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IL-6 is a predictive biomarker for stroke associated infection and future mortality in the elderly after an ischemic stroke

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ABSTRACT

Background and purpose: Stroke associated infection (within the first seven days) occurs in approximately half of stroke patients and is associated with a worse prognosis, especially in the elderly. It is uncertain what factors predict stroke associated infection, yet identification of a suitable biomarker for infection may allow early and appropriate intervention with antibiotics. The aims of this study were to: a) identify independent risk factors for stroke associated infection, and b) test relationships between these risk factors and mortality at 2 years.
Methods: Eight-two elderly patients were assessed within 72 h of stroke. Data on stroke severity (Barthel Index), stroke associated infection and mortality at 2 years were collected. Inflammatory biomarkers at baseline and 6 months were measured by ELISA. Logistic regression was used to identify risk factors for stroke associated infection and death.
Results: Patients with stroke associated infection, especially pneumonia, had increased IL-6, more severe strokes, and higher mortality. IL-6 was independently associated with stroke associated infection (OR = 19.2, [95%CI 3.68, 100], $p < 0.001$), after adjustment for other risk factors and cytokines. IL-6 was also independently associated with 2 year mortality (OR = 9.2, [1.0, 85.1], $p = 0.031$).
Conclusions: These data suggest that IL-6 may be a key biomarker for predicting stroke associated infection and mortality in the first two years post stroke.

1. Introduction

1.1. Why do we need a biomarker to predict stroke associated infection?

The main objective of this study was to identify one or more biomarkers to enable the early prediction of stroke associated infection. The effects of stroke-associated infections (SAI) on short-term outcome are well known, increasing the risk of early death and institutionalization, especially in the elderly. For example, patients with a SAI were three times more likely to die within the first 5 days compared to those who did not have a SAI (Kwan and Hand, 2007). The effect of SAI on long term outcome was demonstrated more recently in a prospective study which showed that the risk of dying over 3 years was significantly higher in patients who had a SAI in the first 2 weeks after a stroke (Kwan et al., 2013). Despite the detrimental effects of SAI, there is no easy way of predicting which patients will develop an infection, or which patients are at increased risk of mortality. Previous studies have demonstrated several parameters associated with SAI, including stroke severity and use of nasal gastric tubes. However, if a simple, quick and inexpensive test to measure one or more biomarkers could be developed to identify those patients most at risk, this may allow early and appropriate intervention with antibiotics. The effect of prophylactic antibiotic use in SAI is currently under investigation (Harms et al., 2008; Nederkoorn et al., 2011; Vargas et al., 2006) and therefore identification of appropriate biomarkers is particularly timely.

1.2. Inflammation in ischemic stroke

Acute stroke initiates an inflammatory response in the brain and peripherally, whilst response to infection also involves inflammation and the acute phase response. Recently there has been evidence that

Abbreviations: BI, Barthel Index; BP, Blood pressure; BMI, Body mass index; CT, Computed tomography; 95% CIs, 95% confidence intervals; HPAA, Hypothalamic–pituitary–adrenal axis; mRS, Modified Rankin score; NIHSS, National Institute of Health Stroke Score; OR, Odds ratios; SAI, Stroke associated infection.

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stroke results in suppression of the immune system which may also predispose towards infection (Braun et al., 2007; Chamorro et al., 2006, 2007a, 2007b; Emsley and Hopkins, 2008; Haeusler et al., 2008; Klehmet et al., 2009; Prass et al., 2003; Vogelgesang et al., 2008). There are several candidate biomarkers that are worth exploring as risk factors for SAI. Proinflammatory cytokines may be early biomarkers for infection, as well as having plausible biological actions that predispose towards infection. For example, IL-6 is an important acute phase mediator that is elevated during infection (Smith et al., 2006), with multiple effects that may be important. Plasma IL-6 levels are elevated in acute stroke, and higher levels of IL-6 are associated with larger infarct volumes and poor outcome (Smith et al., 2004, 2006; Whiteley et al., 2012). TNFα is also a good candidate biomarker as there is increasing evidence that inflammation may suppress both innate and adaptive cellular immunity in stroke via TNFα (Haeusler et al., 2008; Urra et al., 2009) which suggests that a proinflammatory state may increase risk of infection. A third candidate cytokine is IL-1β which has multiple effects on cytokine and acute phase protein gene expression, as well as both innate and adaptive immunity via lymphocyte stimulation and an involvement in clearance of bacterial infections (Miller et al., 2007). These novel biomarkers may be used in combination with more traditional parameters, such as blood pressure and age, to produce a sensitive and predictive model for SAI.

1.3. Aims of the study

Therefore, given the link between SAI and poor outcome, the current concern over inappropriate antibiotic use, and a lack of a robust predictive model for SAIs, the aims of this study were to: a) identify independent risk factors for SAI and b) test relationships between these risk factors and mortality at 2 years.

2. Materials and methods

2.1. Participants

This study complied with the Declaration of Helsinki, the local ethics committee approved the research protocol, and informed consent was obtained from the subjects (or their guardians). Eighty two volunteers who had an acute ischemic stroke in the previous 72 h were recruited. Exclusion criteria and data collection methods have previously been described (Englyst et al., 2008). Presence of ischemic stroke was confirmed by CT within two weeks of admission for suspected stroke (this data was collected between 2003 and 2008 when CT scans were not carried out quickly as part of routine practice). Stroke subtypes were categorized according to the Oxfordshire Community Stroke Project classification scheme, identifying clinical features (Bamford et al., 1991). Stroke subtype was confirmed by computed tomography (CT) scan and infarct volume calculated using standardized software (GE dvantage Workstation). Within 72 h of stroke onset, baseline neurological deficits were assessed using the National Institute of Health Stroke Score (NIHSS) scale (Brott et al., 1989) and functional disability was assessed using the Barthel Index (BI) (Mahoney and Barthel, 1965) and modified Rankin score (mRS) (van Swieten et al., 1988). In this study, moderate–severe strokes were defined as having a BI of less than 15, whilst mild strokes were defined as having a BI of 15 to 20 (Patel et al., 2002).

SAI was defined as an infection occurring during the first week post stroke. The definitions of specific infections were similar to those used in previously published studies (Davenport et al., 1996; Langhorne et al., 2000). In particular, urinary tract infection (UTI) was defined as the presence of relevant clinical symptoms and/or signs such as dysuria, urinary frequency with positive microbiological cultures, or negative cultures with leucocytosis ($>11 \times 10^9$/L), fever (temperature 37.5 °C), or both. Pneumonia was defined as the presence of relevant clinical symptoms and/or signs (such as purulent cough, unilateral inspiratory crackles, bronchial breath sounds) with at least one of: leucocytosis, fever, or a positive chest radiograph. Data on SAIs were abstracted from case notes by a consultant stroke specialist. Every patient was examined for signs and symptoms of SAIs daily as part of the routine ward round by the stroke team; hence all infections were prospectively identified, investigated, treated and recorded systematically and according to unit guidelines.

Retrospective mortality data collection using hospital notes and local and national databases was also conducted to determine mortality at 6 months and 2 years. Fig. 1 gives a schematic representation of the timing of the different measurements in this study.

2.2. Laboratory measurements

Venous blood was collected within the first 72 h and at 6 months for measurement of IL-6, TNFα and IL-1β (all R&D Systems, UK; minimum detectable doses were 0.7 pg/mL, 15.4 pg/mL and 1 pg/mL and 0.739 pg/mL respectively). All samples were taken between 8 am and 12 noon to minimise the effect of circadian rhythms. All samples were analysed blinded in the laboratory.

2.3. Statistical analysis

Data were analysed using SPSS version 17. Data that were not normally distributed were converted to a normal distribution by logarithmic transformation. Data represent mean ± s.d. (or median ± interquartile range for Rankin score). Differences in frequencies between groups were examined using X^2-tests (Fisher's exact test, p-values are shown). Differences in measurements across the stroke subgroups were analysed by ANOVA. Differences in measurements between patients with and without infection were assessed by unpaired t-tests. Binary logistic regression was undertaken and odds ratios (ORs) and 95% CIs calculated, to identify factors associated with: a) risk of SAI or b) mortality over the first two years after stroke. $P < 0.05$ was considered statistically significant in all these tests.

3. Results

3.1. Patients with a stroke associated infection had higher levels of inflammation and more severe strokes

Table 1 shows the baseline characteristics and outcomes of patients with and without SAI. In the first 7 days after having a stroke, 55% of patients had an infection (of these, 38% had confirmed pneumonia, 20% had confirmed UTI, 42% had other types of infections that were

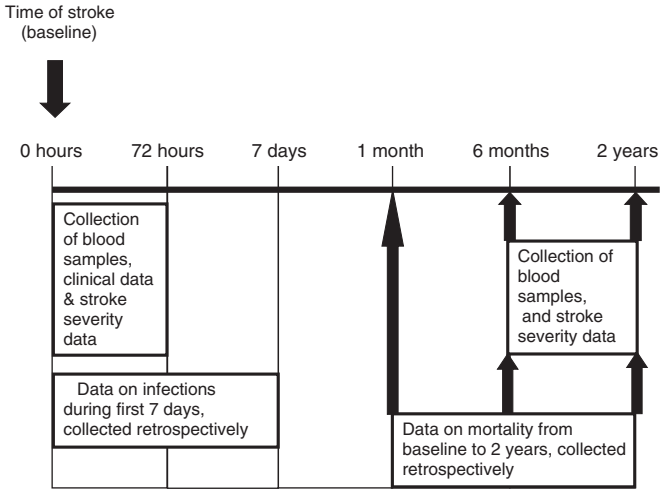

Fig. 1. A schematic representation of the timing of the different measurements.