Author’s response to reviews

Title: Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome.

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Dear Editor

RE: Peak plasma interleukin-6 and other peripheral markers of inflammation in the first week of ischaemic stroke correlate with brain infarct volume, stroke severity and long-term outcome (manuscript number: 3855307812132361)

Thank you for your recent e-mail regarding the above manuscript. We are very grateful for the reviewers’ interesting and constructive comments. We have carefully considered all of the comments, and have responded or modified the manuscript accordingly, whilst attempting to keep the manuscript concise.

Reviewer 1

Discretionary revisions

2. The relationship between inflammatory markers and outcome measures was determined using Spearman rank correlation. This statement has been added to the Statistical analysis section, page 8.

Major compulsory revisions

1. The relatively small sample size in our study was mainly limited by our rigorous study design, explained as follows. Most previous studies performed a single measurement of plasma IL-6/CRP within the first 24 to 72 hours after symptom onset and related this to outcome. We believe that a particular strength of our study design was to recruit stroke patients early after symptom onset within 12 hours, and perform serial plasma sampling in order to assess the magnitude of the inflammatory response (peak) in individual patients. Patients were not recruited if the time of symptom onset could not be reliably determined, or if there was any improvement in symptoms, which may have indicated a TIA, thereby excluding a large proportion of consecutive patients screened.
As discussed in the manuscript, our sample size precluded the use of multifactorial analysis to determine whether there was an independent relationship between peak plasma IL-6 and infarct volume or mRS. To address this issue using our current study design, and taking into account multiple variables affecting outcome after stroke (e.g., age, ethnicity, sex, stroke severity, blood pressure, blood glucose, previous stroke, diabetes, atrial fibrillation, coronary artery disease), would require approximately 200-300 patients recruited within 12 hours, with serial plasma samples. However, we feel we have been robust in defining clear primary analyses with respect to peak plasma IL-6, and have drawn appropriate conclusions from the data. We did not measure fibrinogen, but did measure several other markers of the acute phase response that were considered in secondary analyses.

2. Five to seven day plasma sampling and CT brain scans were performed on the same day, except in five cases where plasma was sampled, and CT scans performed, within 24 hours of each other. This statement has been added to the Measurement of CT brain infarct volume section, page 7.

3. We appreciate the reviewer's concern regarding measurement of infarct volume. We evaluated the inter-observer and intra-observer agreement of our semi-automated technique using the method of Bland and Altman (Lancet 1986, 1: 307-310), which has been submitted as a separate manuscript (Gavin et al, 2003, submitted for publication). As the limits of agreement between observers were generally wide, infarct volume was expressed as a mean of the observers' independent measurements.

4. Of the 28 patients with infarct volumes measured, 6 had discrete areas of previous infarction when admission and 5 to 7 days scans were compared. Of these, 2 had ipsilateral, 2 contralateral and 2 bilateral areas of previous infarction. The presence of previous infarction may well confound infarct volume measurements, although in our experience, definition of the recent infarct perimeter was the main factor consistently limiting reliability between observers.

The mRS and BI were also measured at presentation (for the four weeks preceding the index stroke), in addition to 3 months and 12 months. This has been explained further by modification of the Assessment of stroke severity and outcome section, page 6. The median presentation mRS and BI data have been added to Table 2.

5. The kinetics of plasma IL-6, plasma cortisol, ESR, peripheral white blood count and aural temperature, compared to controls matched for age, sex and degree of atherosclerosis have been presented elsewhere (Emsley et al, J Neuroimmunol 2003, 139: 93-101).

6. Ischaemic stroke patients with atrial fibrillation were treated according to UK National Guidelines (http://www.rcplondon.ac.uk/pubs/books/stroke/index.htm).

7. In Table 2, the scores for NIHSS, BI or mRS (non-linear, ordinal scales) are the medians, with the minimum and maximum values in brackets, for the survivors at each timepoint. The NIHSS score was formulated specifically to measure neurological impairment in patients with acute stroke. However, the BI and mRS were not originally devised specifically for stroke patients, and measure performance in activities of daily living, and independence respectively. In the present study, NIHSS score in survivors at 3 months correlated strongly (p<0.001) with BI at 3 months (r = -0.80), or mRS a 3 months (r = 0.79). Furthermore, NIHSS score in survivors at 12 months correlated strongly (p<0.001) with BI at 12 months (r = -0.87), or mRS at 12 months (r = 0.75). These data can be incorporated into the results section if required.

Reviewer 2

We are grateful for the very positive response from this reviewer.
Discretionary revisions

1. We acknowledge the interesting study by Kostulas et al (1999) which reported a correlation between peripheral blood IL-1, IL-8 or IL-17 mRNA expression, and neurological impairment in acute stroke patients. As our present manuscript focuses on plasma IL-6 and other acute phase markers in plasma, rather than cytokine production, we didn't feel it was appropriate to add reference to this here. However, as discussed further below, we do have a manuscript in preparation which focuses on peripheral cytokine mRNA and protein production. The study by Kostulas et al (1999) will clearly be important to include when these data are reported.

Major compulsory revisions

1. Please see the response to reviewer 1, Major Compulsory Revisions, point 1.

2. Elispot and flow cytometry measure cytokine expression by peripheral blood cells. Our supposition was that cytokines were either being produced in the central nervous system, unless they represented a peripheral focus of inflammation in tissues. Data from Kostulas et al (1999), did suggest cytokines were produced by peripheral blood cells. However, our own data (in preparation), suggest that unstimulated blood cells from acute stroke patients produce little cytokine protein (ELISA) or cytokine mRNA (quantitative PCR), although this production is massively enhanced in the presence of LPS.

3. Plasma IL-6 was studied as the primary measure since it is induced by the proinflammatory cytokines IL-1 and TNF, is a major stimulus of the acute phase response, and can be reliably measured in plasma. Although we did measure several other plasma cytokines, including TNF, IL-1, their soluble receptors, leptin and IL-10, we were cautious not to invalidate statistical analysis by performing multiple primary analyses. These other measures, along with the cytokine production data, are to be reported elsewhere.

We appreciate the opportunity to improve our manuscript by addressing the points raised by the reviewers and hope we have adequately dealt with the issues raised.

Sincerely

Dr Craig J. Smith