Introduction

Outcome after aneurysmal subarachnoid haemorrhage (SAH) remains poor; half the patients die and 20% remain dependent for activities of daily life [21]. The initial impact of the haemorrhage, as reflected by the clinical condition on admission and the amount of extravasated blood, is the strongest prognostic factor for a poor outcome [5, 24]. These two measures reflect the severity of the initial cerebral ischaemia caused by cessation of cerebral blood flow at the time of aneurysmal rupture [16, 19]. Secondary cerebral ischaemia is the most important complication after treatment of the aneurism [5, 20]. There are large interindividual differences in recovery after aneurysmal SAH. These may be partly genetic in origin.

Cerebral ischaemia is a potent stimulus for gene expression in animal models [1, 26, 36]. In these laboratory animals, the gene products of the expressed genes influence recovery after ischaemia [17, 23, 25, 43]. In man, some of the corresponding genes have different variants (polymorphisms) leading to different protein isoforms or differences in level of protein expression. These include apolipoprotein E (APOE) [27], insulin-like growth factor-1 (IGF-1) [34], tumor necrosis factor-A (TNF-A)
 interleukin-1A (IL-1A) [31], interleukin-1B (IL-1B) [33], and interleukin-6 (IL-6) [12]. Therefore, the different polymorphic variants of APOE, IGF-1, TNF-A, IL-1A, IL-1B and IL-6 may have different effects on recovery after cerebral ischaemia.

We hypothesized that in patients with aneurysmal SAH, initial or secondary cerebral ischaemia leads to expression of the APOE, IGF-1, TNF-A, IL-1A, IL-1B and IL-6 genes. The aim of our study was to investigate whether the APOE, IGF-1, TNF-A, IL-1A, IL-1B and IL-6 genotypes are associated with outcome after aneurysmal SAH.

Methods

Patient recruitment

From February 1999 to February 2002 we obtained informed consent for genotyping from 167 consecutive patients with aneurysmal SAH admitted to the University Medical Centre Utrecht or from their relatives. Patients with aneurysmal SAH were defined by symptoms suggestive of SAH combined with subarachnoid blood on CT and a proven aneurysm on CT angiography or conventional angiography. Blood samples were taken during the first four days of the hospital admission. All patients were treated according to our standard protocol, which includes oral nimodipine, refraining from antihypertensive medication, and intravenous administration of fluid until a positive fluid balance of at least 750 cc. The ethical review board approved our study protocol.

Data collection

We recorded the patient’s age at time of SAH, sex, clinical condition on admission, amount of blood on initial CT scan, and any episodes of rebleeding or secondary cerebral ischaemia. For the clinical condition on admission we used the World Federation of Neurological Surgeons’ (WFNS) scale [9]. The amount of blood on the CT scan on admission (within 72 hours after the initial symptoms) was graded on a scale of 0 to 30 as defined by Hijdra et al. [18]. Rebleeding was defined as a sudden deterioration in the level of consciousness or a sudden increase in headache, combined with an increase of blood on CT compared with the previous CT. Secondary cerebral ischaemia was defined as a gradual decline in the level of consciousness or a gradual development of new focal deficits or both, with confirmation of a new hypodensity on CT, and no evidence for rebleeding, hydrocephalus or metabolic disturbances.

Laboratory analyses

Genotyping of the APOE (APOE ε2, ε3 and ε4 alleles; APOE ε3 allele wild type) [27], IGF-1 (variable CA repeat; allele with 19 CA repeats/192 base pairs (bp) wild type) [34], TNF-A (–863 C/A) [38], IL-1A (–889 C-T) [31], IL-1B (–511 C-A) [33], and IL-6 (–174 G-C) [12] polymorphisms was performed on coded DNA samples so that the patients’ charactersitics and outcomes remained unknown. Polymerase chain reaction (PCR) was performed using previously described primers for APOE [44], IGF-1 [34], TNF-A [38], IL-1A [31], IL-1B [33], and IL-6 [12]. The assay conditions are available on request. We used well-established negative and positive quality controls for all these reactions.

Outcome assessment

Outcome was assessed approximately three months (with a range from eight to 16 weeks) after SAH by means of the Glasgow Outcome Scale (GOS) [22]. Outcome was assessed before the laboratory analyses were done, i.e. without knowledge of the patient’s genotype.

Data analyses

The clinical condition on admission was determined as ‘good’ (WFNS I–III) or ‘poor’ (WFNS IV–V). For the amount of SAH we dichotomised the scores at the median of the Hijdra scores. The amount of subarachnoid blood could not be assessed in 16 patients (10%) because they had not been admitted within 72 hours of the initial symptoms; for them a score was computed instead with the likelihood approach [33]. We split the outcome into ‘good’ (independent [GOS 4 and 5]) or ‘poor’ (dependent [GOS 2–3] or dead [GOS 1]). Using logistic regression, we assessed the risk of a poor outcome three months after SAH as an odds ratio (OR) with corresponding 95% confidence intervals (CI).

For the analyses on the APOE genotype we grouped carriers of ε2/2 and ε2/3 as well as carriers of ε4/4, ε3/4 and ε2/4. For the analyses of the IGF-1, TNF-A, IL-1A, IL-1B and IL-6 polymorphisms, we compared patients homozygous for the wild type allele (WT) with patients homozygous or heterozygous for the non-wild type allele(s) (non-WT). Since the frequencies of the 9 non-wild type alleles of the IGF-1 polymorphism are low, these alleles were pooled as described in other studies [13, 34, 41]. We adjusted for the known prognostic factors for outcome after SAH: sex, age at time of SAH, clinical condition on admission, amount of blood on initial CT scan, and episodes of rebleeding or secondary cerebral ischaemia.

Results

The distribution of all genotypes was in Hardy-Weinberg equilibrium. The genotype frequencies are shown in Table 1. The patients’ characteristics and outcome are summarized in Table 2. Compared to those carrying the IL-6 non-WT allele patients homozygous for the IL-6 WT allele had less often a poor clinical condition on admission (10% versus 27%, p = 0.006) and more often a large amount of cisternal blood (62% versus 45%, p = 0.03). The remaining characteristics did not show differences between patients with WT and non-WT alleles (data not shown).

Table 3 shows the adjusted and unadjusted odds ratios for poor outcome three months after SAH according to genotype. Patients carrying any IGF-1 non-wild type allele had a lower risk of a poor outcome (adjusted OR 0.4, 95% CI 0.2–2; p = 0.05) or in other words homozygotes for the IGF-1 wild type allele had a higher risk, whereas patients carrying the TNF-A non-wild type allele had a higher risk (adjusted OR 2.3, 95% CI 1–5.4; p = 0.05). When we made additional adjustments for the covariates pneumonia, hydrocephalus and the intervention clipping versus coiling, the odds ratios did not change. Although the risk of poor outcome was lower for patients carrying the APOE ε4 allele and higher for carriers of the IL-1A non-wild type allele, these associations were not statistically significant (APOE ε4 adjusted OR