

# Differential immunological profiles herald magnetic resonance imaging-defined perioperative cerebral infarction

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## Abstract

**Background:** The perioperative period is associated with a high risk for human ischaemic stroke. Although inflammatory mechanisms are known to have an important role in cerebral infarction in the nonoperative setting, their role in modulating perioperative risk remains unclear.

**Methods:** In this prospective case-control study, we compared 10 patients (cases) who developed magnetic resonance imaging (MRI) evidence of cerebral infarction following transcatheter aortic valve implantation with 10 patients (controls) who underwent the same procedure without neurological complication. Blood sampling was performed preoperatively (baseline) and at 24 h, 48 h and 72 h postoperatively and analysed for specific cytokines, chemokines and complement factors.

**Results:** Baseline serum assessments identified significant differences between the two cohorts for levels of complement C3, complement C4b, granulocyte-macrophage colony-stimulating factor, interleukin-15 and macrophage inflammatory protein-1 $\beta$ . Longitudinal regression analysis and best-fit polynomial curves of postoperative analyte profiles identified significantly higher levels of complement C3 and matrix metalloproteinase-9, and lower levels of interferon- $\gamma$  and macrophage inflammatory protein-1 $\beta$  levels in cases *versus* controls.

**Conclusions:** These results support a potentially important role for inflammatory mechanisms in MRI-defined perioperative stroke and reveal a potentially important role for complement components in this process.

**Keywords:** clinical trials observational study, embolism, immunology, infarction, inflammation, magnetic resonance imaging

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## Introduction

Over the past decades, there have been substantial advances in our understanding of the immunological mechanisms that result from, contribute to, and modify cerebrovascular disease. This has revealed a complex cascade of events, with inflammatory signalling an active and integral participant in all stages.<sup>1</sup> Following ischaemic brain injury, deleterious events such as oxidative stress, neuronal excitotoxicity, blood-brain barrier dysfunction, microvascular injury and postischaemic inflammation transpire. These changes are

orchestrated by a complex interplay between neurons, microglial cells, astrocytes, endothelial cells, and the innate and adaptive immune system, all mediated by synergy between pro-inflammatory prostaglandins, cytokines and chemokines (Figure 1). However, this understanding has been primarily derived from preclinical (predominantly murine) models, with data from human investigations comparatively scarce. Significant immunological differences between these two species are well described,<sup>2</sup> which could explain why the

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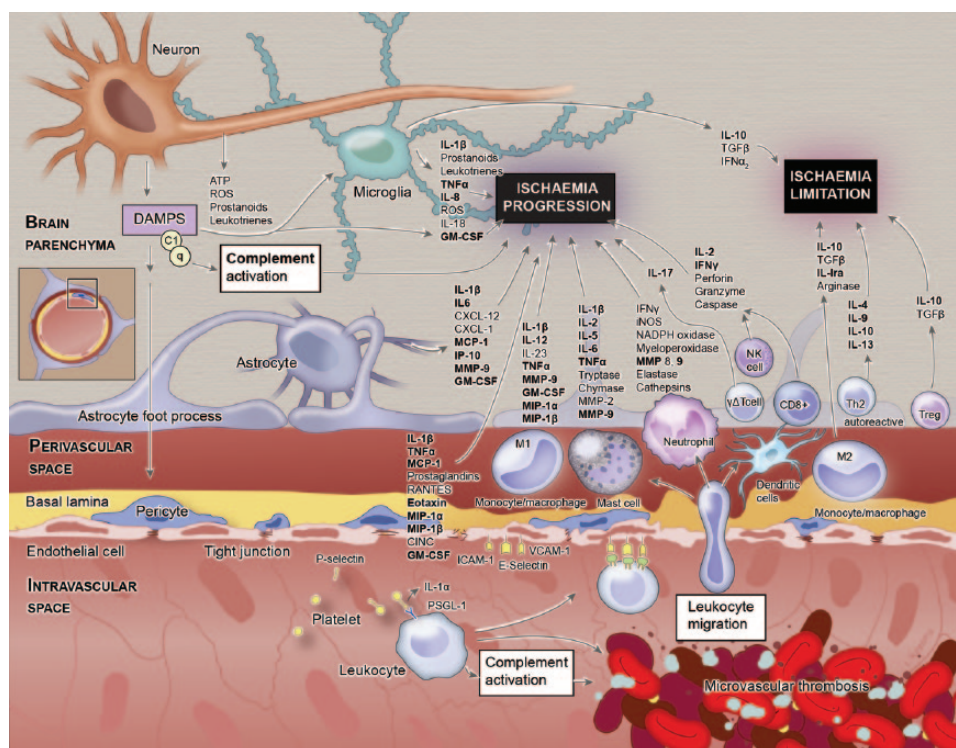
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**Figure 1.** Inflammatory disequilibrium in stroke. The components shown in bold were analysed in this study. (Copyright © 2016 Studio Kayama.)

GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP-10, interferon gamma-induced protein 10; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; MMP-9, matrix metalloproteinase-9; TNF- $\alpha$ ; tumour necrosis factor alpha.

successful translation of novel therapeutic targets to manage ischaemic stroke in humans has, to date, proven elusive.

The limited data specifically from humans show that acute inflammation is a major aetiological factor for ischaemic stroke. For example, acute infection is associated with a subsequent eight-fold increase in repeat stroke risk that persists for at least 3 months.<sup>3</sup> Furthermore, levels of several inflammatory markers are altered in the postischaemic period, and these levels seem to be independently associated with clinical outcome.<sup>4</sup> No such studies have investigated the role of immunological and inflammatory associations with cerebral infarction in the perioperative period.

Unpredictability in the occurrence and timing of stroke in the clinical setting has hampered the study of the subsequent acute response in humans. These issues are largely overcome in the perioperative setting, where there is a high risk of stroke occurring within a relatively short and well-defined timeframe. For example, following the

transcatheter aortic valve implantation (TAVI) procedure that is used to treat severe aortic stenosis, there is a greater than 60% incidence of new cerebral infarcts detected with magnetic resonance imaging (MRI) in postoperative relative to preoperative scans.<sup>5</sup> This injury has been presumed to be primarily embolic in nature but the exact aetiology remains uncertain, as hypoperfusion and inflammation probably also precipitate or exacerbate embolic injury.

Given the important and potentially modifiable role of inflammation in cerebral infarction in non-operative settings, we investigated whether inflammatory mechanisms also modulate the risk for, and occurrence of, peri-interventional cerebral infarction.

## Methods

### Experimental design

A total of 20 patients were prospectively enrolled at The Prince Charles Hospital between March

2014 and October 2016 and included in this an *a priori* prospective case-control sub-study of a larger study assessing perioperative neurological injury in patients undergoing TAVI.<sup>6</sup> All 20 patients underwent TAVI with a SAPIEN-XT valve (Edwards Lifesciences, Irvine, CA, USA) *via* transfemoral access and under standardized general anaesthesia for the management of severe aortic stenosis. Subsequent to the TAVI procedure, consecutive participants with complete blood samples (see below) were grouped according to whether they had postoperative MRI evidence of cerebral infarction (cases), or did not (controls). Patients were excluded from enrolment if any of the following criteria were met: one or more previous cerebrovascular events; any autoimmune or chronic inflammatory disease (including vascular disease); primary or secondary immunodeficiency (from disorders including diabetes, renal failure, liver failure, malignancy and asplenia/hyposplenism); a significant history of atopy or thrombosis; any recent infection; the recent use of immune-modulating or antibiotic medications. This last criterion did not include the dual antiplatelet thromboprophylaxis regimen (aspirin and clopidogrel) that all participants commenced preoperatively, or the heparin/protamine and cephazolin regimen that was routinely administered intraoperatively. Prior to any data collection, protocol approval was granted by the institution's Human Research and Ethics Committee (HREC/12/QPCH/291). All participants provided written informed consent prior to their study participation.

### *Radiological assessment*

MRI examinations were performed at baseline (within the 24 h prior to the procedure) and at day  $3 \pm 1$  postoperatively, using a 1.5 Tesla MAGNETOM Aera (Siemens Healthcare, Erlanger, Germany). Perioperative cerebral infarction was defined as any "new focus of restricted diffusion (high diffusion weighted imaging signal and low apparent diffusion coefficient) occurring in either white or grey matter, located in the cerebrum, cerebellum or brain-stem" on the postoperative MRI scan that was not apparent at baseline.<sup>7</sup>

### *Clinical assessments*

The National Institutes of Health Stroke Scale was used to detect the occurrence of clinically apparent stroke and the modified Rankin Scale

was used to categorize stroke severity. These tests were administered within 24 h prior to the TAVI procedure, and daily for the first 3 days postoperative.

### *Blood sampling and processing*

In the 24 h prior to surgery, and 24 h, 48 h and 72 h postoperatively, blood samples were collected by peripheral phlebotomy and transferred immediately to the in-house laboratory for processing. One 3 ml ethylenediamine tetraacetic acid and one serum separator tube (SST) 5 ml, were processed immediately for 'standard' laboratory blood tests, including a full blood count (haemoglobin, platelets, white cell count and differential), and assessments of renal (serum creatinine and urea) and hepatic function (aspartate and alanine aminotransferase, alkaline phosphatase, gamma-glutamyl transferase, lactate dehydrogenase and albumin). A second SST tube was centrifuged at  $3000 \times g$  for 15 min upon its arrival in the laboratory; the serum then was aliquoted and samples frozen at  $-80^{\circ}\text{C}$  until the time of assay at the completion of subject recruitment.

### *Immunological profiling of serum*

Serum levels of high-sensitivity C-reactive protein were measured using a commercial enzyme-linked immunosorbent assay kit (DE740011, Demeditec Diagnostics GmbH, Kiel, Germany). Measures for the remaining immunological analytes were determined on a Millipore MAGPIX System (Merck, Darmstadt, Germany) using the following dedicated panels, as per the manufacturer's instructions:

- (1) human complement panels (HCMP1MAG19K08 and HCMP2MAG19K02, Merck) were used to measure serum levels of mannose-binding lectin, factor D, factor I, factor B, factor H, properdin and complement components 1q (C1q), C2, C3, C3b/iC3b, C4, C4b, C5, C5a and C9;
- (2) a human cytokine/chemokine panel (HCYTOMAG60K23, Merck) was used to measure levels of the following analytes: interleukin-1 $\beta$  (IL-1 $\beta$ ), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IL-1 receptor-alpha (IL-1R $\alpha$ ), tumour necrosis factor alpha (TNF- $\alpha$ ), interferon alpha-2 (IFN- $\alpha_2$ ), interferon gamma

**Table 1.** Baseline characteristics of study participants.

Variable	Controls (n = 10)	Cases (n = 10)
<b>Patient characteristics</b>		
Age, years	83.3 ± 9.4	84.2 ± 3.2
Female	4 (40%)	5 (50%)
Body mass index, kg/m <sup>2</sup>	31.4 ± 8.4	27 ± 5.9
EuroSCORE II, %	5.9 ± 2.6	5.1 ± 3.1
STS, %	6.6 ± 3.6	5.5 ± 2.7
Arteriovenous anastomosis, cm <sup>2</sup>	0.8 ± 0.1	0.8 ± 0.1
Peak jet velocity	4.3 ± 0.4	4.4 ± 0.6
Mean pressure gradient	49.2 ± 11.4	47 ± 16.2
Pre-operative left ventricular ejection fraction	58.4 ± 9.2	51.3 ± 16.7
<b>Procedural characteristics</b>		
Device success	10 (100%)	10 (100%)
Average procedure time, min	93 ± 68	61 ± 13
STS, Society of Thoracic Surgeons.		

(IFN- $\gamma$ ), interferon gamma-induced protein 10 (IP-10) also known as C-X-C motif chemokine 10 (CXCL10), monocyte chemoattractant protein 1 (MCP-1) also known as chemokine C-C motif ligand 2, macrophage inflammatory protein 1 alpha (MIP-1 $\alpha$ ), macrophage inflammatory protein 1 beta (MIP-1 $\beta$ ), eotaxin and granulocyte-macrophage colony-stimulating factor (GM-CSF);

- (3) a human matrix metalloproteinase-9 (MMP-9) bead panel (HMMP2MA-G55K01, Merck) was used to measure serum levels of MMP-9.

### Statistical analysis

Group means and 95% confidence intervals (CIs) were calculated for all baseline variables. Student's *t* test was performed to determine the statistical significance of any difference between MRI-positive cases and MRI-negative controls. As the kinetic profile is different for each analyte and the exact sampling time varied for each participant, changes from baseline were plotted as changes in concentration *versus* time. A smoothing model was then used to select the best-fitting polynomial curve over time for cases *versus* controls. Six polynomial curves were tested for each group and the best

combination chosen using the selecting model with the smallest deviance (best fit to the data). The best fit was plotted using the mean and 95% CI. For each variable, inferential testing to identify statistically significant mean differences in the two curves was performed at the 24 h postoperative time point. As a last step, longitudinal regression was used to test for overall differences between cases and controls over all time points. A random intercept was used to account for repeated results from the same patient. The dependent variable of interest was, once again, change from baseline (time 0), to either three or four follow-up time points (depending on the duration of follow up). Three regression parameters were determined: (a) the measured difference between those who had new cerebral infarction *versus* those who did not; (b) a linear time trend, scaled to 10 h (used to control for any general change over time in all patients); (c) the constant (or intercept), which is the estimated value at time zero for all patients (a positive value indicates that values generally increased from baseline).

## Results

### Baseline data

**Patient and procedural characteristics.** Of the 20 patients enrolled, 10 had postoperative MRI



evidence of cerebral infarction (cases), whereas 10 did not (controls). Baseline patient and procedural characteristics for cases and controls are summarized in Table 1. No significant differences in either patient or procedure characteristics were identified that might have confounded subsequent comparisons between groups.

**Radiological assessments.** The participants with MRI-defined cerebral infarction typically had several lesions with a median  $\pm$  interquartile range of  $3 \pm 3$  (95% CI 1–8) lesions/participant and a volume of  $197 \pm 776$   $\mu$ l/participant (data not shown). Where perioperative cerebral infarcts were sustained, 83% of the volume of infarction occurred within the cortical grey matter, with just 2% in subcortical grey matter, 4% in subcortical white matter, and 9% within the cerebellar cortex.

**Clinical assessments.** No clinically apparent symptoms of stroke were evident from the serial neurological assessments.

**Blood and serum assessments.** Baseline blood and serum assessments are summarized in Table 2. Note that no statistically or clinically significant differences were apparent in any blood cell counts or any marker of renal or liver function. Significant differences between MRI-positive (cases) and MRI-negative (controls) were identified for serum levels of complement C3 ( $38 \pm 22$  versus  $73 \pm 40$ , respectively;  $p = 0.005$ ), complement C4b ( $4011 \pm 783$  versus  $2357 \pm 804$ ;  $p = 0.048$ ), GM-CSF ( $0.98 \pm 0.35$  versus  $0.56 \pm 0.25$ ;  $p = 0.009$ ), IL-15 ( $5.42 \pm 6.7$  versus  $2.575 \pm 0.15$ ;  $p = 0.047$ ) and MIP-1 $\beta$  ( $39 \pm 32$  versus  $14.9 \pm 6.50$ ;  $p = 0.038$ ).

### Postoperative changes

**Longitudinal regression.** The three regression parameters (difference, time and constant/intercept) are presented for each variable in Supplementary Table 1. Of note, C3 (mean difference 25.79; 95% CI 1.19–42.24) levels were significantly higher ( $p < 0.05$ ) whereas IFN- $\gamma$  (mean difference  $-14.17$ ; 95% CI  $-27.8$  to  $-0.57$ ) and MIP-1 $\beta$  levels (mean difference  $-10.6$ ; 95% CI  $-20.67$  to  $-0.47$ ) were significantly lower ( $p < 0.05$ ) postoperatively over time in the MRI-positive group than in the control group.

**Smoothing model analysis.** The best-fit polynomial curves for the change from baseline data for

select analytes of interest are presented in Figure 2, with the remainder available in Supplementary Figure 1. Results of inferential testing performed 24 h postoperatively are shown in Table 3. At this time point, significantly higher levels of MMP-9 (mean difference 72,435; 95% CI 6401–135,310;  $p = 0.0272$ ) and complement C3 (mean difference 25.9; 95% CI 4.46–47.8;  $p = 0.0288$ ) were identified in MRI-positive cases, whereas platelets (mean difference  $-26.7$ ; 95% CI  $-52.3$  to  $-3.74$ ;  $p = 0.0272$ ), and IFN- $\gamma$  (mean difference  $-14.3$ ; 95% CI  $-27.5$  to  $-2.1$ ;  $p = 0.0288$ ) were lower.

### Discussion

In this study, we tested serum from a select group of prospectively enrolled TAVI patients during the perioperative period for a panel of immune markers, including cytokines, chemokines and complement components that have been implicated in the inflammatory response to ischaemic stroke.<sup>8</sup> Serum levels from patients with MRI-documented cerebral infarction were compared with those from patients with no evidence of infarction. Over and above the inflammatory changes seen across the entire cohort, secondary to underlying aortic stenosis and the perioperative period, we detected significant differences in baseline levels of complement C3 and C4b, as well as cytokines GM-CSF, IL-15 and MIP-1 $\beta$ . Postoperatively, there were significant differences in serum MMP-9, complement C3, IFN- $\gamma$  and MIP-1 $\beta$  between patients who sustained cerebral infarction and those who did not.

Over the past decade, matrix metalloproteinases, especially MMP-9, have been widely investigated for their role in the acute pathogenesis of blood–brain barrier disruption, vasogenic oedema and haemorrhage transformation following stroke.<sup>9–11</sup> Immunohistochemical analysis of human stroke tissue has shown an overproduction of MMP-9 during the early and pro-inflammatory phases of stroke, as well as an increase in the number of neutrophils (the primary source of MMP-9), for up to 1 week post-stroke.<sup>12</sup> MMP-9 has also been investigated as a biomarker for stroke, as it is measurably elevated in the serum of stroke patients, correlates positively with infarct volume, and is an effective marker of worse outcomes.<sup>13</sup> Peak levels of MMP-9 generally occur between 12 h and 24 h after the stroke, but elevated levels persist for up to 5 days.<sup>9</sup> These data are consistent with these present results for the perioperative

**Table 2.** Summary statistics for baseline blood analyte measurements in cases *versus* controls.

Analyte	Controls		Cases	
	Mean	SD	Mean	SD
<b>Routine laboratory tests</b>				
White blood cell count	6.820	2.205	6.460	2.667
Neutrophils	4.371	2.227	3.892	2.245
Lymphocytes	1.646	0.413	1.717	0.467
Monocytes	0.582	0.124	0.565	0.185
Creatinine	101.300	33.330	107.400	28.640
Haemoglobin	126.700	10.478	121.600	12.076
Platelets	173.300	60.369	201.200	51.441
C2	5212.300	1413.629	8723.800	5881.982
C4b*	3256.600	803.701	4010.560	783.449
C5	90,399.900	33,301.216	1,11,882.100	59,453.757
C5a	4912.400	1177.679	5797.600	1877.560
C9	4278.200	1147.436	3867.500	1123.123
Factor D	1120.800	11.361	1125.200	11.361
MBL	3527.278	4822.419	3443.722	3548.954
C1q	44.723	14.821	39.727	18.430
C3**	72.862	39.904	38.026	22.024
C3b/iC3b	350.452	125.924	428.461	242.295
C4	544.511	199.553	459.482	136.783
Factor B	147.966	64.978	117.032	37.082
Factor H	313.943	86.472	266.233	93.867
Factor I	11,162.800	65.583	11,188.200	65.583
Properdin	18.307	6.733	17.135	8.379
<b>Cytokines/chemokines</b>				
Eotaxin	134.869	53.173	164.066	48.438
GM-CSF**	0.559	0.247	0.975	0.348
IFN- $\alpha_2$	3.922	1.153	43.972	89.830
IFN- $\gamma$	3.170	1.613	22.689	29.608
IL-10	6.121	5.843	6.338	6.361
IL-12p40	4.790	1.110	23.614	37.595
IL-12p70	2.914	0.201	13.216	32.658
IL-13	6.597	10.657	97.719	275.431
IL-15*	2.575	0.145	5.420	6.703
IL-17	2.466	0.134	5.281	6.155

**Table 2.** (continued)

Analyte	Controls		Cases	
	Mean	SD	Mean	SD
IL-1 $\alpha$	65.631	175.526	144.218	358.130
IL-1b	2.970	0.129	4.944	6.454
IL-2	1.791	0.987	10.513	17.697
IL-4	7.150	5.637	27.545	58.181
IL-5	2.394	1.299	33.162	92.903
IL-6	6.007	5.886	7.344	9.446
IL-7	2.458	1.627	9.904	16.032
IL-8	15.445	14.992	13.820	17.883
IP-10	509.487	288.678	486.979	235.094
MCP-1	627.069	139.342	562.416	269.159
MIP-1 $\alpha$	3.671	1.046	10.757	11.650
MIP-1 $\beta$ *	14.902	6.516	39.008	32.052
TNF- $\alpha$	12.716	7.090	17.411	12.572
<b>Other inflammatory markers</b>				
MMP-9	1,95,266.800	95,023.810	1,52,542.000	85,619.562
hs-CRP	5484.9	5035.4	2075.2	2362.8

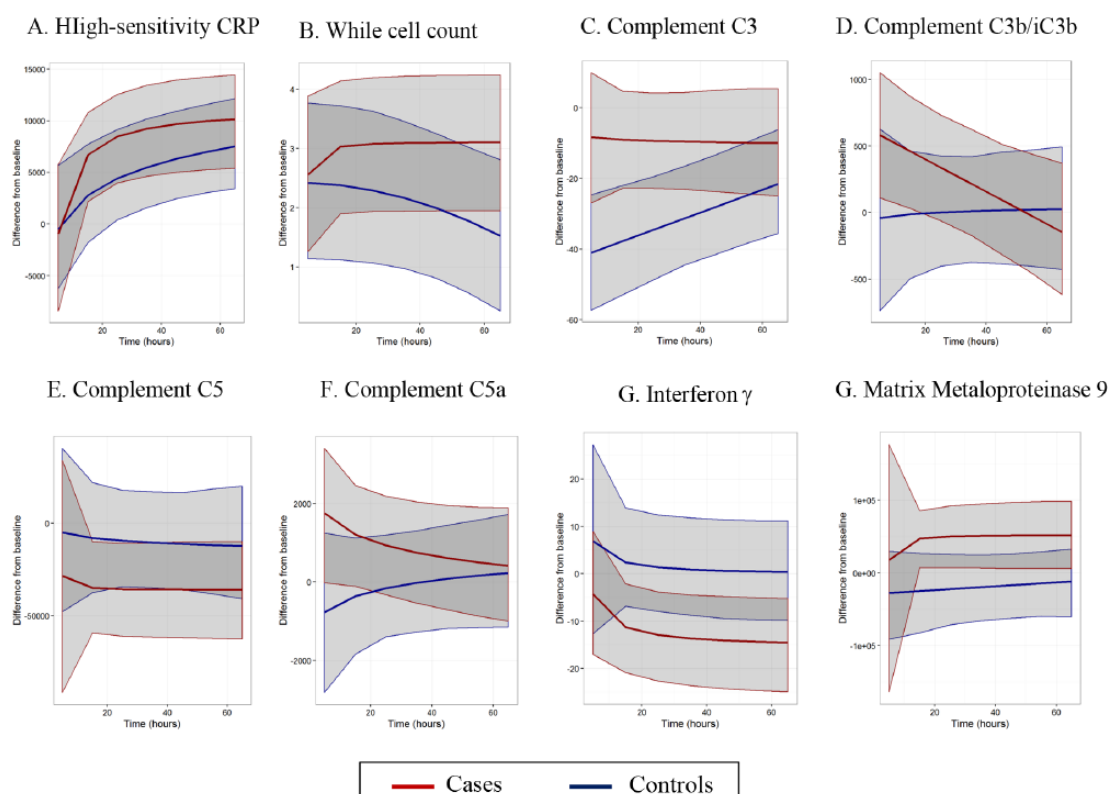
\* $p < 0.05$ ; \*\* $p < 0.001$ . hs-CRP, high-sensitivity C-reactive protein; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP-10, interferon gamma-induced protein 10; MBL, mannose-binding lectin; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; MMP-9, matrix metalloproteinase-9; SD, standard deviation; TNF- $\alpha$ , tumour necrosis factor alpha.

period, which show significantly higher serum MMP-9 levels at 24 h postoperatively in those who sustain cerebral infarction.

Similarly, our data on changes in the perioperative complement profile agree with previously published findings in nonoperative settings. The complement system is a primordial and highly conserved component of the innate immune response to specific pathogen- and damage-associated molecular patterns, and accounts for approximately 10% of the globulin fraction within serum.<sup>14,15</sup> It has multiple neurological functions in both health (e.g. elimination of synapses from maturing and axotomized neurons, neurogenesis and neuroprotection) and various disease states, and is implicated in the inflammatory disequilibrium and cerebral tissue damage that occurs during ischaemic stroke.<sup>1,16,17</sup> In acute ischaemic stroke, complement activation results in the release of pro-inflammatory cytokines, leucosequestration,

opsonization and membrane attack complex deposition on damaged and surrounding cells within the ischaemic penumbra (Figure 3).

As the central component for all activating pathways, changes in C3 levels can be considered a surrogate marker that reflects the global status of complement function. Our detection of higher C3 levels in the postoperative period among our cerebral infarction cohort is therefore noteworthy (Figure 2c). In fact, this positive difference reflects a less marked drop from baseline in the cerebral infarction group than in controls. Concurrently, on visual inspection of the polynomial curves, complement C3b/iC3b, the immediate by-product of C3 breakdown, is substantially elevated from baseline in cerebral infarction cases *versus* controls during the early postoperative phase (Figure 2d). The postoperative drop in C5 was more marked in the cases *versus* control group (Figure 2e), with corresponding separation between the two curves early postoperatively for the C5 by-product C5a



**Figure 2.** Cases *versus* controls polynomial best-fit curves for select analytes.

**Table 3.** Results of 24 h significance testing for differences between groups extracted from the polynomial curve of best-fit smoothing models.

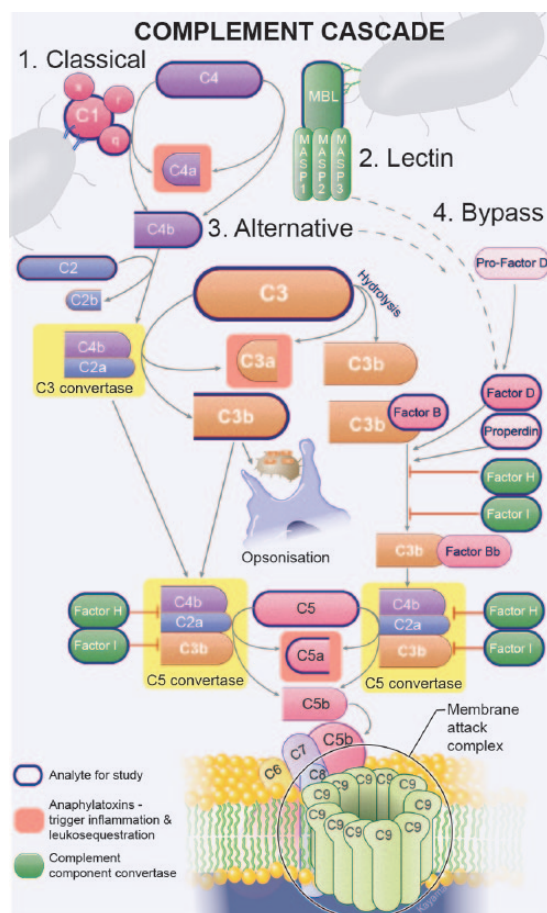
Analyte	Difference between groups	
	Mean difference	95% confidence interval
<b>Routine laboratory tests</b>		
White blood cell count	0.711	-1.67, 3.1
Neutrophils	0.318	-2.27, 2.93
Lymphocytes	0.0238	-0.477, 0.504
Monocytes	0.0504	-0.325, 0.399
Creatinine	-0.604	-17, 15.3
Haemoglobin	1.98	-7.55, 11
Platelets*	<b>-26.7</b>	<b>-52.3, -3.74</b>
<b>Complement cascade components</b>		
C2	-1028	-6632, 4740
C4b	-766	-2311, 766
C5	-26,221	-62,308, 9688
C5a	1131	-653, 2986
C9	210	-364, 793
Factor D	12.8	-26.5, 52.9
MBL	-427	-3089, 2298



**Table 3.** (continued)

Analyte	Difference between groups	
	Mean difference	95% confidence interval
<b>C1q</b>	1.62	-13.1, 16.3
<b>C3*</b>	<b>25.9</b>	<b>4.46, 47.8</b>
<b>C3b/iC3b</b>	352	-232, 958
<b>C4</b>	23.4	-118, 159
<b>Factor B</b>	14.8	-38.9, 68.9
<b>Factor H</b>	18	-53.4, 90
<b>Factor I</b>	394	-233, 1011
<b>Properdin</b>	-1.32	-7.17, 4.47
<b>Cytokines/chemokines</b>		
<b>Eotaxin*</b>	<b>-36.8</b>	<b>-74.2, 0.384</b>
<b>GM-CSF</b>	-6.8	-27.7, 14.1
<b>IFN-<math>\alpha_2</math></b>	-17.7	-65, 30.8
<b>IFN-<math>\gamma</math>*</b>	-14.3	-27.5, -2.1
<b>IL-10</b>	16.7	-9.57, 44.2
<b>IL-12p40</b>	-8.06	-24.6, 8.44
<b>IL-12p70</b>	-4.85	-17.8, 8.01
<b>IL-13</b>	-20.6	-47.9, 6.58
<b>IL-15</b>	-1.41	-4.73, 1.98
<b>IL-17</b>	-0.767	-1.84, 0.364
<b>IL-1<math>\alpha</math></b>	19.2	-51.4, 86.5
<b>IL-1b</b>	-1.25	-4.04, 1.68
<b>IL-2</b>	-6.96	-15.3, 1.58
<b>IL-4</b>	-5.45	-25.9, 16.1
<b>IL-5</b>	-7	-16, 2.42
<b>IL-6</b>	25.8	-28.9, 84.1
<b>IL-7</b>	-6.51	-13.7, 0.943
<b>IL-8</b>	5.82	-3.05, 14.3
<b>IP-10</b>	-4.05	-213, 213
<b>MCP-1</b>	-13.6	-153, 131
<b>MIP-1<math>\alpha</math></b>	-2.67	-5.76, 0.473
<b>MIP-1<math>\beta</math></b>	-8.25	-18.8, 2.43
<b>TNF<math>\alpha</math></b>	-1.45	-5.76, 2.87
<b>Other inflammatory markers</b>		
<b>hs-CRP</b>	3261	-2924, 870
<b>MMP9*</b>	<b>72,435</b>	<b>6401, 1,35,310</b>

\* $p < 0.05$ . hs-CRP, high-sensitivity C-reactive protein; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFN, interferon; IL, interleukin; IP-10, interferon gamma-induced protein 10; MBL, mannose-binding lectin; MCP-1, monocyte chemoattractant protein 1; MIP, macrophage inflammatory protein; MMP-9, matrix metalloproteinase-9; TNF- $\alpha$ , tumour necrosis factor alpha.



**Figure 3.** Overview of the complement cascade. (Copyright © 2016 Studio Kayama.)

(Figure 2f). Together, these observations support a distinct pattern of complement activation associated with perioperative cerebral infarction.

Despite the appearance of the polynomial curves, statistically significant differences were only evident for the C3 component of the complement system. However, this is not surprising given the short *in vivo* half-lives of complement activation products. For example, C5a has a half-life of approximately 1 min, due to rapid receptor binding, and although C3 activation product kinetics are slightly more favourable, they are still short-lived and their half-life of 2–3 h is much less than the 24 h sampling intervals used in this study.<sup>18,19</sup> A more sensitive indicator of *in vivo* activation would therefore be a reduction in native component levels rather than the activation products alone.<sup>20,21</sup> Consistent with this, we found that the aforementioned differences in complement components between cerebral infarct and control groups predicted by the best-fit polynomial curve

smoothing model were most evident at the earliest time points.

Interestingly, the general pattern of expression for the cytokines/chemokines in this study was an initial transitory rise from baseline measured in the cerebral infarct *versus* control group, followed by a rapid reduction, often with levels dropping markedly lower than baseline levels. Three important exceptions to this were the pro-inflammatory cytokines IL-6 and IL-8, and the anti-inflammatory cytokine IL-10, for which cerebral infarct patients averaged more positive values than controls. Longitudinal regression analysis showed that the observed changes were statistically significant only for IFN- $\gamma$  and MIP-1 $\beta$ , and only for IFN- $\gamma$  at the 24 h time point in the smoothing model.

This pattern of expression and the notable drop in pro-inflammatory cytokines were unexpected, given that these markers are typically elevated following ischaemic stroke in preclinical models.<sup>1</sup> However, such inconsistencies have been a common theme among human studies in this field, with the kinetic profiles of key pro-inflammatory cytokines often producing conflicting results. Although a small number of studies have demonstrated elevated levels of TNF- $\alpha$  and IL-1 $\beta$  in patients following ischaemic stroke,<sup>22,23</sup> others have detected no change.<sup>24–27</sup> IL-6 elevation is well documented but the timing of this elevation has varied widely, with peaks reported as early as 10 h and as late as 7 days post-stroke.<sup>26,28,29</sup> In addition to their circulation half-life, factors that might influence the serum levels of these and other cytokine/chemokines assessed in this study include their location of expression, their presence in cerebrospinal fluid, disruption of the blood–brain barrier, extra-cranial release and a systemic acute phase response (including a hepatic chemokine response).

In addition to the changes we observed subsequent to cerebral infarction, our baseline results indicate key differences between those who ultimately would go on to sustain infarction and those who would not, suggesting potential risk factors or predictors of their occurrence. The most significant baseline differences, both statistically and biologically, were evident for C3 (case mean:  $38 \pm 22$  *versus* control mean:  $73 \pm 40$ ;  $p = 0.005$ ) and for GM-CSF (case mean:  $1 \pm 0.3$  *versus* control mean:  $0.6 \pm 0.2$ ;  $p = 0.009$ ).

As discussed above, complement factor C3 is both one of the most common blood proteins and the central component of the complement cascade (Figure 3). We observed baseline levels of C3 that were approximately 50% less in patients who go on to sustain cerebral infarction following TAVI compared with those who do not. Low C3 has been attributed to both depressed synthesis and increased consumption/hypercatabolism.<sup>21</sup> Several autoimmune diseases have been implicated in acquired deficiencies of complement, secondary to accelerated consumption by immune complexes (e.g. systemic lupus erythematosus, antiphospholipid antibody syndrome, cryoglobulinaemia, various vasculitides). However, the rarity of these disorders means this is an unlikely explanation for the observations made in this study. C3 consumption may also result from coagulation and a prothrombotic state. Several interactions exist between the complement and coagulation cascades, including, most appreciably, platelet-mediated immune activation (Figure 1). This is most apparent clinically in the established anti-inflammatory effects of many of the antiplatelet medications, for example, clopidogrel.<sup>30</sup> They are also both inextricably linked in the pathobiology of the response to cerebral ischaemic infarction.<sup>1</sup> Indeed, during the postoperative period, platelet counts dropped significantly more in the cerebral infarction group, suggesting thromboses and increased consumption of C3 (the mean case-control difference on longitudinal regression analysis was  $-26.7$ ; 95% CI  $-52.3$  to  $-3.74$ ;  $p = 0.027$ ). Consumption of C3 might also be expected to result in comparatively low levels of C4 in those with cerebral infarction. Although this reduction was evident in the cerebral infarction group (mean:  $459 \pm 137$  versus control mean:  $545 \pm 199$ ), this difference was not statistically significant ( $p = 0.281$ ). Usually, C3 levels are three- to six-fold higher than C4 levels.<sup>14,15</sup> Therefore, the same percentage change in both (as would occur with consumption) could reduce C3 to a statistically significant extent, but not C4. Supporting this theory of baseline complement hyper-catabolism/consumption, the C4 activation product C4b was significantly higher in the cerebral infarction group (mean:  $4011 \pm 783$  versus control mean:  $3257 \pm 804$ ;  $p = 0.048$ ).

Several factors with the potential to alter a patient's inflammatory status were present in our study cohort. First, inflammation is a key propagator of calcific aortic stenosis.<sup>31</sup> Complement activation probably plays a critical role, and has

been demonstrated in stenotic aortic valves leading to increases in pro-inflammatory cytokines IL-6, IL-8 and MCP-1.<sup>32</sup> Furthermore, the myocardium has been shown to be a major source of pro-inflammatory cytokines; as such, the increased left ventricular mass common in patients with severe aortic stenosis might also be a contributing factor.<sup>33</sup> However, no differences existed between the aetiology (non-rheumatic, degenerative calcific) or severity of aortic stenosis between our two subject groups, or in their pre-treatment health status. Second, all patients were on dual antiplatelet therapy (aspirin and clopidogrel) and statins, both of which have anti-inflammatory properties. However, this was consistent across the entire cohort of assessed patients and, as such, is unlikely to have confounded comparisons between the two groups.

Although we were able to detect some notable immunological differences between the MRI-positive and MRI-negative groups, our study was limited by the fact that we sampled blood every 24 h and for only 3 days of follow up, which could have resulted in peak serum level changes from baseline being missed. In addition, our study exclusively recruited a homogeneous group of highly selected patients limiting extrapolation of these findings to other settings (both operative and nonoperative). Furthermore, all cerebral infarcts considered were iatrogenic and subclinical limiting extrapolation of data to out-of-hospital clinically apparent stroke. Moreover, although the timing of cerebral infarction in this setting is assumed to be predominantly intraprocedural, the exact timing remains unknown. It is possible that infarcts occurred at any time between the patients' baseline MRI scan and the day 3 post-procedure follow-up scan. Finally, the relatively small sample size of just 10 subjects per group is also likely to have limited our ability to detect significant intergroup differences.

## Conclusion

This study provides evidence for notable differences in the complement activation and cytokine/chemokine responses associated with perioperative cerebral infarction. The significant baseline and postoperative differences in complement C3 between those who sustained cerebral infarction and those who did not is a particularly major finding, especially as this protein constitutes a major fraction of total blood protein. Our results should inspire further investigation focused on these

identified complement and cytokine/chemokine signals and their potential to predict, detect and modulate neuroinflammation after perioperative cerebral infarction.

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### Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

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