

BMJ Open

Relative risk values of age, acrolein, IL-6, and CRP as markers of periventricular hyperintensities

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005598
Article Type:	Research
Date Submitted by the Author:	09-May-2014
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Primary Subject Heading:	Diagnostics
Secondary Subject Heading:	Neurology, Diagnostics
Keywords:	Stroke < NEUROLOGY, Neurology < INTERNAL MEDICINE, Stroke medicine < INTERNAL MEDICINE

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4 1 **Relative risk values of age, acrolein, IL-6, and CRP as markers of periventricular**
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7 2 **hyperintensities: a cross-sectional study**
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12 4 Arata Abe*, Yasuhiro Nishiyama, Mina Harada-Abe, Seiji Okubo, Masayuki Ueda, Masahiro
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30 10 **Keywords:** Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity, White matter
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For peer review only

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4 22 **Abstract**

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6 23 Objective: Brain white matter hyperintensities (WMHs) can be divided into periventricular
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10 24 hyperintensity (PVH) and deep-and-subcortical white matter hyperintensity (DSWMH), and the
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12 25 former contribute more to cognitive dysfunction and infarction risk. We conducted the present
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15 26 investigation to define the relationship between PVH and DSWMH.

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18 27 Design: Cross-sectional study.

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21 28 Setting: University hospital

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24 29 Participants: We prospectively enrolled 228 healthy Japanese volunteer subjects with relative risk
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27 30 values (RRVs) > 0.5.

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30 31 Primary outcome measures: We investigated whether it is possible to use the RRV to predict PVH
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33 32 and DSWMH.

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36 33 Results: Among 228 subjects, 103 (45.1%) and 157 (68.8%) exhibited PVH and DSWMH,
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39 34 respectively. Age, body mass index (BMI), and PVH were significant independent determinants of
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42 35 RRV. A significant odds ratio (OR) for PVH was noted in the highest RRV tertile compared with the
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45 36 lowest one after adjusting for potential confounding factors. A significant OR for high predicted
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48 37 PVH risk was also found for RRV level as well.

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51 38 Conclusion: Elevated RRV levels were significantly associated with increased predicted PVH,
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54 39 suggesting that measuring the plasma PCAcro, IL-6, and CRP levels may be useful for identifying
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57 40 Japanese at high risk for PVH.

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4 41 Strengths and limitations of this study:

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7 42 Using the RRV at clinical level, we investigate to evaluate WMHs.

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10 43 We provided the first evidence that RRV is associate with PVH rather than DSWMH.

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12 44 These data are obtained from cases of cautious health care in Asian people and may not be applicable
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16 45 to populations of poor health.

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21 47 **Keywords:** Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity

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27 49 **1. Introduction**

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30 50 A number of large-scale clinical studies have demonstrated that white matter hyperintensities

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33 51 (WMHs) are associated with high stroke risk¹⁻³. The results of the large-scale, multicenter open trial

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36 52 PICA study⁴ conducted in Japan suggest that the Fazekas-classified periventricular hyperintensities

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39 53 (PVHs) and deep-and-subcortical white matter hyperintensities (DSWMHs)⁵ are related to the risk of

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42 54 symptomatic brain infarction (SBI). In the Rotterdam Scan study on elderly subjects with no history

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45 55 of stroke, conducted by magnetic resonance imaging (MRI) for 4.2 years, the proportional hazard

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48 56 ratio of stroke occurrence after adjustment of comorbid factors was 4.7 (95% confidence interval

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51 57 [CI], 2.0–11.2) in PVH and 3.6 (CI, 1.4–9.2) in DSWMH⁶. Unlike DSWMH, PVH is associated with

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54 58 cognitive dysfunction⁷. In other studies, associations were separately assessed for PVH and DSWMH

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57 59 and was significant only for PVH, which was related to decreased processing speed and executive

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4 60 function^{8,9}. Additionally, PVH predicted poorer functional outcome after stroke both in the acute
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7 61 and chronic phases, independently of DSWMH^{10,11}. A Chiba University group reported that the
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10 62 relative risk value (RRV) measured based on protein-conjugated acrolein (PC-Acro) together with
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13 63 interleukin-6 (IL-6) and C-reactive protein (CRP) can be used to predict the stroke risk factors of
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16 64 silent brain infarction (SBI), carotid atherosclerosis (CA), and WMH with high sensitivity and
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19 65 specificity¹². We measured plasma PCAcro, IL-6 and CRP, analyzed the measurements in
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22 66 conjunction with age to determine whether it is possible to use the RRV to predict PVH and
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25 67 DSWMH.

26 27 68 2. Materials and methods

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33 70 We examined 228 elderly volunteers (78 women and 150 men, age 65.0 ± 7.0 years, range 31–83
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36 71 years). All these subjects were healthy volunteers living independently at home without apparent
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39 72 history of stroke, cardiovascular disease, or malignancy. Subjects with $RRV > 0.5$ were enrolled
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42 73 prospectively. Informed consent was provided by each subject, and our study protocol was approved
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45 74 by the Ethics Committees of Nippon Medical School Hospital. Experiments were carried out in
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48 75 accordance with the Declaration of Helsinki principles. Blood samples were collected into tubes
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51 76 containing 3 U/mL heparin and centrifuged at $1500 \times g$ for 10 min at 4°C.
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53 54 55 56 78 2.2. PC-Acro, IL-6, and CRP Measurements

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4 79 Blood samples were drawn from the antecubital vein after overnight fasting. PC-Acro
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7 80 [*N*-(3-formyl-3,4-dehydropiperidino)-lysine (FDPLysine) in protein] was determined as previously
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10 81 described¹³ using an ACR-LYSINE ADDUCT ELISA SYSTEM (NOF Corporation, Tokyo, Japan)
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13 82 and 0.01 mL plasma. IL-6 and CRP were quantified using an Endogen Human IL-6 ELISA kit
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16 83 (Pierce Biotechnology, Inc., Rockford, IL, USA) and a human CRP ELISA kit (Alpha Diagnostic
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19 84 International, San Antonio, TX, USA), respectively, according to the manufacturers' protocols. After
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22 85 the reaction was terminated, absorbance was measured at 450 nm using a microplate reader
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25 86 (MTP-800APC, Hitachi, Tokyo, Japan). The biochemical markers from each subject were measured
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28 87 by an investigator who was blinded to the MRI results (Amine Pharma Research Institute, Chiba,
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31 88 Japan). Relative risk value (RRV) was calculated with artificial neural networks by back propagation
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34 89 method using NEUROSIM/L software version 4 (Fujitsu, Tokyo, Japan)¹⁴. Using the report by a
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37 90 Chiba University group, we worked out predictive RRV in the range of 0–1^{15 16}, with the nil as the
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40 91 lowest value as an index of the degree of tissue damage. Values > 0.5 were considered to indicate
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43 92 WMH risk.

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46 94 Standard enzymatic methods were used to measure the levels of serum total cholesterol,
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49 95 triglycerides, creatinine, and plasma glucose. Serum high-density lipoprotein (HDL) cholesterol level
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52 96 was measured with a direct method, and serum low-density lipoprotein (LDL) cholesterol level was
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55 97 calculated using Friedewald's formula in the 228 subjects with serum triglyceride levels < 400
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58 98 mg/dL¹⁷. Diabetes was defined as a fasting plasma glucose level ≥ 126 mg/dL or the use of

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3 99 glucose-lowering medications. Dyslipidemia was defined as total cholesterol level ≥ 220 mg/dL,
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6 100 HDL cholesterol level < 40 mg/dL and a triglyceride level ≥ 150 mg/dL, as well as the use of
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9 101 lipid-lowering medications. The estimated glomerular filtration rate (eGFR) was calculated for
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11 102 Japanese men as recommended by the Japanese Society of Nephrology¹⁸ and represented as: eGFR
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14 103 $(\text{mL}/\text{min}/1.73 \text{ m}^2) = 193 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287}$.

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20 105 All 228 subjects underwent T1- and T2-weighted MRI and fluid-attenuated inversion recovery
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23 106 (FLAIR) at the Nippon Medical School Hospital, Japan, within 1 month after blood sampling. MRI
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26 107 was performed as described previously [10]. PVH and DSWMH were defined as hyperintense areas
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29 108 on T2 and FLAIR images without any abnormality on T1 [Fazekas,16] in subjects without
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32 109 neurological signs and/or symptoms. The 228 subjects were classified into 103 PVH subjects (38
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35 110 women and 65 men, aged 68.2 ± 6.0 years, RRV 0.75 ± 0.11) and 157 WMH subjects (61 women
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38 111 and 96 men, aged 66.7 ± 5.8 years, RRV 0.71 ± 0.12).

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46 114 All statistical tests were performed using the JMP9.02 software program (SAS Institute, Cary, NC,
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49 115 USA). Continuous variables except for triglyceride levels were expressed as means \pm standard
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52 116 deviation (SD). Triglyceride levels were transformed to the common logarithm for statistical analysis
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55 117 and are expressed as the geometric mean because of their skewed distribution. Categorical data are
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4 118 expressed as the number of subjects (percent of total). The clinical characteristics for each RRV
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7 119 tertile were compared by analysis of variance (ANOVA) for continuous variables and χ^2 test for
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10 120 categorical variables. The RRVs between the two groups were compared by Student's t-tests or by
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13 121 ANOVA followed by multiple comparisons with the Bonferroni correction between the two groups.
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16 122 Correlations between RRV and other variables were evaluated with the Pearson's moment correlation
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19 123 coefficient. Factors with a P value < 0.20 as determined by Pearson's correlation analysis were
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22 124 included in a multiple linear regression analysis to identify independent determinants of the RRV.
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25 125 Logistic regression analysis was performed to obtain the odds ratios (ORs) for PVH and DSWMH in
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28 126 the three tertiles. All statistical tests were two-sided, and a P value < 0.05 was considered as
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39 130 The study subjects were divided into tertiles according to RRV (0.50–0.62, 0.63–0.79, and 0.80–0.90
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42 131 from the lowest to highest tertile, respectively). The subjects' clinical characteristics are summarized
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45 132 in Table 1. The mean RRV of the entire subject population was 0.71 ± 0.13 , and the mean age was 65
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48 133 ± 7 years. Age, body mass index (BMI), diastolic blood pressure (BP), HDL cholesterol level,
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51 134 triglyceride level, eGFR, and current smoking status were significantly different among the groups.
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54 135 RRVs were significantly higher in subjects with older age, lower eGFR, or PVH (Table 1). A simple
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57 136 correlation analysis showed that RRV was significantly correlated with age, systolic BP, eGFR, and

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4 137 PVH (Table 2). Multiple linear regression analysis indicated that BMI ($\beta = 0.0026$, $P = 0.044$) and
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7 138 PVH ($\beta = 0.0380$, $P < 0.0001$) were significant independent determinants of RRV.
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10 139 The results of logistic regression analysis of the association between PVH and RRV are shown in
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12 140 Table 3. Significant, unadjusted ORs for PVH were noted in the third RRV tertile (5.26 [95% CI,
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15 141 2.66–10.78], $P < 0.0001$), compared to the first tertile. After adjusting for model 1 (BMI, systolic BP,
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18 142 triglycerides, eGFR, and current smoking status) the ORs in the third RRV tertile remained
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21 143 significant (4.75 [95% CI, 2.33–10.05], $P < 0.0001$). After adjusting for model 2, we found that the
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24 144 OR in the third RRV tertile 3 was significant (5.26 [95% CI, 2.65–10.83], $P < 0.0001$). A significant
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27 145 relationship was observed between RRV and PVH ($P < 0.05$) but no such significance was found
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30 146 between RRV and DSWMH (figure 1).
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38 149 The present study demonstrated a significant, positive correlation between RRV and PVH in healthy
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41 150 Japanese volunteers. Notably, the highest RRV level tertile showed a significantly higher OR for a
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44 151 high predicted PVH risk in comparison to the lowest tertile, even after adjusting for multiple
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47 152 confounding factors. These results suggest that RRV is associated with the estimated risk of PVH in
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50 153 healthy Japanese volunteers. A number of clinical and epidemiological studies have examined the
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53 154 predictive value of RRV for the presence of WMH^{15 16}. However, those studies assessed WMH
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56 155 prevalence; no studies have shown any significant association of RRV with PVH and DSWMH
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4 156 separately. In this regard, our results raise the possibility that RRV predicts the risk of PVH in the
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7 157 healthy Japanese population. With respect to age, these biochemical markers provide a good index of
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10 158 the presence of tissue damage related to PVH.

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13 159 More recent studies focused on WMH location have reported that functional impairment within 1–3
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16 160 months after stroke correlated with PVH but not with DSWMH^{10 19}. PVH WMH, especially PVH,
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19 161 has impacts on early functional recovery after ischemic stroke regardless of the initial stroke severity
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22 162 and other cardiovascular risk factors¹¹. Other groups found a significant association between PVH
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25 163 and decreases in processing speed and executive function, but there was no such relationship with
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28 164 DSWMH^{8 9}. Why PVH and DSWMH have different relationships with stroke outcome remains
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31 165 unclear, but several theories have been put forward. DSWMH predominantly disrupt short
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34 166 association fibers that link adjacent gyri, while PVH affects long association fibers that connect the
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37 167 more distant cortical areas²⁰. Thus, lesions in various white matter locations may disconnect from
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40 168 different neural networks that affect neural repair processes after stroke²¹. In addition, PVHs are
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43 169 related to diminished cerebral vasomotor reactivity and subsequent occurrence of cerebral
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46 170 hypoperfusion²², while DSWMHs are generally associated with microangiopathy²³. It is clear that
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49 171 regional hypoperfusion is a good predictor of functional outcome²⁴. These findings shed light on
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52 172 why PVH can predict functional stroke outcome and specific cognitive functions¹¹.

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55 173 Acrolein induces IL-6 production in astrocytes, macrophages, and endothelial cells, while IL-6
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58 174 induces CRP production in hepatocytes. Then, CRP stimulates IL-6 production, and IL-6 decreases

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4 175 acrolein toxicity ²⁵. Acrolein was thought to be one of the toxic compounds produced from
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7 176 unsaturated fatty acids by active oxygen species such as superoxide anion radical, hydrogen peroxide,
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10 177 and hydroxyl radical¹³. These findings may partially explain the pathophysiological mechanisms
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13 178 underlying the association between PVH and the three biomarkers assessed in the present study.
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15 179 Further investigation will be needed for a better understanding of their interrelationship.
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181 Our multiple linear regression analysis showed that RRV was independently associated with BMI
182 and PVH. Although obesity is believed to be an independent cardiovascular risk factor ²⁶, it is still
183 controversial whether BMI is a significant risk factor for stroke ^{27 28}. BMI was previously reported to
184 be correlated with high RRV ²⁹ which may be caused by vascular degeneration and endothelial
185 dysfunction associated with hypertension and metabolic disorders. Subjects with metabolic
186 syndrome are generally defined as those who have abdominal obesity and two additional metabolic
187 disorders including hypertension, dyslipidemia, and hyperglycemia ^{30 31}.

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189 Our study has some potential limitations. Because it was a cross-sectional investigation, we could not
190 determine a causal relationship between increased RRV and PVH risk. In addition, the population
191 included healthy Japanese volunteers only. Therefore, it is unclear whether the results can be
192 extrapolated to other populations of poor health, patients with cardiovascular diseases, or other
193 ethnic groups. Despite these potential limitations, our findings support the conclusion that elevated

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4 194 RRV is significantly associated with PVH in healthy Japanese volunteers. These results suggest that
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7 195 RRV measurement may be useful for identifying PVH in the general population. This would allow
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10 196 clinicians to follow patients who may be at risk for stroke and cognitive dysfunction.

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16 198 **Acknowledgments**

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18 199 We thank Drs. Mari Adachi and Sadaji Kura at Katsushika Health Center for assisting with the data
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21 200 analyses.

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24 201 **Contributorship Statement**

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27 202 Conception and design: AA

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30 203 Analysis and interpretation: YN, MHA, SO, MU

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33 204 Writing the article: AA

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36 205 Critical revision of the article: YN, MM, YK

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39 206 Final approval of the article: YN, MHA, SO, MU, MM, YK

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42 207 Statistical analysis: YN, MM

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45 208 Overall responsibility: AA

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47 209 **Competing Interests**

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53 211 **Data Sharing Statement**

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56 212 No additional data available

Table 1. Characteristics of the study subjects due to their stroke risk marker level tertile

Item	All	Silent brain infarction RRV tertile			P value*
		Tertile 1	Tertile 2	Tertile 3	
Subjects (n)	228	76	73	79	-
Silent brain infarction relative risk value	0.71 ± 0.13	0.51–0.07**	0.69–0.05**	0.86–0.03**	-
Age (years)	65 ± 7	59 ± 7	66 ± 3	70 ± 6	<0.0001
Male Sex	150 (65.7)	51 (67.1)	52 (71.2)	47 (59.4)	0.299
Body mass index, kg/m ²	24.2 ± 6.4	23.2 ± 2.9	24.6 ± 6.3	24.8 ± 8.6	0.133
Systolic BP, mmHg	123 ± 15	121 ± 14	122 ± 14	126 ± 17	0.275
Diastolic BP, mmHg	75 ± 11	75 ± 9	75 ± 10	75 ± 13	0.947
Hypertension, n (%)	81 (35.6)	23 (30.6)	20 (27.4)	38 (48.1)	0.015
Total cholesterol, mg/dL	208 ± 32	208 ± 30	208 ± 32	207 ± 34	0.903
LDL cholesterol [†] , mg/dL	123 ± 29	123 ± 25	123 ± 29	122 ± 33	0.848
HDL cholesterol, mg/dL	58 ± 15	59 ± 17	57 ± 13	60 ± 16	0.385
Triglycerides ^{††} , mg/dL	125 (113, 138)	141 (111, 172)	123 (105, 142)	112 (99, 124)	0.425

Dyslipidemia, n (%)	55 (24.1)	19 (25.0)	13 (17.8)	23 (29.1)	0.259
Fasting plasma glucose, mg/dL	100 ± 16	100 ± 14	99 ± 18	100 ± 15	0.754
Diabetes, n (%)	18 (7.8)	3 (3.9)	6 (8.2)	9 (11.3)	0.226
eGFR (mL/min/1.73 m ²)	67.6 ± 12.5	70.2 ± 15.3	67.3 ± 10.6	65.4 ± 10.6	0.027
Current smoking status, n (%)	40 (17.5)	16 (21.0)	12 (16.4)	12 (15.1)	0.603
PVH, n (%)	103 (45.1)	18 (23.6)	36 (49.3)	49 (62.0)	<0.0001
DSWMH, n (%)	157 (68.8)	52 (68.4)	50 (68.4)	55 (69.6)	0.983

*Analysis of variance or chi-square test among the ADMA tertile. **Range of a minimum-to-maximum stroke risk marker in each tertile. †n =

228. ††Geometric mean (95% confidence intervals). ‡Statistical analysis was not conducted because of the extremely small number of subjects in each category. BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; RRV, relative risk value

Table 2. Correlation coefficients and multiple linear regression analysis of relative risk value with the clinical parameters

Item	Simple correlation analysis		Multiple linear regression analysis	
	Correlation coefficient (r)	P-value	Standardized regression coefficient (β)	P value
Age	0.60	<0.0001	†	
Male Sex	0.05	0.347	††	
BMI	0.11	0.075	0.0026	0.044
Systolic BP	0.14	0.026	0.0009	0.075
Diastolic BP	0.05	0.382	††	
Total cholesterol	-0.03	0.618	††	
LDL cholesterol*	-0.02	0.669	††	
HDL cholesterol	0.02	0.731	††	
Triglycerides**	-0.10	0.130	-0.0001	0.083
Fasting plasma glucose	0.01	0.841	††	
eGFR	-0.13	0.041	-0.0008	0.235
Current smoking status (Yes = 1)	-0.10	0.115	-0.0161	0.158
PVH (Yes = 1)	0.26	<0.0001	0.0380	<0.0001
DSWMH (Yes = 1)	0.02	0.689	††	

* n = 228. ** Log-transformed value. † Not included in the multiple linear regression analysis to avoid multicollinearity with PVH relative risk value.

†† Not included in the multiple linear regression analysis because their P values were ≥ 0.20 in the simple correlation analysis. BMI, body mass index; BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3. Unadjusted and adjusted PVH ORs in each silent brain infarction relative risk value tertile

Item	Unadjusted			Adjusted*			Adjusted**		
	OR	95% CI	P-value	OR	95% CI	P value	OR	95% CI	P value
Tertile 1	1.00	reference	-	1.00	reference	-	1.00	reference	-
Tertile 2	3.13	1.57–6.41	0.0014	3.68	1.77–7.97	0.0004	2.95	1.11–8.35	0.02
Tertile 3	5.26	2.66–10.78	<0.0001	4.75	2.33–10.05	<0.0001	5.26	2.65–10.83	<0.0001

* Adjusted for BMI, systolic BP, triglycerides, eGFR, and current smoking status

** Adjusted for BMI

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; OR, odds ratio; PVH, periventricular hyperintensity

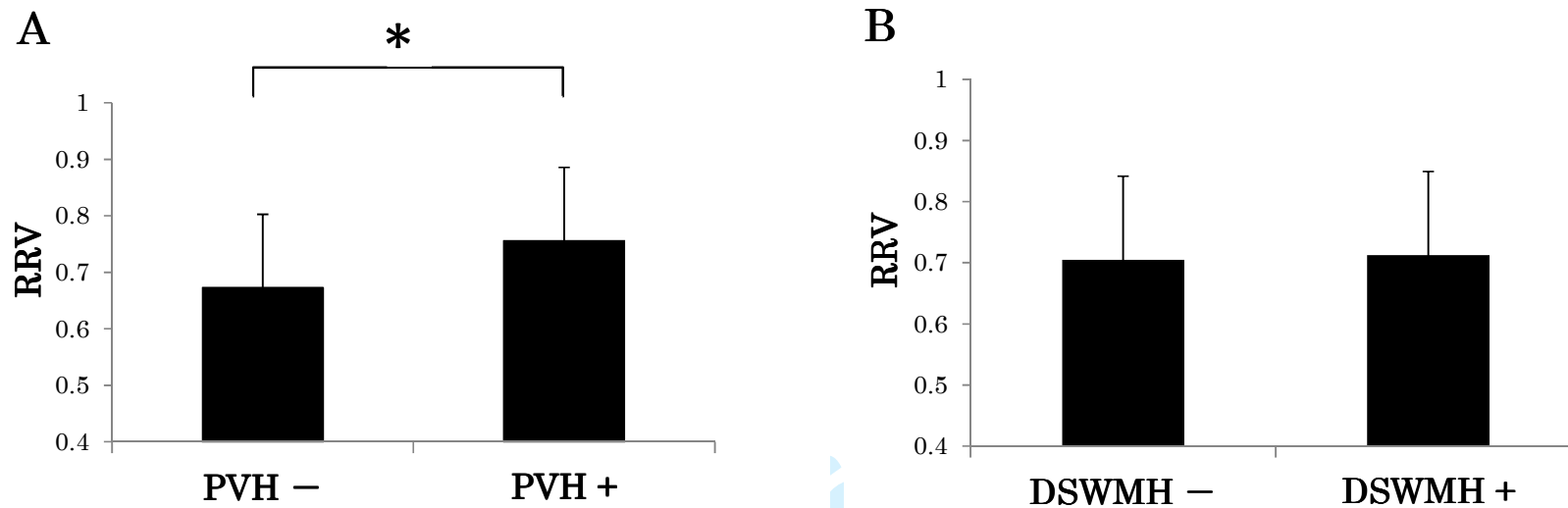


Figure 1. Correlation between the RRV and the PVH (A). Correlation between the RRV and DSWMH (B)

*Significant at $P < 0.05$

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Page3
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page4
Methods			
Study design	4	Present key elements of study design early in the paper	Page5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	Page5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page6
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page7-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Page7-8
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	NA
		(d) If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page4
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	NA
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page8-9
		(b) Report category boundaries when continuous variables were categorized	Page8-9
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page8-9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page8-9
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page11
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

Relative risk values of age, acrolein, IL-6, and CRP as markers of periventricular hyperintensities: a cross-sectional study

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005598.R1
Article Type:	Research
Date Submitted by the Author:	04-Jul-2014
Complete List of Authors:	Abe, Arata; Nippon Medical School, epartment of Neurological Science, Graduate School of Medicine Nishiyama, Y; Nippon Medical School, epartment of Neurological Science, Graduate School of Medicine Abe-Harada, Mina; Nippon Medical School, epartment of Neurological Science, Graduate School of Medicine Okubo, Seiji; Nippon Medical School, epartment of Neurological Science, Graduate School of Medicine Ueda, Masayuki; Nippon Medical School, epartment of Neurological Science, Graduate School of Medicine Mishina, Masahiro; Graduate School of Medicine, Nippon Medical School, Department of Neurological Science Katayama, Yasuo; Nippon Medical School, epartment of Neurological Science, Graduate School of Medicine
Primary Subject Heading:	Diagnostics
Secondary Subject Heading:	Neurology, Diagnostics
Keywords:	Stroke < NEUROLOGY, Neurology < INTERNAL MEDICINE, Stroke medicine < INTERNAL MEDICINE

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4 1 **Relative risk values of age, acrolein, IL-6, and CRP as markers of periventricular**
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12 4 Arata Abe*, Yasuhiro Nishiyama, Mina Harada-Abe, Seiji Okubo, Masayuki Ueda, Masahiro
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30 10 **Keywords:** Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity, White matter
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7 21 **Abstract**

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10 22 Objective: Brain white matter hyperintensities (WMHs) can be divided into periventricular
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12 23 hyperintensity (PVH) and deep-and-subcortical white matter hyperintensity (DSWMH), and the
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15 24 former contribute more to cognitive dysfunction and infarction risk. We conducted the present
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18 25 investigation to define the relationship between PVH and DSWMH.

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21 26 Design: Cross-sectional study.

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24 27 Setting: University hospital

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27 28 Participants: We prospectively enrolled 228 healthy Japanese volunteer subjects with relative risk
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30 29 values (RRVs) > 0.5.

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33 30 Primary outcome measures: We investigated whether it is possible to use the RRV to predict PVH
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36 31 and DSWMH.

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39 32 Results: Among 228 subjects, 103 (45.1%) and 157 (68.8%) exhibited PVH and DSWMH,
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41
42 33 respectively. Age, body mass index (BMI), and PVH were significant independent determinants of
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45 34 RRV. A significant odds ratio (OR) for PVH was noted in the highest RRV tertile compared with the
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48 35 lowest one after adjusting for potential confounding factors. A significant OR for high predicted
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51 36 PVH risk was also found for RRV level as well.

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54 37 Conclusion: Elevated RRV levels were significantly associated with increased predicted PVH,
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57 38 suggesting that measuring the plasma PCAcro, IL-6, and CRP levels may be useful for identifying

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4 39 Japanese at high risk for PVH.
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7 40 **Strengths and limitations of this study:**
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10 41 Using the RRV at clinical level, we investigate to evaluate WMHs.
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12 42 We provided the first evidence that RRV is associate with PVH rather than DSWMH.
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15 43 These data are obtained from cases of cautious health care in Asian people and may not be applicable
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59 1. Introduction

60 A number of large-scale clinical studies have demonstrated that white matter hyperintensities
61 (WMHs) are associated with high stroke risk¹⁻³. The results of the large-scale, multicenter open trial
62 PICA study⁴ conducted in Japan suggest that the Fazekas-classified periventricular hyperintensities
63 (PVHs) and deep-and-subcortical white matter hyperintensities (DSWMHs)⁵ are related to the risk of
64 symptomatic brain infarction (SBI). In the Rotterdam Scan study on elderly subjects with no history
65 of stroke, conducted by magnetic resonance imaging (MRI) for 4.2 years, the proportional hazard
66 ratio of stroke occurrence after adjustment of comorbid factors was 4.7 (95% confidence interval
67 [CI], 2.0–11.2) in PVH and 3.6 (CI, 1.4–9.2) in DSWMH⁶. Unlike DSWMH, PVH is associated with
68 cognitive dysfunction⁷. In other studies, associations were separately assessed for PVH and DSWMH
69 and was significant only for PVH, which was related to decreased processing speed and executive
70 function^{8,9}. Additionally, PVH predicted poorer functional outcome after stroke both in the acute
71 and chronic phases, independently of DSWMH^{10,11}. A Chiba University group reported that the
72 relative risk value (RRV) measured based on protein-conjugated acrolein (PC-Acro) together with
73 interleukin-6 (IL-6) and C-reactive protein (CRP) can be used to predict the stroke risk factors of
74 silent brain infarction (SBI), carotid atherosclerosis (CA), and WMH with high sensitivity and
75 specificity¹². We measured plasma PCAcro, IL-6 and CRP, analyzed the measurements in
76 conjunction with age to determine whether it is possible to use the RRV to predict PVH and

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4 77 DSWMH.
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7 78 2. Materials and methods
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10 79 2.1. Subjects and blood sampling
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12 80 We examined 228 adult volunteers (78 women and 150 men, age 65.0 ± 7.0 years, range 31–83
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15 81 years). All these subjects were healthy volunteers living independently at home without apparent
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18 82 history of stroke, cardiovascular disease, or malignancy. Subjects with RRV > 0.5 were enrolled
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21 83 prospectively. Informed consent was provided by each subject, and our study protocol was approved
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24 84 by the Ethics Committees of Nippon Medical School Hospital. Experiments were carried out in
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27 85 accordance with the Declaration of Helsinki principles. Blood samples were collected into tubes
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30 86 containing 3 U/mL heparin and centrifuged at $1500 \times g$ for 10 min at 4°C .
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36 88 2.2. PC-Acro, IL-6, and CRP Measurements
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38 89 Blood samples were drawn from the antecubital vein after overnight fasting. PC-Acro
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41 90 [*N*-(3-formyl-3,4-dehydropiperidino)-lysine (FDPLysine) in protein] was determined as previously
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44 91 described¹³ using an ACR-LYSINE ADDUCT ELISA SYSTEM (NOF Corporation, Tokyo, Japan)
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47 92 and 0.01 mL plasma. IL-6 and CRP were quantified using an Endogen Human IL-6 ELISA kit
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50 93 (Pierce Biotechnology, Inc., Rockford, IL, USA) and a human CRP ELISA kit (Alpha Diagnostic
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53 94 International, San Antonio, TX, USA), respectively, according to the manufacturers' protocols. After
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56 95 the reaction was terminated, absorbance was measured at 450 nm using a microplate reader
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4 96 (MTP-800APC, Hitachi, Tokyo, Japan). The biochemical markers from each subject were measured
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7 97 by an investigator who was blinded to the MRI results (Amine Pharma Research Institute, Chiba,
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10 98 Japan). Relative risk value (RRV) was calculated with artificial neural networks by back propagation
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12 99 method using NEUROSIM/L software version 4 (Fujitsu, Tokyo, Japan)¹⁴. Using the report by a
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15 100 Chiba University group, we worked out predictive RRV in the range of 0–1^{12 15}, with the nil as the
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18 101 lowest value as an index of the degree of tissue damage. Values > 0.5 were considered to indicate
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21 102 WMH risk.
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26 104 Standard enzymatic methods were used to measure the levels of serum total cholesterol,
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32 106 was measured with a direct method, and serum low-density lipoprotein (LDL) cholesterol level was
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35 107 calculated using Friedewald's formula in the 228 subjects with serum triglyceride levels < 400
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37 108 mg/dL¹⁶. Diabetes was defined as a fasting plasma glucose level ≥ 126 mg/dL or the use of
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40 109 glucose-lowering medications. Dyslipidemia was defined as total cholesterol level ≥ 220 mg/dL,
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43 110 HDL cholesterol level < 40 mg/dL and a triglyceride level ≥ 150 mg/dL, as well as the use of
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46 111 lipid-lowering medications. The estimated glomerular filtration rate (eGFR) was calculated for
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49 112 Japanese men as recommended by the Japanese Society of Nephrology¹⁷ and represented as: eGFR
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51 113 $(\text{mL}/\text{min}/1.73 \text{ m}^2) = 193 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287}$.
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4 115 All 228 subjects underwent T1- and T2-weighted MRI and fluid-attenuated inversion recovery
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7 116 (FLAIR) at the Nippon Medical School Hospital, Japan, within 1 month after blood sampling. MRI
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10 117 was performed as described previously¹⁰. PVH and DSWMH were defined as hyperintense areas on
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13 118 T2 and FLAIR images without any abnormality on T1¹⁸ in subjects without neurological signs and/or
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16 119 symptoms. The 228 subjects were classified into 103 PVH subjects (38 women and 65 men, aged
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19 120 68.2 ± 6.0 years, RRV 0.75 ± 0.11) and 157 WMH subjects (61 women and 96 men, aged 66.7 ± 5.8
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22 121 years, RRV 0.71 ± 0.12). In more detail, 76 among all the subjects had both PVH and DSWMH, the
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25 122 other 23 having silent brain infarction. Also, 22 subjects had a complication of PVH and silent brain
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28 123 infarction, while in 20 subjects there was complication of DSWMH and silent brain infarction.
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36 126 All statistical tests were performed using the JMP9.02 software program (SAS Institute, Cary, NC,
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39 127 USA). Continuous variables except for triglyceride levels were expressed as means \pm standard
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45 129 and are expressed as the geometric mean because of their skewed distribution. Categorical data are
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48 130 expressed as the number of subjects (percent of total). The clinical characteristics for each RRV
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51 131 tertile were compared by analysis of variance (ANOVA) for continuous variables and χ^2 test for
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54 132 categorical variables. The RRVs between the two groups were compared by Student's t-tests or by
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57 133 ANOVA followed by multiple comparisons with the Bonferroni correction between the two groups.
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4 134 Correlations between RRV and other variables were evaluated with the Pearson's moment correlation
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7 135 coefficient. Factors with a P value < 0.05 as determined by Pearson's correlation analysis were
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10 136 included in a multiple linear regression analysis to identify independent determinants of the RRV.
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13 137 Logistic regression analysis was performed to obtain the odds ratios (ORs) for PVH and DSWMH in
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16 138 the three tertiles. All statistical tests were two-sided, and a P value < 0.05 was considered as
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27 142 The study subjects were divided into tertiles according to RRV (0.50–0.62, 0.63–0.79, and 0.80–0.90
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30 143 from the lowest to highest tertile, respectively). The subjects' clinical characteristics are summarized
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33 144 in Table 1. The mean RRV of the entire subject population was 0.71 ± 0.13 , and the mean age was 65
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36 145 ± 7 years. Age, body mass index (BMI), diastolic blood pressure (BP), HDL cholesterol level,
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39 146 triglyceride level, eGFR, and current smoking status were significantly different among the groups.

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42 147 RRVs were significantly higher in subjects with older age, lower eGFR, or PVH (Table 1). A simple
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45 148 correlation analysis showed that RRV was significantly correlated with age, systolic BP, eGFR, and
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48 149 PVH (Table 2). Multiple linear regression analysis indicated that BMI ($\beta = 0.0026$, $P = 0.044$) and
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51 150 PVH ($\beta = 0.0380$, $P < 0.0001$) were significant independent determinants of RRV.

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54 151 The results of logistic regression analysis of the association between PVH and RRV are shown in
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57 152 Table 3. Significant, unadjusted ORs for PVH were noted in the third RRV tertile (5.26 [95% CI,

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4 153 2.66–10.78], $P < 0.0001$), compared to the first tertile. After adjusting for model 1 (BMI, systolic BP,
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7 154 triglycerides, eGFR, and current smoking status) the ORs in the third RRV tertile remained
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10 155 significant (4.75 [95% CI, 2.33–10.05], $P < 0.0001$). After adjusting for model 2, we found that the
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13 156 OR in the third RRV tertile 3 was significant (5.26 [95% CI, 2.65–10.83], $P < 0.0001$). A significant
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16 157 relationship was observed between RRV and PVH ($P < 0.05$) but no such significance was found
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19 158 between RRV and DSWMH (figure 1).

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27 161 The present study demonstrated a significant, positive correlation between RRV and PVH in healthy
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30 162 Japanese volunteers. Notably, the highest RRV level tertile showed a significantly higher OR for a
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33 163 high predicted PVH risk in comparison to the lowest tertile, even after adjusting for multiple
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36 164 confounding factors. These results suggest that RRV is associated with the estimated risk of PVH in
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39 165 healthy Japanese volunteers. A number of clinical and epidemiological studies have examined the
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42 166 predictive value of RRV for the presence of WMH^{12 15}. However, those studies assessed WMH
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45 167 prevalence; no studies have shown any significant association of RRV with PVH and DSWMH
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48 168 separately. In this regard, our results raise the possibility that RRV predicts the risk of PVH in the
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51 169 healthy Japanese population. With respect to age, these biochemical markers provide a good index of
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54 170 the presence of tissue damage related to PVH.

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56 171 More recent studies focused on WMH location have reported that functional impairment within 1–3
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4 172 months after stroke correlated with PVH but not with DSWMH^{10 19}. PVH WMH, especially PVH,
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7 173 has impacts on early functional recovery after ischemic stroke regardless of the initial stroke severity
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10 174 and other cardiovascular risk factors¹¹. Other groups found a significant association between PVH
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13 175 and decreases in processing speed and executive function, but there was no such relationship with
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16 176 DSWMH^{8 9}. Why PVH and DSWMH have different relationships with stroke outcome remains
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19 177 unclear, but several theories have been put forward. DSWMH predominantly disrupt short
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22 178 association fibers that link adjacent gyri, while PVH affects long association fibers that connect the
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25 179 more distant cortical areas²⁰. Thus, lesions in various white matter locations may disconnect from
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28 180 different neural networks that affect neural repair processes after stroke²¹. In addition, PVHs are
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31 181 related to diminished cerebral vasomotor reactivity and subsequent occurrence of cerebral
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34 182 hypoperfusion²², while DSWMHs are generally associated with microangiopathy²³. It is clear that
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37 183 regional hypoperfusion is a good predictor of functional outcome²⁴. These findings shed light on
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40 184 why PVH can predict functional stroke outcome and specific cognitive functions¹¹.
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43 185 Acrolein induces IL-6 production in astrocytes, macrophages, and endothelial cells, while IL-6
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46 186 induces CRP production in hepatocytes. Then, CRP stimulates IL-6 production, and IL-6 decreases
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49 187 acrolein toxicity²⁵. Acrolein was thought to be one of the toxic compounds produced from
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52 188 unsaturated fatty acids by active oxygen species such as superoxide anion radical, hydrogen peroxide,
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55 189 and hydroxyl radical¹³. These findings may partially explain the pathophysiological mechanisms
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58 190 underlying the association between PVH and the three biomarkers assessed in the present study.
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4 191 Further investigation will be needed for a better understanding of their interrelationship.
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10 193 Our multiple linear regression analysis showed that RRV was independently associated with BMI
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12 194 and PVH. Although obesity is believed to be an independent cardiovascular risk factor ²⁶, it is still
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14 195 controversial whether BMI is a significant risk factor for stroke ^{27 28}. BMI was previously reported to
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16 196 be correlated with high RRV ²⁹ which may be caused by vascular degeneration and endothelial
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18 197 dysfunction associated with hypertension and metabolic disorders. Subjects with metabolic
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20 198 syndrome are generally defined as those who have abdominal obesity and two additional metabolic
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22 199 disorders including hypertension, dyslipidemia, and hyperglycemia ^{30 31}.
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33 201 Our study has some potential limitations. Because it was a cross-sectional investigation, we could not
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35 202 determine a causal relationship between increased RRV and PVH risk. In addition, the population
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37 203 included healthy Japanese volunteers only. Therefore, it is unclear whether the results can be
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39 204 extrapolated to other populations of poor health, patients with cardiovascular diseases, or other
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41 205 ethnic groups. Despite these potential limitations, our findings support the conclusion that elevated
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43 206 RRV is significantly associated with PVH in healthy Japanese volunteers. These results suggest that
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45 207 RRV measurement may be useful for identifying PVH in the general population. This would allow
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47 208 clinicians to follow patients who may be at risk for stroke and cognitive dysfunction.
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4 210 **Acknowledgments**
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7 211 We thank Drs. Mari Adachi and Sadaji Kura at Katsushika Health Center for assisting with the data
8
9
10 212 analyses.

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12 213 **Contributorship**
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14 214
15 215 Conception and design: AA
16 216 Analysis and interpretation: YN, MHA, SO, MU
17 217 Writing the article: AA
18 218 Critical revision of the article: YN, MM, YK
19 219 Final approval of the article: YN, MHA, SO, MU, MM, YK
20 220 Statistical analysis: YN, MM
21 221 Overall responsibility: AA
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24 223 **Competing Interests**
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26 224
27 225 None
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30 227 **Data Sharing Statement**
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32 228
33 229 No additional data available
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Table 1. Characteristics of the study subjects due to their stroke risk marker level tertile

Item	All	Silent brain infarction RRV tertile			P value*
		Tertile 1	Tertile 2	Tertile 3	
Subjects (n)	228	76	73	79	-
Silent brain infarction relative risk value	0.71 ± 0.13	0.51 ± 0.07**	0.69 ± 0.05**	0.86 ± 0.03**	-
Age (years)	65 ± 7	59 ± 7	66 ± 3	70 ± 6	<0.0001
Male Sex	150 (65.7)	51 (67.1)	52 (71.2)	47 (59.4)	0.299
Body mass index, kg/m ²	24.2 ± 6.4	23.2 ± 2.9	24.6 ± 6.3	24.8 ± 8.6	0.133
Systolic BP, mmHg	123 ± 15	121 ± 14	122 ± 14	126 ± 17	0.275
Diastolic BP, mmHg	75 ± 11	75 ± 9	75 ± 10	75 ± 13	0.947
Hypertension, n (%)	81 (35.6)	23 (30.6)	20 (27.4)	38 (48.1)	0.015
Total cholesterol, mg/dL	208 ± 32	208 ± 30	208 ± 32	207 ± 34	0.903
LDL cholesterol [†] , mg/dL	123 ± 29	123 ± 25	123 ± 29	122 ± 33	0.848
HDL cholesterol, mg/dL	58 ± 15	59 ± 17	57 ± 13	60 ± 16	0.385
Triglycerides ^{††} , mg/dL	125 (113, 138)	141 (111, 172)	123 (105, 142)	112 (99, 124)	0.425

Dyslipidemia, n (%)	55 (24.1)	19 (25.0)	13 (17.8)	23 (29.1)	0.259
Fasting plasma glucose, mg/dL	100 ± 16	100 ± 14	99 ± 18	100 ± 15	0.754
Diabetes, n (%)	18 (7.8)	3 (3.9)	6 (8.2)	9 (11.3)	0.226
eGFR (mL/min/1.73 m ²)	67.6 ± 12.5	70.2 ± 15.3	67.3 ± 10.6	65.4 ± 10.6	0.027
Current smoking status, n (%)	40 (17.5)	16 (21.0)	12 (16.4)	12 (15.1)	0.603
PVH, n (%)	103 (45.1)	18 (23.6)	36 (49.3)	49 (62.0)	<0.0001
DSWMH, n (%)	157 (68.8)	52 (68.4)	50 (68.4)	55 (69.6)	0.983

*Analysis of variance or chi-square test among the ADMA tertile. **Range of a minimum-to-maximum stroke risk marker in each tertile. †n = 228. ††Geometric mean (95% confidence intervals). ‡Statistical analysis was not conducted because of the extremely small number of subjects in each category. BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; RRV, relative risk value

Table 2. Correlation coefficients and multiple linear regression analysis of relative risk value with the clinical parameters

Item	Simple correlation analysis		Multiple linear regression analysis	
	Correlation coefficient (r)	P-value	Standardized regression coefficient (β)	P value
Age	0.60	<0.0001	†	
Male Sex	0.05	0.347	††	
BMI	0.11	0.075	††	
Systolic BP	0.14	0.026	0.0009	0.078
Diastolic BP	0.05	0.382	††	
Total cholesterol	-0.03	0.618	††	
LDL cholesterol*	-0.02	0.669	††	
HDL cholesterol	0.02	0.731	††	
Triglycerides**	-0.10	0.130	††	
Fasting plasma glucose	0.01	0.841	††	
eGFR	-0.13	0.041	-0.0009	0.173
Current smoking status (Yes = 1)	-0.10	0.115	††	
PVH (Yes = 1)	0.26	<0.0001	0.0384	<0.0001
DSWMH (Yes = 1)	0.02	0.689	††	

* n = 228. ** Log-transformed value. † Not included in the multiple linear regression analysis to avoid multicollinearity with PVH relative risk value.

†† Not included in the multiple linear regression analysis because their P values were ≥ 0.05 in the simple correlation analysis. BMI, body mass index; BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

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Table 3. Unadjusted and adjusted PVH ORs in each silent brain infarction relative risk value tertile

Item	Unadjusted			Adjusted*		
	OR	95% CI	P-value	OR	95% CI	P value
Tertile 1	1.00	reference	-	1.00	reference	-
Tertile 2	3.13	1.57–6.41	0.0014	3.01	1.50–6.20	0.0018
Tertile 3	5.26	2.66–10.78	<0.0001	4.87	2.43–10.08	<0.0001

* Adjusted for systolic BP and eGFR

BP, blood pressure; eGFR, estimated glomerular filtration rate; OR, odds ratio; PVH, periventricular hyperintensity

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Figure 1. Correlation between the RRV and the PVH (A). Correlation between the RRV and DSWMH (B)

*Significant at $P < 0.05$

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4 1 **Relative risk values of age, acrolein, IL-6, and CRP as markers of periventricular**
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7 2 **hyperintensities: a cross-sectional study**
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12 4 Arata Abe*, Yasuhiro Nishiyama, Mina Harada-Abe, Seiji Okubo, Masayuki Ueda, Masahiro
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24 8 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan
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30 10 **Keywords:** Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity, White matter
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4 **22 Abstract**

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7 23 Objective: Brain white matter hyperintensities (WMHs) can be divided into periventricular
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10 24 hyperintensity (PVH) and deep-and-subcortical white matter hyperintensity (DSWMH), and the
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13 25 former contribute more to cognitive dysfunction and infarction risk. We conducted the present
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16 26 investigation to define the relationship between PVH and DSWMH.

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19 27 Design: Cross-sectional study.

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22 28 Setting: University hospital

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25 29 Participants: We prospectively enrolled 228 healthy Japanese volunteer subjects with relative risk
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27 30 values (RRVs) > 0.5.

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30 31 Primary outcome measures: We investigated whether it is possible to use the RRV to predict PVH
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33 32 and DSWMH.

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36 33 Results: Among 228 subjects, 103 (45.1%) and 157 (68.8%) exhibited PVH and DSWMH,
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39 34 respectively. Age, body mass index (BMI), and PVH were significant independent determinants of
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42 35 RRV. A significant odds ratio (OR) for PVH was noted in the highest RRV tertile compared with the
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45 36 lowest one after adjusting for potential confounding factors. A significant OR for high predicted
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48 37 PVH risk was also found for RRV level as well.

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51 38 Conclusion: Elevated RRV levels were significantly associated with increased predicted PVH,
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54 39 suggesting that measuring the plasma PCAcro, IL-6, and CRP levels may be useful for identifying
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57 40 Japanese at high risk for PVH.

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4 41 Strengths and limitations of this study:

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7 42 Using the RRV at clinical level, we investigate to evaluate WMHs.

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10 43 We provided the first evidence that RRV is associate with PVH rather than DSWMH.

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12 44 These data are obtained from cases of cautious health care in Asian people and may not be applicable
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16 45 to populations of poor health.

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21 47 **Keywords:** Protein-conjugated acrolein, IL-6, CRP, Periventricular hyperintensity

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27 49 **1. Introduction**

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30 50 A number of large-scale clinical studies have demonstrated that white matter hyperintensities

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33 51 (WMHs) are associated with high stroke risk¹⁻³. The results of the large-scale, multicenter open trial

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36 52 PICA study⁴ conducted in Japan suggest that the Fazekas-classified periventricular hyperintensities

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39 53 (PVHs) and deep-and-subcortical white matter hyperintensities (DSWMHs)⁵ are related to the risk of

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42 54 symptomatic brain infarction (SBI). In the Rotterdam Scan study on elderly subjects with no history

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45 55 of stroke, conducted by magnetic resonance imaging (MRI) for 4.2 years, the proportional hazard

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48 56 ratio of stroke occurrence after adjustment of comorbid factors was 4.7 (95% confidence interval

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51 57 [CI], 2.0–11.2) in PVH and 3.6 (CI, 1.4–9.2) in DSWMH⁶. Unlike DSWMH, PVH is associated with

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54 58 cognitive dysfunction⁷. In other studies, associations were separately assessed for PVH and DSWMH

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57 59 and was significant only for PVH, which was related to decreased processing speed and executive

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4 60 function^{8 9}. Additionally, PVH predicted poorer functional outcome after stroke both in the acute
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7 61 and chronic phases, independently of DSWMH^{10 11}. A Chiba University group reported that the
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10 62 relative risk value (RRV) measured based on protein-conjugated acrolein (PC-Acro) together with
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13 63 interleukin-6 (IL-6) and C-reactive protein (CRP) can be used to predict the stroke risk factors of
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16 64 silent brain infarction (SBI), carotid atherosclerosis (CA), and WMH with high sensitivity and
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19 65 specificity¹². We measured plasma PCAcro, IL-6 and CRP, analyzed the measurements in
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22 66 conjunction with age to determine whether it is possible to use the RRV to predict PVH and
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25 67 DSWMH.

26 27 68 2. Materials and methods

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33 70 We examined 228 **adult** volunteers (78 women and 150 men, age 65.0 ± 7.0 years, range 31–83
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36 71 years). All these subjects were healthy volunteers living independently at home without apparent
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39 72 history of stroke, cardiovascular disease, or malignancy. Subjects with $RRV > 0.5$ were enrolled
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42 73 prospectively. Informed consent was provided by each subject, and our study protocol was approved
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45 74 by the Ethics Committees of Nippon Medical School Hospital. Experiments were carried out in
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48 75 accordance with the Declaration of Helsinki principles. Blood samples were collected into tubes
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51 76 containing 3 U/mL heparin and centrifuged at $1500 \times g$ for 10 min at 4°C.
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53 54 55 56 78 2.2. PC-Acro, IL-6, and CRP Measurements

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4 79 Blood samples were drawn from the antecubital vein after overnight fasting. PC-Acro
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7 80 [*N*-(3-formyl-3,4-dehydropiperidino)-lysine (FDPLysine) in protein] was determined as previously
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10 81 described¹³ using an ACR-LYSINE ADDUCT ELISA SYSTEM (NOF Corporation, Tokyo, Japan)
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12 82 and 0.01 mL plasma. IL-6 and CRP were quantified using an Endogen Human IL-6 ELISA kit
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14 83 (Pierce Biotechnology, Inc., Rockford, IL, USA) and a human CRP ELISA kit (Alpha Diagnostic
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17 84 International, San Antonio, TX, USA), respectively, according to the manufacturers' protocols. After
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21 85 the reaction was terminated, absorbance was measured at 450 nm using a microplate reader
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24 86 (MTP-800APC, Hitachi, Tokyo, Japan). The biochemical markers from each subject were measured
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27 87 by an investigator who was blinded to the MRI results (Amine Pharma Research Institute, Chiba,
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30 88 Japan). Relative risk value (RRV) was calculated with artificial neural networks by back propagation
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33 89 method using NEUROSIM/L software version 4 (Fujitsu, Tokyo, Japan)¹⁴. Using the report by a
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36 90 Chiba University group, we worked out predictive RRV in the range of 0–1^{12 15}, with the nil as the
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39 91 lowest value as an index of the degree of tissue damage. Values > 0.5 were considered to indicate
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42 92 WMH risk.

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46 94 Standard enzymatic methods were used to measure the levels of serum total cholesterol,
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49 95 triglycerides, creatinine, and plasma glucose. Serum high-density lipoprotein (HDL) cholesterol level
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52 96 was measured with a direct method, and serum low-density lipoprotein (LDL) cholesterol level was
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55 97 calculated using Friedewald's formula in the 228 subjects with serum triglyceride levels < 400
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58 98 mg/dL¹⁶. Diabetes was defined as a fasting plasma glucose level ≥ 126 mg/dL or the use of

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3 99 glucose-lowering medications. Dyslipidemia was defined as total cholesterol level ≥ 220 mg/dL,
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6 100 HDL cholesterol level < 40 mg/dL and a triglyceride level ≥ 150 mg/dL, as well as the use of
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9 101 lipid-lowering medications. The estimated glomerular filtration rate (eGFR) was calculated for
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11 102 Japanese men as recommended by the Japanese Society of Nephrology¹⁷ and represented as: eGFR
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14 103 $(\text{mL}/\text{min}/1.73 \text{ m}^2) = 193 \times \text{serum creatinine}^{-1.094} \times \text{age}^{-0.287}$.

17 104 2.3. Imaging

20 105 All 228 subjects underwent T1- and T2-weighted MRI and fluid-attenuated inversion recovery
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23 106 (FLAIR) at the Nippon Medical School Hospital, Japan, within 1 month after blood sampling. MRI
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26 107 was performed as described previously [10]. PVH and DSWMH were defined as hyperintense areas
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29 108 on T2 and FLAIR images without any abnormality on T1¹⁸ in subjects without neurological signs
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32 109 and/or symptoms. The 228 subjects were classified into 103 PVH subjects (38 women and 65 men,
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35 110 aged 68.2 ± 6.0 years, RRV 0.75 ± 0.11) and 157 WMH subjects (61 women and 96 men, aged 66.7
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38 111 ± 5.8 years, RRV 0.71 ± 0.12). In more detail, 76 among all the subjects had both PVH and DSWMH,
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41 112 the other 23 having silent brain infarction. Also, 22 subjects had a complication of PVH and silent
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44 113 brain infarction, while in 20 subjects there was complication of DSWMH and silent brain infarction.

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49 115 2.4. Statistics

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53 116 All statistical tests were performed using the JMP9.02 software program (SAS Institute, Cary, NC,
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56 117 USA). Continuous variables except for triglyceride levels were expressed as means \pm standard

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4 118 deviation (SD). Triglyceride levels were transformed to the common logarithm for statistical analysis
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7 119 and are expressed as the geometric mean because of their skewed distribution. Categorical data are
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10 120 expressed as the number of subjects (percent of total). The clinical characteristics for each RRV
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12 121 tertile were compared by analysis of variance (ANOVA) for continuous variables and χ^2 test for
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15 122 categorical variables. The RRVs between the two groups were compared by Student's t-tests or by
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18 123 ANOVA followed by multiple comparisons with the Bonferroni correction between the two groups.
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21 124 Correlations between RRV and other variables were evaluated with the Pearson's moment correlation
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24 125 coefficient. Factors with a P value < 0.05 as determined by Pearson's correlation analysis were
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27 126 included in a multiple linear regression analysis to identify independent determinants of the RRV.
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30 127 Logistic regression analysis was performed to obtain the odds ratios (ORs) for PVH and DSWMH in
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33 128 the three tertiles. All statistical tests were two-sided, and a P value < 0.05 was considered as
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36 129 significant.

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41 131 3. Results

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44 132 The study subjects were divided into tertiles according to RRV (0.50–0.62, 0.63–0.79, and 0.80–0.90
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47 133 from the lowest to highest tertile, respectively). The subjects' clinical characteristics are summarized
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50 134 in Table 1. The mean RRV of the entire subject population was 0.71 ± 0.13 , and the mean age was 65
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53 135 ± 7 years. Age, body mass index (BMI), diastolic blood pressure (BP), HDL cholesterol level,
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56 136 triglyceride level, eGFR, and current smoking status were significantly different among the groups.
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4 137 RRVs were significantly higher in subjects with older age, lower eGFR, or PVH (Table 1). A simple
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7 138 correlation analysis showed that RRV was significantly correlated with age, systolic BP, eGFR, and
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10 139 PVH (Table 2). Multiple linear regression analysis indicated that BMI ($\beta = 0.0026$, $P = 0.044$) and
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13 140 PVH ($\beta = 0.0380$, $P < 0.0001$) were significant independent determinants of RRV.

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16 141 The results of logistic regression analysis of the association between PVH and RRV are shown in
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19 142 Table 3. Significant, unadjusted ORs for PVH were noted in the third RRV tertile (5.26 [95% CI,
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21 143 2.66–10.78], $P < 0.0001$), compared to the first tertile. After adjusting for model 1 (BMI, systolic BP,
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24 144 triglycerides, eGFR, and current smoking status) the ORs in the third RRV tertile remained
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27 145 significant (4.75 [95% CI, 2.33–10.05], $P < 0.0001$). After adjusting for model 2, we found that the
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30 146 OR in the third RRV tertile 3 was significant (5.26 [95% CI, 2.65–10.83], $P < 0.0001$). A significant
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33 147 relationship was observed between RRV and PVH ($P < 0.05$) but no such significance was found
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36 148 between RRV and DSWMH (figure 1).

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44 151 The present study demonstrated a significant, positive correlation between RRV and PVH in healthy
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47 152 Japanese volunteers. Notably, the highest RRV level tertile showed a significantly higher OR for a
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50 153 high predicted PVH risk in comparison to the lowest tertile, even after adjusting for multiple
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53 154 confounding factors. These results suggest that RRV is associated with the estimated risk of PVH in
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56 155 healthy Japanese volunteers. A number of clinical and epidemiological studies have examined the
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4 156 predictive value of RRV for the presence of WMH^{12 15}. However, those studies assessed WMH
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7 157 prevalence; no studies have shown any significant association of RRV with PVH and DSWMH
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10 158 separately. In this regard, our results raise the possibility that RRV predicts the risk of PVH in the
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13 159 healthy Japanese population. With respect to age, these biochemical markers provide a good index of
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16 160 the presence of tissue damage related to PVH.

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19 161 More recent studies focused on WMH location have reported that functional impairment within 1–3
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22 162 months after stroke correlated with PVH but not with DSWMH^{10 19}. PVH WMH, especially PVH,
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25 163 has impacts on early functional recovery after ischemic stroke regardless of the initial stroke severity
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28 164 and other cardiovascular risk factors¹¹. Other groups found a significant association between PVH
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31 165 and decreases in processing speed and executive function, but there was no such relationship with
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34 166 DSWMH^{8 9}. Why PVH and DSWMH have different relationships with stroke outcome remains
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37 167 unclear, but several theories have been put forward. DSWMH predominantly disrupt short
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40 168 association fibers that link adjacent gyri, while PVH affects long association fibers that connect the
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43 169 more distant cortical areas²⁰. Thus, lesions in various white matter locations may disconnect from
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46 170 different neural networks that affect neural repair processes after stroke²¹. In addition, PVHs are
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49 171 related to diminished cerebral vasomotor reactivity and subsequent occurrence of cerebral
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52 172 hypoperfusion²², while DSWMHs are generally associated with microangiopathy²³. It is clear that
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55 173 regional hypoperfusion is a good predictor of functional outcome²⁴. These findings shed light on
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58 174 why PVH can predict functional stroke outcome and specific cognitive functions¹¹.

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4 175 Acrolein induces IL-6 production in astrocytes, macrophages, and endothelial cells, while IL-6
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7 176 induces CRP production in hepatocytes. Then, CRP stimulates IL-6 production, and IL-6 decreases
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10 177 acrolein toxicity ²⁵. Acrolein was thought to be one of the toxic compounds produced from
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13 178 unsaturated fatty acids by active oxygen species such as superoxide anion radical, hydrogen peroxide,
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16 179 and hydroxyl radical¹³. These findings may partially explain the pathophysiological mechanisms
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19 180 underlying the association between PVH and the three biomarkers assessed in the present study.
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21 181 Further investigation will be needed for a better understanding of their interrelationship.
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27 183 Our multiple linear regression analysis showed that RRV was independently associated with BMI
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30 184 and PVH. Although obesity is believed to be an independent cardiovascular risk factor ²⁶, it is still
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33 185 controversial whether BMI is a significant risk factor for stroke ^{27 28}. BMI was previously reported to
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36 186 be correlated with high RRV ²⁹ which may be caused by vascular degeneration and endothelial
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39 187 dysfunction associated with hypertension and metabolic disorders. Subjects with metabolic
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42 188 syndrome are generally defined as those who have abdominal obesity and two additional metabolic
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45 189 disorders including hypertension, dyslipidemia, and hyperglycemia ^{30 31}.

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50 191 Our study has some potential limitations. Because it was a cross-sectional investigation, we could not
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53 192 determine a causal relationship between increased RRV and PVH risk. In addition, the population
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56 193 included healthy Japanese volunteers only. Therefore, it is unclear whether the results can be
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4 194 extrapolated to other populations of poor health, patients with cardiovascular diseases, or other
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7 195 ethnic groups. Despite these potential limitations, our findings support the conclusion that elevated
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10 196 RRV is significantly associated with PVH in healthy Japanese volunteers. These results suggest that
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13 197 RRV measurement may be useful for identifying PVH in the general population. This would allow
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16 198 clinicians to follow patients who may be at risk for stroke and cognitive dysfunction.

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21 200 Acknowledgments

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24 201 We thank Drs. Mari Adachi and Sadaji Kura at Katsushika Health Center for assisting with the data
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27 202 analyses.
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Table 1. Characteristics of the study subjects due to their stroke risk marker level tertile

Item	All	Silent brain infarction RRV tertile			P value*
		Tertile 1	Tertile 2	Tertile 3	
Subjects (n)	228	76	73	79	-
Silent brain infarction relative risk value	0.71 ± 0.13	0.51 ± 0.07**	0.69 ± 0.05**	0.86 ± 0.03**	-
Age (years)	65 ± 7	59 ± 7	66 ± 3	70 ± 6	<0.0001
Male Sex	150 (65.7)	51 (67.1)	52 (71.2)	47 (59.4)	0.299
Body mass index, kg/m ²	24.2 ± 6.4	23.2 ± 2.9	24.6 ± 6.3	24.8 ± 8.6	0.133
Systolic BP, mmHg	123 ± 15	121 ± 14	122 ± 14	126 ± 17	0.275
Diastolic BP, mmHg	75 ± 11	75 ± 9	75 ± 10	75 ± 13	0.947
Hypertension, n (%)	81 (35.6)	23 (30.6)	20 (27.4)	38 (48.1)	0.015
Total cholesterol, mg/dL	208 ± 32	208 ± 30	208 ± 32	207 ± 34	0.903
LDL cholesterol [†] , mg/dL	123 ± 29	123 ± 25	123 ± 29	122 ± 33	0.848
HDL cholesterol, mg/dL	58 ± 15	59 ± 17	57 ± 13	60 ± 16	0.385
Triglycerides ^{††} , mg/dL	125 (113, 138)	141 (111, 172)	123 (105, 142)	112 (99, 124)	0.425

Dyslipidemia, n (%)	55 (24.1)	19 (25.0)	13 (17.8)	23 (29.1)	0.259
Fasting plasma glucose, mg/dL	100 ± 16	100 ± 14	99 ± 18	100 ± 15	0.754
Diabetes, n (%)	18 (7.8)	3 (3.9)	6 (8.2)	9 (11.3)	0.226
eGFR (mL/min/1.73 m ²)	67.6 ± 12.5	70.2 ± 15.3	67.3 ± 10.6	65.4 ± 10.6	0.027
Current smoking status, n (%)	40 (17.5)	16 (21.0)	12 (16.4)	12 (15.1)	0.603
PVH, n (%)	103 (45.1)	18 (23.6)	36 (49.3)	49 (62.0)	<0.0001
DSWMH, n (%)	157 (68.8)	52 (68.4)	50 (68.4)	55 (69.6)	0.983

*Analysis of variance or chi-square test among the ADMA tertile. **Range of a minimum-to-maximum stroke risk marker in each tertile. †n = 228. ††Geometric mean (95% confidence intervals). ‡Statistical analysis was not conducted because of the extremely small number of subjects in each category. BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PVH, periventricular hyperintensity; RRV, relative risk value

Table 2. Correlation coefficients and multiple linear regression analysis of relative risk value with the clinical parameters

Item	Simple correlation analysis		Multiple linear regression analysis	
	Correlation coefficient (r)	P-value	Standardized regression coefficient (β)	P value
Age	0.60	<0.0001	†	
Male Sex	0.05	0.347	††	
BMI	0.11	0.075	††	
Systolic BP	0.14	0.026	0.0009	0.078
Diastolic BP	0.05	0.382	††	
Total cholesterol	-0.03	0.618	††	
LDL cholesterol*	-0.02	0.669	††	
HDL cholesterol	0.02	0.731	††	
Triglycerides**	-0.10	0.130	††	
Fasting plasma glucose	0.01	0.841	††	
eGFR	-0.13	0.041	-0.0009	0.173
Current smoking status (Yes = 1)	-0.10	0.115	††	
PVH (Yes = 1)	0.26	<0.0001	0.0384	<0.0001
DSWMH (Yes = 1)	0.02	0.689	††	

* n = 228. ** Log-transformed value. † Not included in the multiple linear regression analysis to avoid multicollinearity with PVH relative risk value.

†† Not included in the multiple linear regression analysis because their P values were ≥ 0.05 in the simple correlation analysis. BMI, body mass index; BP, blood pressure; DSWMH, deep-and-subcortical white matter hyperintensity; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3. Unadjusted and adjusted PVH ORs in each silent brain infarction relative risk value tertile

Item	Unadjusted			Adjusted*		
	OR	95% CI	P-value	OR	95% CI	P value
Tertile 1	1.00	reference	-	1.00	reference	-
Tertile 2	3.13	1.57–6.41	0.0014	3.01	1.50–6.20	0.0018
Tertile 3	5.26	2.66–10.78	<0.0001	4.87	2.43–10.08	<0.0001

* Adjusted for systolic BP and eGFR

BP, blood pressure; eGFR, estimated glomerular filtration rate; OR, odds ratio; PVH, periventricular hyperintensity

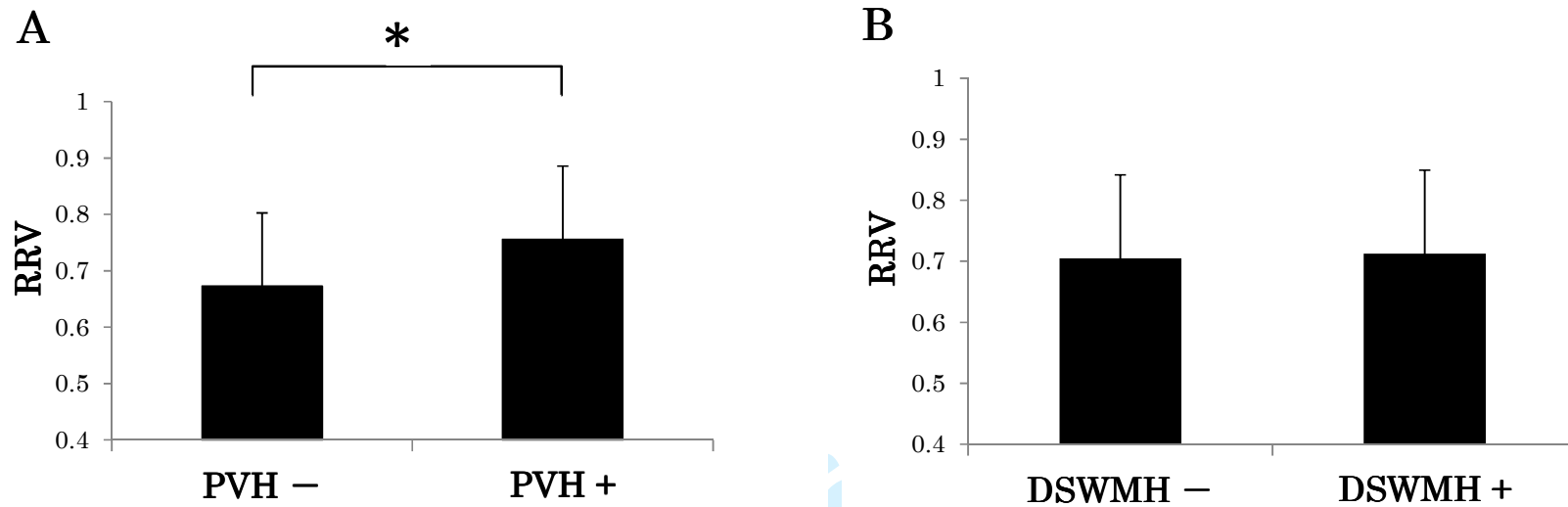


Figure 1. Correlation between the RRV and the PVH (A). Correlation between the RRV and DSWMH (B)

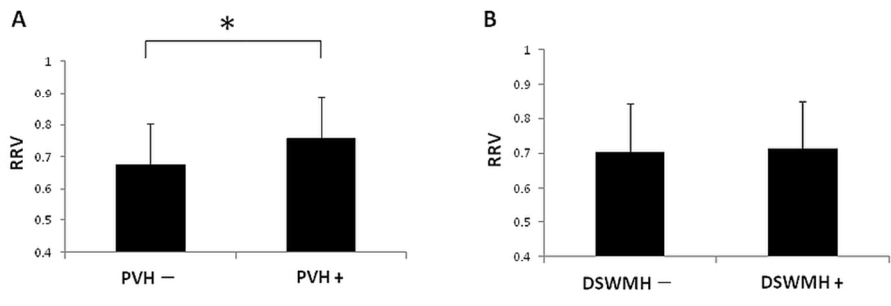
*Significant at $P < 0.05$

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of *cohort studies*

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	Page3
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	Page3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Page4
Objectives	3	State specific objectives, including any prespecified hypotheses	Page4
Methods			
Study design	4	Present key elements of study design early in the paper	Page5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Page5
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	Page5
		(b) For matched studies, give matching criteria and number of exposed and unexposed	Page5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Page5-7
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Page6
Bias	9	Describe any efforts to address potential sources of bias	NA
Study size	10	Explain how the study size was arrived at	NA
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Page7-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	Page7-8
		(b) Describe any methods used to examine subgroups and interactions	NA
		(c) Explain how missing data were addressed	NA
		(d) If applicable, explain how loss to follow-up was addressed	NA
		(e) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	NA
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Page4
		(b) Indicate number of participants with missing data for each variable of interest	NA
		(c) Summarise follow-up time (eg, average and total amount)	NA
Outcome data	15*	Report numbers of outcome events or summary measures over time	NA
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Page8-9
		(b) Report category boundaries when continuous variables were categorized	Page8-9
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	Page8-9
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Page8-9
Discussion			
Key results	18	Summarise key results with reference to study objectives	Page8-9
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Page11
Generalisability	21	Discuss the generalisability (external validity) of the study results	Page11
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	NA

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.