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ACUTE STROKE BIOMARKERS: ARE WE THERE YET?

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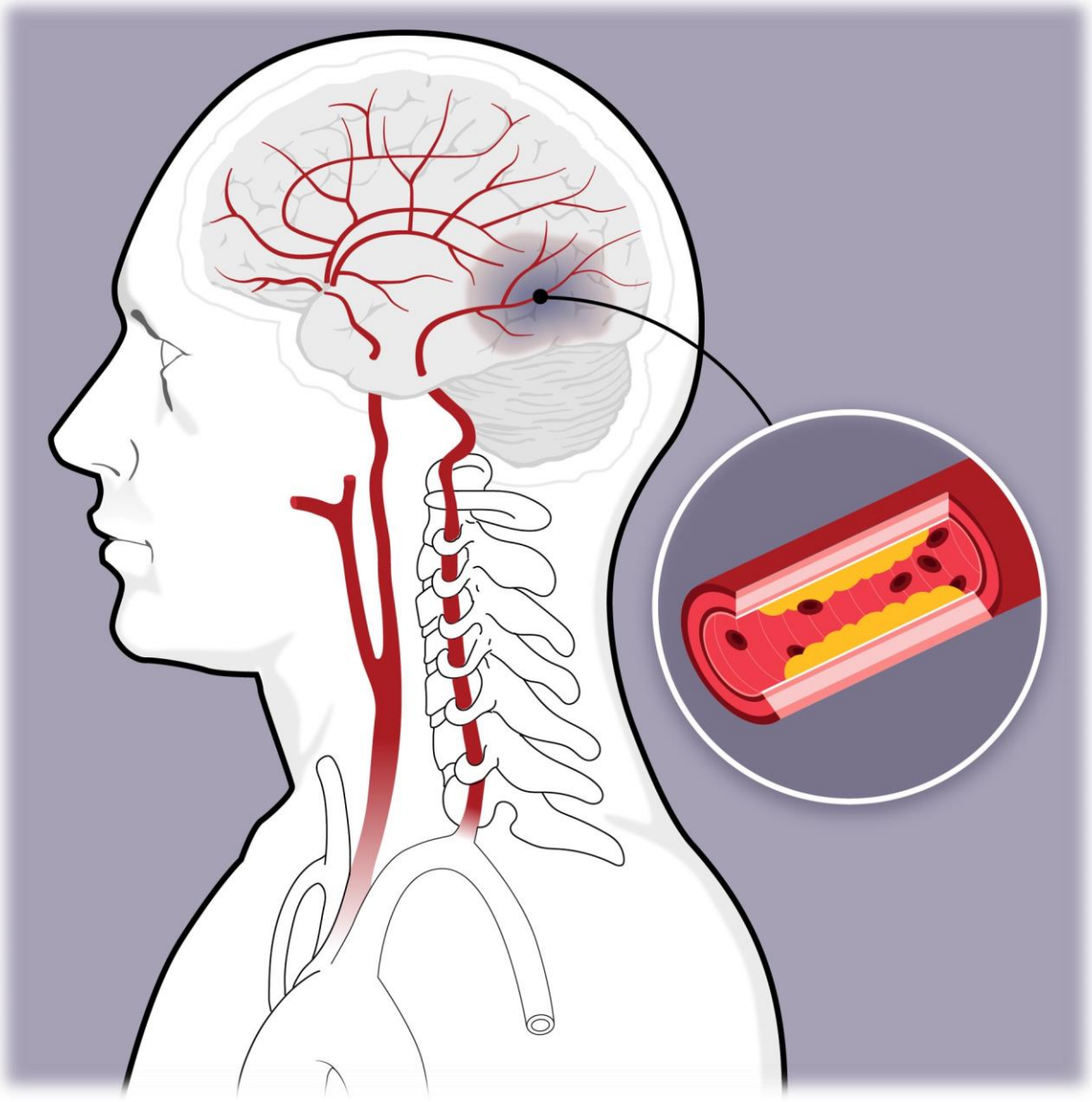


Acute stroke biomarkers: Are we there yet?

Introduction:

"Never reduce a target, instead increase actions"

Stroke is one of the leading causes of death and disability in India. The estimated adjusted prevalence rate of stroke ranges, 84-262/100,000 in rural and 334-424/100,000 in urban areas. The incidence rate is 119-145/100,000 based on the recent population-based studies. (1) Distinguishing between stroke subtypes and knowing the time of stroke onset are critical in clinical practice as it determines the treatment modality, survival, post-stroke complications and thereafter rehabilitation. Diagnosis of acute stroke has been challenging as always and mostly it is dependent on imaging studies. However, noticing the current healthcare scenario in the remotest location where imaging studies are not available, development of biomarkers seemed to be of utmost importance.

Background: how incidence of stroke has changed?

Stroke units are available mostly in the private hospitals in India, government run tertiary care hospitals are now emerging as stroke management centres which mainly focuses on treating acute patients of stroke stroke over telemedicine with the help of physicians with basic stroke management trainings. Unavailability of imaging facility in several primary health care centres or even secondary centres impose a serious challenge in such telemedicine based management . Intravenous (IV) and intra-arterial thrombolysis (IA) are commonly used in India. In the on-going Indo USA National stroke registry the rate of IV thrombolysis is 11%. (1) Treatment of stroke is not limited to only this but an organized stroke centre has a vast menu to cater to the affected population which needs to be addressed at the earliest. Stroke rehabilitation is also an unorganized endevour at many centre till date. Hence requirement of 'Biomarker' has been a question in everyone's mind.

If we take the statistics of burden of stroke, we will find the incidence to have increased in the recent decade which may be attributed to the change of lifestyle of the population in the recent times.

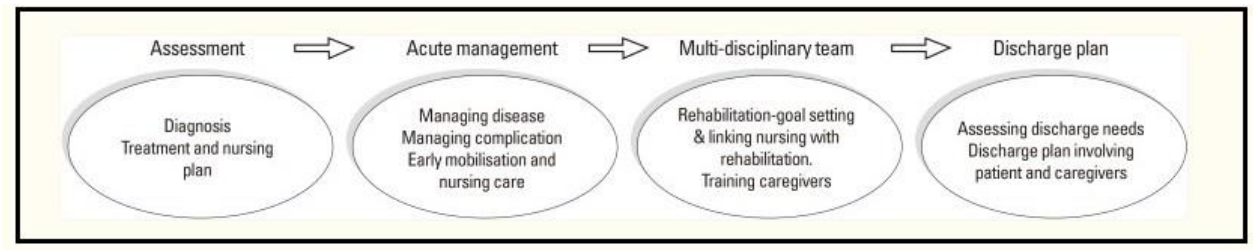
Stroke Epidemiology between the 1990s and 2020s

A stroke study conducted in Kolkata from 1998 to 1999 showed a crude prevalence rate of 147/100,000 and an annual incidence rate of 36/100,000. (5)When adjusted to the 1996 US population, the age-adjusted prevalence rate was 334/100,000 and the age-adjusted annual incidence rate was 105/100,000. Compared to men, women had substantially higher age-adjusted prevalence rate (564/100,000 for women versus 196/100,000 for men) and incidence rate (204/100,000 for women versus 36/100,000 for men). In a prospective community based study conducted in Kolkata , out of the screened population of 52 377 (27 626 men, 24 751 women), the age standardized prevalence rate of stroke to world standard population is 545.10 (95% CI, 479.86 to 617.05) per 100 000 persons. The age standardized average annual incidence rate to world standard population of first-ever-in-a-lifetime stroke is 145.30 (95% CI, 120.39 to 174.74) per 100 000 persons per year. Thirty-day case fatality rate is 41.08% (95% CI, 30.66 to 53.80). Women have higher incidence and case fatality rates. (6) From 1990 to 2019, the burden (in terms of the absolute number of cases) increased substantially ,70.0% increase in incident strokes, 43.0% deaths from stroke, 102.0% prevalent strokes, and 143.0% DALYs), with the bulk of the global stroke burden (86.0% of deaths and 89.0% of DALYs) residing in lower-income and lower-middle-income countries . (7) Statistics show that in 2020, there were 7.08 million deaths attributable to cerebrovascular disease worldwide (3.48 million deaths from ischemic stroke, 3.25 million deaths from intracerebral hemorrhage (ICH), and 0.35 million from subarachnoid hemorrhage).

Stroke unit and need for Biomarkers:

The studies cited above clearly indicate the increase in incidence in our country affecting younger population imposing a serious economic burden on the family. Hence, this requires urgent steps to identify stroke, its type and manage as early as possible thereby reducing deaths, increasing survival, decreasing post stroke complication and providing proper of post stroke rehabilitation. This requires developing stroke units with all equipment and facilities at the grass root level for reaching out to patients in remotest areas. Stroke unit is a multidisciplinary team comprising of medical, nursing, physiotherapy, occupational therapy, speech therapy.

Figure 1: Components of stroke unit

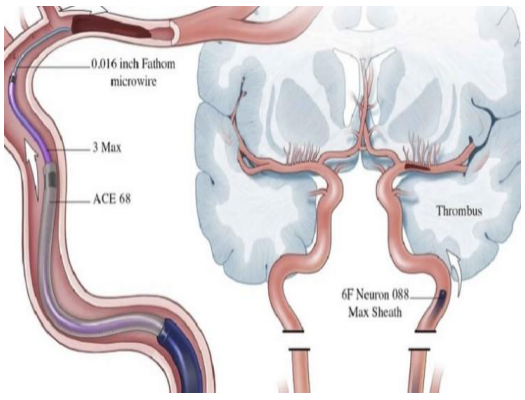


Amongst all the medical team is of utmost importance and it should be aided with facilities like imaging (CT Scan , MRI , CT angiography , DWI) and a properly equipped cath lab with trained technicians and qualified doctors . However, imaging studies cost, availability, contraindications, as well as the level of expertise required to interpret advanced imaging results have restricted its wide use at the PHC level thereby, instigating research for blood parameters which can act as Biomarker for identifying stroke , its type and also giving insight to therapeutic monitoring of such patients .

Need for biomarkers in stroke patients:

The dearth need for acute stroke biomarkers focuses on the area of actions stated below:

- Distinguish between stroke subtypes & knowing the time of stroke onset
- Neuroimaging though helps to decide whom to treat, how to treat is contraindicated in many
- Underuse of reperfusion therapies –thrombolysis or thrombectomy??



What is a biomarker ?

A characteristic that is objectively measured and evaluated as an indicator of normal biological process or pathogenic/ pharmacologic response to a therapeutic intervention .

Characteristic of a biomarker

- Cheap & widely catered test
- very minimum invasive approach to procure sample
- Specific and sensitive
- Non overlapping
- Stability of parameters in samples
- Reliable --- change with disease severity / prognosis indicator/therapeutic monitoring aid

Biomarker categories for stroke

Physical

• Neuroimaging

• Cardiac imaging

• Electrophysiological

• Histological

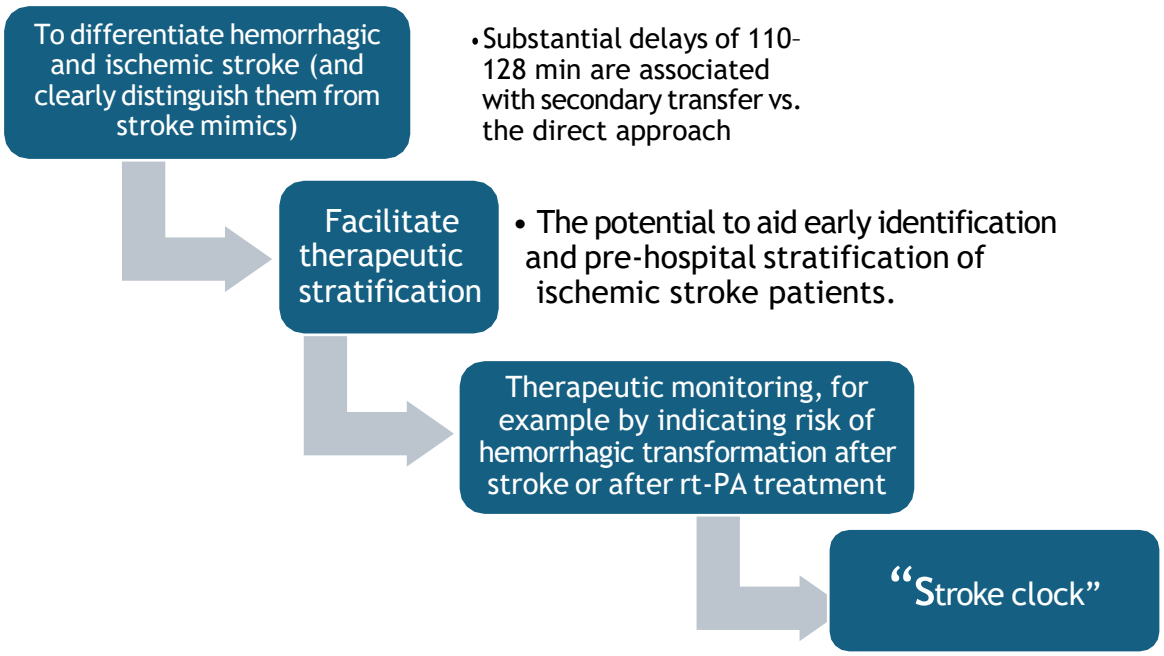
Genetic

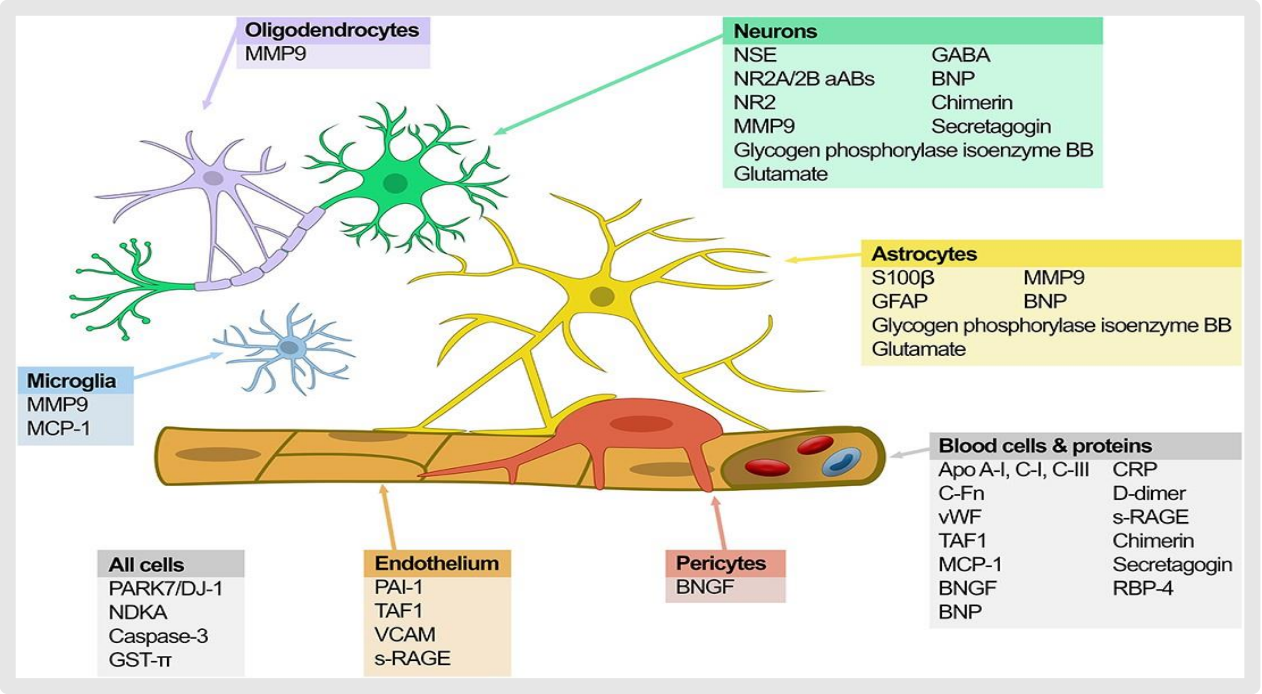
• Serum/plasma/cerebrospinal fluid

– Systemic

– Neuronal

Areas of biomarker identification





Candidate Diagnostic Biomarkers

- **S100B**
- **GFAP**
- **MMP-9**
- **NMDA-R**
- **Apo-lipoproteins**
- **PARK7 and NDKA (nucleoside diphosphate kinase A)**
- **C-reactive protein (CRP), P-selectin and homocysteine**

Biomarkers of Disease Progression

- MMP-9, NSE, and S100B are linked with increased bleeding risk after IS.
- Plasma levels of c-Fn (cellular-fibronectin), which reflect vascular damage, have been associated with the development of hemorrhagic transformation following t-PA use.
- Several biomarkers have been associated with **early neurological deterioration (END).** This has been defined as neurological worsening between 48 and 72 h after admission and occurs in one third ischemic stroke patients .

- Cytotoxic mechanisms mediated by **glutamate, nitric oxide, cytokines and endothelial-leukocyte adhesion molecules** have been proposed as mediators of progression of tissue damage

- High plasma glutamate concentrations have been correlated with neurological worsening and infarct growth at 72 h after stroke onset. Plasma glutamate concentrations of >200 μmol/l on admission have a positive predictive value for neurological deterioration at 48 h after lacunar infarction of 67% .

- Plasma GABA levels <240 nmol/l on admission also had a positive predictive value for neurological deterioration at 48 h after lacunar infarction of 84% .

- Higher levels of inflammatory markers such as **ferritin, IL-6 (interleukine-6), TNF-α (tumor necrosis factor-α) and ICAM-1 (intercellular adhesion molecule-1)** were also shown to be associated with early neurological worsening.

- Space-occupying brain oedema (also called malignant oedema), an early life-threatening problem in patients with large hemispheric stroke, has been shown to be predicted by an elevated plasma S100B level (>0.35 g/l) with a 75% sensitivity and a 80% specificity at 12 h after stroke and even more at 24 h (94 and 83% sensitivity and specificity, respectively).

- c-Fn and MMP-9 concentrations have also been found to be significantly higher in patients with malignant MCA (m-MCA) infarction than in controls. c-Fn concentrations of >16.6 μg/ml provided a 90% sensitivity and 100% specificity with 89 and 100% negative and positive predictive values, respectively, for prediction of m-MCA infarction

Biomarker panels: In the pipeline

- The combination **of S100B, B-type neurotrophic growth factor (BNGF), von Willebrand factor (vWF), MMP-9, and monocyte chemotactic protein-1 (MCP-1)** provided diagnosis of stroke within 12 h after symptom onset with a 91% sensitivity and a 97% specificity.

- A related panel of **S100B, MMP-9, vWF, and vascular cell adhesion molecule (VCAM)** studied by the same group of researchers in 65 suspected ischemic stroke patients and 157 controls within 24 h of symptoms provided a sensitivity and specificity of 90% .

- Patients with **acute focal neurologic deficits** admitted within 6 h of onset of symptoms, a panel including **D-dimer, CRP, B-type natriuretic protein (BNP), MMP-9, and S100B** was predictive of ischemic stroke with sensitivity and specificity of 81 and 70%, respectively

Stroke vs. mimics: Biomarker panel to distinguish

- **Montaner et al.** tested, in an ED setting, a panel of blood biomarkers including CRP, S100B, MMP-9, a soluble receptor for advanced glycation end products (sRAGE), D-Dimer, brain natriuretic peptide (BNP), caspase-3, neurotrophin-3, chimerin, and secretagogin. They identified levels of caspase-3, D-dimer, sRAGE, chimerin, secretagogin, and MMP-9 as independent predictors of **stroke vs. mimics**. Moreover, they reported a predictive probability for identifying stroke of 99.01% by combining set cut-off values of these six biomarkers .(44)

IS VS ICH: Biomarker panel

- The same team have also demonstrated, in a cohort of 915 stroke patients, that just **S100B and sRAGE**, could **distinguish between IS and ICH** with an AUC of 0.76 for blood samples obtained within 3 h after symptom onset. This was confirmed in blood samples obtained within 6 h of symptom onset

- Measurement of **retinol binding protein 4 (RBP4)** (with a cut off value >61 g/ml) and **GFAP** (with a cut off value of <0.07 ng/ml) was shown to distinguish IS from ICH with a specificity of 100% in a cohort of 38 IS and 28 ICH samples

SMARTchip study (june 10 2016 to nov 23,2020) : In progress

- A simple blood test of substances (the purines) that result from cellular metabolism and are produced in excess when brain cells are starved of oxygen and glucose (as occurs during a stroke) is proposed. The sensors designed by the investigators are used to measure blood purines during a procedure in which blood flow to the brain is reduced to allow surgical interventions on the major arteries that supply the brain. Previous studies by the investigators have shown that as soon as blood flow to the brain is reduced, purines are produced within minutes and are detectable in systemic arterial blood.(43)

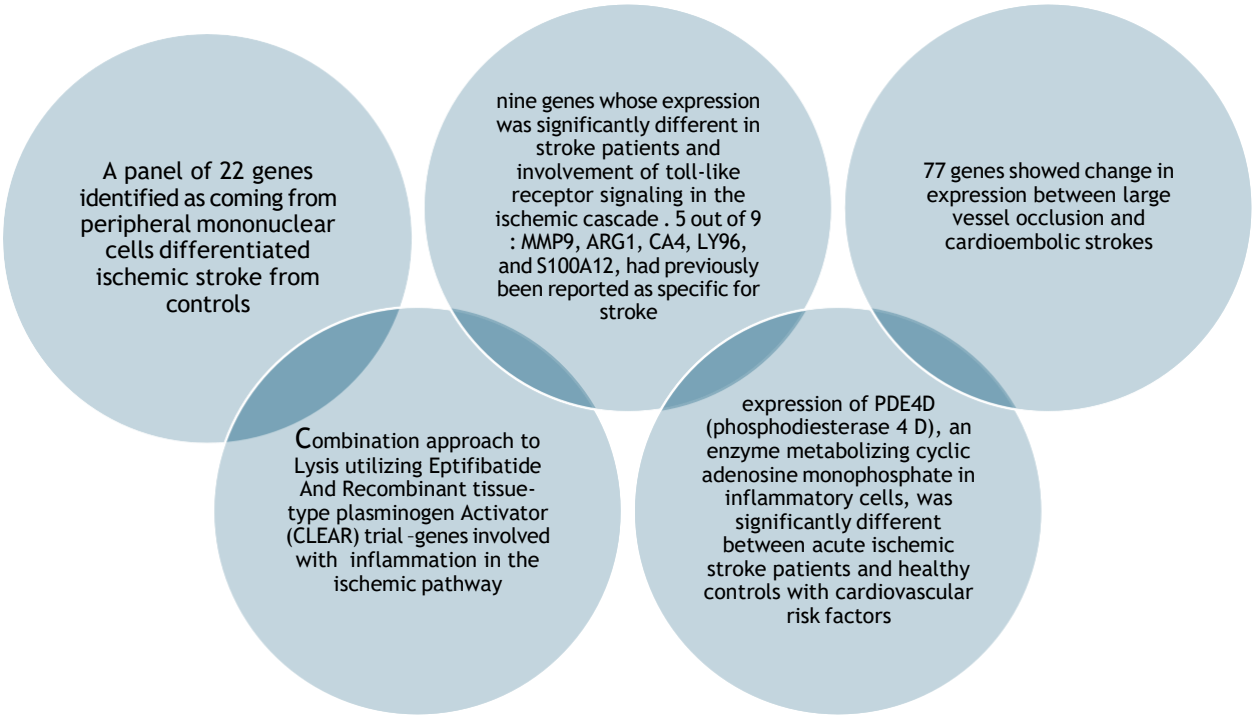
- The current project will now compare the levels of purines in the blood of stroke patients and controls. The purines will be measured on admission to hospital and 24 hours later. The occurrence and magnitude of a stroke will be determined by an MRI scan given between 24 and 72hrs after admission. This study will establish whether purines are elevated in the blood of stroke patients on admission to hospital compared to healthy controls, and whether this correlates with the size of the stroke and damage to the brain.

The mRNA Revolution : "TIME IS BRAIN."

- Oligonucleotide microarray techniques, and more recently RNAseq

- RNA shed from damaged or communicating cells, or contained within the cells of the immune system, the body's own "first responders" to injury.

- Changes in mRNA expression occur very quickly often before changes of protein expression can be detected



Biomarkers of stroke clock

- In serial blood samples collected at 3, 6, 12, 18, 24, 48, 72, 96, and 120 h after onset of stroke symptoms, NSE concentration, measured by immune-assay, rose in the first 2–3 h, then fell until 12 h before a second elevation that was maintained until measurement ended on day 5.
- Tau concentration showed a continuous increase from admission onward.
- In plasma samples collected at 12, 24, and 48 h after symptoms onset in 39 patients with ischemic stroke, while MMP-9 concentrations were greater in stroke patients than the reference interval for healthy controls, no significant changes were reported over time

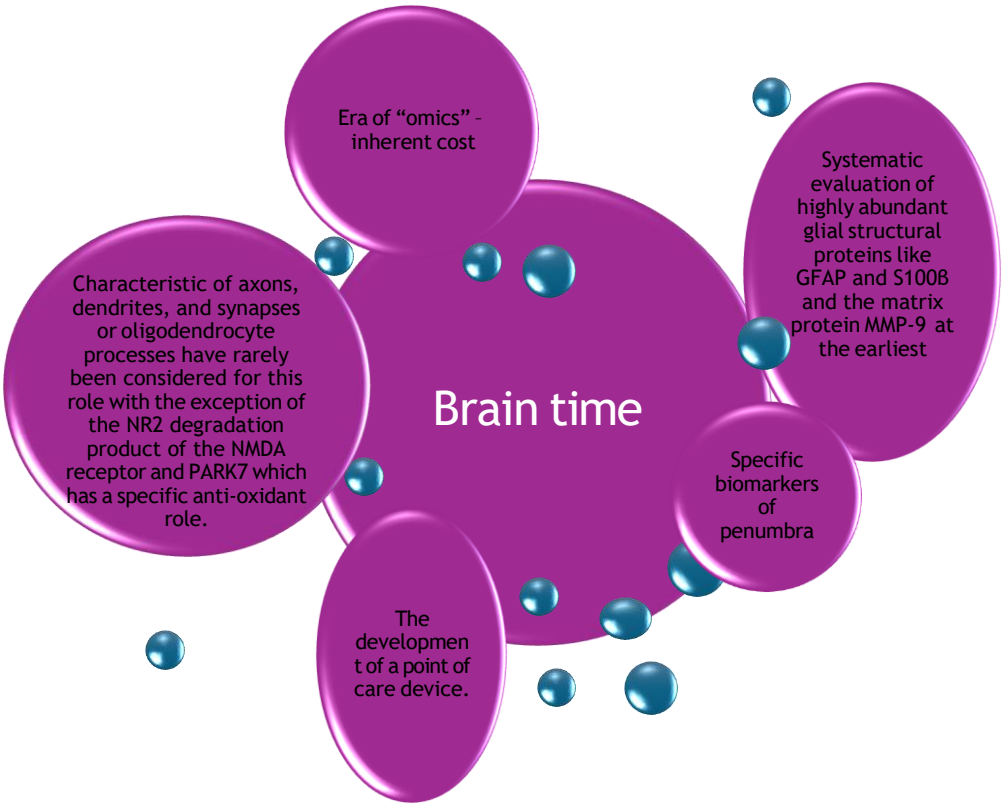
Biomarkers of Acute Stroke Etiology (BASE) study

- Study aims to identify biomarkers defining acute IS etiology and patients presenting within 24 h of symptom onset are recruited . Blood samples are being obtained on arrival and 24, and 48 h later, and gene expression profiling is being used to identify biomarker candidates of stroke

Blood And Clot Thrombectomy Registry And Collaboration (BACTRAC) trial

- Fraser et al. aim to collect intracranial thrombus material and arterial blood collected before, after and during mechanical thrombectomy to allow gene expression and proteomic analysis of the early human molecular response to ischemic stroke .

Critical appraisal : how far feasible ?



Food for thought :the mothership vs. drip and ship dilemma

- Timing of biomarker measurement, particularly early when decision making is most important, requires urgent and systematic study.
- The kinetics of change may be revealing in their own right and, if a biomarker stroke clock can be constructed, might dramatically broaden the utility of thrombolysis and thrombectomy ,combining imaging and biological biomarkers
- point of care immune assays for biomarker detection (rapid immunoassays)
- Miniaturization now also makes highly sensitive and selective and rapid analyte detection by a range of mass spectrometry protocols possible, even at the bedside .Even nucleic acid biomarkers can now be detected within minutes, with recent publications reporting completion of 30 qPCR cycles within 54 s
- the promise of portable devices that might be used at the bedside are
- nanotechnology offers the promise of highly multiplexed biosensors capable of rapid simultaneous analysis of large panels of biomarkers

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