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# Characteristics of Vitamin B12 Deficiency in Adult Chinese Patients with Type 2 Diabetes and the Implication of Metformin

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## 1. Introduction

Vitamin B12 deficiency remains a worldwide health problem in the 21<sup>st</sup> century. The advent of metabolite assays in the last few decades further expands the population with subclinical or tissue deficiency and thus the magnitude of the problem. The increasing recognition of its association with the use of metformin raises another controversy for clinicians regarding detection and treatment of vitamin B12 deficiency. Since the clinical use of metformin in 1957, malabsorption of vitamin B12 in diabetic patients treated with metformin was first noted in 1969 (Berchtold et al., 1969). Subsequent studies revealed both short-term and long-term use of metformin induces malabsorption of vitamin B12 and causes decreased serum vitamin B12 concentrations in 10% to 30% of diabetic patients (Tomkin et al., 1971; Bauman et al., 2000; Wulffele et al., 2003; Hermann et al., 2004). A randomized placebo controlled Dutch trial recently reconfirmed this finding and demonstrated treatment with metformin for a mean of 4.3 years resulted not only in a 19% persistent and progressive reduction of mean serum vitamin B12 concentrations, but also raised serum homocysteine concentrations (de Jager et al., 2010). Other case-control and cross-sectional studies identified duration and dosage of metformin use as risk factors for vitamin B12 deficiency (Ting et al., 2006). It is believed that if individuals with type 2 diabetes receiving metformin develop low serum vitamin B12 concentrations, a stage of asymptomatic tissue deficiency, would eventually progress to symptomatic clinical deficiency, as evidenced by reports of megaloblastic anaemia caused by metformin-related vitamin B12 malabsorption, unless they are duly treated (Gilligan, 2002; Filioussi et al., 2003; Liu et al., 2006).

Metformin is now extensively used as the first line pharmacological agent for glycaemic control especially following the favourable results of the United Kingdom Prospective Diabetes Study (UKPDS 34) in 1998. This coupled with the rising incidence of type 2 diabetes in many parts of the world constitutes a driving force for relevant parties to make appropriate recommendations or guidelines concerning the detection and treatment of vitamin B12 deficiency among diabetic patients on metformin. Although the haematological and neurological manifestations of overt or clinical vitamin B12 deficiency are easy to diagnose, they are often overlooked and attributed to diabetic complications or aging among diabetic patients.

The present study attempts to address the issue from the end spectrum of the disorder - florid vitamin B12 deficiency in type 2 diabetic patients. Through description and analysis of the clinical manifestations of overt vitamin B12 deficiency in a cohort of adult Chinese patients with type 2 diabetes, and delineation of the underlying aetiology of vitamin B12 deficiency, the study aims to highlight the characteristics of vitamin B12 deficiency in type 2 diabetic patients, and to evaluate the implications of metformin use in relation to the development of vitamin B12 deficiency of undetermined origin in diabetic patients.

## **2. Materials and methods**

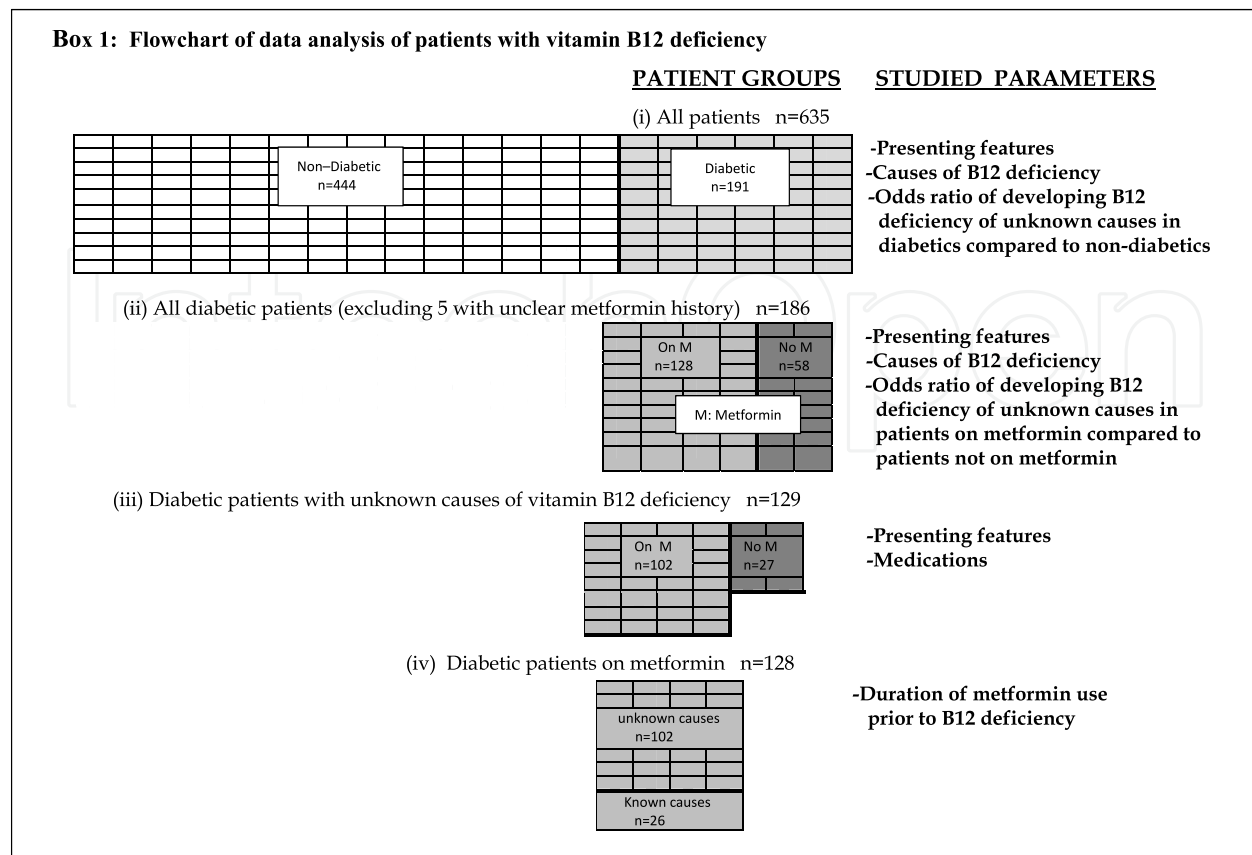
### **2.1 Patient source and stratification**

The patient source was from a longitudinal study of adult Chinese patients ( $\geq 18$  years) presenting with megaloblastic anaemia and/or neurological deficits in association with low serum vitamin B12 levels serially encountered in a regional hospital in Hong Kong between May 1994 and October 2010 (Chan et al., 2008). According to whether they had type 2 diabetes as shown by the diagnostic codes in the medical records at the time of diagnosis of vitamin B12 deficiency, patients were stratified into diabetic and non-diabetic groups. Documentation of diagnosis of diabetes was at the discretion of in-charge physicians and the diagnostic criteria of World Health Organization (WHO, 1999) with fasting plasma glucose  $\geq 7.0$  mmol/L or 2-hour post prandial plasma glucose  $\geq 11.1$  mmol/L were adopted. Diabetes both with and without complications were included. Diabetic patients were further stratified according to whether they were on metformin at the time of diagnosis of vitamin B12 deficiency. Diabetic patients who had been on metformin for one or more years immediately before the diagnosis of vitamin B12 deficiency were counted as metformin users and assigned to the "on metformin" group. Other diabetic patients on dietary control, insulin, sulphonylureas, metformin for less than one year or longer period but having stopped in the one year before the diagnosis of vitamin B12 deficiency, were assigned to the "not on metformin" group.

### **2.2 Data collection and analysis**

Baseline data were retrieved from patients' medical records and investigators' file of adult Chinese patients with vitamin B12 deficiency. Data entry included presenting clinical and laboratory features at the time of diagnosis of vitamin B12 deficiency; causes of vitamin B12 deficiency; medications including metformin, sulphonylureas, insulin, proton pump inhibitors (PPI), histamine 2 blockers (H2B), lipid lowering agents, antiplatelet agents, antihypertensive and cardiac agents; duration of metformin use; and duration of diabetic history.

The sequence of data analysis was shown in Box 1. (i) The presenting features and the causes of vitamin B12 deficiency were compared between patients with and without type 2 diabetes. The odds ratio (OR) of developing vitamin B12 deficiency of undetermined origin among diabetic patients to non-diabetic patients was determined. (ii) The presenting features and the causes of vitamin B12 deficiency were compared between type 2 diabetic patients "on metformin" and diabetic patients "not on metformin". The OR of developing vitamin B12 deficiency of undetermined origin among diabetic patients "on metformin" to patients "not on metformin" was determined. (iii) For diabetic patients with undetermined aetiology of vitamin B12 deficiency, the presenting features and the medications prescribed at the time of diagnosis of vitamin B12 deficiency were compared between those "on metformin" and those "not on metformin". (iv) The duration of metformin use in relation to the onset of vitamin B12 deficiency was studied among all diabetic patients on metformin, irrespective of the underlying aetiology of vitamin B12 deficiency.



### 2.3 Definitions

Known causes of vitamin B12 deficiency include (i) pernicious anaemia (PA) defined by the presence of serum intrinsic factor (IF) antibody and/or by abnormal Schilling test compatible with immune loss of IF; (ii) probable PA (PPA) defined by the absence of demonstrable IF antibody, no performance of Schilling test, and the presence of at least 2 out of 3 immune features – gastric parietal cell (GPC) antibody, antithyroid antibodies, and histological evidence of autoimmune gastropathy (J.C.W. Chan & F.H.Y. Chan, 2011); (iii) gastrointestinal disorders such as total gastrectomy and terminal ileal resection or diseases e.g. Crohn’s disease, ulcerative colitis; (iv) nutritional deficiency in strict vegetarians. Only after excluding PA and PPA, the first two overriding causes, gastrointestinal and nutritional causes are considered.

The cause of vitamin B12 deficiency is considered to be undetermined if none of the above conditions are present. Common predisposing factors for the development of vitamin B12 deficiency of undetermined causes include food cobalamin malabsorption, idiopathic gastric atrophy, Helicobacter pylori (HP) associated gastritis, use of PPI and H2B, metformin related malabsorption of vitamin B12, and a multitude of other conditions all of which currently lack easily available confirmatory tools and are diagnosed largely by exclusion. Known causes of vitamin B12 deficiency and the diagnostic approach to arrive at the conclusion of undetermined aetiology of vitamin B12 deficiency are summarized in Box 2.

### 2.4 Measurements

Serum cobalamin was measured with a fluorometric method using Abbott IMX analyzer (Abbott Laboratory, Chicago, IL) from 1994 to 2002; and with a chemiluminescent

immunoassay using paramagnetic particles of Access Immunoassay System (Beckman Coulter, Fullerton, CA) from 2003 to 2010. Reference range for serum vitamin B12 level was 132–835 pmol/L from 1994 to 2002, and 180–914 ng/L from 2003 to 2010. To facilitate analysis, serum vitamin B12 levels in pmol/L were converted to ng/L by using the equation, 1 pmol/L=1.355 ng/L, (molecular mass of vitamin B12=1355 mol/g). Vitamin B12 deficiency was defined by serum vitamin B12 levels below 132 pmol/L or below 180 ng/L.

Box 2: Aetiology of vitamin B12 deficiency	
<u>Known causes</u>	<u>Undetermined causes (diagnostic steps)</u>
(i) pernicious anaemia	(i) exclusion of known causes
(ii) probable pernicious anaemia	(ii) examples of predisposing factors:
(iii) gastrointestinal disorders	food cobalamin malabsorption, gastric atrophy,
(iv) malnutrition	Helicobacter pylori infection, long-term ingestion of metformin, proton pump inhibitor, histamine 2 blocker

## 2.5 Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Inc, Chicago, Illinois, USA), version 15. Data were reported as means and standard deviation (SD), or as medians and interquartile range (IQR). The chi-square test was used for comparison between categorical variables, Student's t test for continuous variables of normal distribution, and Mann-Whitney U test for continuous data of skewed distribution. P values of less than 0.05 were considered to indicate statistical significance. Among patients with B12 deficiency, a chi-square analysis (two-tailed; two-by-two table) was used to estimate the crude OR of having B12 deficiency of undetermined origin among patients with type 2 diabetes to those without diabetes, and another chi-square analysis (two-tailed; two-by-two table) was used to calculate the crude OR of having B12 deficiency of undetermined origin among patients taking metformin to the diabetic patients not taking metformin. Multivariate analysis using stepwise forward logistic regression were used to identify risk factors independently associated with vitamin B12 deficiency of undetermined origin in all patients and in diabetic patients; and in diabetic patients on metformin and not on metformin. Extended Mantel-Haenzel chi-square analysis was performed for linear trend.

## 3. Results

Between May 1994 and October 2010, 635 adult Chinese patients (excluding 1 patient with type 1 diabetes and PA diagnosed at the age of 14 years old) were diagnosed to have overt vitamin B12 deficiency in a regional hospital in Hong Kong. At the time of diagnosis of vitamin B12 deficiency, 191 (30%) patients had type 2 diabetes, and 444 (70%) were non-diabetics.

### 3.1 Vitamin B12 deficiency: Type 2 diabetic and non-diabetic patients

Concerning the demographic features (Table 1), 37% of 191 diabetic patients and 44% of 444 non-diabetic patients were male ( $p=0.079$ ), and the mean ages of diabetic and non-diabetic patients were 75.6 years (SD 9.1) and 72.3 years (SD 14.5) respectively ( $p=0.001$ ). The clinical manifestations of vitamin B12 deficiency were less symptomatic in diabetic than non-diabetic patients, as evidenced by 34% of diabetic and 47% of non-diabetic patients having anaemia ( $p=0.002$ ), and 3% of diabetic and 7% of non-diabetic patients having peripheral

	Number of patients (%)		P	adjusted OR	95% CI
	With diabetes (n=191)	No diabetes (n=444)			
<b>Demographics</b>					
Sex, male	70/191 (37)	196/444 (44)	0.079		
Mean age [SD], years	75.6 [9.1]	72.3 [14.5]	<b>0.001</b>		
<b>Presenting features</b>					
Anaemia	65/191 (34)	210/444 (47)	<b>0.002</b>		
PN/SCD <sup>1</sup>	5/191 (3)	30/444 (7)	<b>0.036</b>		
Dementia	15/191 (8)	41/444 (9)	0.574		
<b>Laboratory findings</b>					
Mean Haemoglobin [SD], g/L	9.8 [2.5]	9.0 [2.9]	<b>0.001</b>		
Mean MCV <sup>2</sup> [SD], fl	110.0 [12.1]	113.4 [15.4]	<b>0.003</b>		
Mean serum B12 level [SD], ng/L	85.8 [38.1]	80.0 [41.9]	<b>0.106</b>		
Intrinsic Factor Antibody +ve	49/191 (26)	248/444 (56)	<b>&lt;0.001</b>		
GPC <sup>3</sup> Antibody +ve	73/191 (38)	245/444 (56)	<b>&lt;0.001</b>		
Anti-thyroid antibody +ve	26/159 (16)	126/337 (37)	<b>&lt;0.001</b>		
<b>Thyrogastric features</b>					
History of AITD <sup>4</sup>	4/191 (2)	29/444 (7)	<b>0.021</b>		
Biopsy proven gastric atrophy	25/80 (31)	86/196 (44)	<b>0.052</b>		
ECH <sup>5</sup>	0/80 (0)	6/196 (3)	0.114		
HP <sup>6</sup> +ve histology	10/80 (13)	16/196 (8)	0.263		
<b>Aetiology of B12 deficiency</b>					
Undetermined	129/191 (68)	132/444 (30)	<b>&lt;0.001</b>	5.54	3.66 - 8.38
Known	62/191 (32)	312/444 (70)			
Pernicious anaemia	54/191 (28)	265/444 (60)	<b>&lt;0.001</b>		
Probable pernicious anaemia	4/191 (2)	15/444 (3)	0.384		
Gastrointestinal surgery/disorder	2/191 (1)	24/444 (5)	<b>0.011</b>		

<sup>1</sup>peripheral neuropathy/subacute combined degeneration <sup>2</sup>mean corpuscular volume

<sup>3</sup>Gastric Parietal Cell, <sup>4</sup>autoimmune thyroid disease, <sup>5</sup>Enterochromaffin Cell Hyperplasia, <sup>6</sup>Helicobacter pylori

Table 1. Comparison of presenting features and causes of vitamin B12 deficiency: type 2 diabetic and non-diabetic patients

neuropathy and/or subacute combined degeneration of cord (p=0.036). Dementia was the presenting feature in 8% of diabetic and 9% of non-diabetic patients (p=0.574). The mean haemoglobin (Hb) concentrations of diabetic and non-diabetic patients were 9.8 g/L (SD 2.5) and 9.0 g/L (SD 2.9) respectively (p=0.001), and the mean mean corpuscular volume (MCV) of the two groups were 110.0 fl (SD 12.1) and 113.4 fl (SD 15.4) respectively (p=0.003). The mean serum vitamin B12 levels of diabetic and non-diabetic patients were 85.8 ng/L (SD 38.1) and 80.0 ng/L (SD 41.9) respectively (p=0.106). Serum IF antibody was detected in 26% of diabetic and 56% of non-diabetic patients (p<0.001); GPC antibody in 38% of diabetic and 56% of non-diabetic patients (p<0.001); and anti-thyroid antibodies in 16% of diabetic and 37% of non-diabetic patients (p<0.001). History of autoimmune thyroid disease (AITD) was obtained in 2% of diabetic and 7% of non-diabetic patients (p=0.021). Endoscopically proven gastric atrophy was detected in 31% of diabetic and 44% of non-diabetic patients (p=0.052),

enterochromaffin cell hyperplasia (ECH) in 0% of diabetic and 3% of non-diabetic patients ( $p=0.114$ ), and HP organisms in 13% of diabetic and 8% of non-diabetic patients ( $p=0.263$ ). The underlying aetiology of vitamin B12 deficiency was undetermined in 68% of diabetic and 30% of non-diabetic patients ( $p<0.001$ ); due to PA in 28% of diabetic and 60% of non-diabetic patients ( $p<0.001$ ); PPA in 2% of diabetic and 3% of non-diabetic patients ( $p=0.384$ ); related to gastrointestinal surgery or disorders in 1% of diabetic and 5% of non-diabetic patients ( $p=0.011$ ); and malnutrition in 1% of diabetic and 2% of non-diabetic patients ( $p=0.484$ ). The percentages of patients with vitamin B12 deficiency of undetermined origin, due to PA, and other known causes (PPA, gastrointestinal disorders, malnutrition) among diabetic and non-diabetic patients were shown in Figure 1. The crude OR of developing vitamin B12 deficiency of undetermined origin in type 2 diabetic patients compared with non-diabetic patients was 4.90 (95% CI, 3.41- 7.08). The adjusted OR (adjusted for sex, age, use of PPI/H2B) of developing vitamin B12 deficiency of undetermined origin in type 2 diabetic patients compared with non-diabetic patients was 5.54 (95% CI, 3.66 – 8.38).

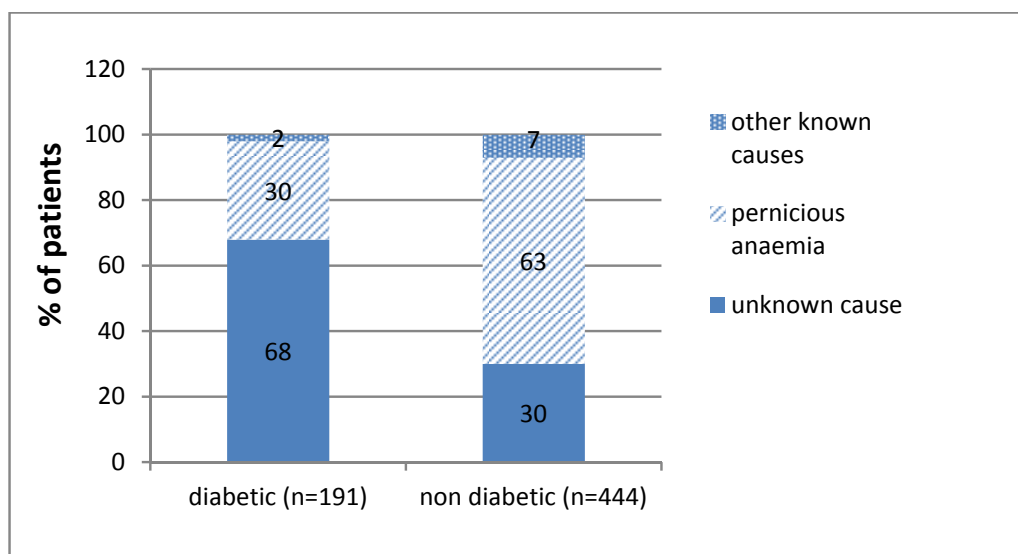


Fig. 1. Comparison of causes of vitamin B12 deficiency: type 2 diabetic and non-diabetic patients

### 3.2 Vitamin B12 deficiency: type 2 diabetic patients “on metformin” and “not on metformin”

Among the 191 patients with type 2 diabetes, 5 patients with unclear metformin history were excluded from analysis, and 186 patients – 128 (69%) “on metformin” and 58 (31%) “not on metformin” were analysed (Table 2). Of the 128 patients “on metformin”, 30% were male, and of the 58 patients “not on metformin”, 50% were male ( $p=0.008$ ). The mean ages of patients “on metformin” and “not on metformin” were 75.3 years [SD 9.2] and 76.3 years [SD 9.2] respectively ( $p=0.463$ ). Thirty one percent of patients “on metformin” and 36% of patients “not on metformin” presented with anaemia ( $p=0.505$ ); 0.8% of patients “on metformin” and 7% of patients “not on metformin” had peripheral neuropathy and/or subacute combined degeneration of cord ( $p=0.017$ ), and 9% of patients “on metformin” and 3% of “not on metformin” had dementia ( $p=0.156$ ). The mean Hb concentrations of patients “on metformin” and “not on metformin” were 9.9 g/L [SD 2.4] and 9.7 g/L [SD 2.6] respectively ( $p=0.498$ ), and the mean MCV 108.5 fl [SD 12.0] and 112.1 fl [SD 11.9] respectively ( $p=0.058$ ). The mean serum

	Number of diabetic patients (%)			adjusted OR	95% CI
	On metformin (n=128)	Not on metformin (n=58)	p		
<b>Demographics</b>					
Sex, male	38/128 (30)	29/58 (50)	<b>0.008</b>		
Mean age [SD], years	75.3 [9.2]	76.3 [9.2]	0.463		
<b>Presenting features</b>					
Anaemia	40/128 (31)	21/56 (36)	0.505		
PN/SCD <sup>1</sup>	1/128 (0.8)	4/58 (7)	<b>0.017</b>		
Dementia	12/128 (9)	2/58 (3)	0.156		
<b>Laboratory findings</b>					
Mean Haemoglobin [SD], g/L	9.9 [2.4]	9.7 [2.6]	0.498		
Mean MCV <sup>2</sup> [SD], in fl	108.5 [12.0]	112.1 [11.9]	0.058		
Mean serum B12 level [SD], ng/L	90.3 [37.6]	76.7 [38.6]	<b>0.026</b>		
Intrinsic Factor Antibody +ve	20/128 (16)	24/58 (41)	<b>&lt;0.001</b>		
Gastric Parietal Cell Antibody +ve	41/127 (32)	30/58 (52)	<b>0.012</b>		
Anti-thyroid Antibody +ve	15/115 (13)	11/42 (26)	<b>0.050</b>		
<b>Thyrogastric features</b>					
History of AITD <sup>3</sup>	3/128 (2.3)	1/58 (1.7)	0.787		
Biopsy proven gastric atrophy	8/48 (17)	13/28 (46)	<b>0.005</b>		
Enterochromaffin Cell Hyperplasia	0/48 (0)	0/28 (0)	-		
Helicobacter pylori +ve histology	8/48 (17)	2/28 (7)	0.236		
<b>Aetiology of B12 deficiency</b>					
Undetermined	102/128 (80)	27/58 (47)	<b>&lt;0.001</b>	5.20	2.33-11.60
Known	26/128 (20)	31/58 (53)			
Pernicious anaemia	22/128 (17)	27/58 (47)	<b>&lt;0.001</b>		
Probable pernicious anaemia	1/128 (0.8)	3/58 (5)	0.384		
Gastrointestinal surgery/disorder	1/128 (0.8)	1/58 (1.7)	0.011		
Nutritional	2/128 (1.6)	0/58 (0)	0.484		
<b>Median diabetic duration [IQR], years</b>	<b>10 [6.5-11.5] (n=121)</b>	<b>4 [1.5-8.5] (n=41)</b>	<b>&lt;0.001</b>		

<sup>1</sup>peripheral neuropathy/subacute combined degeneration

<sup>2</sup>mean corpuscular volume <sup>3</sup>autoimmune thyroid disease

Table 2. Comparison of presenting features and causes of vitamin B12 deficiency: type 2 diabetic patients “on metformin” and “not on metformin”

B12 levels of patients “on metformin” and “not on metformin” were 90.3 ng/L [SD 37.6] and 76.7 ng/L [SD 38.6] respectively (p=0.026). Frequencies of IF antibody in the “on metformin” and “not on metformin” groups were 16% and 41% respectively (p<0.001), that of GPC antibody 32% and 52% respectively (p=0.012), and that of anti-thyroid antibodies 13% and 26% respectively (p=0.050). History of AITD was obtained in 2.3% of patients “on metformin” and 1.7% of patients “not on metformin” (p=0.787). Histological evidence of gastric atrophy was detected in 17% of patients “on metformin” and 46% of patients “not on metformin” (p=0.005), ECH in none of the patients in the two groups, and HP organisms in



17% of patients “on metformin” and 7% of patients “not on metformin” ( $p=0.236$ ). The median duration of diabetic history for those “on metformin” ( $n=121$ ) and “not on metformin” ( $n=41$ ) were 10 years [IQR 6.5-11.5] and 4 years [IQR 1.5-8.5] respectively ( $p<0.001$ ).

Of the 128 diabetic patients “on metformin” and 58 diabetic patients “not on metformin”, the causes of vitamin B12 deficiency were undetermined in 80% of patients “on metformin” and in 47% of those “not on metformin” ( $p<0.001$ ); due to PA in 17% of patients “on metformin” and 47% of “not on metformin” ( $p<0.001$ ); PPA in 0.8% of patients “on metformin” and 5% of “not on metformin” ( $p=0.384$ ); due to gastrointestinal disorders in 0.8% and 1.6% of patients “on metformin” and “not on metformin” respectively ( $p=0.011$ ), and due to malnutrition in 1.5% and 0% of patients “on metformin” and “not on metformin” respectively ( $p=0.484$ ). The percentages of patients with vitamin B12 deficiency of undetermined origin, due to PA, and other known causes (PPA, gastrointestinal disorders, malnutrition) among non-diabetic patients, diabetic patients “on metformin”, and diabetic patients “not on metformin” were shown in Figure 2. The crude OR of developing vitamin B12 deficiency of undetermined origin in type 2 diabetic patients “on metformin” compared with diabetic patients “not on metformin” was 4.50 (95% CI, 2.30 - 8.82). The adjusted OR (adjusted for sex, age, use of PPI/H2B) of developing vitamin B12 deficiency of undetermined origin in type 2 diabetic patients “on metformin” compared with diabetic patients “not on metformin” was 5.20 (95% CI, 2.33 - 11.60).

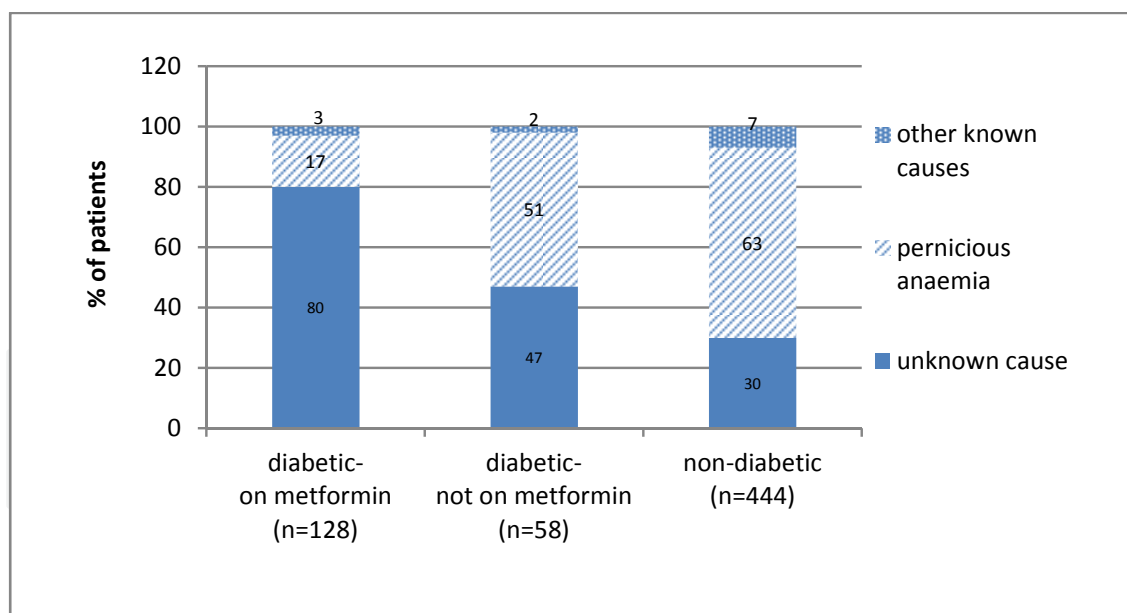


Fig. 2. Comparison of causes of vitamin B12 deficiency: diabetic patients on metformin and not on metformin, non-diabetic patients

### 3.3 Vitamin B12 deficiency of undetermined origin: “on metformin” and “not on metformin”

Of the 191 diabetic patients with vitamin B12 deficiency, 62 patients (including 5 with unclear metformin history) had known causes of vitamin B12 deficiency. Excluding these 62

patients, 129 diabetic patients had vitamin B12 deficiency of undetermined aetiology - 102 (79%) "on metformin" and 27 (21%) "not on metformin" at the time of diagnosis of vitamin B12 deficiency (Table 3). Thirty two percent of patients "on metformin" were male, and 44% of patients "not on metformin" were male (p=0.241). The mean ages of patients "on metformin" and "not on metformin" were 76.4 years [SD 8.2] and 77.1 years [SD 10.3] respectively (p=0.721). Anaemia was the presenting feature in 25% of patients "on metformin"

	Number of patients (%)		P
	On metformin (n=102)	Not on metformin (n=27)	
<b>Demographics</b>			
Sex, male	33/102 (32)	12/27 (44)	0.241
Mean age [SD], in years	76.4 [8.2]	77.1 [10.3]	0.721
<b>Presenting features</b>			
Anaemia	25/102 (25)	8/27 (30)	0.588
PN/SCD <sup>1</sup>	1/102 (1)	3/27 (11)	<b>0.007</b>
Dementia	9/102 (9)	1/27 (4)	0.376
<b>Laboratory findings</b>			
Mean Haemoglobin [SD], g/L	10.2 [2.2]	10.2 [2.4]	0.988
Mean MCV <sup>2</sup> [SD], fl	107.3 [11.1]	108.2 [11.3]	0.706
Mean serum B12 level [SD], ng/L	93.7 [38.1]	86.0 [43.5]	0.369
Gastric Parietal Cell Antibody +ve	24/101(24)	12/27 (44)	<b>0.034</b>
Anti-thyroid Antibody +ve	11/91 (12)	0/18 (26)	0.120
<b>Thyrogastric features</b>			
History of AITD <sup>3</sup>	1/102 (1)	0/27 (0)	0.606
Biopsy proven gastric atrophy	2/39 (5)	3/13 (23)	0.057
Enterochromaffin Cell Hyperplasia	0/39 (0)	0/13 (0)	-
Helicobacter pyloria +ve histology	8/39 (21)	1/13 (8)	0.290
<b>Median diabetic duration [IQR] in years</b>	10 [6-10] (n=99)	3.5 [1.3-6.8] (n=20)	<b>&lt;0.001</b>
<b>Diabetic medications</b>			
Sulph <sup>4</sup> +metformin* Sulph <sup>4</sup> alone**	54/102 (53)*	11/27 (41)**	0.260
Insulin+metformin* Insulin alone**	9/102 (9)*	1/27 (4)**	0.376
Metformin alone	39/102 (38)	-	
Nil anti-diabetic medication	-	15/27 (55)	
<b>Other concomitant medications</b>			
PPI/H2B <sup>5</sup>	26/101 (26)	9/26 (35)	0.815
Anti-platelet agents	42/101 (42)	6/26 (23)	0.083
Lipid lowering agents	28/101 (28)	3/26 (12)	0.087
Hypertensive/cardiac agents	88/101 (87)	18/26 (69)	<b>0.028</b>

<sup>1</sup>peripheral neuropathy/subacute combined degeneration <sup>2</sup>mean corpuscular volume

<sup>3</sup>autoimmune thyroid disease

<sup>4</sup>sulphonylurea <sup>5</sup>proton pump inhibitor/ histamine 2 blocker

Table 3. Comparison of presenting features and medications of diabetic patients with vitamin B12 deficiency of undetermined aetiology: "on metformin" and "not on metformin"

and 30% of patients “not on metformin” ( $p=0.588$ ), peripheral neuropathy/subacute combined degeneration of cord in 1% of patients “on metformin” and 11% of patients “not on metformin” ( $p=0.007$ ), and dementia in 9% of patients “on metformin” and 4% of patients “not on metformin” ( $p=0.376$ ). The mean Hb concentrations of those “on metformin” and “not on metformin” were 10.2 g/L [SD 2.2] and 10.2 g/L [SD 2.4] respectively ( $p=0.988$ ), the mean MCV of the two groups were 107.3 fl [SD 11.1] and 108.2 fl [SD 11.3] respectively ( $p=0.706$ ), and the mean serum vitamin B12 levels were 93.7 ng/L [SD 38.1] and 86.0 ng/L [SD 43.5] respectively ( $p=0.369$ ). GPC antibody was detected in 24% of patients “on metformin” and 44% of patients “not on metformin” ( $p=0.034$ ), and anti-thyroid antibodies in 12% and 26% of the two groups respectively ( $p=0.120$ ). AITD occurred in 1% of patients “on metformin” and none of the patients “not on metformin” ( $p=0.606$ ). Histology of gastric atrophy was detected in 5% of patients “on metformin” and 23% of patients “not on metformin” ( $p=0.057$ ), ECH in none of the patients in the two groups, and histological evidence of HP organisms in 21% and 8% of the two groups respectively ( $p=0.290$ ).

The median duration of diabetes for the “on metformin” ( $n=99$ ) and “not on metformin” ( $n=20$ ) patients were 10 years [IQR 6-10] and 3.5 years [IQR 1.3-6.8] respectively ( $p<0.001$ ). Fifty three percent of the “on metformin” patients were also on sulphonylureas, and 41% of the “not on metformin” patients were on sulphonylurea alone ( $p=0.260$ ). Nine percent of the “on metformin” patients were also on insulin, and 4% of the “not on metformin” patients were on insulin alone ( $p=0.376$ ). Thirty eight percent of the “on metformin” patients were on metformin alone, and 55% of the “not on metformin” patients were not on any diabetic agents - 4 chronic renal insufficiency, 2 early deaths, 9 early stage of diabetes on dietary control alone (Table 3). Other concomitant medications of 101 (99%) “on metformin” and 26 (96%) “not on metformin” diabetic patients at the time of diagnosis of vitamin B12 deficiency were available for analysis. Twenty six percent of “on metformin” and 35% of “not on metformin” diabetic patients were on either PPI or H2B ( $p=0.815$ ), 42% of “on metformin” and 23% of “not on metformin” diabetic patients were on antiplatelet agents, ( $p=0.083$ ), 28% of “on metformin” and 12% of “not on metformin” diabetic patients were on lipid lowering agents ( $p=0.087$ ), and 87% of “on metformin” and 69% of “not on metformin” diabetic patients were on antihypertensive agents and/or medications for ischaemic heart disease ( $p=0.028$ ).

### 3.4 Duration of metformin use in relation to development of vitamin B12 deficiency

Of the 128 diabetic patients on metformin, 47 (37%) patients developed vitamin B12 deficiency of all causes within 1 to 5 years of metformin use, 71 (55%) within 6 to 10 years, and 10 (8%) after 10 years, as shown in Figure 3 (i). Among these 128 patients, 102 (80%) patients had vitamin B12 deficiency of undetermined aetiology, and 26 (20%) had known causes. Of the 102 patients with undetermined aetiology, 31 (30%) developed overt vitamin B12 deficiency within 1 to 5 years of metformin use, 63 (62%) within 6 to 10 years, and 8 (8%) beyond 10 years, up to 30 years. Of the 26 patients with known causes, 16 (62%) developed vitamin B12 deficiency within 1 to 5 years of metformin use, 8 (31%) within 6 to 10 years, and 2 (8%) after 10 years. The relationship between the duration of metformin use and the development of vitamin B12 deficiency of undetermined and known causes was shown in Figure 3 (ii). The Extended Mantel-Haenzel chi-square analysis for linear trend was 4.74 ( $p = 0.029$  with one degree of freedom).

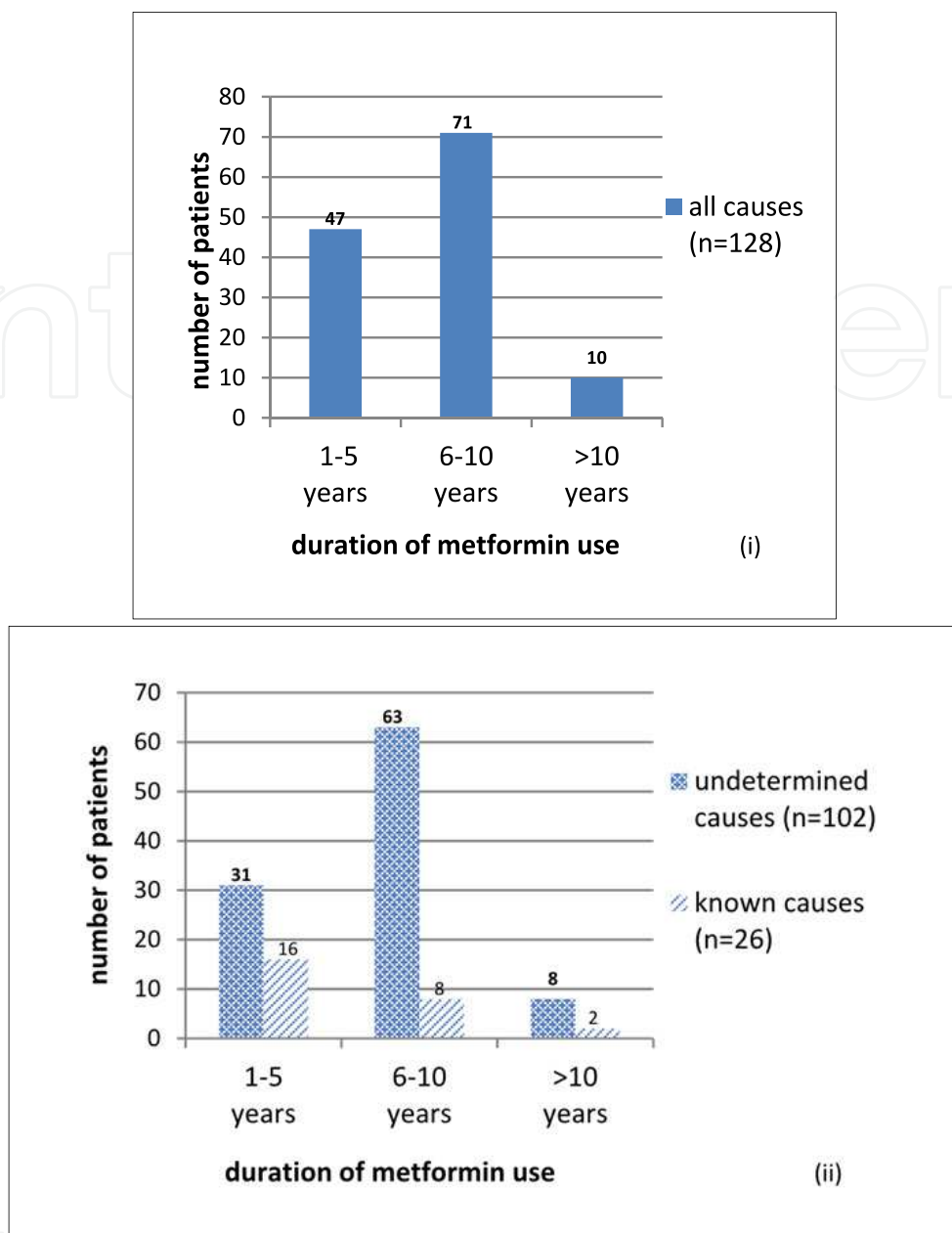


Fig. 3. Relationship between duration of metformin use and development of vitamin B12 deficiency (i) of all causes (ii) of undetermined and known causes

## 4. Conclusions

### 4.1 Characteristics of vitamin B12 deficiency in type 2 diabetic patients

The present study illustrates certain characteristics of vitamin B12 deficiency in adult Chinese patients with type 2 diabetes. Majority of the common causes of vitamin B12 deficiency encountered in the general population such as PA, gastrointestinal diseases and nutritional deficiency also occur in type 2 diabetic patients. The frequencies of occurrence of these causes however differ between diabetic and non-diabetic patients. Vitamin B12 deficiency of undetermined origin is more frequently encountered in diabetic than non-diabetic patients (68% versus 30%), whereas PA, the most common definitive cause of

vitamin B12 deficiency, is more frequently detected in non-diabetic than diabetic patients (63% versus 30%). This preponderance of vitamin B12 deficiency of undetermined origin is even more conspicuous in diabetic patients on metformin than diabetic patients not on metformin (80% versus 47%). In this cohort of adult Chinese patients, the risk of developing vitamin B12 deficiency of undetermined origin is 5.5-fold higher in diabetic than non-diabetic patients, and diabetic patients taking metformin have a 5.2-fold higher risk of developing vitamin B12 deficiency of undetermined origin than patients not taking metformin.

This remarkable difference in the aetiological pattern of vitamin B12 deficiency between diabetic and non-diabetic patients accounts for the differences in the clinical manifestations of vitamin B12 deficiency between the two groups of patients. PA as an autoimmune disease causes profound gastric atrophy, IF depletion and vitamin B12 malabsorption, and is often accompanied by immune features; whereas conditions causing vitamin B12 deficiency of undetermined origin are often non-immune processes. They tend to cause a less severe degree of vitamin B12 malabsorption and have a paucity of immune features. Diabetic patients with vitamin B12 deficiency of undetermined origin thus generally have a less severe degree of vitamin B12 deficiency, a less severe degree of anaemia, and less autoimmune features such as GPC and antithyroid antibodies, AITD, and autoimmune gastropathy compared with non-diabetic patients, as shown in the study.

## **4.2 Implications of metformin use in type 2 diabetic patients**

### **4.2.1 Evidence of association of metformin use with vitamin B12 deficiency**

The finding of a greater proportion of type 2 diabetic patients especially those on metformin having vitamin B12 deficiency of undetermined origin in comparison with non-diabetic subjects implies the association of metformin use with vitamin B12 deficiency of undetermined origin. However, not all cases of vitamin B12 deficiency of undetermined origin are related to metformin. Vitamin B12 deficiency of undetermined origin actually has causative factors. Lack of easily available diagnostic tools bars the clarification or confirmation of these factors. In general, diagnostic approach to define the causes of vitamin B12 deficiency is first to look for nutritional deficiency and malabsorption syndromes notably PA and structural lesions of the stomach and intestine. These are the classical known causes of vitamin B12 deficiency. The previously less common or less well defined aetiologies of vitamin B12 deficiency, such as atrophic gastritis, gastric disease associated with *Helicobacter pylori* infection, gastric bypass for obesity, pancreatic insufficiency due to alcohol abuse, long-term use of metformin and acid-suppressive drugs like cimetidine, ranitidine, omeprazole, intestinal bacterial overgrowth, or even ageing and idiopathic, are principally diagnosed on clinical grounds and are grouped as vitamin B12 deficiency of undetermined origin, or now increasingly known as food cobalamin malabsorption, to be distinguished from the classical known causes (Clarke & Brown, 2003; Andres, 2008). Metformin-induced vitamin B12 deficiency is among one of the miscellaneous causes which are classified as “unknown” or “undetermined” simply because of lack of objective means to prove it as the sole agent or condition in the causation of vitamin deficiency.

The present cohort study, comprising 186 type 2 diabetic patients with 128 on metformin, serves only to provide indirect evidence that metformin use constitutes a risk factor for the development of vitamin B12 deficiency, among other “unknown” factors which may also be present in these diabetic patients. The methodology adopted by the study is traditional - exclusion of the classical known causes. In this context, the study has the advantage that as

part of a previously reported longitudinal study of PA (Chan et al., 2008), all patients had documented vitamin B12 deficiency, had IF and GPC antibody assay performed, and had Schilling test done during the period when it was available. This diagnostic approach to arrive at the conclusion of metformin-induced vitamin B12 deficiency is similar to that cited in the other reports (Table 4, Table 5).

In addition, the study attempted to include medications other than metformin in the analysis of risk factors. Two principal categories of drugs that have been commonly reported to have interaction with vitamin B12 are biguanides and acid suppressive therapy. PPI and H2B have been shown to suppress acid secretion by parietal cells and impair the absorption of protein bound dietary vitamin B12 and thus contribute to the development of vitamin B12 deficiency (Howden, 2000; Ruscini et al., 2002; Force et al., 2003; den Elzen et al., 2008; Ali et al., 2009). The current evidence of vitamin B12 deficiency associated with long-term PPI use however is based on small nonrandomized retrospective studies (Steinberg et al., 1980; Marcuard et al., 1994; Termanini et al., 1998; Hirschowitz et al., 2008). Overall the current collective body of information supports the notion that prolonged use of PPI notably in the elderly and in persons on high doses of PPI is associated with increased risk of vitamin B12 deficiency (Thomas et al., 2010). In the present study, evaluation of medications prescribed at the time of diagnosis of vitamin B12 deficiency showed no significant differences in the utilization rates of PPI/H2B, between diabetic and non-diabetic patients, and diabetic patients "on metformin" and "not on metformin". There were also no significant differences in the utilization rates of other medications such as lipid lowering agents, anti-platelet agents, sulphonylureas and insulin between diabetic patients "on metformin" and "not on metformin". As all patients in one group were exposed to metformin compared to none in the other group, metformin in the study emerged as the most likely culprit for medication-related vitamin B12 malabsorption. The antihypertensive and cardiovascular agents were significantly more frequently used by diabetic patients "on metformin" than those "not on metformin" in the study. This could be related to the longer diabetic history and the associated more cardiovascular complications in the group of patients on metformin. Although there are studies reporting longer diabetic history might predispose to the development of vitamin B12 deficiency (Pflipsen et al., 2009), there is insufficient data for a definite conclusion to be drawn.

Literature search found further evidence of metformin related vitamin B12 malabsorption. Impaired vitamin B12 absorption in patients who had been treated with metformin for up to 3 months were first reported in 1969 (Berchtold et al., 1969). In the ensuing 4 decades, both observational and interventional studies unequivocally demonstrated the association between metformin use and vitamin B12 deficiency (Table 4). An observational study described malabsorption of vitamin B12 in 30% of diabetic patients during biguanide therapy (Adams et al., 1983). A cross-sectional study demonstrated a 22% prevalence of metabolically confirmed vitamin B12 deficiency in type 2 diabetic patients, with those on metformin exhibiting a statistically higher risk for B12 deficiency (Pflipsen et al., 2009). Another cross-sectional study demonstrated 6.9% of diabetic patients on metformin had low serum vitamin B12 level (Nervo et al., 2011). A case-control study demonstrated impaired gastrointestinal absorption in 30% and decreased serum B12 levels in 6% of patients in the metformin arm compared to none in the control arm (Tomkin et al., 1971). Another case-control study similarly demonstrated significant falls in the mean serum B12 levels in the metformin arm compared to the control arm (Bauman et al., 2000). A nested case-control study comparing diabetic patients on metformin with and without vitamin B12 deficiency

Authors Report year	Metformin duration	Metformin dosage	Outcome parameters	No. of patients on metformin	Study type
Tomkin 1971	4-6 y <sup>1</sup>	1.5-6g/d	*absorption B12↓ **serum B12↓	*21/71 (30%) **4/71 (6%)	[2]
		<i>control</i>	<i>no change</i>	19 on S <sup>5</sup>	
Adams 1983	1-6 y	1-3g/d	absorption B12 ↓	14/46 (30%)	[1]
Bauman 2000	3 months	0.85-2.55g/d	mean serum B12↓ serum holoTCII <sup>2</sup> ↓	14	[2]
		<i>control</i>	<i>no change</i>	7 on S <sup>5</sup>	
Wulffele 2003	16 weeks	0.85-2.55g/d	mean serum B12↓: 16% HCy <sup>3</sup> ↑: 8%	171	[3]
		<i>placebo</i>	B12↓: 2% HCy↑: 4%	182 on I <sup>6</sup>	
Hermann 2004	mean 5.2 y (range 1-18)	mean 2.2 g/d (range 0.85-3.4)	*serum B12↓ **serum HCy↑	*12/53 (23%) **7/53 (13%)	[2]
		<i>control</i>	<i>no change</i>	31 on S <sup>5</sup> I <sup>6</sup> D <sup>7</sup>	
Pongchaidecha 2004	>6months	-	mean serum B12 } P=0.011 serum B12 }	88 64§	[2]
Ting 2006	median 4 y (IQR 2-5) median 2 y (IQR 1-4)	2.0±0.7g/d 1.4±0.7g/d	mean serum B12↓ serum B12 normal	155 310	[2]
Sahin 2007	6 weeks	- <i>control</i>	mean serum B12↓ <i>no change</i>	165	[2]
Pflipsen 2009	-	-	serum B12↓ ±MMA <sup>4</sup> , HCy↑	44/195†(22%)	[1]
Stehouwer 2010	4.3 y	2.05g/d <i>placebo</i>	mean serumB12↓:19% serum HCy↑:26% mean serum B12↓:0% serum HCy↑:20%	134 146 on I <sup>6</sup>	[3]
Nervo 2011	median 4 y (IQR 2-8.1)	1.7-2.55g/d	serum B12↓	10/144(6.9%)	[1]

<sup>1</sup>years <sup>2</sup>holotranscobalamin II <sup>3</sup>homocysteine <sup>4</sup>methylmalonic acid <sup>5</sup>sulphonylurea <sup>6</sup>insulin <sup>7</sup>diet  
[1] observational / cross-sectional study [2] case control cohort study [3] randomized placebo  
controlled study

† ± metformin § no metformin

Table 4. Studies investigating associations between metformin use and vitamin B12 deficiency

indicated both the dose and the duration of metformin use are risk factors for vitamin B12 deficiency (Ting et al., 2006). Other cross-sectional cohort studies in Sweden, Thailand and Turkey also pointed to patients on metformin had lower serum B12 levels compared with thenon-exposed control group (Hermann et al., 2004; Pongchaidecha et al., 2004; Sahin et al., 2007). Effects of short-term (16 weeks) and long-term (52 months) treatment with metformin on the serum concentrations of vitamin B12 in type 2 diabetic patients were investigated by a Dutch randomized placebo controlled trial and showed both short- and long-term use of metformin increased the risk of vitamin B12 deficiency (Wulffele et al., 2003; Stehouwer et al., 2010). All these studies defined vitamin B12 deficiency based on biochemical markers and did not evaluate the clinical importance.

Concerning reports on the clinical manifestations of metformin-related vitamin B12 deficiency in diabetic patients (Table 5), there are case reports of megaloblastic anaemia related to long-term metformin treatment causing vitamin B12 deficiency (Callaghan et al., 1980; Gilligan, 2002; Liu et al., 2006; Lin et al., 2007). A case series showed metformin-related megaloblastic anaemia accounted for 6% (10/162) of all vitamin B12 deficiency patients (Andres et al., 2002), and another case series revealed a 9% prevalence (54/600) of megaloblastic anemia in diabetic patients taking long-term biguanides (Filioussi et al., 2003). Neurological deficits due to vitamin B12 deficiency manifest chiefly as peripheral neuropathy, subacute combined cord degeneration, and cognitive impairment. There are case reports of subacute combined degeneration and peripheral neuropathy related to metformin therapy (Liu et al., 2006; Bell, 2010), and a prospective case-control study demonstrating diabetic patients with concurrent symptomatic peripheral neuropathy had lower serum B12 levels and more severe neuropathy in the metformin treatment group in comparison with non-metformin treated patients (Wile & Toth, 2010).

Authors Report year	Metformin duration	Metformin dosage	Presenting features	No. of patients on metformin	Tests	Metformin off/ continued	B12	Study type
Callaghan 1980	8 y <sup>1</sup>	1g/d	MA <sup>2</sup>	1	Schill <sup>5</sup>	continued	inj <sup>10</sup>	[4]
Gilligan 2002	5 y	-	MA	1	Schill IF <sup>6</sup>	off	oral	[4]
Andres 2002	8.9±3.4 y range 3-10	2.015± 0.45g/d range 1.4-2.5	MA	10	Schill IF	continued:8 off:2	inj:4 oral:6	[5]
Filioussi 2003	11.8±3.6 y	-	MA	54/600 (9%)	GPC <sup>7</sup>	continued	Route NM	[5]
Liu 2006	*>10 y **8 y	-	*SCD <sup>3</sup> , dementia **MA	1 1	IF IF GPC	off off	inj inj	[4]
Lin 2007	6 y	1g/d	MA DVT <sup>4</sup>	1	GPC	off	inj	[4]
Wile 2010	>6 months	total 3389.5g  control	mean B12 ↓, more severe neuropathy normal B12, less severe neuropathy	59  63 †				[6]
Bell 2010	3 y	-	peripheral neuropathy	1	IF CD <sup>8</sup>	NM <sup>9</sup>	inj	[4]

<sup>1</sup>years <sup>2</sup>megaloblastic anaemia <sup>3</sup>subacute combined degeneration <sup>4</sup>deep vein thrombosis <sup>5</sup>Schilling test <sup>6</sup>intrinsic factor antibody <sup>7</sup>gastric parietal cell antibody <sup>8</sup>celiac disease ruled out <sup>9</sup>not mentioned <sup>10</sup>injection †on sulphonylurea/insulin [4] case report [5] case series [6] prospective case control study

Table 5. Reports of metformin-related symptomatic vitamin B12 deficiency

B12 malabsorption associated with long-term metformin treatment (Callaghan et al., 1980; Gilligan, 2002; Liu et al., 2006; Lin et al., 2007). A case series showed metformin-related megaloblastic anaemia accounted for 6% (10/162) of all established vitamin B12 deficiency patients (Andres et al., 2002), and another case series revealed a 9% prevalence (54/600) of megaloblastic anemia in diabetic patients taking long-term biguanides (Filioussi et al., 2003).



Neurological deficits due to vitamin B12 deficiency manifest chiefly as peripheral neuropathy, subacute combined cord degeneration, and cognitive impairment. There are case reports of subacute combined degeneration and peripheral neuropathy related to metformin therapy (Liu et al., 2006; Bell, 2010), and a prospective case-control study demonstrating diabetic patients with concurrent symptomatic peripheral neuropathy had lower serum B12 levels and more severe neuropathy in the metformin treatment group compared with non-metformin treated patients (Wile & Toth, 2010).

#### **4.2.2 Duration and dosage of metformin use in relation to onset of vitamin B12 deficiency**

From the present study, for diabetic patients on metformin, about 30% developed vitamin B12 deficiency of undetermined origin within 5 years of metformin use, and 60% within 6 to 10 years; and for those with known causes especially those with PA, 60% within the first 5 years, and 30% within 6 to 10 years. The main determining factor in the latter group of patients was probably the known cause, e.g. PA, and whether metformin use contributed to or hastened the development of vitamin B12 deficiency in these patients is difficult to determine at this stage. Due to the retrospective nature of data collection concerning diabetic control and medications, precise analysis of metformin duration and dose in relation to the development of vitamin B12 deficiency is beyond the capacity of the present study. One practical message is that clinicians should be aware of the development of vitamin B12 deficiency with both short-term and long-term use of metformin ranging from 1 year to beyond 10 years. An equally important message corollary to this is that known causes of vitamin B12 deficiency such as PA occur in diabetic patients as in non-diabetic subjects, and the same diagnostic approach to delineate the underlying cause of vitamin B12 deficiency should be adopted for diabetic patients as for other patients in general.

From the reports in the literature (Table 4), diminished absorption of vitamin B12, manifested as decreased serum B12 levels, occurred as early as 3 to 4 months after the use of metformin (Bauman et al., 2000; Wulffele et al., 2003). Symptomatic deficiency of the vitamin, according to most reports (Table 5), occurred 5 to 10 years after the use of metformin. This is in line with the established knowledge that the body store of vitamin B12 is huge (2500 µg) in comparison to daily loss/requirement (1-2 µg) (Carmel, 2008), and depletion of the pre-existing body store vitamin thus takes 12 to 15 years. The process of exhaustion of body store will be hastened in the presence of other predisposing factors for vitamin B12 deficiency such as partial gastrectomy.

Two recent reports concerning metformin-related vitamin B12 deficiency suggested need for improved management of such patients. The increased risk of vitamin B12 deficiency with metformin use was re-emphasized by a nested case-control study of Chinese patients (Ting et al., 2006). It showed correlation between the dose and duration of metformin use with vitamin B12 deficiency. Compared with those receiving metformin for less than 3 years, the adjusted OR was 2.39 (95% CI, 1.46-3.91) for those using metformin for 3 years or more. After excluding subjects with borderline vitamin B12 concentrations, the metformin dose remained the strongest independent predictor of vitamin B12 deficiency. Each 1-g/day increment in metformin dose conferred an OR of 2.88 (95% CI, 2.15-3.87) of developing vitamin B12 deficiency. In a prospective randomized placebo controlled trial, the Dutch scientists found the fall in serum B12 levels with metformin persists and becomes more apparent with time (Stehouwer et al., 2010). It is reasonable to assume that harm will eventually occur in some patients with metformin-induced low vitamin B12 levels.

#### 4.2.3 Treatment of metformin-induced vitamin B12 deficiency

There is no consensus on optimal treatment of metformin-induced vitamin B12 deficiency. In the present study, metformin was continued unless there were other reasons for discontinuation or switching to other anti-diabetic therapy such as renal insufficiency or poor glycaemic control. Vitamin B12 replacement was given intramuscularly, daily, monthly, and then 3-monthly as maintenance. Most authors of the case reports/series (Table 5) treated their patients' metformin-induced megaloblastic anaemia with vitamin B12 either orally or parenterally at 1000 µg daily or on alternate days initially, and continued metformin therapy (Callaghan et al., 1980; Andres et al., 2002; Filioussi et al., 2003). In all these cases the anaemia responded but the neuropathy of one patient persisted (Bell, 2010).

In general, two aspects of treatment have to be considered. Vitamin B12 replacement is mandatory and can be given either intramuscularly or orally. Parenteral route may be preferred for subjects with neurological deficits due to the potential risk of irreversibility. The rationale for oral vitamin B12 replacement is that there is evidence for an alternative pathway of vitamin B12 absorption that does not require IF or the presence of an intact ileum (Berlin et al., 1968; Kuzminski et al., 1998; Andres et al., 2008). For both routes of administration, large doses of crystalline cobalamin in the range of 1000 µg should be given. The duration of vitamin B12 supplementation depends on the removal of the triggering factor. If metformin is continued, appropriate vitamin B12 replacement should be administered. Although malabsorption of vitamin B12 is reversible 2 to 8 weeks after stopping metformin therapy (NeLM, 2005), withdrawal of metformin is not mandatory in view of its efficacy and safety, and most of the complications of vitamin B12 deficiency are remediable apart from irreversible damage to the central and peripheral nervous system which may occur if treatment is delayed.

Up to now, the mechanism of metformin-related vitamin B12 malabsorption is still not fully understood. Metformin causes fall in blood glucose primarily through suppression of hepatic gluconeogenesis, enhancement of insulin sensitivity, and reduction of gastrointestinal absorption of glucose. This action is achieved through reduction of efficiency of ion exchange across cell membranes which is brought about through activation of AMP-activated protein kinase and perhaps other enzymes. This mode of action also underlies the untoward side effects of increased intestinal motility and lactic acidosis. Whether the process of malabsorption of vitamin B12 is also similarly mediated is unclear. Currently the most likely mechanism is impaired uptake of vitamin B12-intrinsic factor complex at the ileal cell membrane receptors. This uptake has been shown to be a calcium dependent process (Bauman et al., 2000). The hydrophobic tail of metformin extends into the hydrocarbon core of cell membrane and the protonated metformin group gives a positive charge to the membrane surface and displaces divalent cations. Thus by altering the membrane potentials and affecting the divalent cation membrane functions, especially those calcium-dependent ones, metformin may act as a calcium channel blocker. This postulation is supported by studies showing reversal of malabsorption by increasing calcium intake (Bauman et al., 2000). Other studies showed the malabsorption might be related to decreased IF secretion (Adams et al., 1983), or delay in intestinal transit and bacterial overgrowth in diabetic patients. Some studies however failed to show bacterial overgrowth in such patients (Bauman et al., 2000). Further research is required to clarify the mode of metformin-induced malabsorption of vitamin B12.

### 4.3 Future direction

The prevalence of vitamin B12 deficiency is expected to increase with time in view of the ageing population and the more widespread use of sensitive metabolite assays to detect subtle cobalamin deficiency. Among the population over the age of 60, the prevalence of metabolically confirmed vitamin B12 deficiency ranges from 12% to 23% (Pennypacker et al., 1992; Lindenbaum et al., 1994; Johnson et al., 2003). Type 2 diabetes, at the same time, is becoming more common almost everywhere in the world. The global prevalence of type 2 diabetes is 2.8% (171 million people) in 2000 (Wild et al., 2004), 6.6% (285 million) in 2010, and will be reaching 7.8% (438 million) in 2030 (The Diabetes Atlas, 2009). The global prevalence of age-standardized adult diabetes, according to a recent large international study, was 9.8% (8.6-11.2) for men, and 9.2% (8.0-10.5) for women in 2008 (Danaei et al., 2011). In Hong Kong, the prevalence of type 2 diabetes in elderly subjects above the age of 60 is estimated to range between 9.8% (Woo et al., 1987) and 10.7% (Kung et al., 1996), and the age-standardized prevalence of type 2 diabetes for the 35-64 age group was 9.5% for men and 10.2% for women (Lam et al., 2000).

(new paragraph) Concerning anti-diabetic therapy, metformin remains one of the two oral anti-diabetic drugs in the WHO model list of essential medicine in 2010, in spite of a large array of new therapeutic agents available in the pharmaceutical market. It is the only anti-diabetic drug that has been conclusively shown to prevent the cardiovascular complications of diabetes. The drug remains active after months or years of therapy and its efficacy is little dependent on the residual effective  $\beta$ -cell mass. It causes less weight gain and fewer hypoglycaemic attacks compared with sulphonylureas and insulin. It is now recommended as the initial therapy for type 2 diabetes by the National Institute for Health and Clinical Excellence of the United Kingdom, the American Diabetes Association, and the European Association for the Study of Diabetes. As metformin has been available in the United Kingdom since 1958, in Canada since 1972, in the United States since 1995, and for over 40 years in Hong Kong, the pool of diabetic patients with metformin-induced vitamin B12 deficiency, diagnosed or undiagnosed, could be considerable in the present era. Concomitant occurrence of two comorbid conditions, diabetes and vitamin B12 deficiency, in one subject is apparently undesirable. Florid deficiency of vitamin B12 is accompanied and actually preceded by reciprocal rise of homocysteine levels imposing an increased risk of cardiovascular complications (Hoogeveen et al., 2000; The Homocysteine Studies Collaboration, 2002). Neurological deficits, one of the major manifestations of vitamin B12 deficiency can often be diagnostically confused with diabetic neuropathy, one of the long-term microangiopathic complications of diabetes. Clinicians should therefore have a high index of suspicion for metformin-induced vitamin B12 deficiency in diabetic patients.

To prevent vitamin B12 deficiency, it is reasonable to consider formulating appropriate screening strategy for diabetic patients on metformin. According to the WHO's criteria, a condition is worthwhile screening if it is an important health problem, if there are simple tests available to detect the condition at an early and treatable stage, and if effective and safe treatment is available (Wilson & Junger, 1968). The condition of vitamin B12 deficiency fulfills these criteria. Before such screening guidelines are available, appropriate preventive measures may be instituted. These may include monitoring of vitamin B12 levels at regular intervals such as annually or biannually; or giving vitamin B12 prophylactically as dietary supplement or more conveniently as annual injection at 1000  $\mu$ g, a dose sufficient to cover vitamin B12 needs for at least a year.

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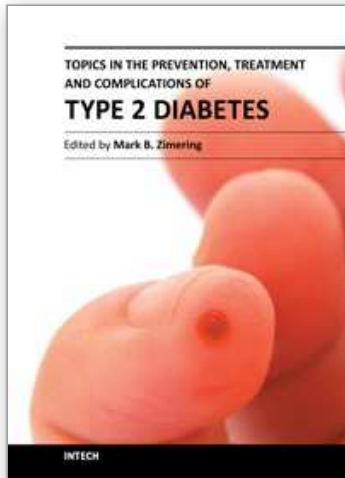
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Edited by Prof. Mark Zimering

ISBN 978-953-307-590-7

Hard cover, 340 pages

**Publisher** InTech

**Published online** 04, November, 2011

**Published in print edition** November, 2011

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### **How to reference**

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Chan Chee Wun Joyce, Chan Hoi Yan Florence, Wong Ho Nam Howard and Yeung Chun Yip (2011). Characteristics of Vitamin B12 Deficiency in Adult Chinese Patients with Type 2 Diabetes and the Implication of Metformin, Topics in the Prevention, Treatment and Complications of Type 2 Diabetes, Prof. Mark Zimering (Ed.), ISBN: 978-953-307-590-7, InTech, Available from: <http://www.intechopen.com/books/topics-in-the-prevention-treatment-and-complications-of-type-2-diabetes/characteristics-of-vitamin-b12-deficiency-in-adult-chinese-patients-with-type-2-diabetes-and-the-imp>

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