



Vitamin B12 deficiency: Cognitive impairment and neuroimaging correlates

Min-Chien Tu^{1,4,†}, Chung-Ping Lo^{2,4}, Ching-Yuan Chen^{3,4,5}

ABSTRACT

Vitamin B12 deficiency has been associated with various neuropsychiatric symptoms, and to be a reversible cause of dementia. As the negative impact of low vitamin B12 status on cognition can range from subclinical neuronal metabolic derangement to an overt debilitating state in line with permanent brain structural changes, prompt recognition of vitamin B12 deficiency is of paramount clinical importance as it is a treatable condition. Several studies have investigated the pathogenesis of vitamin B12 deficiency.

Keywords

Vitamin B12 deficiency, Cognitive impairment, Neuroimaging, Clinical importance, Neuropsychiatric symptoms

Introduction

Vitamin B12 deficiency has been associated with various neuropsychiatric symptoms, and it has also been reported to be a reversible cause of dementia [1]. As the negative impacts of low vitamin B12 status on cognition can range from subclinical neuronal metabolic derangement to an overt debilitating state associated with permanent structural changes in the brain [1], prompt recognition of vitamin B12 deficiency is of paramount clinical importance as it is a treatable condition. Several studies have investigated the pathogenesis of vitamin B12 deficiency. While the clinical implications of biomarkers such as homocysteine, methylmalonic acid, and holotranscobalamin related to a low vitamin B12 status have been discussed [1], neuroimaging studies of patients with predefined vitamin B12

deficiency are limited. We hypothesized that neuroimaging studies could reveal associations between neural substrates and vitamin B12 deficiency through neurobiological interactions.

Discussion

Our team recently conducted two neuroimaging studies in patients with a low vitamin B12 status. In the structural neuroimaging study, cognitive performance and morphometric indices of brain magnetic resonance imaging (MRI) were compared between 34 consecutive patients with vitamin B12 deficiency (serum level ≤ 250 pg/ml) and 34 demographically-matched controls [2]. The majority of these patients had mild cognitive impairment based on structuralized cognitive evaluations [Mini-Mental State Examination (MMSE)= 23.03 ± 6.21 ; number of patients with

¹Department of Neurology, Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, No. 88, Sec. 1, Fengxing Rd., Tanzi Dist., Taichung City 427, Taiwan (R.O.C.)

²Department of Radiology, Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, No. 88, Sec. 1, Fengxing Rd., Tanzi Dist., Taichung City 427, Taiwan (R.O.C.)

³Department of Nuclear Medicine, Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Taichung, Taiwan

⁴School of Medicine, Tzu Chi University, Hualien, Taiwan

⁵Graduate Institute of Medical Imaging and Radiological Sciences, Central Taiwan, University of Science and Technology, Taichung, Taiwan

[†]Author for correspondence: Dr. Min-Chien Tu, Department of Neurology, Taichung Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, No. 88, Sec. 1, Fengxing Rd., Tanzi Dist., Taichung City 427, Taiwan (R.O.C.); Tel: +886-4-3606-0666 ext.4913; Fax: +886-4-3606-5928; email: tmctmc3@yahoo.com.tw

Clinical Dementia Rating 0/0.5/1=5/24/5]. The therapeutic response rate was 60.8% by re-testing the MMSE 3 months after normalization of serum vitamin B12 level. The vitamin B12 deficiency group had lower total MMSE and Cognitive Abilities Screening Instrument (CASI) scores, mainly due to significant deficits in the domains of language, orientation, and mental manipulation. Interestingly, the patients also had a greater frontal horn ratio than the controls, while there were no significant differences in other neuroimaging parameters targeting regional assessments. On analyzing the correlation between cognitive performance and neuroimaging parameters, the bicaudate ratio appeared to show the most pronounced results due to a significant inverse correlation with the total scores of the MMSE/CASI as well as the domains of long-term memory, mental manipulation, orientation, language, and verbal fluency. As both a greater frontal horn ratio and bicaudate ratio reflect reductions in volume of frontal regions, these findings imply that dysfunction of fronto-subcortical circuits may be the fundamental pathogenesis underlying vitamin B12 deficiency. Therefore, we suggest that serum vitamin B12 evaluations should be considered among patients with non-amnesic presentations and predominant frontal atrophy in structural neuroimaging.

As neuronal dysfunction would be expected to coexist or even predate overt structural changes in neurodegenerative diseases, we also conducted another study using Tc-99m ethyl cysteinate dimer single-photon emission computed tomography (Tc-99m-ECD SPECT) as the functional neuroimaging tool to investigate its clinical relevance [3]. We enrolled 12 symptomatic patients with low serum vitamin B12 status (< 250 pg/ml), and investigated associations between Tc-99m-ECD SPECT and clinical presentations as well as performance on neuropsychological tests. The patients in this study were screened using brain MRI, and were devoid of major intracranial artery occlusion or infarcts as potential confounders for the Tc-99m-ECD SPECT interpretation. Our segregated regional assessments revealed several interesting findings. First, we identified the neuronal substrates responsible for psychiatric symptoms and cognitive impairment related to vitamin B12 deficiency. All of the patients (100%) had temporal hypoperfusion on Tc-99m-ECD SPECT. In those with hypoperfusion restricted within temporal regions and deep

nuclei ($n=5$), psychiatric symptoms with spared cognition were the main presentations, whereas among those with additional frontal hypoperfusion ($n=7$), six (86%) had dysexecutive syndrome. When incorporating neuropsychiatric assessments, six patients (86%) were confirmed to have impaired cognition, and two were diagnosed as having dementia. The psychiatric symptoms and dysexecutive syndrome were therefore attributed to impaired limbic and dorsolateral prefrontal circuits originating from basal ganglia, respectively. Second, the temporospatial distribution of Tc-99m-ECD SPECT was consistent with the cognitive impairment stage and therapeutic response. Most of the cases with hypoperfusion restricted within temporal and deep nuclei showed normal or nearly normal achievements in cognitive evaluations, while the patients with additional frontal hypoperfusion were prone to cognitive deficits. In addition, the degree and extent of signal reversal, especially within fronto-temporal regions, was correlated with cognitive changes after vitamin B12 replacement therapy in follow-up Tc-99m-ECD SPECT studies. Third, the negative impact of low vitamin B12 status on the central nervous system may have occurred in the very early stage, as evidenced by our Tc-99m-ECD SPECT findings. Two patients with a CDR of 0 and younger age (29 and 41 years) had neuropsychiatric symptoms (e.g., anxiety, dysthymia, and sleep disorders) and hypoperfusion confined within temporal regions and basal ganglia. These findings confirmed the detrimental impact of vitamin B12 deficiency, as the presentations of these two patients were atypical for endogenous mood disorders, and they were much younger than typical patients with neurodegenerative diseases. Taken together, our Tc-99m-ECD SPECT findings provide important evidence of neurobiological changes within basal ganglia and fronto-temporal regions in conjunction with disease severity among patients with vitamin B12 deficiency. A previous SPECT study reported that asymmetric hypoperfusion over frontal regions and the hippocampus occur in association with non-amnesic and amnesic subtypes of mild cognitive impairment, respectively [4], and therefore symmetric hypofrontality in SPECT in the context of dysexecutive syndrome may serve as a distinguishing feature of non-amnesic mild cognitive impairment attributed to vitamin B12 deficiency.

We also performed a literature review of previous neuroimaging studies to provide a

more comprehensive understanding of the pathogenesis related to vitamin B12 deficiency or relatively low vitamin B12 status (**Table 1**). In one MRI study including 107 community-dwelling volunteers aged 61 to 87 years without cognitive impairment at enrollment conducted over a 5-year observation period, those with the lowest tertile of serum vitamin B12 level were associated with an increased rate of brain volume loss [5]. Although there were no significant differences in the number of patients with cognitive impairment in subgroup analysis, the brain volume loss underpinned the negative impact related to a relatively low vitamin B12 status. Another cohort study including younger patients with subacute combined degeneration reported widespread changes in parameters in diffusion tensor imaging (predominantly in the right hemisphere tracts) but no volume reduction in white or gray matter [6]. Furthermore, another study including patients with mild cognitive impairment suggested that a vitamin B12 level in the low-normal range

(≥ 150 and < 304 pmol/L) was associated with poorer memory performance, and that this was partially mediated through the negative effect on the microstructure of cornu ammonis 4 and dentate gyrus region subfields [7]. These studies appear to describe a picture of diffuse changes with some regional/cognitive domains being more vulnerable to a low vitamin B12 status. The concept of a potential regional vulnerability was also reported in an earlier functional imaging study including patients with vitamin B12 deficiency with heterogeneous subtypes of dementia and superimposed delirium, in which a predominant post-central cerebral blood flow decrement with better preserved central and prefrontal flow was observed [8]. A novel MRI study reported findings relating microstructural changes to cerebral blood flow by combining diffusion tensor imaging and pseudo-continuous arterial spin labelling techniques, in which cerebral blood flow was noted to vary according to different regions and gray/white matter properties [9]. Of note, alterations in cerebral

Table 1: Summary of selected studies related to both neuropsychiatry and neuroimaging assessment.

First author Year/[Ref. No.]	Imaging tools	Method	Major findings	
			Neuropsychiatry assessment	Neuroimaging assessment
Vogiatzoglou A 2008/[5]	MRI	Structural image evaluation using normalization of atrophy	No subgroup differences (1st and 2nd vs. 3rd tertile of percentage of brain volume loss; $n=71$ vs. 36) over a 5-year period was noted.	The brain volume loss was greater among those with lower VitB12 and holotranscobalamin levels.
Gupta PK 2013/[6]		Voxel-based morphometry and DTI (tract-based statistics)	Pts with subacute combined degeneration ($n=51$) showed poorer visuospatial capacity and visuomotor speed than the controls ($n=46$).	Pts showed no significant changes in gray and white matter volumes but significant microstructural changes in multiple brain regions compared with the controls.
Köbe T 2016/[7]		DTI (voxel-based analysis)	Pts with low-normal VitB12 (< 304 pmol/L; $n=50$) showed poorer learning ability and recognition memory than Pts with high-normal VitB12 ($n=50$).	Impaired microstructure integrity of the hippocampus, mainly in the cornu ammonis 4 and dentate gyrus region, was noted in Pts with low-normal VitB12.
Hsu YH 2016/[2]		Morphometric index assessments	Pts with VitB12 deficiency had poorer general cognition and deficits in the domains of language, orientation, and mental manipulation than the control group (both $n=34$).	Pts had preferential atrophy in frontal regions than the controls.
Roy B 2015/[9]		DTI (tract-based statistics) and pseudo-continuous arterial spin labelling	Pts with VitB12 deficiency showed altered neuropsychological tests scores than the controls (both $n=16$).	Micro-structural changes in some regions were found in pre- and post-treated Pts as compared to the controls. Micro-structural recovery lagged behind cerebral blood flow and cognition recovery at 6 weeks post therapy.
K. Nilsson 2000/[8]	SPECT	133 Xe inhalation	Pts with mild to moderate dementia ($n=15$) but not those with severe dementia ($n=9$) improved clinical delirium state after treatment.	All Pts showed predominantly post-central flow pathology with better preserved central and pre-frontal flow values. Concomitant increase of general cerebral blood flow was noted in Pts with mild to moderate dementia but not those with severe dementia.
Tu MC 2015/[3]		Tc-99m ethyl cysteinate dimer	Psychiatric symptoms and/or dysexecutive syndrome were the main features.	All Pts (100%) had temporal hypoperfusion within temporal regions, including 5 Pts (42%) with hypoperfusion restricted within temporal regions and deep nuclei and 7 Pts (58%) with additional hypoperfusion within frontal regions. Signal reversal within bilateral fronto-temporal regions correlated with clinical improvement after treatment.

MRI: Magnetic Resonance Imaging; SPECT: single-photon emission computed tomography; DTI: diffusion tensor imaging; Pts: patients; VitB12: vitamin B12.

blood flow were concluded to be an early predictor of complete recovery in patients with B12 deficiency, as they normalized more quickly than diffusion tensor imaging parameters.

In another perspective, neuroimaging assessments also delineated neuronal substrates with meaningful symptomatological correlation (Table 2). One study highlighted the association between widespread microstructural changes within white matter and performance of visuospatial capacity and visuomotor speed [6]. Another study identified significant relationship between hippocampus structural integrity and memory performance [7]. Some other researches, however, proposed dysfunction within certain circuits and/or regions contributed specific clinical presentations. For example, we associated impaired fronto-subcortical circuits with cognitive decline on the basis that the morphometric parameters aiming on the frontal regions showed divergent correlations with multiple cognitive domains [2]. Consistently,

two SPECT studies supported the critical role of the frontal regions in cognitive prognosis and/or performance [3,8]. Specifically, although the temporal regions were associated with variable psychiatric symptoms or the state of delirium, intact function of the frontal regions appeared to govern the orchestration of global cognitive process.

Two biological pathways may account for the neuroimaging changes related to a low vitamin B12 status [1]. First, impaired L-methylmalonyl-CoA mutase can result in the accumulation of methylmalonic acid and a decrease in succinyl-CoA level. This pathway is regarded to be essential for myelin integrity as well as lipid and protein metabolism. Second, functional loss of methionine synthase can result in hyperhomocysteinemia, a decrease in S-adenosylmethionine level, and a functional folate-deficiency state. Therefore, this pathway is critical for the synthesis of monoamine neurotransmitters and red blood cells in addition

Table 2: Summary of important neuronal correlates related to neuropsychiatric presentations of vitamin B12 deficiency.

First author Year/[Ref. No.]	Imaging tools	Method	Major findings		
			Association measure or clinical relevance	Neuronal correlates	Specific presentations
Gupta PK 2013/[6]	MRI	Voxel-based morphometry and DTI (tract-based statistics)	The Digit Symbol test correlated with FA ($r=0.63, p < 0.001$) and RD ($r=-0.54, p < 0.001$). The number connection test correlated with FA ($r=-0.56, p < 0.001$) and RD ($r=0.57, p < 0.001$).	Widespread cerebral white matter changes	Poorer visuospatial capacity and visuomotor speed
Köbe T 2016/[7]		DTI (voxel-based analysis)	A significant indirect effect of the VitB12 group difference in learning ability ($\beta=2.46$; 95% CI: 0.38, 5.12) and recognition memory performance ($\beta=1.00$; 95% CI: 0.22, 2.07) through the mean diffusivity of the hippocampus cornu ammonis 4 and dentate gyrus subfields in mild cognitive impairment Pts.	The hippocampus microstructure changes	Poorer memory performance
Hsu YH 2016/[2]		Morphometric index assessments	Bicaudate ratio, fronto-occipital ratio, uncotemporal index, and normalized interuncal distance all showed a strong correlation with general cognition ($p < 0.01$). The bicaudate ratio showed the most pronounced correlations with long-term memory, mental manipulation, orientation, language, and verbal fluency (all $p < 0.01$).	Dysfunction of fronto-subcortical circuits	Cognitive impairment
K. Nilsson 2000/[8]	SPECT	133 Xe inhalation	Clinical improvement ($n=15$) was associated with a general increase in cerebral blood flow, most marked in the temporal lobe. The clinically non-improved Pts ($n=9$) only showed a hyperperfusion in the sensory motor cortex but hypoperfusion in the precentral association cortex.	The temporoparietal region hypoperfusion	Delirium
				The frontotemporal region hypoperfusion	Widespread cortical dysfunction and poor prognosis
Tu MC 2015/[3]		Tc-99m ethyl cysteinate dimer	Hypoperfusion restricted within the thalamus/basal ganglia and temporal regions may be seen in the earlier state of VitB12 deficiency ($n=5$), when psychiatric symptoms predominated. Hypoperfusion beyond the thalamus/basal ganglia and involving the frontal regions ($n=7$) appeared when dysexecutive syndromes were manifested.	Impaired limbic circuits and basal ganglia	Psychiatric symptoms
				Impaired dorsolateral prefrontal circuits and basal ganglia	Dysexecutive syndrome

MRI: Magnetic Resonance Imaging; SPECT: single-photon emission computed tomography; DTI: diffusion tensor imaging; Pts: patients; FA: fractional anisotropy; RD: radial diffusivity; VitB12: vitamin B12; CI: confidence interval.

to the maintenance of myelin integrity. In patients with cognitive impairment due to a low vitamin B12 status, we previously reported that a hastening ageing process through impaired myelin integrity and subsequent volume reduction as seen in structural imaging findings may be the pathogenesis [2]. In addition, the derangement of neurotransmitters, neuronal dysfunction, and damage of vessels and myelin secondary to the accumulation of homocysteine and methylmalonic acid may contribute to the mechanisms related to our functional imaging observations [3]. Consistent with a previous study [10], we also demonstrated a dissociative involvement between neurological and hematological systems in our previous cohort study [2]. Of 34 patients with vitamin B12 deficiency in our structural imaging study, only one had macrocytic anemia, yet not confirmed as having pernicious anemia. Future studies are warranted to investigate whether a dissociative involvement exists between these two biological pathways.

Conclusion

There are several potential limitations to our two neuroimaging reports. The main concern is the possibility of coexisting neurodegenerative diseases such as Alzheimer's disease. However,

both of our cohorts presented with cognitive profiles and neuroimaging features distinct from those typical for Alzheimer's disease. A considerable number of our patients also demonstrated a robust improvement in follow-up studies of cognitive evaluations and/or Tc-99m-ECD SPECT. These observations corroborate that the underlying pathogenesis in both of these cohorts was related to vitamin B12 deficiency. Second, the structural and functional imaging findings may be related to ageing. To further clarify the association between low vitamin B12 status and neuroimaging changes and the existence of a threshold effect related to vitamin B12 deficiency, if any, subgroup comparisons between patients with comparable cognitive profiles but different ranges of vitamin B12 level would be of interest. Finally, as a low vitamin B12 status may be related to global changes within the cerebrum, further studies are warranted to evaluate the application of different neuroimaging parameters/tools (e.g., voxel-based morphometry, diffusion tensor imaging, and graph theory network analysis) and serum biomarkers related to vitamin B12 status with regards to their clinical significance. Nevertheless, our studies highlight that neuroimaging changes and dysexecutive syndrome may be the key underlying neurobiological mechanism of cognitive impairment related to vitamin B12 deficiency.

References

- Lachner C, Steinle NI, Regenold WT. The neuropsychiatry of vitamin B12 deficiency in elderly patients. *J. Neuropsychiatry. Clin. Neurosci* 24(1), 05-15 (2012).
- Hsu YH, Huang CF, Lo CP, *et al.* Vitamin B12 deficiency: Characterization of psychometrics and MRI morphometrics. *Nutr. Neurosci* 19(2), 47-54 (2016).
- Tu MC, Lo CP, Chen CY, *et al.* Correlation of Tc-99 m ethyl cysteinate dimer single-photon emission computed tomography and clinical presentations in patients with low cobalamin status. *BMC. Neurol* 15(1), 251 (2015).
- Nobili F, Frisoni GB, Portet F, *et al.* Brain SPECT in subtypes of mild cognitive impairment. Findings from the DESCRIPA multicenter study. *J. Neurol* 255(9), 1344-1353 (2008).
- Vogiatzoglou A, Refsum H, Johnston C, *et al.* Vitamin B12 status and rate of brain volume loss in community-dwelling elderly. *Neurology* 71(11), 826-832 (2008).
- Gupta PK, Gupta RK, Garg RK, *et al.* DTI correlates of cognition in conventional MRI of normal-appearing brain in patients with clinical features of subacute combined degeneration and biochemically proven vitamin B(12) deficiency. *AJNR. Am. J. Neuroradiol* 35(5), 872-877 (2014).
- Köbe T, Witte AV, Schnelle A, *et al.* Vitamin B-12 concentration, memory performance, and hippocampal structure in patients with mild cognitive impairment. *Am. J. Clin. Nutr* 103(4), 1045-1054 (2016).
- Nilsson K, Warkentin S, Hultberg B, *et al.* Treatment of cobalamin deficiency in dementia, evaluated clinically and with cerebral blood flow measurements. *Aging. Clin. Exp. Res* 12(3), 199-207 (2000).
- Roy B, Trivedi R, Garg RK, *et al.* Assessment of functional and structural damage in brain parenchyma in patients with vitamin B12 deficiency: A longitudinal perfusion and diffusion tensor imaging study. *Magn. Reson. Imaging* 33(5), 537-543 (2015).
- Lindenbaum J, Healton EB, Savage DG, *et al.* Neuropsychiatric disorders caused by cobalamin deficiency in the absence of anemia or macrocytosis. *N. Engl. J. Med* 318(26), 1720-1728 (1988).