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# Correlation between images of silent brain infarction, carotid atherosclerosis and white matter hyperintensity, and plasma levels of acrolein, IL-6 and CRP

Madoka Yoshida[a,b], Kyohei Higashi[a,b], Eiichi Kobayashi[c], Naokatsu Saeki[c], Kenji Wakui[d], Tadafumi Kusaka[e], Hirotaka Takizawa[f], Koichi Kashiwado[f], Norikazu Suzuki[g], Kazumasa Fukuda[h], Takao Nakamura[h], Sakae Watanabe[i], Kei Tada[i], Yoshiki Machi[a], Mutsumi Mizoi[b], Toshihiko Toida[a], Tetsuto Kanzaki[j], Hideyuki Tomitori[j], Keiko Kashiwagi[j], Kazuei Igarashi[a,b,*]

[a] *Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana Chuo-ku, Chiba 260-8675, Japan*
[b] *Amine Pharma Research Institute, Innovation Plaza at Chiba University, Chiba, Japan*
[c] *Graduate School of Medicine, Chiba University, Chiba, Japan*
[d] *Chiba Neurosurgical Clinic, Chiba, Japan*
[e] *Kusaka Hospital, Chiba, Japan*
[f] *Portsquare Kashiwado Clinic, Chiba, Japan*
[g] *Suzuki Neurosurgical Clinic, Chiba, Japan*
[h] *Chiba Central Medical Center, Chiba, Japan*
[i] *Saisei Hospital, Chiba, Japan*
[j] *Faculty of Pharmacy, Chiba Institute of Science, Japan*

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ABSTRACT

*Objective:* We found previously that the measurement of plasma levels of protein-conjugated acrolein (PC-Acro) together with IL-6 and CRP can be used to identify silent brain infarction (SBI) with high sensitivity and specificity. The aim of this study was to clarify how three biochemical markers are correlated to SBI, carotid atherosclerosis (CA) and white matter hyperintensity (WMH).
*Methods:* The levels of PC-Acro, IL-6 and CRP in plasma were measured by ELISA. SBI and WMH were evaluated by MRI, and CA was evaluated by duplex carotid ultrasonography.
*Results:* A total of 790 apparently healthy volunteers were classified into 260 control, 214 SBI, 263 CA and 245 WMH subjects, which included 187 subjects with two or three pathologies. When the combined measurements of PC-Acro, IL-6 and CRP were evaluated together with age, using a receiver operating characteristic curve and artificial neural networks, the relative risk value (RRV), an indicator of tissue damage, was in the order SBI with CA (0.90) > SBI (0.80) > CA (0.76) > WMH with CA (0.65) > WMH (0.46) > control (0.14). RRV was also correlated with severity in each group of SBI, CA and WMH.
*Conclusion:* The RRV supports the idea that the degree of risk to develop a stroke is in the order SBI > CA > WMH.



## 1. Introduction

Polyamines (putrescine, spermidine and spermine) are essential for normal cell growth and are present in cells at millimolar concentrations [1]. However, when cells are damaged, the toxic compounds acrolein ($CH_2$=CHCHO) and $H_2O_2$ are produced from polyamines, in particular from spermine, by polyamine oxidases (PAO, spermine oxidase and acetylpolyamine oxidase) [2]. When the toxicities of acrolein and $H_2O_2$ were compared in a cell-culture system, the major toxic factor produced from polyamines was acrolein [3]. We examined whether the levels of PAO and protein-conjugated acrolein (PC-Acro) in plasma are correlated with pathologies that involve tissue damage, and found that levels of PAO and PC-Acro in plasma are well correlated with the severity of chronic renal failure [4] and stroke [2]. The size of stroke was

*Abbreviations:* PC-Acro, protein-conjugated acrolein; SBI, silent brain infraction; CA, carotid atherosclerosis; IMT, intima-medial thickness; WMH, white matter hyperintensity; DSWMH, deep and subcortical white matter hyperintensity; PVH, periventicular hyperintensity; RRV, relative risk value; IL-6, interleukin-6; CRP, C-reactive protein; MRI, magnetic resonance imaging; FLAIR, fluid-attenuated inversion recovery; ELISA, enzyme-linked immunosorbent assay; ROC, receiver operating characteristic; PPV and NPV, positive- and negative-predictive values; LR+ and LR−, positive- and negative-likelihood.

* Corresponding author at: Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan.
Tel.: +81 43 224 7500; fax: +81 43 224 7500.
*E-mail address:* iga16077@p.chiba-u.ac.jp (K. Igarashi).

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nearly paralleled with the multiplied value of PC-Acro by PAO [2]. It was also shown that the induction of brain infarction in model mice was well correlated with the increase in PC-Acro at the locus of infarction and in plasma [5].

There are reports that silent brain infarction (SBI) increases the risk of subsequent stroke [6–8], dementia [8] and mild cognitive impairment [9]. It is, therefore, valuable to estimate SBI at an early period by biochemical markers, because measurement of biochemical markers in blood is common and economical compared to diagnostic imaging such as magnetic resonance imaging (MRI) and computed tomography. We have reported that measurement of PC-Acro together with interleukin-6 (IL-6) and C-reactive protein (CRP) makes it possible to identify SBI with high sensitivity and specificity [10]. It has been also reported that carotid atherosclerosis (CA) is a risk factor for stroke and SBI [11,12], and that SBI and marked white matter hyperintensity (WMH) are risk factors of stroke [13]. Thus, we tested how these biochemical markers are correlated with SBI, WMH and CA as determined by imaging, and whether these markers can evaluate the severity of CA and WMH in addition to SBI.

## 2. Materials and methods

### 2.1. Subjects and collection of blood

We examined 790 elderly volunteers (330 women and 460 men, age 61.0 ± 7.0 years, range 40–88 years). All participants were healthy volunteers, living independently at home without apparent history of stroke or dementia. Informed consent was given by each participant, and our study protocol was approved by the Ethics Committees of Chiba University Hospital. Experiments were conducted in accordance with the Declaration of Helsinki principles. Blood containing 3 U/mL heparin was centrifuged at 1500 × *g* for 10 min at 4 °C.

### 2.2. Measurement of PC-Acro, IL-6 and CRP in plasma

PC-Acro [$N^{\varepsilon}$-(3-formyl-3,4-dehydropiperidino)-lysine (FDP-lysine) in protein] was determined by the method of Uchida et al. [14] using ACR-LYSINE ADDUCT ELISA SYSTEM (NOF Corporation) and 0.01 mL plasma. IL-6 and CRP were measured using Endogen Human IL-6 ELISA kit (Pierce Biotechnology, Inc.) and human CRP ELISA kit (Alpha Diagnostic), respectively, according to the manufacturer's protocol. After the reaction was terminated, absorbance at 450 nm was measured by a microplate reader Hitachi MTP-800APC. All biochemical markers of each subject were measured by an investigator blind to the results of the MRI.

### 2.3. Imaging

All 790 subjects underwent T1- and T2-weighted MRI and FLAIR in parallel with the collection of blood samples. MRI was performed as described previously [10]. The diagnosis of SBI was made as follows: (1) spotty areas ≥3 mm in diameter showing in high intensity in the T2 and FLAIR images and low intensity in the T1 image, (2) lack of neurological signs and/or symptoms that can be explained by the MRI lesions, and (3) no medical history of clinical stroke [6,15]. The subjects with WMH [deep and subcortical white matter hyperintensity (DSWMH) and periventricular hyperintensity (PVH)] were defined as subjects with spots showing in high intensity in the T2 and FLAIR images without any consistent finding in the T1 image [16], and subjects who lack neurological signs and/or symptoms. Carotid ultrasound examination was performed using a LOGIQ S6 (GE Healthcare) or a LOGIQ 500 MD (GE Yokogawa Medical Systems) scanner equipped with a 7.5-MHz linear array imaging probe [17,18]. Intima-medial thickness (IMT) was measured as the distance between the luminal–intimal interface and the medial–adventitial interface. The IMT value was measured in three points for each right and left side (observation-possible areas of common carotid artery, carotid bifurcation, and internal carotid artery), and the largest value (max-IMT) in each side was used for analysis. Control subjects were defined as subjects with no spotty areas and CA and no neurological signs and/or symptoms. Through this diagnosis, 790 subjects were classified into 260 control subjects (108 women and 152 men, aged 54.0 ± 6.0 years), 214 SBI subjects (88 women and 126 men, aged 68.0 ± 7.0 years), 263 CA subjects (105 women and 158 men, aged 66.0 ± 6.0 years) and 245 WMH subjects (105 women and 140 men, aged 61.0 ± 5.8 years). There were 187 subjects with two or three pathologies, and of those, 88 subjects had SBI and CA, 9 subjects SBI and WMH, 85 subjects CA and WMH, and 5 subjects SBI, CA and WMH. Accordingly, subjects with SBI and CA, or with WMH and CA consisted of 93 and 90 subjects, respectively.

**Table 1**
Distribution of subjects as control, SBI, CA and WMH as a function of age.

| Age (years) | Number of subjects | Control (%) | SBI (%) | CA (%) | WMH (%) |
|---|---|---|---|---|---|
| 40–49 | 105 | 69.5 | 7.6 | 6.7 | 16.2 |
| 50–59 | 250 | 47.6 | 11.6 | 22.4 | 31.2 |
| 60–69 | 256 | 23.4 | 31.3 | 37.5 | 36.3 |
| ≥70 | 179 | 4.5 | 54.2 | 58.1 | 31.8 |

Since the association of SBI with CA, SBI with WMH and WMH with CA increased with age, total percentage at age 40–49, 50–59, 60–69 and ≥70 became 100%, 112.8%, 128.5% and 148.6%, respectively.

### 2.4. Statistics

Statistical calculations were performed with GraphPad Prism® Software (GraphPad Software). Values are indicated as median ± interquartile deviation. Groups were compared using Wilcoxon rank sum test or Kruskal–Wallis test. Relative risk value (RRV) was calculated with artificial neural networks by back propagation method using NEUROSIM/L software version 4 (Fujitsu) [19]. Age and three biochemical markers of 260 control and 214 SBI subjects were used as prediction output values 0 and 1, respectively, and the rules to build RRV were obtained. Then, RRV (0–1) for control, SBI with CA, SBI, CA, WMH with CA and WMH subjects was calculated according to the rules. Sensitivity and specificity for SBI subjects vs. control subjects were evaluated using a receiver operating characteristic (ROC) curve [20]. Sensitivity, specificity, positive- and negative-predictive values (PPV and NPV), and positive- and negative-likelihood (LR+ and LR−) were calculated with the standard method [21]. Cutoff value was set up as the closest point on ROC curve from the P point, that is sensitivity = 1 and 1 − specificity = 0.

## 3. Results

### 3.1. Relationship between biochemical markers (PC-Acro, IL-6 and CRP) and SBI, CA and WMH determined by imaging

The distribution of subjects as control (260), SBI (214), CA (263) and WMH (245) was classified as a function of age. As shown in Table 1, the incidence of SBI and CA increased with age, but the incidence of WMH was observed at younger ages (40th) and was similar at ages above 50 years. The association of SBI with CA, SBI with WMH and WMH with CA was approximately 40%, 6% and 37%, respectively (Fig. 1S). The results indicate that the association of SBI with WMH is small. The incidence of SBI, CA and WMH was age-

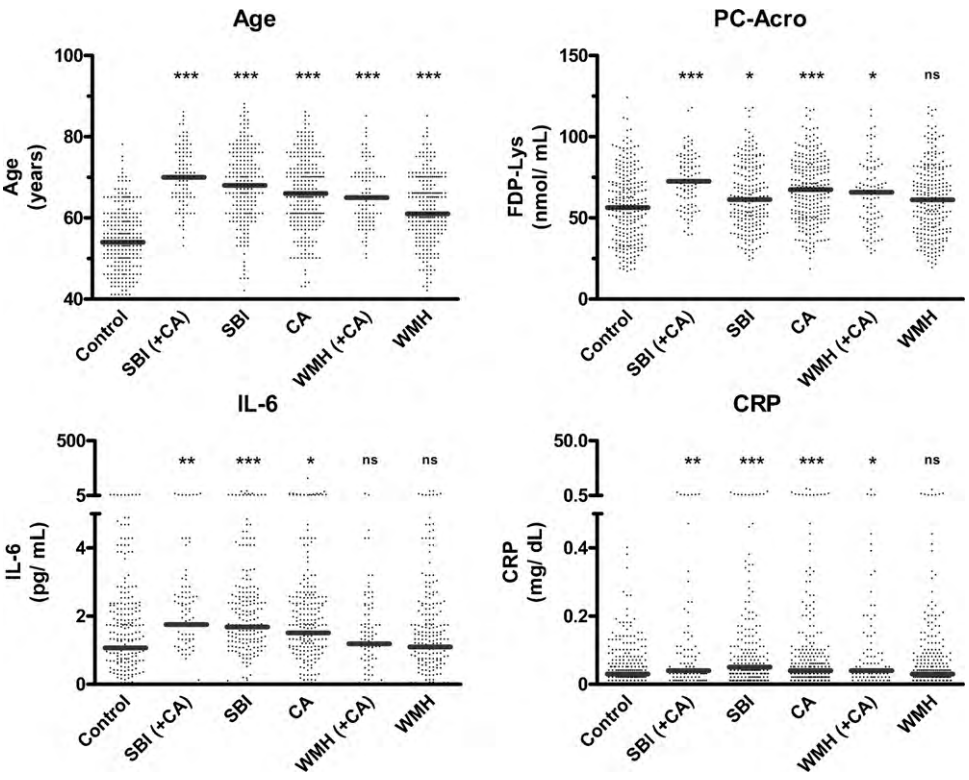


**Fig. 1.** Comparison of age and level of PC-Acro, IL-6 and CRP in the plasma of control, SBI, CA and WMH subjects. Age, PC-Acro, IL-6 and CRP were shown with median (horizontal line). Experiments were performed twice and results were reproducible. The $p$ value was calculated using Wilcoxon rank sum test. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; ns, $p \geq 0.05$ compared with control subjects.

related, in the order SBI > CA > WMH > control (Fig. 1). PC-Acro, IL-6 and CRP were significantly higher in SBI and CA compared to control (Fig. 1). In WMH, levels of PC-Acro, IL-6 and CRP were slightly higher than control (Fig. 1). PC-Acro was most strongly associated with CA, and IL-6 and CRP were with SBI. Thus, it is thought that CA is the first tissue damage related to stroke, in which acrolein is produced. SBI, CA and WMH were often associated with hypertension and hyperlipidemia, but not with hyperuricemia and hyperglycemia (Table 1S).

The ROC curve for the detection of SBI by age, with measurement of PC-Acro plus IL-6 and CRP is shown in Fig. 2A, and these data are consistent with previously published results [10]. Sensitivity and specificity were 84.1% and 83.5%, respectively, which were slightly lower than the previous values [10]. This is probably due to the lowering in the average age of control subjects, because age is a factor for the calculation of ROC curve with artificial neural networks. Relative risk value (RRV) was then calculated for SBI, CA and WMH. A value of 1 is the highest value, and 0 is the lowest value as an index of the degree of tissue damage. The median RRV for SBI with CA (93 subjects), SBI (214 subjects), CA (263 subjects), WMH with CA (90 subjects), WMH (245 subjects) and control (260 subjects) was 0.90, 0.80, 0.76, 0.65, 0.46 and 0.14, respectively (Fig. 2B). In this case, SBI (214 subjects), CA (263 subjects) and WMH (245 subjects) include subjects with single, double and triple pathologies.

In a previous report [10], we have shown that the level of LDH was high, and that of platelets was low in SBI. Various biochemical markers in blood of control, SBI, CA and WMH subjects were also measured (Table 2S). A decrease in platelets was observed in the order SBI ≈ CA > WMH, and significant increase of LDH in SBI. Furthermore, eGFR (estimated glomerular filtration rate) [22] was decreased in the order SBI ≈ CA > WMH, and urea nitrogen in serum was increased in the order SBI > CA ≈ WMH.

### 3.2. Correlation between RRV and severity of CA and WMH

We next studied whether the median RRV is correlated with the severity of CA. The severity of CA was evaluated by the additive value of max-IMT in both right and left carotid arteries. Definitions of "mild" and "severe" are max-IMT values of 1.1–2.9 mm and ≥3 mm, respectively. Age and PC-Acro were significantly increased, and IL-6 and CRP were slightly higher in subjects with severe CA than those in mild CA (Fig. 3A). Accordingly, the median RRV of 175 mild subjects and 88 severe subjects was 0.67 and 0.90, respectively (Fig. 3A). With an increase in severity, from mild to severe, the association with SBI increased from 30% to 45% (data not shown).

The correlation between the severity of WMH and the median RRV was also studied. WMH was sub-classified into 166 subjects with a single PVH or DSWMH and 79 subjects with both PVH and DSWMH. Age and PC-Acro were significantly higher in subjects with both PVH and DSWMH than in subjects with a single PVH or DSWMH (Fig. 3B). Difference of IL-6 and CRP was not significant between two groups. Thus, RRV for the subjects with PVH or DSWMH and with both PVH and DSWMH was 0.34 and 0.55, respectively (Fig. 3B). With an increased severity of WMH, the association with SBI increased from 3.6% to 10% (data not shown).

## 4. Discussion

It has been reported that SBI increases the risk of subsequent stroke, dementia and mild cognitive impairment [6–9]. There are also reports that CA is a risk factor for stroke and SBI [11,12], and that SBI and marked WMH for stroke [13]. In addition, it has been reported that WMH is a modest risk factor of SBI [23]. Thus, we compared biochemical markers (PC-Acro, IL-6 and CRP) among SBI, CA and WMH to determine whether these markers are useful to gauge the severity of SBI, CA and WMH. RRV obtained from age, PC-

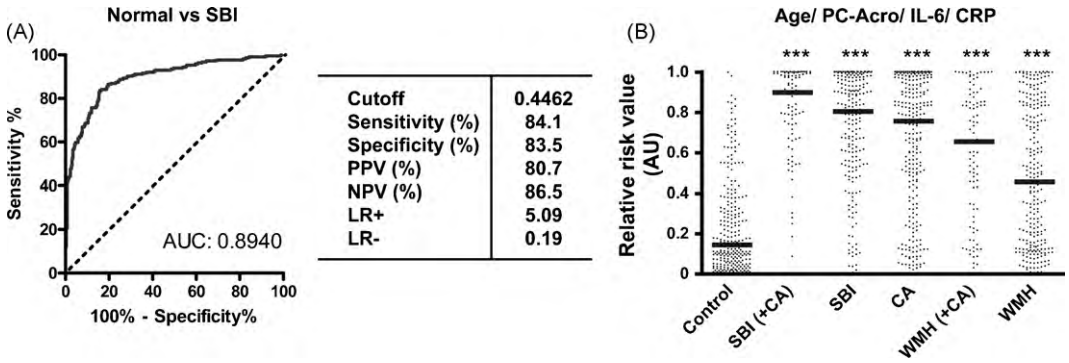


**Fig. 2.** ROC curve of age/PC-Acro/IL-6/CRP for SBI vs. control subjects (A) and relationship between RRV and SBI, CA and WMH (B). (A) ROC curve analysis was performed as described in Section 2. AUC, area under curve. (B) RRV of control, SBI with CA, SBI, CA, CA with WMH and WMH calculated from ROC curve was shown with median (horizontal line). $^{***}p < 0.001$ compared with control subjects.

Acro, IL-6 and CRP was used for this assessment. The RRV was in the order SBI with CA > SBI > CA > WMH with CA > WMH. Furthermore, the severity of CA and WMH was parallel with RRV. The size of brain infarction was also correlated with RRV [2,10]. Thus, these biochemical markers together with age provide a good index of the severity of tissue damage related to stroke.

It has been reported that increased levels of PC-Acro [10], IL-6 [10,15] and CRP [10,15,24] were observed in SBI, and an increase in CRP was observed in CA [17]. The results obtained in this study provide additional data showing increased levels of PC-Acro and IL-6 in CA, and a slight increase of PC-Acro, IL-6 and CRP in WMH. Thus, the combined measurement of PC-Acro, IL-6 and CRP makes it possible to detect SBI and CA with high sensitivity and specificity. Since RRV was high with severe WMH (see Fig. 3B), severe WMH can be also detected by measuring these biochemical markers. RRV is the first reliable marker to evaluate the severity of SBI, CA and WMH. SBI was not well associated with WMH (see Fig. 1B). Some subjects with severe WMH may directly progress to a stroke without an intermediate SBI [6].

Although PC-Acro, IL-6 and CRP are not, by themselves, specific for brain infarction, the early stages of brain infarction could be detected with high specificity by measuring these three biochemical markers. This may reflect, in part, the effective production of IL-6 by astrocytes [25]. Biomarkers specific for brain infarction did not change significantly at the early stage of brain infarction [10,26]. PC-Acro was most strongly associated with CA. This may be explained by the finding that foam cells included at the locus of atherosclerosis contained high levels of PC-Acro and a portion of the PC-Acro releases from them (unpublished results). Data were also analyzed by a multivariable regression analysis. Results were essentially the same as those obtained with artificial neural networks.

Although PC-Acro was shown as the level of FDP-lysine in Figs. 1 and 3, it has been recently found that MP-lysine [$N^{\varepsilon}$-(3-methylpyridinium)lysine] rather than FDP-lysine is a major product in plasma of SBI and control subjects [27]. Since the antibody used to measure PC-Acro recognizes both MP-lysine and FDP-lysine [28], the level of PC-Acro is correctly presented although the unit is presented as FDP-lysine.

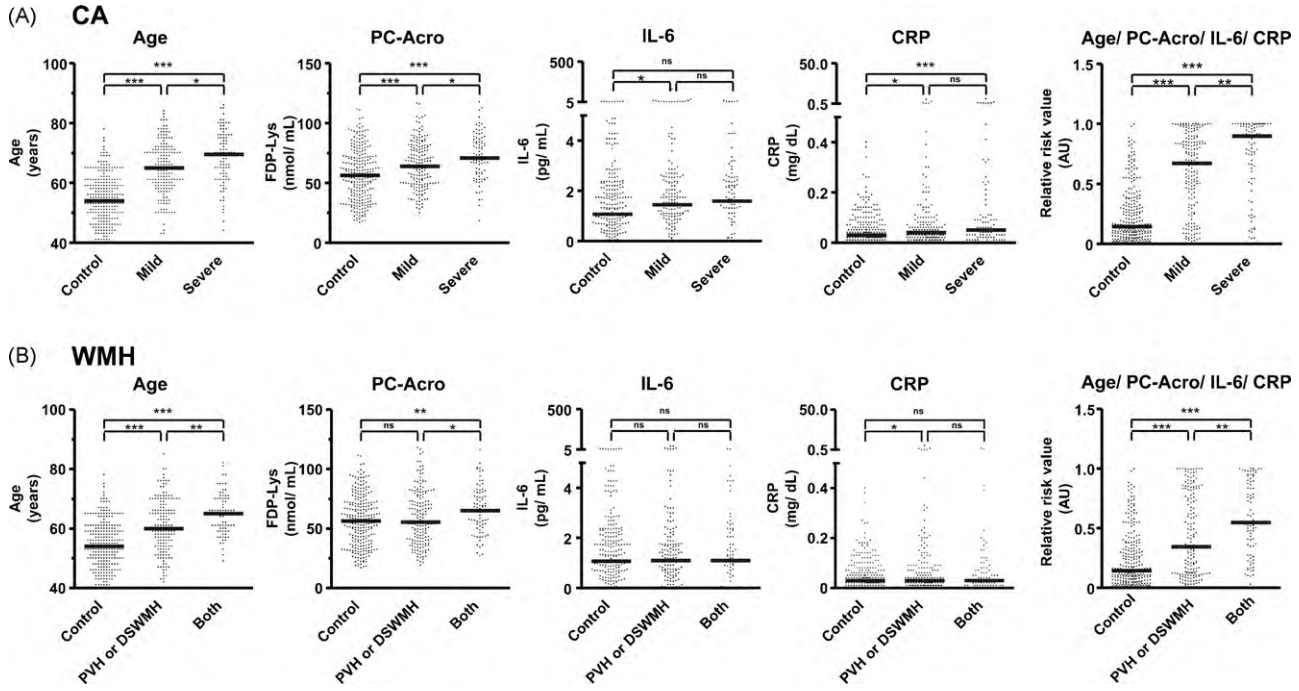


**Fig. 3.** Relationship between severity of CA (A) and WMH (B), and various markers. (A and B) Each value was shown with median (horizontal line). The $p$ value was calculated using Kruskal–Wallis test. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; ns, $p \geq 0.05$.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2010.03.031.

## References

[1] Igarashi K, Kashiwagi K. Modulation of cellular function by polyamines. Int J Biochem Cell Biol 2010;42:39–51.
[2] Tomitori H, Usui T, Saeki N, et al. Polyamine oxidase and acrolein as novel biochemical markers for diagnosis of cerebral stroke. Stroke 2005;36:2609–13.
[3] Sharmin S, Sakata K, Kashiwagi K, et al. Polyamine cytotoxicity in the presence of bovine serum amine oxidase. Biochem Biophys Res Commun 2001;282:228–35.
[4] Sakata K, Kashiwagi K, Sharmin S, et al. Increase in putrescine, amine oxidase, and acrolein in plasma of renal failure patients. Biochem Biophys Res Commun 2003;305:143–9.
[5] Saiki R, Nishimura K, Ishii I, et al. Intense correlation between brain infarction and protein-conjugated acrolein. Stroke 2009;40:3356–61.
[6] Kobayashi S, Okada K, Koide H, Bokura H, Yamaguchi S. Subcortical silent brain infarction as a risk factor for clinical stroke. Stroke 1997;28:1932–9.
[7] Vermeer SE, Hollander M, van Dijk EJ, et al. Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study. Stroke 2003;34:1126–9.
[8] Vermeer SE, Longstreth Jr WT, Koudstaal PJ. Silent brain infarcts: a systematic review. Lancet Neurol 2007;6:611–9.
[9] Lopez OL, Jagust WJ, Dulberg C, et al. Risk factors for mild cognitive impairment in the Cardiovascular Health Study Cognition Study: part 2. Arch Neurol 2003;60:1394–9.
[10] Yoshida M, Tomitori H, Machi Y, et al. Acrolein, IL-6 and CRP as markers of silent brain infarction. Atherosclerosis 2009;203:557–62.
[11] O'Leary DH, Polak JF, Kronmal RA, et al. Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults. New Engl J Med 1999;340:14–22.
[12] Inoue K, Matsumoto M, Shono T, Toyokawa S, Moriki A. Increased intima media thickness and atherosclerotic plaques in the carotid artery as risk factors for silent brain infarcts. J Stroke Cerebrovasc Dis 2007;16:14–20.
[13] Bokura H, Kobayashi S, Yamaguchi S, et al. Silent brain infarction and subcortical white matter lesions increase the risk of stroke and mortality: a prospective cohort study. J Stroke Cerebrovasc Dis 2006;15:57–63.
[14] Uchida K, Kanematsu M, Morimitsu Y, et al. Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. J Biol Chem 1998;273:16058–66.
[15] Hoshi T, Kitagawa K, Yamagami H, et al. Relations of serum high-sensitivity C-reactive protein and interleukin-6 levels with silent brain infarction. Stroke 2005;36:768–72.
[16] Shinohara Y, Tohgi H, Hirai S, et al. Effect of the Ca antagonist nilvadipine on stroke occurrence or recurrence and extension of asymptomatic cerebral infarction in hypertensive patients with or without history of stroke (PICA Study). 1. Design and results at enrollment. Cerebrovasc Dis 2007;24:202–9.
[17] Makita S, Nakamura M, Hiramori K. The association of C-reactive protein levels with carotid intima–media complex thickness and plaque formation in the general population. Stroke 2005;36:2138–42.
[18] Yamagami H, Kitagawa K, Hoshi T, et al. Associations of serum IL-18 levels with carotid intima–media thickness. Arterioscler Thromb Vasc Biol 2005;25:1458–62.
[19] Ellenius J, Groth T, Lindahl B, Wallentin L. Early assessment of patients with suspected acute myocardial infarction by biochemical monitoring and neural network analysis. Clin Chem 1997;43:1919–25.
[20] Hanley JA, McNeil BJ. The meaning and use of the area under a receiver operating characteristic (ROC) curve. Radiology 1982;143:29–36.
[21] Brenner H, Gefeller O. Variation of sensitivity, specificity, likelihood ratios and predictive values with disease prevalence. Stat Med 1997;16:981–91.
[22] Kobayashi M, Hirawa N, Yatsu K, et al. Relationship between silent brain infarction and chronic kidney disease. Nephrol Dial Transplant 2009;24:201–7.
[23] Gouw AA, van der Flier WM, Fazekas F, et al. Progression of white matter hyperintensities and incidence of new lacunes over a 3-year period: the Leukoaraiosis and Disability study. Stroke 2008;39:1414–20.
[24] Kawamura T, Umemura T, Kanai A, et al. Soluble adhesion molecules and C-reactive protein in the progression of silent cerebral infarction in patients with type 2 diabetes mellitus. Metabolism 2006;55:461–6.
[25] Fujita T, Tozaki-Saitoh H, Inoue K. P2Y1 receptor signaling enhances neuroprotection by astrocytes against oxidative stress via IL-6 release in hippocampal cultures. Glia 2009;57:244–57.
[26] Whiteley W, Tseng MC, Sandercock P. Blood biomarkers in the diagnosis of ischemic stroke: a systematic review. Stroke 2008;39:2902–9.
[27] Yoshida M, Higashi K, Jin L, et al. Identification of acrolein-conjugated protein in plasma of patients with brain infarction. Biochem Biophys Res Commun 2010;391:1234–9.
[28] Furuhata A, Ishii T, Kumazawa S, et al. $N^{\varepsilon}$-(3-Methylpyridinium)lysine, a major antigenic adduct generated in acrolein-modified protein. J Biol Chem 2003;278:48658–65.