

Table 2 shows the prevalence of tHcy ≥14 and of metabolic B_{12} deficiency by age group.

Both increased significantly with age. At ages 70–80, 23.5% of patients had tHcy ≥14, and at age ≥80 it was 35%. Figure 2 shows the distribution of tHcy and serum B_{12} by

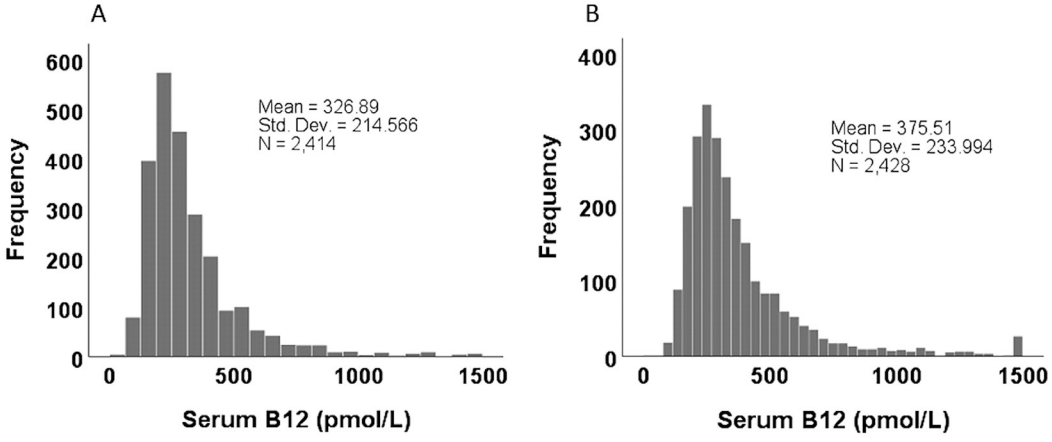

Figure 1 Distribution of serum B_{12} from 2002 to 2008 versus 2009–2017. (A) Distribution of serum B_{12} from 2002 to 2008. (B) Distribution of serum B_{12} from 2009 to 2017. Serum B_{12} is shown for all patients referred to the Urgent TIA (transient ischaemic attack) Clinic with serum B_{12} levels, including those without total homocysteine available. The distributions are significantly different by a Kruskal-Wallis test (p=0.0001).

Table 1 Characteristics of the study population

	Metabolic B_{12} deficiency		
	No, n=3626	Yes, n=429	P value
Continuous variables, mean±SD			ANOVA
Age	64.41±14.46	71.58±12.25	0.0001
Systolic pressure	143.57±21.87	144.25±22.65	0.809
Diastolic pressure	81.75±12.69	80.01±15.12	0.297
Total cholesterol (mmol/L)	4.81±1.19	4.47±1.12	0.054
Triglycerides	1.84±1.26	1.65±0.76	0.296
HDL cholesterol	1.34±0.43	1.25±0.46	0.170
LDL cholesterol	2.67±1.02	2.48±0.99	0.224
Smoking (pack-years)	15.64±20.98	24.46±28.78	0.002
Serum B_{12} (pmol/L)	319.05±128.62	188.68±44.18	0.0001
tHcy (µmol/L)	10.14±4.36	18.93±6.02	0.0001
Plasma creatinine (µmol/L)	84.10±38.85	101.65±43.49	0.0001
eGFR	76.48±21.33	63.11±25.00	0.0001
Categorical variables, n (%)			χ^2
Male	1831 (50.5)	242 (56.4)	0.022

ANOVA, analysis of variance; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; tHcy, plasma total homocysteine.

quartiles of eGFR. It is clear that patients with impaired renal function have higher tHcy, so metabolic B_{12} deficiency would not be appropriately defined in them by a tHcy ≥14. After excluding patients with eGFR <60, metabolic B_{12} deficiency was present in 9.2% of patients at ages 70–80, and at age ≥80 it was 12.3%. Figure 2 shows the serum B_{12} and tHcy by age groups.

Table 3 shows the prevalence of tHcy and metabolic B_{12} deficiency by stroke subtypes. Post-hoc tests for differences are shown in online supplementary material.

Both were significantly more prevalent in patients with large artery disease and cardioembolic stroke, and less prevalent in patients with other rare causes or undetermined causes of stroke. Among the 3410 patients whose stroke subtype was determined, HHcy was present in 18.4% of patients; this differed significantly by stroke subtype: 23.3% of large artery atherosclerosis, 18.1% of cardioembolic, 16.3% of small vessel disease, 10.8% of other unusual aetiologies and 13.6% of undetermined subtypes (p=0.0001) (figure 3).

Table 4 shows the baseline characteristics by stroke subtype.

Figure 4A shows the serum B_{12} levels by year, and figure 4B shows the number of patients with serum B_{12} >700 pmol/L by year. It appears that after the 2009 report,[12] B_{12} supplementation became more common in our referral area. Before 2009, serum B_{12} (mean±SD) was 293.65±135.74 pmol/L; after 2009 it was 356.57±129.18 pmol/L (p=0.001).

DISCUSSION

We found that despite evidence of probable increasing B_{12} supplementation in the community, metabolic B_{12}

Table 2 Age groups, hyperhomocysteinaemia and B_{12} deficiency, n (%)

Age group						
	<50	50–59	60–69	70–79	≥80	P value, χ^2
tHcy >14*	51 (8.0)†	87 (11.3)†	166 (16.4)†	271 (23.5)†	263 (35.1)†	0.0001
Biochemical B_{12} deficiency‡	57 (8.2)	77 (8.8)	80 (7.2)	106 (8.4)	65 (8.5)	0.70
Metabolic B_{12} deficiency§	27 (3.9)†	41 (4.8)†	87 (7.8)†	139 (10.9)	122 (15.1)†	0.0001
Metabolic B_{12} deficiency 2¶	14 (3.4)†	21 (4.1)	39 (6.5)	52 (9.2)	36 (12.3)†	0.0001

Age groups are approximately quintiles of the study population.
*tHcy, plasma total homocysteine.
†Statistically significant difference from other categories in post-hoc testing.
‡Serum B_{12} <156 pmol/L.
§Serum B_{12} <258 and pmol/L and tHcy >14 µmol/L.
¶Excluding patients with eGFR <60.

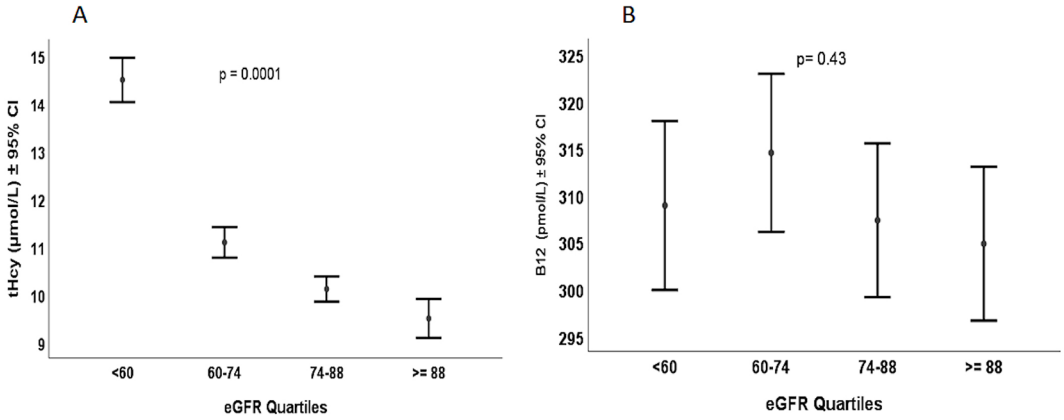

Figure 2 Plasma total homocysteine (tHcy) and serum B_{12} by quartiles of estimated glomerular filtration rate (eGFR). (A) tHcy was clearly elevated with impaired renal function. (B) Serum B_{12} was not significantly affected by renal impairment.

deficiency and HHcy remain common among patients referred for TIA or minor stroke.

The shift to higher serum B_{12} levels over time is unlikely to be due to reasons other than B_{12} supplementation. The high end of the reference range of 698 pmol/L represents the 95th percentile for all patients, including those who received supplements; plasma levels above 700 pmol/L are unlikely to be physiological, and are therefore probably due to supplementation. Figure 4B shows that the percentage of patients with a serum B_{12} >700 pmol/L increased after 2014 from ~5% of patients to 17.5%; this is very unlikely to be due to anything other than supplements. As the principal dietary source of B_{12} is meat, changes to a healthier lifestyle would not account for increases in serum B_{12}. It appears therefore that an increase in awareness and treatment of B_{12} deficiency since 2009 in our referral area has resulted in a decline in recent years in the prevalence of metabolic B_{12} deficiency and HHcy among patients referred with stroke or TIA. This is supported by the decline in HHcy since the 2009 report from this clinic population (from 40% of patients aged >80 to 35%)[12] and the decline in the prevalence of HHcy since the 2006 report (from 30% of patients aged ≥71 to 15.9% aged ≥80).[13] Nevertheless, B_{12} deficiency and HHcy remain common in this patient population.

Including some patients who probably received B_{12} supplementation in the analyses probably resulted in an underestimate of the prevalence of metabolic B_{12} deficiency and HHcy that would be observed among patients with stroke/TIA, absent supplementation.

HHcy and low eGFR were more common in patients with large artery disease, who were older than patients with other stroke subtypes. Plasma tHcy has previously been reported to be associated with carotid total plaque area.[18 19] Renal function declines with age, and tHcy levels increase with age.

We found that despite probable increases in B_{12} supplementation in our community, HHcy and metabolic deficiency are still common in patients with stroke, particularly in older patients. These findings are important on several counts. As discussed above, B vitamins to lower tHcy reduce the risk of stroke. Besides increasing the risk of stroke by raising tHcy, B_{12} deficiency also causes neuropathy, myelopathy and dementia. Cognitive decline is common in patients with stroke, and treating B_{12} deficiency and HHcy can prevent dementia.[20–22] In a recent meta-analysis, patients with higher blood levels of B_{12}, B_6 and folate had a reduced risk of developing atrial fibrillation, a known risk factor for cardioembolic strokes.[23] Falls in the elderly, an important cause of disability, are probably also increased by loss of position sense with B_{12} deficiency.

Table 3 Stroke subtypes, hyperhomocysteinaemia and metabolic B_{12} deficiency, n (%)

	Large artery disease	Cardioembolic	Small vessel disease	Other rare or unusual aetiologies	Undetermined	P value
tHcy >14*	256 (23.3)†	238 (18.1)	57 (16.3)	21 (10.8)†	72 (13.6)†	0.0001
Biochemical B_{12} deficiency‡	111 (10.1)	104 (7.9)	28 (8.5)	23 (11.3)	45 (8.5)	0.27
Metabolic B_{12} deficiency§	139 (12.7)†	121 (9.2)	31 (8.8)	11 (5.4)†	44 (8.1)	0.002
Metabolic B_{12} deficiency 2¶	49 (10.9)	48 (6.8)	11 (6.2)	4 (4.3)	15 (5.2)†	0.001

Stroke subtypes were as defined among 3466 of the cases in a previous study[14].
*tHcy, plasma total homocysteine µmol/L.
†Statistically significant difference from other categories in post-hoc testing.
‡Serum B_{12} <156 pmol/L.
§Serum B_{12} <258 and pmol/L and tHcy >14 µmol/L.
¶Excluding patients with estimated glomerular filtration rate <60.

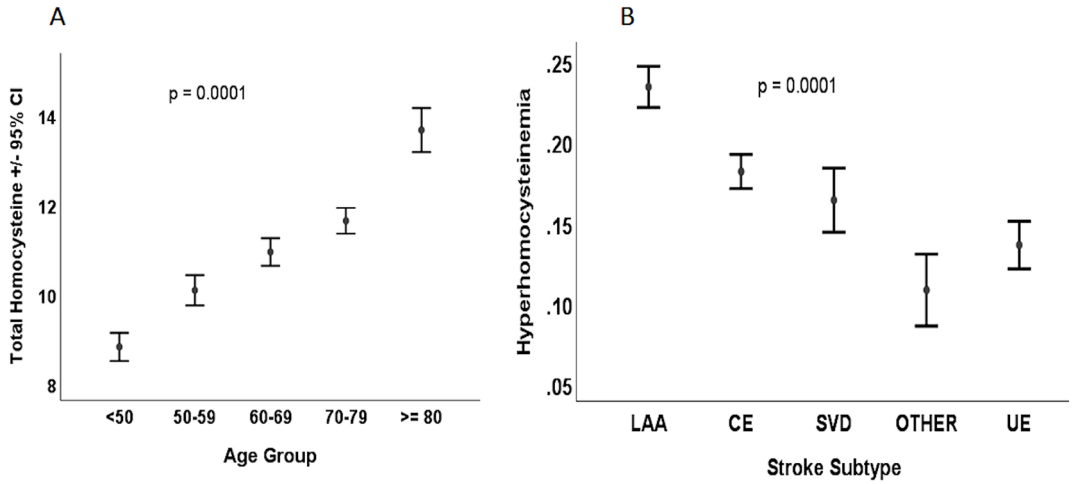

Figure 3 Plasma tHcy by age group and frequency of hyperhomocysteinaemia by stroke subtype. (A) tHcy clearly increases with age (ANOVA p=0.0001). (B) Stroke subtypes are shown for patients referred between 2002 and 2012; hyperhomocysteinaemia differed significantly by stroke subtype (χ^2 p=0.0001). ANOVA, analysis of variance; CE, cardioembolic; LAA, large artery atherosclerosis; Other, other unusual aetiologies; SVD, small vessel disease; tHcy, total homocysteine; UE, undetermined aetiology.

It also seems likely that patients in other regions and patients with more severe strokes may have a greater prevalence of both these conditions. In the Newcastle 85+ study, the lowest quartile of plasma B_{12} was <170 pmol/L, with tHcy 19.9 (95% CI 16.3 to 24.6); that is, 25% of UK patients whose age in 2006 was 85 years had metabolic B_{12} deficiency. B_{12} deficiency is very common in India, in part because of a high prevalence of vegetarianism. Even in adolescents, B_{12} deficiency was reported in 32% of study participants in the Haryana region.[24] In Pakistan 58.3% of patients with ischaemic stroke were reported to have HHcy defined as tHcy >15 µmol/L[25]; in China the prevalence of tHcy >30 µmol/L among patients with hypertension was 16.7%.[26] It would therefore be important to assess the prevalence of HHcy and metabolic B_{12} deficiency in other stroke populations. It would also be very important to assess serum B_{12} and tHcy in patients with cognitive impairment.[20 27 28]

CONCLUSION

Despite a probable increase in B_{12} supplementation in our referral area in recent years, metabolic B_{12} deficiency and

Table 4 Baseline characteristics by stroke subtype for cases with serum B_{12} <700 pmol/L

	Large artery	Cardioembolic	Small vessel	Other unusual	Undetermined	P value
Continuous variables, mean±SD						(ANOVA)
Age	70.08±10.93	62.23±15.96	65.94±13.11	53.32±14.39	64.02±14.84	0.0001
Systolic pressure (mm Hg)	143.82±20.81	135.40±19.41	157.52±24.37	140.67±20.69	142.70±21.13	0.0001
Diastolic pressure (mm Hg)	80.26±12.44	79.16±12.34	86.48±14.54	81.77±12.74	81.15±11.16	0.001
Total cholesterol (mmol/L)	4.71±1.21	4.74±1.12	4.93±1.20	5.04±1.20	4.96±1.18	0.003
Triglycerides (mmol/L)	1.94±1.30	1.69±1.16	2.03±1.78	1.87±0.94	1.85±1.14	0.001
HDL cholesterol (mmol/L)	1.28±0.40	1.35±0.43	1.34±0.44	1.29±0.39	1.37±0.42	0.009
LDL cholesterol (mmol/L)	2.60±1.04	2.64±0.95	2.71±0.98	2.89±1.07	2.79±1.01	0.019
Smoking (pack-years)	25.29±23.34	17.62±19.89	21.03±22.16	11.06±16.28	16.16±17.07	0.0001
Serum B_{12} (pmol/L)	298.44±129.85	300.47±125.20	294.10±122.67	291.95±122.63	302.71±132.71	0.785
tHcy (µmol/L)	11.96±5.34	10.91±5.25	11.04±4.71	9.20±4.14	10.28±4.91	0.0001
Plasma creatinine (µ mol/L)	94.29±32.21	82.72±34.31	88.00±52.04	79.12±20.25	83.92±41.67	0.0001
eGFR	68.04±20.77	76.78±21.28	73.70±21.67	80.05±22.07	76.84±23.90	0.0001
Categorical value, n (%)						χ^2
Male	668 (62.2)*	571 (44.3)*	158 (48.0)	77 (38.1)*	238 (46.2)*	0.0001

Post-hoc testing for continuous variables is shown in online supplementary material.
*Statistically significant difference from other categories in post-hoc testing.
ANOVA, analysis of variance; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; tHcy, plasma total homocysteine.

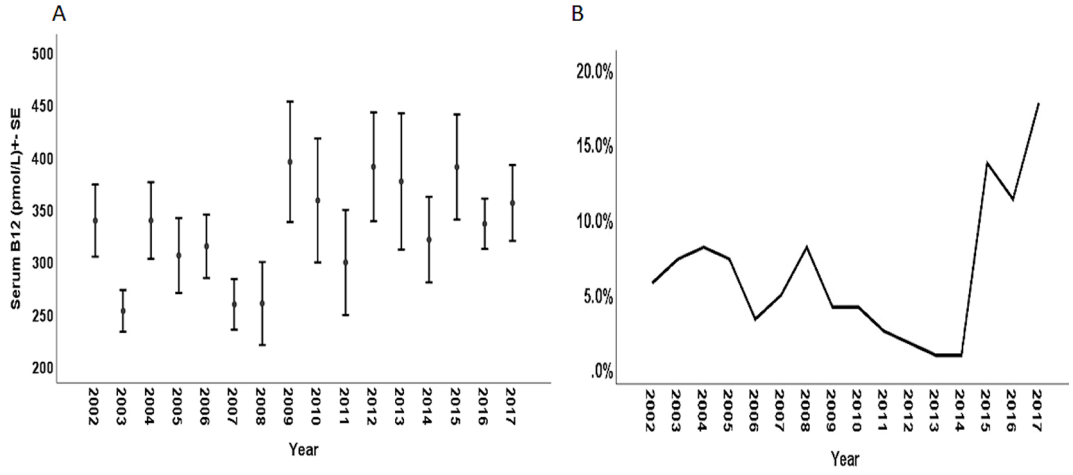

Figure 4 Serum B_{12} by year of referral and per cent of patients with serum B_{12} >700 pmol/L at the time of referral. (A) Serum B_{12} levels increased among patients referred to our clinic after the 2009 report of a high prevalence of hyperhomocysteinaemia in our patients. Before 2009 the mean serum B_{12} (SD) was 326.62 (214.68); thereafter it was 374.90 (234.29) (p=0.0001). (B) The per cent of patients with serum B_{12} >700 pmol/L increased markedly in recent years, indicating probable B_{12} supplementation.

HHcy remain common in patients with TIA or stroke. As these conditions have serious consequences and are easily treated, serum B_{12} and tHcy should be measured in all patients with ischaemic stroke.

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Contributors JDS conceived of the study. CB collected the data on patients referred before 2012 and assigned the stroke subtypes under the supervision of DGH and JDS, and SA collected the data from the patients referred since 2012, during a 3-month elective rotation. Statistical analyses were performed by SA and JDS, and ACR supervised the measurements of total homocysteine and B_{12} and provided information on the methods. SA wrote the first draft; all authors contributed to revisions of the manuscript. JDS wrote revisions based on comments from the other authors and completed the final draft of the manuscript. The other authors (DGH, LAS, AK, JM, MRA and VH) also cared for the patients, ordered the laboratory tests and obtained approval for the study from the University Ethics Committee. The corresponding author accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish, that all listed authors meet the authorship criteria and that no others meeting the criteria have been omitted.

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Disclaimer JDS affirms that the manuscript is an honest, accurate and transparent account of the study being reported; that no important aspects of the study have been omitted; and that there are no discrepancies from the study as originally planned.

Competing interests After this paper was written, JDS became a consultant to Orphan Technologies, a company that makes a truncated human cystathionine beta synthase for treatment of classical homocystinuria.

Patient consent for publication Not required.

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Data sharing statement Data may be shared if permission is first obtained from the University of Western Ontario Health Sciences Ethics Review Board.

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