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厚生労働科学研究委託費

認知症研究開発事業

アルブミンの劣化に主眼をおいたアルツハイマー病発症前診断及びその治療応用に関する研究

平成２６年度　委託業務成果報告書

業務主任者　山本　圭一

平成２７（２０１５）年　３月

本報告書は、厚生労働省の認知症研究開発事業による委託業務として、山本圭一が実施した平成２６年度「アルブミンの劣化に主眼をおいたアルツハイマー病発症前診断及びその治療応用に関する研究」の成果を取りまとめたものです。

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厚生労働科学研究委託費（認知症研究開発事業）
委託業務成果報告（総括・業務項目）

アルブミンの劣化に主眼をおいたアルツハイマー病発症前診断及びその治療応用に関する研究

業務主任者又は担当責任者　山本　圭一　大阪市立大学医学部神経内科

研究要旨

アルツハイマー病(AD)発症前段階からの治療介入を目指し、申請者らが発見したアルブミンAβ複合体の多寡を抗アルブミンAβ複合体抗体により測定、またAD及びその前段階でのアルブミンの性質の変化も調べる。

A．研究目的

AD 発症前段階から治療介入できれば、より効果的に認知症発症を抑制できる可能性が高く、そのため申請者が発見したアルブミン Aβ複合体の低下やアルブミンの変化について、次の点を明らかにしたい。

①現在抗Aβ抗体と抗アルブミン抗体とのサンドイッチ法にて複合体を測定しているが、抗アルブミンAβ複合体抗体と抗アルブミン抗体とのサンドイッチ法の方が感度、特異度とも高いであろう。大阪市立大学工学部と協力し抗アルブミンAβ複合体抗体を作成し、複合体の濃度を測定する。

②アルブミンの性質の変化に関しては、酸化や糖化などが知られているが、酸化型は加齢にて増加し、糖化型は高血糖にて増加する。加齢も糖尿病も AD のリスクファクターとして知られている為、これらのファクターによりアルブミンが修飾を受け Aβと結合しにくい形態となり、Aβをクリアランスできず AD が発症すると考えている。AD 患者ではアルブミンはどのような修飾を受けているのか検証する。

B．研究方法

①感度、特異度のより高い検査法の開発

現在抗Aβ抗体と抗アルブミン抗体とのサンドイッチ法にて複合体を測定しているが、感度、特異度とも 80%弱である。更に感度、特異度を向上させる為、抗アルブミンAβ複合体抗体と抗アルブミン抗体とのサンドイッチ法を開発する。抗アルブミンAβ複合体抗体は大阪市立大学工学部准教授立花太郎先生と協力し作成する。具体的には、ヒト血清アルブミンにAβ1-42 ペプチドを加え、複合体を形成させた後、ゲル濾過クロマトグラフィーで精製し、マウスに投与しモノクローナル抗体を作製。Aβ単独やアルブミン単独に反応せず、アルブミン Aβ複合体のみを認識する抗体をスクリーニングする。その抗体を用い、血清アルブミンAβ複合体の測定値と、AD の発症リスクにつき検証する前向きコホート研究を大阪市立大学医学部老年科神経内科にて行う。大阪市立大学では平成 19 年度より PIB-PET を導入しており、すでに 300 例以上撮影を行っており、また PIB-PET 撮影者の血液検体が保管されている。PIB-PET 陽性でかつ AD を発症していない患者をピックアップ

し、血清アルブミンAβ複合体の値により、血清採取時より2年間以内にどの群が有意にADを発症しやすいかを、年齢、性別、家族歴、教育歴などを加味した多変量解析を行う。解析は大阪市立大学血液内科講師康秀男先生と協力して行う。

②アルブミン修飾解析

MCIやAD患者の脳には、Aβが凝集した老人斑を認めるが、その中にはCuやZnなど2価の金属が多く含まれ（Journal of the Neurological Sciences 158: 47-52: 1998）、CuやZnはAβを凝集させ、神経毒性を持たせる（Br J Pharmacol. 163: 211-219: 2011）ことが報告されている。また、アルブミンはCuと結合しやすい蛋白である。以上から、MCIやAD患者では非認知症群に比べアルブミンがCuと結合しにくい状態になっており、そのためCuを介したAβとの結合ができず複合体量が減少し、その結果Aβ及びCuが脳内に留まりし神経毒性を発揮すると仮定し、銅イオンと結合できる正常アルブミンの量を測定する方法で、軽度認知障害とアルツハイマー型認知症を、非認知症群と鑑別できるかについて横断的に検証した。

（倫理面への配慮）

本研究は、ヒトを対象とする医学研究の倫理的原則(ヘルシンキ宣言)および疫学研究の倫理指針を遵守し、試験審査委員会を設置し、研究計画書、同意文書の作成を行い、当該施設の倫理審査委員会の承認を得る。本臨床研究の開始に際して、担当医は対象となる患者に対して同意説明文を参考に治療法、検査および遺伝学的な解析データの使用について、患者に十分な説明を行った上で患者本人の自由意思による同意を書面により得ることとする。本研究への参加の有無によって患者にはいかなる不利益もかかることはなく、また患者はいつでも同意を撤回することができる。患者データは定期的に試験審査委員会で検討し、重篤な有害事象が生じる可能性がある場合には、一時中断し、試験方法の改正を行う。プライバシーや遺伝子に関わる情報の守秘義務を徹底する。また、本研究プロトコールについては大阪市立大学大学院医学研究科の倫理委員会に提出し、受理されている。

C．研究結果

①抗アルブミンAβモノクローナル抗体作製

マウスに抗原投与、及びハイブリドーマは作製した。今後、目的とする抗体ができているかを確認する予定である。

検体に関しては、PIB陽性MCI患者より血清を集めている。複合体濃度の測定は、まだ行っていない。

②アルブミン修飾解析

当科に通院された14例の認知症を有さない他疾患患者(Control)、22例のPIB陽性MCI患者、26例のPIB陽性AD患者を対象に血液を採取し、銅イオン親和性アルブミンの濃度を測定し、一元配置分散分析及び多変量解析にて比較し、銅イオン親和性アルブミン濃度は、Control＞MCI>AD の順で、低下することが分かった。

D．考察

抗アルブミンAβモノクローナル抗体が作製できれば、アルブミンAβ複合体の

測定を開始する。また、アルブミン Aβ複合体測定以外で、MCI を診断する方法として、血中銅イオン親和性アルブミンが、MCI(due to AD)及び AD のバイオマーカーになる可能性がある。

E．結論

アルブミン Aβモノクローナル抗体作製、検体採取を継続して行っていく。併せて、血中銅イオン親和性アルブミンについても、更なる検証を行う。

F．健康危険情報

なし

G．研究発表

1. 論文発表

Serum levels of albumin-amyloid beta complexes are decreased in Alzheimer's disease.
Yamamoto K, Shimada H, Koh H, Ataka S, Miki T.
Geriatr Gerontol Int. 2014; 14(3): 716-23.

Cerebral infarction in the left hemisphere compared with the right hemisphere increases the risk of aspiration pneumonia.
Yamamoto K, Koh H, Shimada H, Takeuchi J, Yamakawa Y, Kawamura M, Miki T.
Osaka City Med J. 2014; 60: 81-86.

2. 学会発表

なし

H．知的財産権の出願・登録状況

（予定を含む。）

1. 特許取得

特願 2014-139665
（出願日：2014 年 7 月 7 日）
発明の名称：銅イオンと結合するアルブミンを測定することでの、軽度認知障害及びアルツハイマー型認知症を診断する方法及びキット
出願人：公立大学法人大阪市立大学
発明者：山本圭一

2. 実用新案登録

なし

3.その他

なし

学 会 等 発 表 実 績

委託業務題目「アルブミンの劣化に主眼をおいたアルツハイマー病発症前診断及びその治療応用に関する研究」

機関名　公立大学法人大阪市立大学

１．学会等における口頭・ポスター発表

発表した成果（発表題目、口頭・ポスター発表の別）	発表者氏名	発表した場所（学会等名）	発表した時期	国内・外の別

２．学会誌・雑誌等における論文掲載

掲載した論文（発表題目）	発表者氏名	発表した場所（学会誌・雑誌等名）	発表した時期	国内・外の別
Serum levels of albumin-amyloid beta complexes are decreased in Alzheimer's disease.	Yamamoto K, Shimada H, Koh H, Ataka S, Miki T.	Geriatr Gerontol Int.	2014年7月	国外
Cerebral infarction in the left hemisphere compared with the right hemisphere increases the risk of aspiration pneumonia.	Yamamoto K, Koh H, Shimada H, Takeuchi J, Yamakawa Y, Kawamura M, Miki T.	Osaka City Med J.	2014年12月	国内

ORIGINAL ARTICLE: BIOLOGY

Serum levels of albumin-amyloid beta complexes are decreased in Alzheimer's disease

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Aim: Decreased amyloid β (Aβ) clearance from the brain to blood might play a key role in the development of Alzheimer's disease (AD). Aβ is normally bound to and transported by albumin in blood, thus possibly maintaining constant concentration of free Aβ in the blood. We therefore hypothesized that decreased blood levels of albumin-Aβ complexes could be associated with decreased Aβ removal from the brain to blood, resulting in Aβ accumulation in the brain.

Methods: We carried out a cross-sectional investigation of the association between serum levels of albumin-Aβ complexes (SLAAC) and AD prevalence in 89 patients who visited our outpatient clinic, and gave written informed consent between August 2008 and May 2012.

Results: We confirmed 45 cases of AD. Low SLAAC was associated with an increased prevalence of AD (OR 0.27; 95% CI 0.14–0.51) in a univariable logistic model and multivariable logistic models. In addition, decreased SLAAC was associated with decreased levels of Aβ42 in CSF ($r = 0.38$, $P = 0.0221$) and increased levels of p-tau in CSF ($r = -0.43$, $P = 0.0090$), findings that have been shown to be associated with AD progression.

Conclusions: This novel method might be very useful for monitoring of the progression of AD. **Geriatr Gerontol Int 2014; 14: 716–723.**

Keywords: albumin, Alzheimer's disease, amyloid β, biomarkers, serum.

Introduction

The incidence of Alzheimer's disease (AD) is increasing rapidly in many developed countries, and is a serious and growing medical, social, and economic problem that requires urgent resolution.[1] It is well known that the pathological changes in AD, characterized by neocortical neurofibrillary tangles and senile plaques composed of amyloid β (Aβ), precede the occurrence of clinical symptoms by several years.[2] In order to carry out earlier therapeutic interventions for AD, it is important to diagnose AD at an earlier stage.[3] The diagnosis of AD is currently based on clinical symptoms and neuropsychological testing.[4] However, by the time a clinical diagnosis is made, significant and irreversible pathological changes have already occurred in the brain, and as a result several therapeutic interventions for AD have failed.[5,6] There is therefore an increasing need for earlier and more specific diagnosis.

Several epidemiological studies have reported that the specific neuropathological findings of AD are strongly associated with increased levels of total tau and p-tau, and decreased levels of the 42 amino acid isoform of amyloid β (Aβ42) in the cerebrospinal fluid (CSF) of patients with AD.[7,8] Recently, it has been reported that use of positron emission tomography to image brain Aβ plaques with the amyloid tracer, Pittsburgh compound B, might be a useful tool to diagnose asymptomatic AD.[9] However, a spinal tap and/or a positron emission tomography scan are less feasible when screening large populations because of the invasive nature of the procedure and high cost. Although there are many reports on the utility of plasma levels of free Aβ or apolipoprotein E (ApoE) phenotype/genotype in the diagnosis of AD, their usefulness has not been established.[10–15] Therefore development of a novel, non-invasive method for diagnosing AD is warranted.

Although the mechanisms underlying the development of AD remain unclear, several hypotheses have been proposed and debated.[16] Recent evidence suggests that decreased Aβ clearance from the brain might be

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involved in the development of the late-onset form of AD, leading to Aβ accumulation in the central nervous system.[17] Of the supposed mechanisms of Aβ clearance from the brain, the "sink hypothesis" has been proposed: Aβ shifts from the brain into the blood in the direction of the Aβ concentration gradient between the brain and blood decreasing.[18,19] Based on this hypothesis, only one clinical trial has been carried out on the efficacy of lowering peripheral Aβ levels in patients with AD.[20] However, to date, the efficacy of this intervention has not been confirmed.

On the basis of these observations, Aβ clearance from the central nervous system might play a pivotal role in the development of AD. Under physiological conditions, Aβ is normally bound to and transported by albumin in the blood, thus possibly maintaining a constant free Aβ concentration in the blood.[21] In addition, it is widely known that the mean level of serum albumin does not differ between AD patients and the healthy population.[22] Taking these factors into consideration, a reduction in the ability of albumin to bind Aβ in blood can be pathophysiologically considered to be one cause of decreased Aβ clearance from the brain. We thus hypothesized that decreased levels of albumin-Aβ complexes in the blood might be associated with the development of AD. To our knowledge, no study has examined the relationship between levels of serum albumin-Aβ complex and AD. Here, we first carried out a cross-sectional investigation into whether serum levels of albumin-Aβ complex were associated with AD in patients who visited a Japanese neurology outpatient clinic.

Methods

Study participants and sample collection

The study population consisted of 45 consecutive AD patients and 44 consecutive age-matched patients without cognitive impairment who visited the neurology outpatient clinic at Osaka City University Hospital, Osaka, Japan, between August 2008 and May 2012, and provided written informed consent to undergo genetic studies. Blood and/or CSF samples from the participants were collected and preserved at −80°C at enrolment. The stored samples consisted of 89 serum samples, and 36 plasma and CSF samples. Of the 36 CSF samples, five samples were insufficient for us to carry out all the necessary tests. The protocol for the present study was approved by the Human Subjects Review Committee at Osaka City University.

Definition

Cognitive function and Mini-Mental State Examination (MMSE) were clinically assessed in all patients. AD was recognized if patients fulfilled the clinical criteria for probable AD proposed by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association.[23] The control group consisted of patients with non-dementia diseases, including osteoporosis, hypertension and hyperlipidemia, none of whom complained of forgetfulness or cognitive decline.

Data measurements

ApoE phenotyping and albumin level in serum and CSF

ApoE phenotyping was determined by isoelectric focusing carried out at SRL, Tokyo, Japan.[24] The serum albumin level was measured using the improved BCP method, and the CSF albumin level was measured using immunonephelometry, carried out at SRL, Tokyo, Japan.[25,26]

Aβ40 and Aβ42 levels in plasma and CSF, and total tau and phosphorylated tau 181 levels in CSF

Levels of Aβ40 and Aβ42 in plasma and CSF, and levels of total tau and p-tau in CSF were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits (Human βAmyloid1-40 ELISA Kit, Human βAmyloid1-42 ELISA Kit, Wako Pure Chemical Industries, Japan; Innotest hTau Ag, Innotest Phosphotau [181P]; Innogenetics, Gent, Belgium) carried out at SRL, Tokyo, Japan, according to the manufacturer's protocol.

Immunoprecipitation to prove the existence of albumin-Aβ complexes in serum

Serum (2 μL) from the control group, as well as patients with AD, was diluted 1:100 with Tris-buffered saline and incubated with 0.6 μmol/L Aβ1–42 peptide (Peptide, Osaka, Japan) or equivalent Tris-buffered saline at 37°C for 1 h. Albumin-Aβ complexes in the samples were immunoprecipitated with 1 μg of 6E10 antibody (or control mouse immunoglobulin G as the negative control) and 20 μL of protein G agarose (Thermo, Yokohama, Japan) at 4°C overnight. After centrifugation for 2 min at 2500 × *g*, the precipitates were washed four times with 0.05% Tween 20 in Tris-buffered saline and once with Tris-buffered saline, and then boiled for 5 min in sodium dodecylsulfate sample buffer. The elutes were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis with 10% Tricine gel and transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA, USA). Non-specific binding was blocked with 5% skimmed milk solution in Tris-buffered saline containing Tween 20. Albumin-Aβ complexes were probed with purified

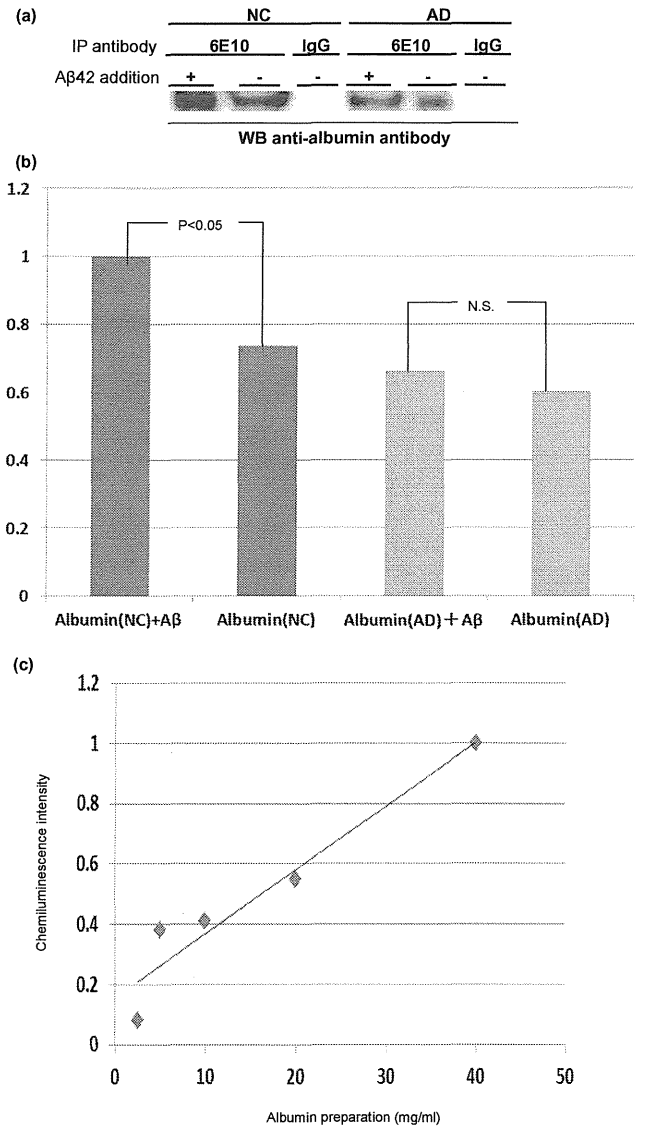

Figure 1 (a) Representative image of immunoblotting analysis of immunoprecipitated albumin-Aβ complexes in the serum of normal control individuals and patients with Alzheimer's disease, and (b) the densitometric measurements. (c) Representative standard curve for sandwich enzyme-linked immunosorbent assay (ELISA). Diluted serum of control individuals and AD patients were incubated with Aβ1–42 peptide or equivalent Tris-buffered saline. Albumin-Aβ complexes in the samples were immunoprecipitated with 6E10 antibody (or control mouse immunoglobulin G as negative control) and protein G agarose. The precipitates were washed and eluted. The elutes were subjected to sodium dodecylsulfate-polyacrylamide gel electrophoresis and transferred to membranes. (a) Albumin-Aβ complexes were probed with anti-human albumin polyclonal antibody, followed by horseradish peroxidase-labeled second antibody and the chemiluminescent substrate. The densitometric measurements were carried out in three persons per group. (b) The value of density of albumin (NC) + Aβ was set to one. To create a standard curve for ELISA, we prepared albumin for intravenous injection as a reference standard. Sandwich ELISA detected a signal from the albumin preparation, which decreased in a linear fashion in the serially diluted standard. (c) Levels of albumin-Aβ complexes detected were expressed as albumin preparation equivalent concentration.

anti-human albumin polyclonal antibody, followed by horseradish peroxidase (HRP)-labeled second antibody and the chemiluminescent substrate. Signals were visualized using a LAS-3000 luminescent image analyzer (Fujifilm, Tokyo, Japan). Densitometric measurements were carried out by Fotodyne visionary documentation system (Fotodyne, Hartland, WI, USA; Fig. 1).

Albumin-Aβ complex level in serum and CSF

We measured the serum level of albumin-Aβ complexes (SLAAC) using a specific sandwich ELISA that uses anti-human Aβ N-terminal monoclonal antibody (BAN50) and anti-human albumin purified polyclonal antibody. Briefly, serum samples were diluted 1:400 and CSF samples were diluted 1:4 with Tris-buffered saline before loading. A 100-μL aliquot from each diluted sample was loaded into a microwell strip coated with BAN50, contained within a commercial kit (Human βAmyloid1-42 ELISA Kit; Wako Pure Chemical Industries, Osaka, Japan). The plates were covered and incubated for 60 min at 37°C before being washed four times with 0.05% Tween 20 in Tris-buffered saline. After washing, 100 μL of purified, HRP-conjugated anti-human albumin polyclonal antibody (A80-129P, Bethyl Laboratories, Montgomery, TX, USA) diluted 1:30000 with 10% BlockAce (Snow Brand, Tokyo, Japan) was added to the microwell strips, which were incubated for 60 min at 37°C. After subsequent washing, 100 μL of the 3, 3′, 5, 5′-tetramethylbenzidine substrate (Wako Pure Chemical Industries) was added, and the strips were incubated for a further 15 min at 37°C. Acid solution (100 μL; Wako Pure Chemical Industries) was then added to each well to terminate the enzyme reaction and stabilize the developed color. The optical density of each well was then measured at 450 nm using an ARVO-2 microplate reader (Perkin Elmer, Yokohama, Japan). The assay was carried out twice and averaged. The intra- and interassay coefficients of variability was 5.1% and 13.8%, respectively.

Standard curve for albumin preparation in the sandwich ELISA

To create a standard curve for the ELISA, we prepared albumin preparation for intravenous injection (Benesis Corporation, Osaka, Japan) as a reference standard. The

sandwich ELISA successfully detected a signal from the albumin preparation, which decreased in a linear manner in the serially diluted standard. The standard curves in the present study were linear with $r = 0.96–0.99$. The levels of albumin-Aβ complexes in serum and CSF were expressed as the albumin preparation-equivalent concentration from a standard curve based on reference standards. The detection limit in serum and CSF was as low as 2.5 mg/mL and 25μg/mL equivalent to albumin preparation, respectively. The concentration of albumin-Aβ complexes below the lower detection limit were set to zero. (Fig. 1)

Statistical analysis

To examine that difference between AD and the control groups, we used unpaired *t*-tests for continuous variables and χ^2-tests for categorical variables. Pearson's correlation coefficients were calculated to assess the association between two normally distributed variables. Univariable and multiple logistic regression analysis were used to estimate the odds ratio for the prevalence of AD. Non-linear effects of continuous, independent variables were evaluated using quadratic and log transformations. Multicollinearity was assessed with the use of a variance inflation factor. A variance inflation factor exceeding 10 is regarded as indicating serious multicollinearity, and values >5.0 might be a cause for concern.[27] We calculated the 95% confidence interval for each odds ratio. All statistical tests were interpreted at the 5% significance level. All *P*-values and 95% confidence intervals were two-sided. All statistical analyses were carried out using the SPSS 12.0 software package (SPSS, Chicago, IL, USA).

Results

Immunoprecipitation to prove the existence of albumin-Aβ complexes in serum

To prove the presence of albumin-Aβ complexes in serum, we carried out immunoprecipitation with 6E10: mouse anti-human Aβ N-terminal monoclonal antibody (Sigma-Aldrich, St. Louis, MO, USA). We were able to suggest the existence of endogenous albumin-Aβ complexes in sera of both AD patients and control individuals by western blot analysis using anti-human albumin purified polyclonal antibody. In addition, we suggested that the serum albumin of control individuals had higher binding ability to exogenous Aβ1–42 than the serum of AD patients (Fig. 1).

Background characteristics of study participants

The characteristics of study participants are summarized in Table 1. Among the 89 eligible participants, we confirmed 45 (50.6%) cases of AD. The mean value of the MMSE score was significantly lower in the AD group than in the control group. The prevalence of ApoE4 was significantly higher in the AD group than in the control group. No significant differences in age or sex were found between these groups.

The mean values of serum albumin were similar between AD and the control group. The mean SLAAC was significantly lower in the AD group than the control group (Table 1, Fig. S1). The mean values of the serum albumin-Aβ complex and the ratio of serum albumin-Aβ complexes to serum albumin were significantly lower in the AD group than in the control group.

Data on plasma and cerebrospinal fluid samples were assessed in 13 and 23 participants of the control group and the AD group, respectively. In five AD patients, data on both CSF albumin and CSF albumin-Aβ complex levels were not available as a result of low sample volumes. The mean values of plasma Aβ40 and Aβ42 did not differ between the AD and control groups. In these 36 participants, the AD group had significantly lower CSF Aβ42 levels than did the control group. The mean values of CSF Aβ40 levels were similar in the AD and control groups. The mean values of CSF tau and p-tau were significantly higher in the AD group than the control group. In the 31 participants who had complete CSF biomarker data, the mean values for CSF albumin and albumin-Aβ complex levels did not differ between the AD and control groups. Furthermore, the mean values of the CSF albumin-Aβ complex and the ratio of CSF albumin-Aβ complexes to CSF albumin did not differ between these groups. (Table 1)

Univariable and multiple logistic regression analyses

In the logistic regression analyses, use of quadratic or log transformations of all continuous variables in all models did not result in an improvement in fit compared with the linear model. In the univariable logistic models, both higher serum albumin-Aβ complexes and the ratio of serum albumin-Aβ complexes to serum albumin were associated with decreased odds of AD. Serum albumin, plasma Aβ40 and plasma Aβ42 levels were not significantly associated with the prevalence of AD. Higher CSF levels of Aβ42 were associated with a decreased odds of AD. Higher CSF tau and p-tau levels were associated with increased odds of AD. CSF Aβ40, CSF albumin, CSF Albumin-Aβ complex and CSF Albumin-Aβ complex per unit CSF albumin were not significantly associated with the prevalence of AD. (Table 2)

In the multiple logistic models, we examined the relationship between SLAAC and the prevalence of AD. Higher SLAAC was independently associated with a decreased odds of AD, even after adjusting for age, sex,

Table 1 Characteristics of the study participants

Characteristics	Control (n = 44) (n = 44/n = 13)	Alzheimer's disease (n = 45) (n = 45/n = 23)	P-value (n = 89/n = 36)
Age (years)	73.84 ± 8.03/71.08 ± 10.36	73.71 ± 10.48/69.65 ± 11.33	0.9479/0.7112
Sex			
Male (n)	10/5	13/6	0.5068/0.4388
Female (n)	34/8	32/17	
MMSE score	27.34 ± 2.44/25.69 ± 2.18	18.18 ± 4.97/19.78 ± 5.78	<0.0001/0.0012
Serum albumin (g/dL)	4.15 ± 0.30/3.93 ± 0.24	4.13 ± 0.31/4.13 ± 0.28	0.7433/0.0396
Serum albumin-Aβ complex (mg/mL equivalent to albumin preparation)	13.48 ± 8.01/14.69 ± 9.12	6.18 ± 5.15/6.54 ± 5.79	<0.0001/0.0024
Serum albumin-Aβ complex/serum albumin	0.33 ± 0.20/0.38 ± 0.23	0.15 ± 0.13/0.16 ± 0.15	<0.0001/0.0020
Serum ApoE allele			
E4 = 0 (n)	33/10	16/7	0.0004/0.0073
E4 = 1 or 2 (n)	11/3	28/16	
Plasma Aβ40 (pmol/L)	–/47.98 ± 12.39	–/49.65 ± 17.27	–/0.7603
Plasma Aβ42 (pmol/L)	–/5.95 ± 3.42	–/6.02 ± 8.30	–/0.9792
CSF Aβ40 (pmol/L)	–/1514.77 ± 347.96	–/1646.96 ± 386.13	–/0.3144
CSF Aβ42 (pmol/L)	–/166.38 ± 52.35	–/85.44 ± 24.53	–/<0.0001
CSF total tau (pg/mL)	–/208.85 ± 86.24	–/542.65 ± 196.40	–/<0.0001
CSF phosphorylated tau (pg/mL)	–/48.59 ± 17.54	–/111.26 ± 44.33	–/0.0001
CSF albumin (mg/mL)†	–/0.28 ± 0.16	–/0.22 ± 0.10	–/0.2036
CSF albumin-Aβ complex (mg/mL equivalent to albumin preparation)†	–/0.43 ± 0.09	–/0.39 ± 0.10	–/0.2523
CSF albumin-Aβ complex/CSF albumin†	–/1.84 ± 0.77	–/1.99 ± 0.73	–/0.5942

Data are number or mean ± SD (n = 89). Data on plasma and cerebrospinal fluid samples were evaluated in 13 patients in the control group (n = 44) and 23 of the patients with Alzheimer's disease (n = 45). †Data on cerebrospinal fluid (CSF) albumin, albumin-Aβ complex, and albumin-Aβ complex/CSF albumin were evaluated in 13 patients in the control group and 18 patients with Alzheimer's disease. Apo E, apolipoprotein E; MMSE, Mini-Mental State Examination.

ApoE genotypes or other blood biomarkers including serum albumin, plasma Aβ40 and plasma Aβ42. (Model 1–3 Table 3)

Correlation between serum albumin-Aβ complex and CSF p-tau

There was a negative correlation between SLAAC and CSF p-tau levels, and positive correlation between SLAAC and CSF Aβ42 levels or Mini-Mental State Examination scores (Fig. 2).

Discussion

In the present study, we first showed that decreased SLAAC was strongly associated with a higher prevalence of AD. This association was independent of age, sex, and ApoE4 allele. In addition, decreased SLAAC was correlated with decreased levels of Aβ42 in the CSF and increased levels of p-tau in the CSF, findings that have been shown to be associated with specific neuropathological findings and AD progression.[7,8,28]

Our data on serum albumin concurred with previous reports that showed no difference in levels of serum albumin between patients with AD and control individuals.[22] Two recent studies reported that levels of plasma-free Aβ40 and Aβ42 in AD patients and controls did not differ.[12,13] Again, our data on plasma-free Aβ40 and Aβ42 are similar. No study has examined the association between the serum albumin-Aβ complex and AD. In the present study, we investigated this association for the first time, and found that low serum levels of the albumin-Aβ complex were associated with a higher prevalence of AD. In addition, we suggested that the serum of control individuals had higher binding ability to Aβ1–42 than that of AD patients. Some studies have reported that human serum albumin is modified by several factors, such as oxidation and glycation.[29,30] These findings suggest that a decrease in Aβ binding to albumin in the blood could lead to a decrease in the capacity for Aβ excretion from the brain to the blood, resulting in Aβ accumulation in the brain. In contrast, levels of the albumin-Aβ complex were much lower, and free Aβ levels were much higher in CSF than that in

Table 2 Univariable logistic models of prevalence of Alzheimer's disease in relation to baseline variables in all study participants and in the participants with plasma and cerebrospinal fluid samples data

Variables	Odds ratios (95% CI) $n = 89/n = 36$	P value $n = 89/n = 36$
Age	0.99 (0.65–1.50)/0.87 (0.43–1.78)	0.9472/0.7023
Female gender	0.72 (0.28–1.88)/1.77 (0.41–7.58)	0.5076/0.4412
MMSE score	0.01 (0.00–0.07)/0.30 (0.00–0.32)	<0.0001/0.0036
Serum albumin	0.93 (0.61–1.42)/2.21 (0.99–4.91)	0.7400/0.0518
Serum Albumin-Aβ complex	0.27 (0.14–0.51)/0.29 (0.11–0.75)	<0.0001/0.0112
Serum Albumin-Aβ complex/serum albumin	0.28 (0.15–0.54)/0.29 (0.11–0.75)	0.0001/0.0112
Serum ApoE4 allele	4.94 (1.99–12.28)/7.62 (1.59–36.49)	0.0006/0.0111
Plasma Aβ40	–/1.12 (0.56–2.23)	–/0.7523
Plasma Aβ42	–/1.01 (0.50–2.02)	–/0.9785
CSF Aβ40	–/1.48 (0.69–3.17)	–/0.3096
CSF Aβ42	–/0.014 (0.001–0.34)	–/0.0089
CSF total tau	–/105.46 (4.06–2741.18)	–/0.0051
CSF phosphorylated tau	–/624.46 (7.23–53918.09)	–/0.0047
CSF albumin†	–/0.61 (0.29–1.32)	–/0.2117
CSF Albumin-Aβ complex†	–/0.62 (0.27–1.40)	–/0.2501
CSF Albumin-Aβ complex/CSF albumin†	–/1.15 (0.56–2.34)	–/0.7025

Odds ratios for continuous variables reflect a 1-SD-magnitude increase ($n = 89$, data on the left of the slash; $n = 36$, data on the right of the slash), †Data on cerebrospinal fluid (CSF) albumin, albumin-Aβ complex and albumin-Aβ complex/CSF albumin were evaluated in 13 patients in the control group and 18 patients with Alzheimer's disease. Apo E, apolipoprotein E; MMSE, Mini-Mental State Examination.

Table 3 Multivariable models of prevalence of Alzheimer's disease in relation to baseline values including serum albumin-Aβ complexes, ApoE genotype, and other blood biomarkers in all the study participants and in the participants with plasma sample data

Variables in the models	Multiple-adjusted odds ratios (95% CI)	P value
Model 1 ($n = 89$)		
Serum albumin-Aβ complexes	0.26 (0.13–0.50)	<0.0001
Serum ApoE4 allele	5.71 (1.94–16.84)	0.0016
Age	0.97 (0.92–1.03)	0.2956
Female gender	0.72 (0.22–2.42)	0.5975
Model 2 ($n = 89$)		
Serum Albumin-Aβ complexes	0.27 (0.14–0.52)	0.0001
Serum ApoE4 allele	5.73 (1.97–16.69)	0.0014
Serum albumin	0.74 (0.42–1.28)	0.2782
Model 3 ($n = 36$)		
Serum Albumin-Aβ complexes	0.18 (0.051–0.65)	0.0084
Serum ApoE4 allele	30.52 (2.40–388.75)	0.0085
Plasma Aβ40	0.56 (0.19–1.68)	0.2994
Plasma Aβ42	1.93 (0.14–25.81)	0.6189

Odds ratios for continuous variables reflect a 1-SD-magnitude increase ($n = 89$, data shown in Model 1,2; $n = 36$, data shown in Model 3. Apo E, apolipoprotein E.

blood in both AD and control groups in the present study. This might indicate that most Aβ is free in the CSF, and binds to albumin after translocation into blood. These observations are consistent with the mechanism advocated by the sink hypothesis.[19,20]

The present study had some potential limitations. First, we cannot conclusively attribute cause-and-effect relationships because of the cross-sectional nature of our data. Second, we cannot ascertain whether SLAAC is low in other diseases characterized by cognitive

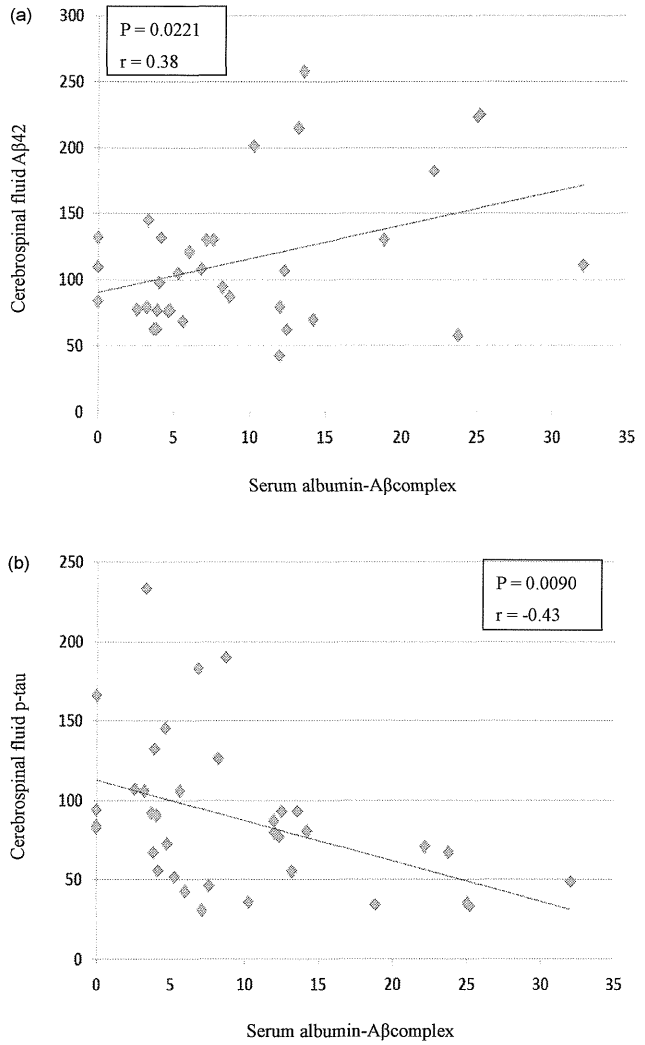

Figure 2 Correlation between (a) serum levels of albumin-Aβ complexes and cerebrospinal fluid Aβ42 level, and (b) between serum levels of albumin-Aβ complexes and cerebrospinal fluid p-tau level in participants with cerebrospinal fluid sample data (n = 36).

decline without an Aβ burden, such as frontotemporal lobar degeneration.[31]

In the present study, we found that low SLAAC is associated with an increased prevalence of AD. In addition, we showed that decreased SLAAC was correlated with increased levels of p-tau in the CSF, which was associated with specific neuropathological findings and progression of AD. Therefore, this non-invasive and convenient novel method might be very useful for monitoring of the progression of AD. Furthermore, these findings might be able to explain the mechanism how Aβ is accumulated in AD brain. Less binding ability of serum albumin to Aβ in AD patients cloud slow the clearance of Aβ from the CNS.

The mechanism by which Aβ binds to albumin remains to be determined. What are included in the serum albumin and Aβ complex? Further research is warranted to confirm the underlying mechanism.

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Disclosures

No potential conflicts of interest were disclosed.

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Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Figure S1 Comparison of serum levels of albumin-Aβ complexes in the controls and those with Alzheimer's disease, for all study participants (n = 89). Each horizontal bar shows the mean value of serum levels of albumin-Aβ complexes (control 13.48; Alzheimer's disease 6.18 mg/mL equivalent to albumin preparation). The concentration of serum albumin-Aβ complexes below the lower detection limit (<2.5 mg/mL equivalent to albumin preparation) were set to zero (control n = 3; Alzheimer's disease n = 10).

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Cerebral Infarction in the Left Hemisphere Compared with the Right Hemisphere Increases the Risk of Aspiration Pneumonia

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Abstract

Background

Aspiration pneumonia (AP) following cerebral infarction (CI) has been considered as one of its most serious complications. Nevertheless, there are no reports on the association between the type or location of CI and the incidence of AP. In addition, the association between dysphagia, which leads to aspiration, and the type or location of CI has never been investigated. Therefore we hypothesized that the laterality of CI affects the development of both dysphagia and AP.

Methods

We performed a retrospective cohort study to examine the association between the laterality of CI and the incidence of dysphagia and AP in 133 patients.

Results

AP was found in 6.0% of the group with left CI and in 0.8% of the group with right CI. A univariate logistic regression analysis revealed that left CI was a significant predictor of AP (hazard ratio, 8.81; 95% confidence interval, 1.07-72.59; p=0.043). Left CI was a significant predictor of AP even after adjusting for age, sex, CI type, or presence of diabetes mellitus. In addition, although the frequency of dysphagia as the direct cause of AP did not differ according to laterality, the frequency of AP that ensued from dysphagia in the left CI group was greater than that observed in the right CI group.

Conclusions

The group with left CI from the motor cortex to the internal capsule complicated by dysphagia exhibited a high risk of AP.

Key Words: Aspiration pneumonia; Cerebral infarction; Laterality

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Introduction

There are several well-known risk factors for aspiration pneumonia (AP), such as advanced age, diabetes, poor oral health, dysphagia, decreased consciousness, or neurological dysfunctions, including cerebral infarction (CI), Alzheimer disease, and Parkinson disease[1]. Among AP caused by these factors, AP following stroke has been considered as one of its most serious complications, because the mortality of stroke patients with AP is reportedly about 3 times higher than that of those without AP[2]. It is considered that an earlier preventive intervention for AP following CI is important for the improvement of the prognosis of this condition. Therefore, the early identification of high-risk groups with AP after stroke would be beneficial. Only one retrospective study has reported age, male sex, dysphagia, and nonlacunar stroke as independent predictors of post-CI pneumonia[3]. However, these findings have not been confirmed.

CI is pathogenetically categorized into 3 subtypes: lacunar ischemic, atheromatous thrombotic, and embolic stroke. Other subtypes are based on the location of CI: cerebral cortex, deep white matter, internal capsule (including the pyramidal tract and corticobulbar tract), brain stem, basal ganglia, and cerebellum. To our knowledge, there are no reports on the association between the type or location of CI and the incidence of AP. In addition, the association between dysphagia, which leads to aspiration, and the type and/or location of CI has never been investigated.

Swallowing and speaking movements are performed in part using the same muscles, which are controlled by the corticobulbar tracts that descend from the cortex to the brain stem. The functional impairment of these movements results in dysphagia and dysarthria, respectively. Only 1 prospective study examined the association between dysarthria and CI type and location[4]. That study showed that the prevalence of dysarthria after left hemisphere CI was higher than that observed after right hemisphere CI, which may indicate a relation between the laterality of CI and the development of dysarthria.

Based on these observations, we hypothesized that the laterality of CI affects the development of both dysphagia and AP. Therefore, we performed a retrospective cohort study to examine the association between the laterality of CI and the incidence of dysphagia and AP. Moreover, we investigated the effect of CI type and location on the development of these complications.

Methods

Study Subjects

This retrospective cohort study included 170 consecutive patients with no history of previous stroke who were admitted to the neurology section of the Japanese Red Cross Society in Wakayama between April 2004 and March 2008.

The background data of patients, including age, sex, type of CI, medical history, medication history, and presence of diabetes, dysphagia, and AP, were collected based on electronic medical records from admission to discharge.

The diagnosis of CI was established using both 1.5 T magnetic resonance imaging (MRI) and the medical report provided by a consultant neuroradiologist after confirmation of the presence and location of any acute CI lesion (high signal on diffusion imaging, low signal on apparent diffusion coefficient map) within 1 week after any neurological event, such as motor paralysis, sensory paralysis, visual field disturbance, ataxia, cranial nerve paralysis (eg, dysarthria, dysphagia, or diplopia), dizziness, or any neuropsychological disorders (eg, aphasia or apraxia).

Lacunar infarcts were defined as round and small (<2 cm in diameter) acute ischemic lesions located in the basal ganglia, internal capsule, centrum semiovale, or brain stem that were accompanied by clinical signs such as pure motor hemiparesis, pure sensory stroke, ataxic hemiparesis, dysarthria/clumsy hand syndrome, or sensorimotor stroke. Dysphagia after CI was diagnosed by asking about swallowing difficulties and observation of food intake. The diagnosis of pneumonia was established based on a combination of clinical features (high fever, coughing, and rales) and radiological findings that occurred within 30 days after stroke onset. We excluded patients with other risk factors of AP, such as disturbance of consciousness (Glasgow Coma Scale <15), Parkinson disease, dementia, malignant disease, periodontal disease, and alcoholism. We also excluded patients with prevention factors for AP, such as use of angiotensin-converting enzyme (ACE) inhibitors. Finally, patients with a history of previous CI, transient CI, or bilateral CI were not included in the study.

The study protocol was approved by the Institutional Review Board (IRB) of the Japanese Red Cross Society in Wakayama. A written informed consent was specifically waived by the approving IRB.

Statistical Analysis

To examine differences between the AP and non-AP groups we used unpaired t tests for continuous variables and χ^2 tests for categorical variables.

Univariate and multiple logistic regression analyses were used to estimate the odds ratio for the risks of AP after CI. The nonlinear effects of continuous, independent variables were evaluated using quadratic and log transformations. Multicolinearity was assessed using a variance inflation factor. A variance inflation factor exceeding 10 is regarded as indicating serious multicolinearity, and values >5.0 may be a cause for concern. We calculated the 95% confidence interval (CI) for each odds ratio. All statistical tests were interpreted at the 5% significance level. All p values and 95% CIs were two-sided. All statistical analyses were performed using the SPSS 12.0 software package (SPSS, Chicago, IL).

Results

Among the 170 consecutive patients, 37 were excluded based on the exclusion criteria mentioned above: 14 patients with bilateral CI, 5 patients with disturbance of consciousness, 5 patients with transient ischemic attack, 5 patients who were heavy drinkers, 3 patients with dementia, 3 patients with Parkinson disease, 1 patient with esophageal cancer, 1 patient with lung cancer, 1 patient with periodontal disease, 1 patient who was taking prednisolone, and 1 patient who was taking the ACE inhibitor lisinopril, with some overlapping.

Patient characteristics are listed in Table 1. Among the 133 patients selected, we confirmed 9 (6.8%) cases of AP and 124 (93.2%) cases of non-AP. AP was found in 6.0% of cases in the left CI group and in 0.8% of cases in the right CI group. Thus, the incidence of AP was significantly lower in the latter group. All patients with AP had dysphagia. The mean age was significantly higher in patients in the AP group compared with those in the group without pneumonia. No differences in other background data, such as sex, CI type, location of CI, and complications (such as diabetes mellitus), were found between these groups. Among the patients who exhibited the complication of dysphagia (n=81), AP was present in 9.9% (n=8) of cases in the left CI group and in 1.2% (n=1) of cases in the right CI group. This difference was significant. There were no differences in background data such as age, sex, CI type, location of CI, and presence of diabetes mellitus.

Table 1. Characteristics of study participants according to the development of aspiration pneumonia

Characteristics	Total	Aspiration pneumonia	No aspiration pneumonia	p value
	(n=133)	(n=9)	(n=124)	
Age, mean (range), years	71.20 (30-90)	78.67 (69-86)	70.65 (30-90)	0.029
Sex				
Male, n (%)	81 (60.9)	5 (3.8)	76 (57.1)	0.734
Female, n (%)	52 (39.1)	4 (3.0)	48 (36.1)	
Type of cerebral infarction				
Lacunar infarcts, n (%)	79 (59.4)	4 (3.0)	75 (56.4)	0.346
Embolism or thrombosis, n (%)	54 (40.6)	5 (3.8)	49 (36.8)	
Location of cerebral infarction				
From the motor cortex to the internal capsule (corticospinal and corticobulbar tracts), n (%)	83 (62.4)	8 (6.0)	75 (56.4)	
Brain stem, n (%)	15 (11.3)	0 (0.0)	15 (11.3)	0.163
Basal ganglia, n (%)	16 (12.0)	1 (0.8)	15 (11.3)	
Cerebellum, n (%)	5 (3.8)	0 (0.0)	5 (3.8)	
Others, n (%)	15 (11.3)	0 (0.0)	14 (10.5)	
Laterality of cerebral infarction				
Right cerebral infarction, n (%)	66 (49.6)	1 (0.8)	65 (48.9)	0.017
Left cerebral infarction, n (%)	67 (50.4)	8 (6.0)	59 (44.4)	
Precerebral infarction complication				
Diabetes mellitus, n (%)	32 (24.1)	1 (0.8)	31 (23.3)	0.348
Non-diabetes mellitus, n (%)	101 (75.9)	8 (6.0)	93 (69.9)	
Postcerebral infarction complication				
Dysphagia, n (%)	81 (60.9)	9 (6.8)	72 (54.1)	0.013
Non-dysphagia, n (%)	52 (39.1)	0 (0.0)	52 (39.1)	

Data are n (%) or the mean (range).

Table 2. Univariate and multivariate analyses of risk of aspiration pneumonia in relation to the laterality of cerebral infarction and other baseline variables (n=133)

	Variable	Odds ratio (95% confidence interval)	p value
Univariate analysis			
	Left cerebral infarction (vs right)	8.81 (1.07-72.59)	0.043
	Age	1.11 (1.01-1.22)	0.034
	Sex	0.73 (0.32-4.95)	0.734
	Lacunar infarction	1.91 (0.49-7.48)	0.351
	Diabetes mellitus	0.38 (0.05-3.12)	0.364
Multivariate analysis			
Model 1	Left cerebral infarction (vs right)	9.49 (1.12-80.07)	0.039
	Age	1.11 (1.01-1.22)	0.032
Model 2	Left cerebral infarction (vs right)	8.78 (1.07-72.33)	0.043
	Sex	1.23 (0.30-4.94)	0.775
Model 3	Left cerebral infarction (vs right)	8.38 (1.01-69.43)	0.049
	Lacunar infarction	1.61 (0.40-6.50)	0.502
Model 4	Left cerebral infarction (vs right)	9.12 (1.10-75.45)	0.040
	Diabetes mellitus	0.34 (0.04-2.91)	0.326