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Association of clinical and aetiologic subtype of acute ischaemic stroke with inflammation, oxidative stress and vascular function: A cross-sectional observational study

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
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Summary

Background: The role of inflammation, vascular dysfunction and oxidative stress in the pathophysiology of different stroke subtypes is not well understood. We aimed to determine if the clinical and aetiologic subtype of acute ischaemic stroke influences systemic markers of vascular function, inflammation and oxidative stress.

Material/Methods: 129 men and women were recruited within 10 days of acute ischaemic stroke or TIA at two tertiary hospitals in this cross-sectional observational study. Stroke severity (NIHSS score and S100B concentration); systemic markers of inflammation (high sensitivity C-reactive protein [hs-CRP] and fibrinogen), endothelial activation (E-selectin), endothelial cell damage (von Willebrand factor activity), and oxidative stress (F_2-isoprostanes) were measured.

Results: Hs-CRP concentrations were higher in total anterior (22.0±24.1 mg/L) than partial anterior circulation (15.3±32.4 mg/L) and lacunar (4.9±4.3 mg/L) syndromes (p=0.01). Hs-CRP concentrations correlated moderately with NIHSS score (r=0.45, p<0.01) and S100B (r=0.48, p<0.01). However aetiologic and clinical subtypes were not independently associated with hs-CRP when included with stroke severity in general linear models.

Conclusions: These data suggest that stroke aetiology and clinical syndrome may not be important independent determinants of the degree of systemic inflammation, oxidative stress or endothelial function in acute ischaemic stroke. Other factors, including stroke severity, pre-morbid inflammation and co-morbidity may explain variations among groups of participants with different subtypes of acute ischaemic stroke.

key words: acute stroke • inflammation • oxidative stress • endothelium

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BACKGROUND

Acute stroke is an heterogeneous disease, comprising various aetiologic and clinical subtypes [1]. The roles of inflammation, vascular dysfunction and oxidative stress in the pathophysiology of different stroke subtypes are not well understood. Overall, acute ischaemic stroke is associated with inflammation [2], which seems to predominantly reflect the severity of the lesion [3]. However other data do not support the hypothesis that markers of the acute phase response simply reflect stroke severity. For example, interleukin-6 has been found to be higher in subjects with small vessel, compared to large vessel, ischaemic stroke [4]. These results suggest a possible particular association between inflammation and small vessel disease. In addition, there is accumulating evidence that inflammation and endothelial activation are relevant to the pathogenesis of small volume and silent brain lesions [5–7].

Acute ischaemic stroke is also associated with oxidative stress. F_2-isoprostanes and ischaemia – modified albumin are elevated in acute ischaemic stroke [8,9]. Small studies show that oxidised LDL is higher in large, compared with small, vessel stroke, suggesting that the degree of oxidative stress may simply reflect infarct size [4]. Markers of oxidative stress correlate inversely with clinical outcome [10]. Cortical infarction is associated with more severe oxidative stress than non-cortical infarction, but this relationship may be confounded by stroke severity [11]. Thus it remains uncertain whether there are important differences in the degree of oxidative stress associated with different subtypes of ischaemic stroke.

We hypothesized that inflammation, vascular function and oxidative stress would be independently associated with stroke sub-type and aetiology among subjects with acute ischaemic stroke. Thus, this study sought to determine if systemic markers of vascular function, inflammation and oxidative stress are influenced by the clinical syndromes and aetiologic subtype of acute ischaemic stroke.

MATERIAL AND METHODS

Participants

Participants with acute ischaemic stroke were recruited from acute stroke wards and TIA clinics within 10 days of onset of acute ischaemic stroke or TIA at two teaching hospitals in Perth, Western Australia between May 2005 and November 2008. Subjects were recruited according to their current clinical diagnosis. Clinical records were reviewed subsequent to the patient's discharge to confirm a final clinical diagnosis of an acute cerebral ischaemic event, classify the clinical syndrome using the Oxfordshire Community Stroke Project classification [12], and assign an aetioloogic subtype using the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) classification [13]. Patients with blood glucose level >13 mmol/L or an acute co-morbid illness were excluded.

Procedures

At enrolment, neurologic impairment, handicap and cognitive function were assessed using the National Institutes of Health Stroke Scale (NIHSS) [14], Modified Rankin Scale (MRS) [15-16], and Mini Mental Status Examination (MMSE) [17]. Laboratory data were collected to assess astroglial injury (S100B concentration [18]), inflammation (high sensitivity C-reactive protein (hs-CRP) and fibrinogen [19]), endothelial activation (E-selectin [20]), endothelial cell damage (Von Willebrand factor (vWF) [20]), and oxidative stress (F_2-isoprostanes [21]). With the exception of F_2-isoprostanes, all assays were performed by the PathWest Laboratory Medicine Units at Royal Perth and Sir Charles Gairdner Hospitals, using routine collection and analysis procedures. For analysis of F_2-isoprostanes, 5 ml of whole venous blood was collected into cold EDTA tubes containing reduced glutathione and centrifuged as soon as possible at 1000g for 10 min at 4°C. The plasma was protected from oxidation by the addition of butylated hydroxytoluene at a final concentration of 20 μg/ml plasma and stored at −80°C until analysis by gas chromatography/mass spectrometry [22,23]. Blood pressure was assessed using validated [24,25] oscillometric ambulatory blood pressure monitors (Oscar 2, SunTech Medical, Morrisville NC USA) worn by participants for 24 hours after enrolment.

Statistical analysis

Data were analysed using SPSS (version 15, SPSS Inc, Chicago, USA). Non-normal data were log transformed prior to analysis. Analysis of variance (ANOVA) was used to compare means, and chi squares to compare proportions of categorical variables. If overall significant differences in means of continuous variables were confirmed, pairwise comparisons were performed using Tukey's adjustment for multiple comparisons. Correlations between markers of stroke severity, inflammation, endothelial function and oxidative stress were then examined. Significant correlations were further examined with partial correlations to determine if the association was independent of blood pressure. General linear models were then used to determine if significant partial correlations were independent of stroke aetiologic and clinical subtype (by entering TOAST and OCSP classifications as fixed factors, S100B or NIHSS as a covariate, and hs-CRP, fibrinogen, e-selectin, vWF or F_2-isoprostanes as the dependent variable) in general linear models. Missing data were excluded listwise.

The research was approved by the Royal Perth and Sir Charles Gairdner Hospital Ethics Committees. Able participants provided written informed consent. If there was uncertainty regarding the person's ability to provide informed consent, agreement to trial participation was also sought from the person's next of kin.

RESULTS

Cohort characteristics (Table 1)

129 patients with acute ischaemic stroke were recruited to the study, a mean of 63 hours (SD 36 hours, minimum 6 hours, and maximum 192 hours) after the onset of symptoms. The sample was predominantly (69%) male. The mean age was 66 years. Most participants had ischaemic stroke of cardioembolic (33%) or unknown (31%) aetiology. The mean NIHSS score was 7, indicating moderate neurologic impairment. However substantial proportions (46%) of participants had severe handicap (MRS 4-5) at the time of assessment.

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Table 1. Cohort characteristics.

Age (yrs)	66.1±12.7
Male (%)	89 (69%)
Enrolled (Hrs)	62.7±35.8
Hypertension	79 (61%)
Atrial fibrillation	16 (12%)
Hypercholesterolaemia	39 (30%)
Diabetes	27 (21%)
Smoking	52 (40%)
Aetiology	
Large vessel	19 (15%)
Small vessel	25 (19%)
Cardioembolic	43 (33%)
Other	2 (2%)
Unknown	40 (31%)
Clinical syndrome	
Total anterior circulation	19 (15%)
Partial anterior circulation	55 (43%)
Lacunar	34 (26%)
Posterior circulation	21 (16%)
Systolic blood pressure (mmHg)	140.8±19.8
Diastolic blood pressure (mmHg)	79.7±11.1
NIHSS Score	7.1±7.6
Modified Rankin Scale Score	
0–3	67 (54%)
4–5	57 (46%)
Glucose (mmol/L)	6.0±1.6
Homocysteine (umol/L)	10.4±4.1
Hs-CRP (mg/L)	12.2±23.9
Fibrinogen (g/L)	4.2±1.0
E-selectin (ng/mL)	26.4±20.2
vWF activity (%)	157.6±61.6
S100B (ug/L)	0.4±0.7
F_2-isoprostanes (nmol/L)	2.9±3.3
Lesion Area (mm²)	981.3±1336.4

NIHSS – NIH Stroke Scale; hs-CRP – high sensitivity C Reactive Protein; vWF – von Willebrand Factor. Values are n (%) or Mean (±SD).

Clinical syndromes (Table 2)

Participants with severe (total anterior circulation) clinical syndromes tended to be older although the difference between groups was not statistically significant. Systolic blood pressure was lower in participants with a partial anterior circulation (129.3±27.2 mmHg) compared to those with lacunar clinical syndromes (142.0±33.7 mmHg; p=0.01). Total anterior circulation syndrome strokes were associated with

the greatest neurologic impairment, handicap and astroglial injury. Hs-CRP concentrations were higher in total (22.0±24.1 mg/L) than partial (15.3±32.4 mg/L) anterior circulation and lacunar (4.9±4.3 mg/L) syndromes (p=0.01). F_2-isoprostanes tended to be higher in subjects with total anterior and lacunar syndromes, although these differences were not significant

Aetiologic subtypes

Only two people had stroke of an "Other" aetiology. Data for the remaining aetiologic subtypes of acute ischaemic stroke are shown in Table 3. Systolic blood pressure was higher in subjects with small vessel (143.5±38.4 mmHg) compared to cardioembolic (131.2±31.5 mmHg) stroke. Astroglial injury (S100B concentration) was greater in large artery (0.4±0.5 ug/L) and cardioembolic (0.6±0.9 ug/L), compared to small vessel 0.1±0.0 ug/L stroke (p<0.01). Hs-CRP concentration tended to be higher in large vessel and cardioembolic stroke, and there was also a trend to higher F2-isoproastane concentrations in subjects with small vessel stroke, although these differences were not statistically significant.

Correlation between markers of stroke severity, inflammation and endothelial function

Hs-CRP concentration correlated moderately with NIHSS score (r=0.45, p<0.01) and S100B concentration (r=0.48, p<0.01). (Table 4) Von-Willebrand factor activity also correlated fairly with S100B concentration (r=0.26, p=0.01). These associations remained significant after controlling for blood pressure. Fibrinogen appeared to correlate weakly with NIHSS (r=0.19) and S100B (r=0.20), but both relationships were of borderline statistical significance (p=0.052 and 0.047 respectively) and the correlations were no longer significant after controlling for blood pressure. There was no correlation between markers of stroke severity and markers of oxidative stress or endothelial activation. Aetiologic and clinical subtypes were not independently associated with markers of inflammation or endothelial damage when included in general linear models also including markers of stroke severity (Table 5).

DISCUSSION

These data suggest that stroke aetiology and clinical syndrome may not be important independent determinants of the degree of systemic inflammation, oxidative stress and endothelial function in acute ischaemic stroke. The apparent finding that inflammation does vary according to stroke clinical subtype appears to be confounded by stroke severity, given that the aetiologic and clinical subtypes were not associated with the degree of systemic inflammation after control for stroke severity. In addition to stroke severity, systolic blood pressure varies in patients with stroke of different aetiologic and clinical subtypes. Measured blood pressure in the setting of acute stroke reflects pre-morbid blood pressure, but is also influenced by haemodynamic changes related to the acute stroke event [26]. Blood pressure confounded an apparent association between stroke severity and fibrinogen concentrations. Other systemic factors (such as co-morbidity, risk factors, and use of medications) may explain inter-individual variation in the systemic acute inflammatory response, and the degree of

Table 2. Markers of inflammation, oxidative stress and vascular function according to stroke clinical syndrome.

	TACI	PACI	LACI	POCI	p	Significant pairwise
n	19 (15%)	55 (43%)	34 (26%)	21 (16%)		
Age (yrs)	72.9±13	64.9±13.4	64.8±10.5	65.3±13.0	0.09	
Male	14 (74%)	34 (62%)	24 (71%)	17 (81%)	0.40	
Enrolled (Hrs)	63.7±44.6	58.5±33.5	67.6±36.9	64.4±32.5	0.74	
Hypertension	10 (53%)	39 (71%)	21 (62%)	9 (43%)	0.14	
Atrial fibrillation	16 (84%)	8 (15%)	1 (3%)	5 (24%)	0.14	
Hypercholesterolaemia	3 (16%)	19 (35%)	11 (32%)	6 (29%)	0.54	
Diabetes	4 (21%)	13 (24%)	9 (26%)	1 (5%)	0.24	
Smoking	8 (42%)	21 (38%)	15 (44%)	8 (38%)	0.92	

Aetiology
  Large vessel
  Small vessel
Cardioembolic
Other
Unknown
	4 (21%)
	0 (0%)
	12 (63%)
	0 (0%)
	3 (16%)
		8 (15%)
		1 (2%)
		22 (40%)
		2 (4%)
		22 (40%)
			1 (3)
			23 (68%)
			2 (6%)
			0 (0%)
			8 (24%)
				6 (29%)
				1 (5%)
				7 (33%)
				0 (0%)
				7 (33%)
					0.00	

SBP* (mmHg)	145.2±23.0	133.2±18.8	148.0±18.6	143.4±14.8	0.01	PA-L
DBP*(mmHg)	80.5±10.9	76.4±9.9	82.5±11.7	82.8±11.6	0.08	
NIHSS* Score	17.4±8.8	6.6±6.8	4.4±4.2	2.7±1.8	0.00	T-PA, T-L, T-PO, PA-PO

MRS
  0–3
  4–5
	2 (11%)
	17 (89%)
		32 (59%)
		22 (41%)
			20 (67%)
			10 (33%)
				13 (62%)
				8 (38%)
					0.00	

Glucose*mmol/L	6.4±1.8	6.0±1.4	6.0±2.0	5.8±1.3	0.73	
Homocysteine umol/L	8.5±3.2	10.9±4.3	10.3±3.6	11.0±4.6	0.14	
hsCRP* mg/L	22.0±24.1	15.3±32.4	4.9±4.3	6.5±5.8	0.01	T-PA,T-L
Fibrinogen g/L	4.3±1.1	4.2±1.2	4.2±0.8	4.1±0.8	0.96	
E-selectin ng/mL	24.0±19.1	26.0±22.5	29.4±21.4	25.3±13.7	0.85	
vWF%	172.7±59.8	146.8±67.4	168.6±59.2	155.9±9.8	0.35	
S100B* ug/L	1.1±1.2	0.2±0.4	0.1±0.1	0.3±0.4	0.00	T-PA, T-L, T-PO
F_2-Isoprostanes nmol/L	3.9±5.4	2.4±2.8	3.5±3.4	2.1±0.4	0.07	

TACI and T – Total Anterior Circulation Syndrome; PACI and PA – Partial Anterior Circulation Syndrome; LACI and L – Lacunar Syndrome; POCI and PO – Posterior Circulation Syndrome; Fib – fibrillation; Hperchol – hypercholesterolaemia; S/DBP – systolic/diastolic blood pressure; NIHSS – NIH Stroke Scale; MRS – Modified Rankin Scale Score; hsCRP – high sensitivity CRP; vWF – von-willebrand factor activity. Values are n (%) or mean ±SD, * log transformed prior to statistical analyses.

oxidative stress and vascular dysfunction, associated with acute ischaemic stroke. This hypothesis is supported by evidence showing that the presence of diabetes is associated with greater oxidative stress in participants with acute ischaemic stroke [27].

Our study has several strengths. We considered vascular risk factors, age, blood pressure and stroke severity (measured both clinically and biochemically) in addition to markers of

the key variables of interest (stroke subtype, inflammation, vascular function and oxidative stress). Stroke is a complex clinical syndrome and clinically "severe" syndromes are sometime caused by small strategically placed lesions. This was adequately accounted for by controlling for both the degree of neurologic impairment (NIHSS Score) and a marker of brain (atroglial) injury (S100B). However, because stroke severity is also associated with stroke sub-type it could be argued that such models over correct for stroke severity. The

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Table 3. Markers of inflammation, oxidative stress and vascular function according to stroke aetiology.

	LA	SA	Cardioemb	Unknown	p	Significant pairwise
n	19 (15%)	25 (19%)	43 (33%)	40 (31%)		
Age (yrs)	62.6±11.2	63.4±10.0	67.3±13.2	69.1±13.7	0.16	
Male (%)	17 (89%)	17 (68%)	30 (70%)	24 (60%)	0.23	
Enrolled (Hrs)	64.5±42.9	60.7±27.7	60.9±35.2	65.4±38.4	0.97	
Hypertension	12 (63%)	15 (60%)	28 (65%)	24 (60%)	0.44	
Atrial fibrillation	0 (0%)	0 (0%)	15 (35%)	1 (2%)	0.00	
Hypercholesterolaemia	7 (37%)	6 (24%)	13 (30%)	12 (30%)	0.87	
Diabetes	5 (26%)	6 (24%)	7 (16%)	9 (22%)	0.82	
Smoking	10 (53%)	10 (40%)	15 (35%)	16 (40%)	0.80	

OCSP
  TAC
  PAC
  LACS
  POCS
	4 (21%)
	8 (42%)
	1 (5%)
	6 (32%)
		0 (0%)
		1 (4%)
		23 (92%)
		1 (4%)
			12 (28%)
			22 (51%)
			2 (5%)
			7 (16%)
				3 (8%)
				22 (55%)
				8 (20%)
				7 (18%)
					0.00	

SBP* (mmHg)	144.4±18.6	151.4±18.8	135.6±22.4	138.0±15.3	0.02	SA-C
DBP*(mmHg)	79.9±10.7	84.1±11.2	79.0±12.0	77.5±10.0	0.22	
NIHSS*	6.9±6.1	4.0±4.0	9.1±9.5	6.9±7.3	0.30	

MRS
  0–3
  4–5
	11 (58%)
	8 (42%)
		17 (71%)
		7 (28%)
			16 (38%)
			26 (62%)
				23 (61%)
				15 (39%)
					0.06	

Glucose*	5.9±1.7	6.1±2.2	6.0±1.2	6.1±1.6	0.89	
Homocysteine umol/L	9.9±4.9	10.1±3.6	10.2±3.9	10.9±4.3	0.81	
hsCRP*mg/L	11.4±25.4	4.6±4.7	14.1±19.7	15.3±32.8	0.08	
Fibrinogen g/L	4.3±1.1	4.1±0.8	4.2±0.9	4.1±1.2	0.85	
E-selectin ng/mL	22.4±17.0	31.5±19.7	25.4±17.6	26.6±24.9	0.46	
vWF%	163.0±67.3	164.3±56.7	158.8±61.0	149.8±64.7	0.83	
S100B* ug/L	0.4±0.5	0.1±0.0	0.6±0.9	0.3±0.4	0.00	SA-LA,SA-C, C-U
F_2-Isoprostanes nmol/L	2.1±0.4	3.8±3.8	3.3±4.8	2.2±0.9	0.06	

LA – Large Artery; SA – Small Artery; Cardioemb and C – cardioembolic; U – unknown; TAC – Total Anterior Circulation Syndrome; PAC – Partial Anterior Circulation Syndrome; LACS – Lacunar Syndrome; POCS – Posterior Circulation Syndrome; S/DBP – systolic/diastolic blood pressure; NIHSS – NIH Stroke Scale; MRS – Modified Rankin Scale Score; hsCRP – high sensitivity CRP; Fibrin – fibrinogen; vWF – von-willebrand factor activity; F2-Iso – F2-Isporostanes. Values are n (%) or mean +SD, * log transformed prior to statistical analyses.

other major limitations of our study are the cross sectional design, and failure to collect detailed information regarding pre-morbid or acute medical therapy and interventions. Baseline (i.e. pre-ictus) systemic inflammation, oxidative stress and vascular dysfunction among participants were also unable to be measured. These could also be important factors, given the evidence that pre-morbid measures of inflammation are associated with stroke risk [28,29]. The markers we have chosen are not entirely specific (for example fibrinogen is not only a marker of inflammation) and vary over time. Furthermore, there is potential for selection bias as

the participants were drawn from hospital populations, and non-consecutive sample of patients were screened (as our centres recruit to multiple trials simultaneously). Thus the study population may not be representative. The study size may have precluded adequate power to detect small independent associations, and future larger studies are required. These factors prevent any causal inferences being made, and limit the conclusions which can be reached.

Our results differ from those in other studies which have supported the hypothesis that there are important

Table 4. Pearson and partial correlations between astroglial injury/neurologic impairment, and markers of inflammation/ endothelial function.

Variable	S100B Concentration	NIHSS Score
Pearson correlations		
Von Willebrand factor activity	0.26, p=0.01, n=104	0.13, p=0.18, n=101
F_2-isoprostane concentration	−0.00, p=0.99, n=103	−0.12, p=0.22, n=102
Hs-CRP concentration	0.48, p=0.00, n=104	0.45, p=0.00, n=101
Fibrinogen concentration	0.19, p=0.052, n=104	0.20, p=0.047, n=102
E-selectin concentration	−0.06, p=0.58, n=104	0.16, p=0.10, n=101
Partial correlations controlling for 24 hour ambulatory systolic and diastolic blood pressure		
Von Willebrand factor activity	0.34, p=0.00, df=70	0.01, p=0.91, df=70
F_2-isoprostane concentration	0.06, p=0.64, df=70	−0.06, p=0.59, df=70
Hs-CRP concentration	0.48, p=0.00, df=70	0.31, p=0.01, df=70
Fibrinogen concentration	0.20, p=0.09, df=70	0.13, p=0.26, df=70
E-selectin concentration	−0.09, p=0.46, df=70	0.00, p=0.97, df=70

Strength of the association: <0.20 poor, 0.21–0.40 fair, 0.41–0.60 moderate, 0.61–0.80 good, >0.80 very good.

Table 5. General Liner Models (GLM) with hs-CRP and vWF as dependent variables.

	df	F	p
GLM with hs-CRP as dependent variable ($R2=0.38$, Adj $R2=0.26$)			
Corrected Model	16	3.050	0.001
Intercept	1	12.835	0.001
S100B concentration	1	11.955	0.001
NIHSS score	1	9.077	0.003
Aetiologic Subtype	4	0.728	0.576
Clinical Syndrome	3	0.350	0.789
Aetiology*syndrome	7	0.786	0.601
GLM with von Willebrand Factor activity as dependent variable ($R2 = 0.24$ Adj $R2 = 0.10$)			
Corrected Model	16	1.745	0.053
Intercept	1	150.631	0.000
S100B concentration	1	8.555	0.004
Aetiologic Subtype	4	1.070	0.377
Clinical Syndrome	3	2.038	0.114
Aetiology*syndrome	8	1.846	0.079

* Interaction term.

differences in inflammation, oxidative stress or vascular function among different stroke subtypes. Our subjects were recruited a mean of 62 hours after the onset of symptoms. Differences in interval from onset of symptoms to collection of data may thus contribute to apparent differences between studies given that the concentrations of biochemical markers may change over time. Some data suggests that oxidative stress peaks around the third day after stroke [10]. However other data found significant elevations in F_2-isoprostanes only in samples collected in the very early phase (median 6 hours) after ictus [8]. Furthermore, some studies have compared only large and small arterial stroke [4,27]. Inclusion of people with stroke of cardioembolic and uncertain aetiology in our study, and the resulting requirement to use ANOVA (rather than independent samples t-tests), may also contribute to the apparent differences in results between studies.

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CONCLUSIONS

These data suggest that stroke aetiology and clinical syndrome may not be important independent determinants of the degree of systemic inflammation, oxidative stress or endothelial function in acute ischaemic stroke. Other factors, including stroke severity, pre-morbid inflammation and co-morbidity may explain variations among groups of participants with different subtypes of acute ischaemic stroke.

Conflicts of interest

Funding sources and donors had no role in the design or conduct of the study. The authors declare that they have no conflicts of interest or disclosures relevant to this work.

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