

A systematic review and meta-analysis of molecular biomarkers associated with early neurological deterioration following acute stroke.

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ABSTRACT

Background: Early neurological deterioration (END) following acute stroke is associated with poorer long-term outcomes. Identification of patients at risk could assist early monitoring and treatment decisions. This review summarised the evidence describing non-radiological biomarkers for END.

Summary: Electronic searches from January 1990-March 2017 identified studies reporting a blood/cerebrospinal fluid (CSF)/urine biomarker measurement within 24 hours of acute stroke and at least two serial assessments of clinical neurological status (<24 hours and <7 days).

Out of 12,895 citations, 82 studies were included, mostly focusing on ischaemic stroke. Using higher neurological thresholds, the n-weighted END incidence for ischaemic stroke was 11.9% (95% confidence interval CI 11.4-12.4%) and 18.6% (17.9-19.2%) for lower thresholds. Incidence decreased with advancing study publication year (Pearson r-squared 0.23 and 0.15 for higher and lower threshold studies). After classification into 3 broad categories, meta-analysis showed that biomarkers associated with increased END risk (n; fixed-effects mean difference; 95% CI) were: “metabolic” [glucose (n=9781; 0.90mmol/l; 0.74-1.06), glycosylated haemoglobin (n=3146; 0.33%; 0.19-0.46), low-density lipoprotein (n=5476; 0.13mmol/l; 0.05-0.20), total cholesterol (n=4822; 0.19mmol/l; 0.10-0.29), triglycerides n=4880; 0.10mmol/l; 0.04-0.15), urea (n=1351; 0.55mmol/l; 0.14-0.96), decreasing albumin (n=513; 0.33g/dl; 0.05-0.61)]; “inflammatory & excitotoxic” [plasma glutamate (n=688 60.13umol/l; 50.04-70.22), CSF glutamate (n=369; 7.50umol/l; 6.76-8.23), homocysteine (n=824; 2.15umol/l; 0.68-3.61), interleukin-6 (n=231; 24.80pg/ml; 21.72-27.88), leucocytes (n=3388; 0.41x10⁹/L; 0.21-0.62), high-sensitivity c-reactive protein (n=1707; 3.79mg/l; 1.23-6.35)]; and “coagulation / haematological” [fibrinogen (n=3132; 0.32g/l; 0.25-0.40); decreasing haemoglobin (n=3586; 2.38g/l; 0.15-4.60)].

Key messages: Declining incidence of END may represent improving care standards but it remains a frequent occurrence. Although statistical associations exist between biomarkers and an increased risk of END, the most promising still need prospective evaluation to determine their additional value relative to baseline radiological and clinical characteristics.

Introduction

Early neurological deterioration (END) describes the worsening of symptoms in the hours and days following acute stroke and is associated with worse outcomes [1]. Although it may reflect natural progression of the underlying pathophysiological process, END is not inevitable and occurs less often when care is provided within a formal stroke unit setting [2]. Associations include age, diabetes, cerebral oedema, haematoma expansion (particularly in the context of hypertension), haemorrhagic conversion of infarction with or without reperfusion treatment and systemic physiological disturbances such as blood pressure variability and pyrexia [1-5].

Variations in case-mix and END definitions have produced wide estimates of incidence, with a previous review reporting frequencies of 2.2% to 37.5% at 24 hours since onset [1]. Fewer patients will be labelled with END at a higher clinical threshold than a lower threshold. It may be also expected that the probability of END will increase with longer time windows between first and second measurements of clinical status. By any definition, prediction of END risk at the point of admission may identify patients in need of additional monitoring, investigations and a lower threshold for clinical intervention.

Clinical and radiological associations with END have already been described in detail [1]. A molecular biomarker is particularly attractive for predicting END as it could be developed into a point-of-care test applied at the beginning of the patient care pathway to provide additional subclinical information about prognosis. A recent horizon scanning review of stroke biomarkers identified associations between END and markers of excitotoxicity (glutamate and glycine), pro-inflammatory cytokine interleukin-6 and fluorescent molecular peroxidation products [3]. However, it did not consider information about possible biomarkers contained within reports from large clinical studies or perform meta-analysis. In view of the increasing focus upon stratification of stroke patients for different emergency treatments and levels of monitoring intensity, we undertook an in-depth review across a broad range of clinical studies to describe END frequency, categorize molecular biomarkers into a simple taxonomy and consider their potential as predictors for END risk, with meta-analysis when possible.

Methods

Medline and Embase were searched from January 1990-March 2017 for interventional or observational studies reporting a blood, CSF or urine biomarker measurement early after acute ischaemic and/or haemorrhagic stroke, including at least two serial assessments of clinical neurological status (baseline <24hrs and repeated within 7 days). The protocol was registered with the PROSPERO review database: ID CRD42017060167.

The search strategy is described in **Error! Reference source not found.** Only studies written in English were included. Studies were initially screened by title and abstract. Full text versions were then sought but authors were not contacted. Data items (Appendix 2) were extracted using a pre-defined proforma including setting, population, participant demographics and baseline characteristics, biomarkers, time of biomarker measurement and author definitions for END, ischaemic END (IS-END) and END following haemorrhagic stroke (ICH-END). Unclear study descriptions were reviewed by both authors. Studies were excluded which specifically included sub-arachnoid haemorrhage or other vascular malformations, and from settings which report highly unusual clinical stroke care practices (e.g. brain imaging was not routinely performed). Due to the heterogeneity of studies and information available, no formal assessment of risk of bias was possible.

Review Manager (Revman) Version 5.3 [4] was used for meta-analysis of individual biomarkers where a mean and standard deviation (SD) were reported for each group. Standard conversions were made to common units. A fixed effects model was used and mean difference (95% CI) is reported. No additional analyses were performed. If a biomarker was described by only one study, the results are presented within the meta-analysis results if the data were normally distributed. All studies reporting data which were not normally distributed, and those which could not be combined because of lack of information about the END definition, are described but are not included in the meta-analysis.

Results

Appendix 3 shows the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [5] flowchart of studies; 86 were included from 12,895 initial citations. Table 1 summarises studies grouped by author-defined neurological thresholds for END.

[TABLE 1]

Using a higher neurological threshold, 35 IS/mixed studies had n-weighted END incidence of 11.9% (95% CI 11.3-12.4%), whereas 43 lower-threshold studies had incidence of 18.0% (95% CI 17.4-18.7%) (Appendix 4). Three higher-threshold ICH studies had an incidence of 12.3% (95% CI 9.2-15.4%), and a single lower threshold ICH study [6] had an incidence of 23.0% (95% CI 17.9-28.1%) (Appendix 4).

There was a trend of declining END incidence with advancing study publication year which persisted when stratified by time window and definition (**Error! Reference source not found., Error! Reference source not found.**).

[FIGURE 1]

[FIGURE 2]

Biomarker data are summarised in three categories: a) metabolic (Table 2); b) inflammatory and excitotoxic (Table 3); c) coagulation and haematological (Table 4). Studies reporting non-parametric biomarker data, results based on a threshold and biomarkers described in single studies only were unsuitable for meta-analysis and are tabulated separately in Appendix 5, 6 and 7 and are referenced by their entry in each Appendix. Citations in bold font concur with the output from the relevant biomarker meta-analysis.

Metabolic biomarkers

[TABLE 2]

Glucose was significantly higher with IS-END [2, 7-36] and ICH-END [37], which may have numerous explanations including poorer collateral flow, endothelial damage, increased oxidative stress and

lactate, haemorrhagic transformation and dysregulation of glucose energy metabolism [38]. This was mirrored in glycosylated haemoglobin (HbA1c), a marker of chronic raised glucose, also predicting END [9, 23, 25, 26, 29, 30, 35, 39] [5K]. One study stratified the effect of glucose by the presence of diabetes mellitus, finding elevated glucose level was associated with END in non-diabetics [38].

Raised total cholesterol, low-density lipoprotein (LDL), triglycerides and triglyceride/HDL ratio were predictive of IS-END, however lower levels of these predicted ICH-END [9, 23, 25, 26, 35, 39-45] [5M, 5S-U]. This concurs with current understanding about cholesterol and the risk of IS/ICH. Hypercholesterolaemia is well described as a modifiable risk factor for IS and lipid-lowering agents are used in stroke prevention. Conversely, possibly because a degree of cholesterol deposition plays a role in maintaining the arterial wall, risk of rupture and haemorrhage is lower in patients with higher total cholesterol [46]. Furthermore, post-hoc analysis of the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial showed early treatment post-IS with high-dose lipid-lowering agents increased ICH risk [47]. There was no association between high-density lipoprotein and END [9, 23, 29, 35, 40-44] [5L].

Increased serum albumin appeared to reduce the risk of END. The included studies did not suggest why [25, 26], however a 2004 study found higher albumin decreased the risk of poor outcome at 3 months indicating a possible link between pre-stroke health and END [48]. A higher ratio of CSF: serum albumin predicted END as a marker of albumin influx into the CSF following blood-brain barrier dysfunction in stroke [49].

The liver enzyme AST metabolises glutamate. Higher levels of liver enzyme AST (aspartate transaminase/glutamate-oxaloacetate transaminase), which metabolises glutamate, were associated with lower incidence of END [9, 11, 25, 26]. Glutamate may be excitotoxic in stroke [50], therefore AST may be neuroprotective by reducing metabolic activity [11]. However, ALT (alanine transaminase/glutamate pyruvate transaminase) was not significantly different in END. Increased bilirubin was associated with END in one study [5F].

Further results from studies which could not be combined into meta-analysis are shown in Appendix 5.

END was associated with higher blood urea nitrogen: creatinine ratio [5H-I], urine specific gravity [5Z], urea [9, 22, 25, 26, 29] and urinary albumin: creatinine ratio (UACR) [5V-X], markers of dehydration/impaired renal function. Of two studies of plasma osmolality/osmolality [5Q-R], one found an association with END, although the one direct study of plasma viscosity did not reach significance [7Q]. Patients with dehydration may suffer END due to increased plasma viscosity and/or lower cerebral perfusion, but these biomarkers for renal function and fluid status may also indicate co-morbidities and limited physiological reserve.

Uric acid (UA), an antioxidant, was found in one study to decrease with time following stroke and a greater decrease by day 7 was associated with END [5Y], possibly because higher baseline UA is associated with a greater vascular risk.

Plasma adrenomedullin was higher in ICH-END but in a single study did not improve the predictive value of the NIHSS score [5B].

Creatinine phosphokinase was associated with ICH-END in one study, however this was only narrowly significant ($p=0.043$) in a study where ~60 variables were analysed and no adjustment for multiple comparisons was made [5I].

Adiponectin [5A], alkaline phosphatase [5D], alanine aminotransferase [9, 11, 25, 26], aspartate [5E], acute decrease in magnesium [5N], methionine [5O], sodium [9, 22, 29], potassium [9, 22, 29], creatinine [9, 22, 26, 29], estimated glomerular filtration rate [10, 18, 29] [5J] and uric acid [12, 25, 26] showed no association with END.

Inflammatory & excitotoxic biomarkers

[TABLE 3]

Excitotoxic amino acids are associated with damage in stroke, particularly glutamate [50], which induces depolarisations around the infarct that disrupt ionic homeostasis, increasing infarct volumes [51]. In meta-analysis, glutamate was associated with END in CSF [24, 52], although this was not corroborated by a further small study [6O]. Plasma glutamate was also associated with IS-END [8, 11,

52, 53] [6N]. Cysteine [6H], homocysteine [23, 35] [6S] and CSF glycine [6Q, 6R] were also associated with END in at least one study. Phosphorylated axonal subunit-H, a marker of axonal injury, was raised in ICH however it did not improve on the PPV of NIHSS alone [6AI]. Glutamine [6P], proline [6AJ] and taurine [6AS] were not associated with END.

Markers of inflammatory immune response were also raised in END, which may suggest ongoing inflammation or an increased risk associated with underlying infection. Standard c-reactive protein (CRP) was significantly different in ICH [37] and in some studies of IS [6G]. However, high-sensitivity CRP (hsCRP) predicted END in IS [12, 23, 41, 43, 54], which is supported by studies showing that raised hsCRP is also associated with IS risk [55]. Leucocyte count was elevated in END in IS [7-9, 11, 16, 17, 22, 25, 26, 29, 35, 36, 39, 45, 53, 56, 57] [AD] and ICH [6, 37]. Neutrophil count was raised in END in one ICH study [6AF], as were segmented neutrophils in IS [6AM]. (s)ICAM-1, which is responsible for neutrophil adhesion [36], was associated with END in two studies [6U, 6AO]. Pro-inflammatory cytokines interleukin-6 [6Z] and TNF α (in plasma and CSF) [6AT-U] were raised in END. TNF α receptors sTNF-R1/R2 were not associated with END [6AP-AQ].

There were several notable findings amongst studies which could not be combined in the meta-analysis (Appendix 6).

Molecules with a role in the inflammatory response included 15-deoxy prostaglandin J2 (15-dPGJ2), which appeared to be neuroprotective against END in atherothrombotic infarcts, but not in other infarct types [6A-B], whereas elevated beta-defensin 2 [6D] and soluble IL-4 receptor [6Y] improved prediction of END over clinical models.

Nitric oxide (NO) has both neuroprotective and neurotoxic roles in brain injury. NO-metabolite predicted END independent of CSF glutamate levels [6AG], whereas L-arginine, a substrate for nitric oxide generation and potential marker of NO-mediated brain damage, was lower in patients with END [6AA-AB].

In a study of monocyte subsets, an increased proportion of CD14^{high}CD16⁻ monocytes, was associated with END by 7 days, potentially because this meant fewer CD14^{high}CD16⁺ monocytes were present

[6E]. The latter produce IL-10, which has been suggested to have a beneficial anti-inflammatory effect in stroke [57]. However, while a study of numerous cytokines and receptors found a significant association between IL-10 and END it was concluded that it did not predict END better than clinical assessment alone [6V]. The study found no association with IL-1B or IL-1RA [6W-X] and END. In a study focussing upon acute penetrating artery stroke, low baseline CD34+ cells, markers of endothelial function, were associated with END [6F].

In a CSF study of monoaminergic neurotransmitters, only elevated 5-hydroxyindoleacetic acid (5-HIAA) was associated with END [6C]. Norepinephrine [6AH], 5-hydroxyindoleacetic acid (5-HIAA) [6C], homovanillic acid (HVA) [6T] and 3,4-dihydroxyphenylacetic acid (DOPAC) [6I] were not.

Two indicators of blood-brain-barrier compromise were associated with END: CSF:serum albumin ratio [5C] and change in matrix metalloproteinase-9 (MMP-9) [6AE]. Fluorescent molecular peroxidation products (FMPPs), markers of oxidative damage hypothesised to promote blood-brain barrier degradation, were significantly associated with END following thrombolysis [6L].

Cysteine [6H], gamma-aminobutyric acid [6M], plasma glycine [6R], p-selectin [6AK], SE-selectin [6AN], segmented & band leucocytes [6AL] and sVCAM-1 [6AR] were not significantly different in END.

A single study showed a non-significant trend towards elevated CSF lactate indicating a higher risk of END [6AC]. Although lactate is generally a useful marker of ischaemia, the low capacity for anaerobic metabolism within neurones may result in the ability to detect only large volumes of vulnerable tissue.

Coagulation and haematological biomarkers

[TABLE 4]

Pro-coagulation factors fibrinogen (In IS [7, 8, 11, 15-17, 28, 31, 35, 36, 45, 53, 56] [7M]; in ICH [6, 37]) and factor VIII [7I] were associated with END, as was INR, a measure of the intrinsic coagulation pathway [29, 36, 42]. A greater 72hr decrease in procarboxypeptidase U, which inhibits fibrinolysis, predicted END, which was hypothesised to be utilised by thrombus growth [7T]. Measures of

endogenous anti-coagulants protein C [7B], antithrombin-III [7E] and thrombin-antithrombin III complex [7Y] did not predict END.

Aspirin non-responsiveness may also play a role in END. Platelet reactivity to aspirin (ARU) at baseline was not associated with END, however a high degree of change in ARU was associated with END [7A]. Poor response to aspirin measured by PFA-100s, a platelet aggregation assay, was also associated with END [7D].

Lower haemoglobin was associated with END in IS [9, 22, 25, 29, 42], which may suggest patients with reduced oxygen-carrying capacity are at greater risk.

Further studies found significant associations which could not be combined in meta-analysis [Appendix 7]. Both CSF and plasma ferritin were associated with END independently of ultimate infarct volume and were hypothesised to be related to excitotoxic mechanisms in stroke, however only the CSF ferritin finding was replicated in a later study [7K-L]. Neither CSF nor plasma iron were associated with END [7O-P].

Higher von Willebrand factor was associated with END [7AA]. In the same study, three markers of thrombin generation (prothrombin F1+2 [7V], fibrin d-dimer [7F] and thrombin-antithrombin complexes [7Y]) were associated with END on univariate analysis. Six studies showed increased D-dimer associated with END, three of which reported the associated as significant.

Activated partial thromboplastin time [7C], factors VIIc and VIII(c) [7H-J] haematocrit [7N], platelets [7R], prolyl carboxypeptidase [7S], prothrombin fragment 1+2 [7U], prothrombin time [7V], soluble fibrin monomer [7W] and thrombin time [7X] were no different with END.

Discussion

Depending upon the threshold used, IS-END incidence ranged from 11.9% (higher threshold definition) to 18.6% (lower threshold definition), indicating that it remains a significant clinical challenge. As only four studies focussed upon ICH in isolation there is limited data about ICH-END incidence in relation to biomarkers, but amongst the three using a higher threshold, 12.2% deteriorated, comparable to

ischaemic stroke. In cohort studies, END rates up to 30% have been reported following ICH [58], but differences are likely to reflect whether the clinical setting is a stroke unit without pre-selection or a specialist neurosurgical unit. Overall, incidence of END fell with advancing study publication year, independently of neurological threshold and time window. This may reflect improvements in stroke care including reperfusion treatments and/or changes in case-mix.

Meta-analysis identified a number of biomarkers, both routinely performed and novel, associated with END. The likely explanations for these associations are diverse and a direct link to mechanisms for END cannot be inferred. Some of the biomarkers described have been considered by trialists as contributors themselves towards the process of neuronal death, but intervention has not consistently shown improved outcomes e.g. insulin for blood glucose lowering [59]. However, there is consistent trial evidence that pro-active assessment for pyrexia, swallowing difficulties and dehydration can reduce dependency, possibly through avoidance of END for some patients. It is therefore not surprising that infection (e.g. leucocytes and high-sensitivity CRP) and fluid status/renal function biomarkers (urea, albumin) may identify patient groups particularly at risk. The link to END may be through the physiological consequences of infection and dehydration such as increased plasma viscosity and fluctuations in cerebral perfusion.

Other biomarkers may reflect the volume of subclinical cerebral tissue injury during acute stroke and hence indicate a greater probability of further clinical progression, particularly those molecules which are specific to neuronal tissue or involved in neurotransmission. For example glutamate, which is also implicated in the progression of cellular death through excitotoxicity [51,53], was associated with END when measured in CSF and plasma. We did not identify any studies which related these biomarkers to advanced imaging techniques such as MRI or CT perfusion, which could provide mechanistic evidence and indicate their potential for further development as rapid near-patient tests. There is already good evidence that perfusion mismatch indicates patients at risk of deterioration [60], which can be averted by reperfusion therapy [61], but a point of care biomarker with sufficient accuracy to alter clinical care is likely to have wider applicability and availability for the global stroke population.

In line with growing evidence that inflammation is key to the development of atherosclerosis and plaque rupture, associations have been shown between biomarkers implicated in inflammatory pathways and END. These may reflect the severity of pre-existing atheroma and so indicate poorer local perfusion and/or collateral supply [62]. Elevated HbA1c, increased LDL, total cholesterol and triglycerides may also indicate a greater burden of background atheroma and vulnerability to tissue ischaemia. The relationship between haematological biomarkers and END is likely to be more direct and immediate. Fibrinogen, a pro-coagulant factor, may reflect the probability of large, thrombin-rich clot formation and progression, whereas impaired penumbral oxygen delivery may explain the association between END and reduced haemoglobin.

A recent review identified numerous challenges when developing biomarkers for stroke diagnosis which are pertinent to predicting END [3]. These include the non-specificity of biomarkers to brain tissue and the inability of molecules to cross the blood-brain barrier. Simultaneous measurement of multiple biomarkers in a single panel has been proposed, combined with recent developments in genomics and proteomics, approaches which have not yet been taken in END. However, as reported by the Stroke-Chip study following evaluation of an assay panel for stroke diagnosis, many biomarkers are not independent of one another and may not be significantly better than clinical judgement and radiological data [63]. Amongst investigations which are already routinely available clinically, the results of the meta-analysis suggest that it would be reasonable to increase monitoring intensity for those acute stroke patients where venepuncture reveals a low haemoglobin or elevated glucose, glycosylated haemoglobin, urea, osmolality, CRP, ESR or leucocytes.

Limitations

This review included only summary results from English language studies indexed in Medline and Embase, and authors were not contacted for data. Most articles provided insufficient information to permit assessment of bias. For example, many studies reported that the biomarker results were concealed from the END assessor, but it was unclear whether they were aware of other results which could cause unblinding such as clinical brain imaging. Formal quality assessment of the included studies was not performed as interpretation would have been difficult due to their early stage, extreme

methodological variability, and the lack of information presented. Sample size estimations were rarely reported. Despite proven associations with patient outcomes, studies did not report other clinical or radiological data which could assist with exploration of mechanisms for biomarker responses and their clinical utility.

Recommendations

The biomarkers identified by this review which have demonstrated strong associations with END in more than one cohort have the potential to be clinically useful, but further studies are required to prove diagnostic utility. These should include reports of standardised clinical and radiological data, such as the Alberta Stroke Program Early CT Score (ASPECTS) [64] score for ischaemic stroke, and haematoma volume for ICH [58]. Other novel biomarkers with a promising pathophysiological basis were considered by single studies, but have not been further developed. Significant investment is needed if affordable and practical tests are to become available for integration into effective clinical monitoring and response protocols.

As END remains a clinical entity with various definitions, standardisation is necessary to allow meaningful comparison and combination of future studies. In the meantime, authors should determine *a priori* which definition is to be used. The timing of biomarker sampling and assessment of END should be at clinically important intervals, for example during thrombolysis/thrombectomy windows. Other factors which may influence END incidence (e.g. time from onset, and thrombolysis/thrombectomy treatment), should be collected within studies that are large enough to allow meaningful analysis of multiple variables.

Conclusions

Although the incidence of END following acute stroke may be decreasing, it remains a common occurrence. This systematic review has identified biomarkers associated with END in predominantly exploratory studies or from incidental observation during clinical trials. Their value in relation to key clinical and radiological characteristics is unclear, and none can yet be recommended for use. Further

studies are required for validation and development of clinically useful tests which stratify patients into higher and lower risk groups, and ultimately improve clinical outcomes through END prediction.

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