Aim: We evaluated the diagnostic value of blood thiamine metabolites for Alzheimer’s disease (AD) by using positron emission tomography with $^{11}$C-Pittsburgh compound B ($^{11}$C-PiB PET) scanning. Methods: Thirty-eight clinically diagnosed AD patients were voluntarily recruited. Blood thiamine metabolites were measured by high-performance liquid chromatography. All the patients received $^{11}$C-PiB PET scanning for the measurement of cerebral amyloid deposition. Results: Thiamine diphosphate (TDP) had 66.7% sensitivity and 80.0% specificity for AD diagnosis, while the $\gamma$-value representing the best combination of thiamine metabolites and age had 24.2% sensitivity and 100.0% specificity according to the cut-off value of our previous study. Conclusion: Blood TDP but not $\gamma$-value exhibited results significant for AD diagnosis.

Lay abstract: In this study, we investigated the diagnostic value of blood thiamine metabolites for Alzheimer’s disease as examined by positron emission tomography with $^{11}$C-Pittsburgh compound B scanning. Our results suggest that the reduction of blood thiamine diphosphate may be a stable biomarker useful for Alzheimer’s disease diagnosis.
been reported as potential AD biomarkers [8-12]. However, because of the low repeatability and instability of these biomarkers tests, none of these are recommended for clinical AD diagnosis. This frustrating situation drives us to explore new peripheral biomarker(s) for AD diagnosis. Our previous multicenter study demonstrated that blood thiamine metabolites detected by high-performance liquid chromatography (HPLC) exhibited significant diagnostic utility for AD [13]. Here, we determined to validate the diagnostic value of the measurement of fasting blood thiamine metabolites in AD patients combining with 11C-PiB PET scanning.

Materials & methods

Participants
This study was approved by the Committee on Medical Ethics of Zhongshan Hospital, Fudan University, Shanghai, China. Thirty-eight clinically diagnosed AD patients were recruited to voluntarily receive 11C-PiB PET scanning and the measurement of blood thiamine metabolites by HPLC from the Dementia Clinic of Neurological Department in Zhongshan hospital from October 2012 to November 2014. Informed consents were obtained from all AD patients or their caregivers. All of them took neuropsychological examinations, including the Mini-Mental Status Examination (MMSE), Clinical Dementia Rating (CDR), Activity of Daily Living (ADL) scales and Hamilton Depression Rating Scale. All patients also received cranial MRI and/or CT scans to exclude the possibilities of vascular dementia or occupied brain lesions. Blood folate, vitamin B12, fasting glucose and thyroid function were also examined. The diagnosis of AD was made by neurologists specialized in dementia according to the criteria of DSM IV and guideline for AD diagnosis published in 2011 (National Institute on Aging – Alzheimer’s Association workgroups) [1]. The inclusion criteria and exclusion criteria of AD subjects were the same as those listed in our previous study [13]. Different from the previous study [13], the current study also recruited patients with age of less than 65 years old. The demographic and clinical data of the current study are summarized in Table 1.

The detection of blood thiamine metabolites

The method of HPLC fluoroscopy for measuring thiamine metabolites was essentially the same as previously described [13]. Briefly, 150 μl of whole blood samples anticoagulated with heparin were immediately mixed with 7.4% perchloric acid and then vibrated for 30 s for deproteinization. After being centrifuged at 10,000 rpm and 4°C for 6 min, the supernatants were collected and stored at -20°C and tested within 2 weeks. The samples were protected from light exposure during the whole process. Potassium ferricyanide was added into the supernatants in order to convert thiamine metabolites into thiochromes that were separated by gradient elution by a C18 reversed-phase analytical column (250 × 4.6 mm). The separated thiochromes were then detected by HPLC (Agilent 1100, CA, USA) with an excitation wavelength of 367 nm and an emission wavelength of 435 nm. The levels of blood thiamine diphosphate (TDP), thiamine monophosphate (TMP) and thiamine were quantified according to the standard curve derived from the standard samples of TDP, TMP and thiamine (Sigma-Aldrich, MO, USA).

11C-PiB & PET imaging

The radiolabeling synthesis of 11C-PiB was based on the method described previously [14]. Approximately 10 mCi radiotracer was injected through the opisthenar vein within 60 s and was flushed with 1 ml saline. Subsequently, a 3D dynamic PET acquisition was performed from 0 to 60 min post injection following an attenuation correction CT using Biograph 64 Truepoint PET/CT scanner (Siemens Medical Solution). The 40–60 min static images were reconstructed using an iterative 3D method with Gaussian filter of 6 mm in Full Width of Half Maximum. The pixel size was 2.0 mm and the slice thickness was 1.5 mm. 11C-PiB PET images were visually assessed as ‘PiB positive’ or ‘PiB negative’ by two experienced experts in PET diagnosis, who were blind to clinical data.

Statistical analysis

The results were shown as mean ± SEM. GraphPad prism software (version 5.01) and SPSS software (version 18.0; SPSS Inc) were used for statistical analysis. Unpaired t-test was used for the comparisons of blood thiamine metabolites between PiB-positive and PiB-negative patients. The diagnostic cut-off point of blood TDP was set up to 99.48 nmol/l, and γ-value was calculated based on the equation: \( \gamma = (1/TDP) *([\text{TDP} + 1]^{[-0.01]} + [T + 1]^{[1/6]} \text{Age}^2) \). And its cut-off point for AD diagnosis was set up to 75.97 according to our previous study [13]. Four-fold table analysis was used to calculate the sensitivity and specificity of blood TDP and γ-value for the differentiation of PiB-positive patients and PiB-negative patients. The statistical significance of four-fold table analysis was examined by Fisher’s exact test. \( p < 0.05 \) was considered statistically significant.
### Table 1. Demographic and clinical data of Alzheimer’s disease subjects recruited in this study.

| Name  | Age | Sex | MMSE | ADL | CDR | \(^{11}\)C-PiB (negative/positive) | TDP   | TMP   | Thiamine | γ-value |
|-------|-----|-----|------|-----|-----|-----------------------------------|-------|-------|----------|---------|
| Case 1| 54  | M   | 17   | 8   | 1   | Negative                          | 116.99| 22.15 | 6.12     | 33.50   |
| Case 2| 63  | M   | 20   | 8   | 1   | Negative                          | 86.48 | 17.29 | 2.90     | 55.94   |
| Case 3| 71  | M   | 25   | 8   | 0.5 | Negative                          | 105.47| 16.71 | 6.40     | 64.83   |
| Case 4| 80  | M   | 17   | 11  | 1   | Negative                          | 149.35| 9.86  | 1.62     | 49.11   |
| Case 5| 86  | M   | 23   | 10  | 0.5 | Negative                          | 130.54| 2.03  | 4.57     | 74.60   |
| Case 6| 48  | M   | 17.5 | 11  | 0.5 | Positive                          | 103.29| 3.99  | 1.79     | 26.05   |
| Case 7| 51  | F   | 16   | 8   | 1   | Positive                          | 96.14 | 17.57 | 6.64     | 36.87   |
| Case 8| 51  | F   | 23   | 9   | 1   | Positive                          | 90.05 | 8.82  | 4.59     | 37.61   |
| Case 9| 52  | F   | 15   | 10  | 1   | Positive                          | 80.50 | 7.90  | 16.03    | 52.71   |
| Case 10| 54  | F   | 11   | 8   | 1   | Positive                          | 49.48 | 37.08 | 0.00     | 56.83   |
| Case 11| 56  | M   | 13   | 11  | 1   | Positive                          | 75.59 | 67.04 | 2.68     | 49.42   |
| Case 12| 57  | M   | 17   | 9   | 1   | Positive                          | 142.92| 11.07 | 3.50     | 28.49   |
| Case 13| 57  | F   | 5    | 15  | 2   | Positive                          | 90.21 | 11.94 | 2.76     | 43.78   |
| Case 14| 58  | M   | 22   | 9   | 0.5 | Positive                          | 80.55 | 8.82  | 1.33     | 47.01   |
| Case 15| 58  | F   | 22   | 8   | 1   | Positive                          | 82.69 | 7.95  | 3.68     | 51.47   |
| Case 16| 61  | M   | 11   | 14  | 2   | Positive                          | 106.09| 15.19 | 2.12     | 41.23   |
| Case 17| 62  | F   | 1    | 19  | 2   | Positive                          | 118.69| 7.42  | 11.17    | 48.09   |
| Case 18| 62  | F   | 12   | 14  | 2   | Positive                          | 70.93 | 9.53  | 0.95     | 59.17   |
| Case 19| 63  | M   | 8    | 11  | 3   | Positive                          | 101.23| 15.88 | 2.83     | 47.68   |
| Case 20| 64  | M   | 9    | 21  | 3   | Positive                          | 65.93 | 2.75  | 0.53     | 65.80   |
| Case 21| 64  | F   | 17   | 14  | 1   | Positive                          | 87.85 | 5.30  | 29.71    | 81.00   |
| Case 22| 66  | M   | 17   | 9   | 1   | Positive                          | 95.95 | 4.08  | 4.05     | 58.50   |
| Case 23| 67  | M   | 25   | 8   | 0.5 | Positive                          | 72.24 | 24.17 | 2.49     | 74.10   |
| Case 24| 68  | F   | 24   | 8   | 0.5 | Positive                          | 123.44| 22.90 | 3.91     | 47.30   |
| Case 25| 68  | M   | 26   | 9   | 0.5 | Positive                          | 121.49| 14.18 | 4.54     | 49.27   |
| Case 26| 69  | M   | 21   | 10  | 1   | Positive                          | 92.25 | 5.29  | 20.09    | 84.22   |
| Case 27| 71  | M   | 23   | 8   | 0.5 | Positive                          | 123.46| 12.58 | 5.36     | 54.15   |
| Case 28| 73  | F   | 22.5 | 8   | 0.5 | Positive                          | 69.66 | 10.01 | 3.63     | 96.43   |
| Case 29| 74  | F   | 19   | 12  | 0.5 | Positive                          | 97.54 | 2.96  | 3.16     | 70.22   |
| Case 30| 74  | F   | 12   | 10  | 1   | Positive                          | 81.20 | 19.76 | 14.57    | 103.38  |
| Case 31| 75  | M   | 16   | 9   | 1   | Positive                          | 68.02 | 38.29 | 3.13     | 100.97  |
| Case 32| 77  | M   | 17   | 8   | 0.5 | Positive                          | 98.02 | 8.52  | 2.11     | 71.43   |
| Case 33| 77  | M   | 20   | 8   | 1   | Positive                          | 92.65 | 17.88 | 2.11     | 75.09   |
| Case 34| 81  | F   | 15.5 | 13  | 1   | Positive                          | 107.89| 7.07  | 2.08     | 71.84   |
| Case 35| 81  | F   | 14   | 10  | 1   | Positive                          | 65.07 | 9.65  | 4.23     | 129.74  |
| Case 36| 82  | M   | 25   | 8   | 0.5 | Positive                          | 111.82| 31.73 | 4.27     | 76.61   |
| Case 37| 83  | M   | 12   | 11  | 1   | Positive                          | 135.02| 16.05 | 3.63     | 64.04   |
| Case 38| 84  | F   | 9    | 11  | 2   | Positive                          | 86.63 | 18.33 | 4.22     | 104.15  |

The γ-value is calculated according to the equation: \( \gamma = \frac{1}{TDP} \times [\text{TMP} + 1]^{-0.01} \times [\text{TF} + 1]^{1/6} \times \text{Age}^2 \) published in our recent study [13].

ADL: Activity of Daily Living; CDR: Clinical Dementia Rating; MMSE: Mini-Mental Status Examination; PiB: Pittsburgh Compound-B; TDP: Thiamine diphosphate; TMP: Thiamine monophosphate.
Results

Reduced blood TDP level in PiB-positive patients as compared with PiB-negative patients

There were 33 PiB-positive patients and five PiB-negative patients within the 38 clinically diagnosed AD patients. The representative images of $^{11}$C-PiB PET in PiB-positive patients showed significant $^{11}$C-PiB deposition in multiple cortical regions as compared with PiB-negative patients. TDP levels in PiB-positive patients (93.47 ± 3.77 nmol/l, n = 33) were significantly reduced as compared with that in PiB-negative patients (117.80 ± 10.70 nmol/l, n = 5; p < 0.05, see Figure 1). There were no significant differences in TMP and thiamine levels between PiB-positive patients (TMP: 15.20 ± 2.26 nmol/l, thiamine: 5.39 ± 1.09 nmol/l) and PiB-negative patients (TMP: 13.61 ± 3.50 nmol/l, thiamine: 4.32 ± 0.92 nmol/l; p > 0.05, Figure 1).

TDP is a stable & reliable biomarker for AD demonstrated by $^{11}$C-PiB PET scanning

There were 22 of 33 PiB-positive patients (66.7%) with blood TDP levels less than 99.48 nmol/l (the cut-off point), whereas only one of five PiB-negative patients (20.0%) manifested blood TDP levels less than 99.48 nmol/l. Thus, the sensitivity and specificity of TDP for the differentiation of PiB-positive patients and PiB-negative patients demonstrated by $^{11}$C-PiB PET scanning were 66.7 and 80.0%, respectively (p = 0.0685; Figure 2).

$\gamma$-value did not show high power for AD diagnosis

There were eight of 33 PiB-positive patients (24.2%) with $\gamma$-value over 75.97 (the cut-off point) and five of five PiB-negative patients with $\gamma$-value less than 75.97. The sensitivity and specificity of $\gamma$-value for the differentiation of PiB-positive patients and PiB-negative patients were 24.2 and 100.0%, respectively (p = 0.5632, Figure 2).

Discussion

Our previous study showed that the reduction of blood TDP and the increase of $\gamma$-value representing the best combination of blood thiamine metabolites (TDP, TMP and thiamine) and age displayed significant power for AD diagnosis, with 77.4% of sensitivity and 78.1% of specificity for TDP with the cut-off point set as 99.48 nmol/l, and 81.4% of sensitivity and 90.5% of specificity for $\gamma$-value when the cut-off point was set to 75.97 [13]. However, our previous results were derived from the data of subjects of age 65 or above. In this study, we validated the diagnostic value of blood thiamine metabolites in AD cases with a wider age range, including those less than 65 years old. The accuracy of clinical diagnosis of AD (nearly 80%) is still not satisfactory. To confirm the diagnostic value of blood thiamine metabolites in AD, we used $^{11}$C-PiB PET scanning to enhance the diagnostic accuracy of AD and reexamine the power of blood TDP and $\gamma$-value for AD diagnosis.

The results showed that blood TDP levels in 33 PiB-positive patients were significantly reduced as compared with that in five PiB-negative patients. It is consistent with the results of our previous study that blood TDP levels in AD patients were significantly lower than that in control subjects with cognitive abilities in the normal range and patients with vascular dementia and frontotemporal dementia [13]. Further, we found 22/33 (66.7%) of PiB-positive patients with TDP levels under the cut-point of 99.48 nmol/l and 4/5 (80.0%) of PiB-negative patients with TDP levels above the cut-point of 99.48 nmol/l. Thus, the sensitivity and specificity of blood TDP for the differentiation of PiB-positive patients and PiB-negative patients were 66.7 and 80.0% (p = 0.0685), respectively. The findings were consistent with the results of our previous study [13]. However, the exact conclusion needs to be further validated using a larger sample of cases examined by $^{11}$C-PiB PET, particularly patients with PiB-negative images, in the future studies. Our current study did not show significant performance of $\gamma$-value for AD diagnosis. The equation for the calculation of $\gamma$-value was established based on 65 years old or above in our previous study [13]. Our current study included AD patients under 65 years. Thus,
the previous formula for $\gamma$-value calculation may be not suitable for this age group of patients (<65 years old) and a better equation for calculating $\gamma$-value without age limitation should be explored in future studies. Overall, owing to the limited sample size of our current preliminary study, we believe that the conclusions we have made require validation in future studies in a larger sample of subjects.

Brain glucose hypometabolism is an invariant feature of AD and closely correlates with cognitive impairment [15,16]. Thus, brain glucose hypometabolism and its associated factor(s) may be potential targets for AD diagnosis and therapy. TDP, a functional thiamine derivative, plays a pivotal role in glucose metabolism as a critical coenzyme of three key enzymes: transketolase, pyruvate dehydrogenase, and $\alpha$-ketoglutarate dehydrogenase. TDP level and the enzymatic activities have been demonstrated to be reduced in AD patients [17–20]. The improvement of brain glucose metabolism by nasal insulin can enhance cognitive function and provide neuroprotection on AD patients. Our previous study has illustrated the beneficial effects of benfotiamine, a thiamine derivative, against AD [21]. Future studies should further clarify not only the mechanism(s) of brain glucose hypometabolism and abnormal thiamine metabolism but also their roles as diagnostic and therapeutic targets for AD.

**Conclusion**

Our results suggest that blood TDP is a stable biomarker for AD diagnosis while $\gamma$-value did not exhibit a significantly diagnostic value for AD, possibly due to the age limitation of participants for the $\gamma$-value calculation.

**Financial & competing interests disclosure**

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References
Papers of special note have been highlighted as:
• of interest; ** of considerable interest

1 McKhann GM, Knopman DS, Chertkow H et al. The diagnosis of dementia due to Alzheimer’s disease: recommendations from the National Institute on Aging – Alzheimer’s Association workgroups on diagnostic guidelines for Alzheimer’s disease. *Alzheimer’s Dement.* 7(3), 263–269 (2011).

2 Lan J, Liu J, Zhao Z et al. The peripheral blood of Abeta binding RBC as a biomarker for diagnosis of Alzheimer’s disease. *Age Ageing* 44(3), 458–464 (2015).

3 Veitinger M, Oehler R, Umlauf E et al. A platelet protein biochip rapidly detects an Alzheimer’s disease-specific phenotype. *Acta Neuropathol.* 128(5), 665–677 (2014).

4 Bermejo-Pareja F, Antequera D, Vargas T, Molina JA, Carro E. Saliva levels of Aβ1–42 as potential biomarker of Alzheimer’s disease: a pilot study. *BMC Neurol.* 10, 108 (2010).

5 Shi M, Sui YT, Peskink ER et al. Salivary tau species are potential biomarkers of Alzheimer’s disease. *J. Alzheimer’s Dis.* 27(2), 299–305 (2011).

6 Mattsson N, Zetterberg H, Janelidze S et al. Plasma tau in Alzheimer disease. *Neurology* 87(17), 1827–1835 (2016).

**Finds that plasma tau partly reflects Alzheimer disease’s (AD) pathology, but the overlap between normal aging and AD is large, thus they did not support plasma tau as an AD biomarker.**

7 Lovheim H, Elgh F, Johansson A et al. Plasma concentrations of free amyloid beta cannot predict the development of Alzheimer’s disease. *Alzheimer’s Dement.* doi:10.1016/j.azd.2016.12.004 (2017) (Epub ahead of print).

8 Kim SM, Song J, Kim S et al. Identification of peripheral inflammatory biomarkers between normal control and Alzheimer’s disease. *BMC Neurol.* 11, 51 (2011).

**Evaluates the value of peripheral inflammatory markers for AD.**

9 Doecke JD, Laws SM, Faux NG et al. Blood-based protein biomarkers for diagnosis of Alzheimer disease. *Arch. Neurol.* 69(10), 1318–1325 (2012).

**Evaluates the value of blood-based protein biomarkers for AD.**

10 Faria MC, Goncalves GS, Rocha NP et al. Increased plasma levels of BDNF and inflammatory markers in Alzheimer’s disease. *J. Psychiatr. Res.* 53, 166–172 (2014).

11 Cunnane SC, Schneider JA, Tangney C et al. Plasma and brain fatty acid profiles in mild cognitive impairment and Alzheimer’s disease. *J. Alzheimers Dis.* 29(3), 691–697 (2012).

12 Muenchhoff J, Poljak A, Song F et al. Plasma protein profiling of mild cognitive impairment and Alzheimer’s disease across two independent cohorts. *J. Alzheimers Dis.* 43(4), 1355–1373 (2015).

13 Pan X, Fei G, Lu J et al. Measurement of blood thiamine metabolites for Alzheimer’s disease diagnosis. *Ebiomedicine* 3, 155–162 (2016).

**Pan et al. reported that the measurement of blood thiamine metabolites by HPLC is a test exhibiting significant value for AD diagnosis with inexpensive, easy to perform and noninvasive merits.**

14 Mathis CA, Wang Y, Holt DP, Huang GF, Debnath ML, Klunk WE. Synthesis and evaluation of 11C-labeled 6-substituted 2-arylbenzothiazoles as amyloid imaging agents. *J. Med. Chem.* 46(13), 2740–2754 (2003).

15 Gibson GE, Hirsch JA, Cirio RT, Jordan BD, Fonzetti P, Elder J. Abnormal thiamine-dependent processes in Alzheimer’s Disease. Lessons from diabetes. *Mol. Cell Neurosci.* 55, 17–25 (2013).

16 Chen Z, Zhong C. Decoding Alzheimer’s disease from perturbed cerebral glucose metabolism: implications for diagnostic and therapeutic strategies. *Progr. Neurobiol.* 108, 21–43 (2013).

**This review systematically and comprehensively discussed the role of impaired glucose metabolism in AD pathogenesis.**

17 Heroux M, Raghavendra Rao VL, Laviole J, Richardson JS, Butterworth RF. Alterations of thiamine phosphorylation and of thiamine-dependent enzymes in Alzheimer’s disease. *Metab. Brain Dis.* 11(1), 81–88 (1996).

18 Mastrogiacoma F, Bettendorff L, Grisar T, Kish SJ. Brain thiamine, its phosphate esters, and its metabolizing enzymes in Alzheimer’s disease. *Ann. Neurol.* 39(5), 585–591 (1996).

19 Butterworth RF, Besnard AM. Thiamine-dependent enzyme changes in temporal cortex of patients with Alzheimer’s disease. *Metab. Brain Dis.* 5(4), 179–184 (1990).

20 Zhao Y, Pan X, Zhao J, Wang Y, Peng Y, Zhong C. Decreased transketolase activity contributes to impaired hippocampal neurogenesis induced by thiamine deficiency. *J. Neurochem.* 111(2), 537–546 (2009).

21 Pan X, Gong N, Zhao J et al. Powerful beneficial effects of benfotiamine on cognitive impairment and beta-amyloid deposition in amyloid precursor protein/presenilin-1 transgenic mice. *Brain* 133(Pt 5), 1342–1351 (2010).