Neuroinflammatory and Neurorepair Biomarkers for Stroke

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Barcelona, SPAIN

Today’s Presentation

• Stroke Biomarkers, what for?

• Stroke Neuroinflammation, but don´t forget Neurorepair!

• What do we know so far.

• What are we doing at the Laboratory.
13.6.2016

7th Kuopio Stroke Symposium

Stroke – there’s treatment if you act FAST.

Face: Is the person’s face drooping? Is the person’s face hanging one-sided?
Arm: Is the person’s arm hanging down?
Speech: Is the person’s speech slurred?
Call 911 now!

Balance, Eyes, Face, Arms, Speech, Time

WORLD STROKE DAY

Youtube Video

F.A.S.T. Song - Stroke Signs: featuring Dee-1 & Tha Hip Hop Doc

41,594 visualizaciones
**Stroke Management & Treatments**

Stroke Units care
BP, Glucose, T° control
(surgery)

Hyperacute thrombolytic and endovascular treatments

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**Tissue Biology of Stroke**

Adapted from: Dimagi U. Ann N Y Acad Sci 2012;1268:21-25

Ischemic Cascade

Cytokines Expression
Neutrophil Recruitment
Proteases Release
BBB damage
Neuron & Glia damage
Infarct Growth

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Ischemic Cascade

Cytokines Expression
Neutrophil Recruitment
Proteases Release
BBB destruction
Neuron & Glia damage
Immune cell trafficking
Infarct Growth

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Ischemic Cascade - Activates Repair!!

Cytokines Expression
Neuroblast prolif. & migr.
Angiogenesis
Vessel remodeling
Proteases
White matter remodeling
Synaptogenesis
Progenitor cells

“Stroke Biomarkers” in Pubmed

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**Biomarker types:**

- Proteins & other molecules
- Genes (mutations & polymorphisms)
- mRNA
- miRNA
- Cells (stem & Progenitor)
- Imaging parameters

**Where?**

- Plasma/serum
- Urine
- Brain Tissue
- CerebroSpinal Fluid
- Brain Microdyalisate
- Arterial blood clots
- Saliva

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**Useful to select patients:**

personalize treatments & decisions.

<table>
<thead>
<tr>
<th>Symptoms onset</th>
<th>hours</th>
<th>24 – 48h</th>
<th>discharge</th>
<th>recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stroke vs. Mimics</td>
<td>Antibiotics</td>
<td>Neurorepair drugs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Relatives information</td>
<td>Ethiology</td>
<td>Rehabilitation programs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HT complications</td>
<td>Clinical trials</td>
<td>Cell therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rt-PA &amp; endovascular treatment</td>
<td>Neuroprotection drugs</td>
<td>Secondary prevention</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stroke unit admission</td>
<td>Surgery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroprotection drugs</td>
<td>Discharge decision</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Early rehabilitation</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Investigating on Stroke Biomarkers

2 Massive genetic tests

3 Genomics
(RNA, miRNA)

1 Select candidate markers

4 Screening Abs.
libraries

5 Proteomics

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Neurovascular Diseases and Biomarkers

Use of biomarkers in clinical practice is getting popular among physicians to aid making diagnostic and prognostic decisions. In certain areas of biomarker literature, biomarker analysis has been incorporated to daily practice as model for lipid lowering therapy (LDL), the diagnosis of acute myocardial infarction (angiography), etc. However, there is a great interest for their use in neurovascular diseases to help physicans in various stages of the evaluation of stroke. These including the risk of further stroke among healthy individuals or in the biochemical diagnosis of stroke or using biomarkers as guides for neurovascular protection.

This initiative attempts to provide a database compilation of published candidate biomarkers in the context of stroke risk, diagnosis, subtypes, etiology and prognosis. It offers updated protocols and to contact research groups with interest in this field.

Indications

Risk  Diagnostic  Subtype  Etiology  Prognosis
Prognosis

Stroke prognosis current face interindividual variability and entails the interaction between several factors (e.g., age, comorbidities, stroke severity). Most of them are not modifiable but others are derived from the neuro-physiological process (such as brain enzyme, post-stroke irritation, ...) or the therapeutic measures (such as post-TRA hemoglobin transformation ...). Early prediction and management of those complications represents a unique opportunity to improve stroke outcome.

Biomarkers might add information at this level, thus optimizing the admission of patients to specialized stroke units, where more aggressive and specific management of stroke complications can be decided. Hence, biomarkers could help in quick decision-making regarding protocols of alternative clinical trials or in the need of hospitalization at discharge.

How will you find information about biomarkers associated with stroke prognosis?

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Association</th>
<th>Endpoint</th>
<th>Genetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRA155</td>
<td>Complement-receptor 1 Coagulate</td>
<td>Complement adds predictive information on functional outcome and mortality at 3 months</td>
<td>Disability, Death</td>
<td></td>
</tr>
<tr>
<td>PON1</td>
<td>Interleukin 1</td>
<td>In stroke patients, many studies have focused on the role of IL-1 in the production of acute neuroinflammatory and proinflammatory cytokine release, thereby, IL-1 blockers have been associated with stroke recurrence and IL-18</td>
<td>Disability, Death</td>
<td></td>
</tr>
</tbody>
</table>

Guidelines and protocols

- NNTN: guidelines for transparent reporting guidelines and tools
- EQUATOR network: resource centre for good reporting of health research studies
- PROSO statement: guidelines for transparent reporting of systematic reviews and meta-analyses
- MOOSE statement: guidelines for meta-analysis of observational studies in epidemiology
- PRISMA: international prospective register of systematic reviews
- STROBE: stroke standards to systematically collect, analyze and share data

Videos

Sample preparation for biomarker studies

World Stroke Day 2012

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Biomarkers associated with Global Outcome

Potential Biomarkers: Literature
- IL-6
- CRP
- Copeptin
- fibronectine, MMP-9
- ICAM-1, VCAM-1, E-selectin
- glutamate...

Matrix Metalloproteinases (MMPs):

Role in Inflammation and Repair

MMPs are enzymes that degrade components of the extracellular matrix, among other functions.

They are produced and secreted in many cell types (neutrophils, endothelial cells, etc.) and are needed for physiological function of most tissues.

However their upregulation regulation after stroke has been related to tissue injury and hemorrhagic complications.

From rodent models of stroke

**Acute administration of MMP inhibitors reduce infarct size.**


**MMP9 knockout mice present reduced infarct and less BBB leakage**

MMP9 is elevated in the ipsilateral hemisphere whereas other MMPS (such as MMP2) remain constitutively expressed

Planas et al. Neurobiology of Disease (2001)

An increase in MMP9 is related to Hemorrhagic transformations


Stroke patients with the more extensive hemorrhagic complications presented higher blood MMP9

Human brain tissue from ischemic strokes

PMNs degrade Collagen IV

Human brain tissue from ischemic strokes

<table>
<thead>
<tr>
<th>Collagen IV</th>
<th>MMP-9</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image1.png" alt="Collagen IV" /></td>
<td><img src="image2.png" alt="MMP-9" /></td>
</tr>
</tbody>
</table>
What happens after the acute phase of stroke?

MMPs are needed for repair and remodelling of the damaged tissue

In a mouse model of stroke the increase of circulating EPCs was delayed in MMP9 deficient mice.

MMP9 deficient EPCs presented abnormal vessel remodelling, in vitro
MOUSE MCAO

Vascular Remodeling induced by Endothelial Progenitor Cells was abolished when brain tissue lacked MMP9

(Morano et al. 2015)

MMP13 deficient presented smaller infarcts and improved forelimb force

(Ma F. et al., 2015)
Ischemia-induced angiogenesis did not occurred in MMP13 deficient mica related to a previous decrease in pro-angiogenic and thropic growth factors in the cortex.
EPCs function was aberrant when MMP13 gene expression was inhibited, in vitro

A. Rosell; SERMEF, 2015.

Cellular Markers: Endothelial Progenitor Cells

Bone-marrow derived CD34+ circulating cells which can migrate towards areas of damage and differentiate into an endothelial phenotype.

Identified in 1997 they show clonogenic capacity with stemness characteristics. Offering a new paradigm for endothelial repair and vascular remodelling.

Table 1. Endothelial progenitor cells—definitions and requirements for therapeutic applications

<table>
<thead>
<tr>
<th>Definition</th>
<th>Therapeutic requirements</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Non-endothelial cell that can give rise to endothelial cells</td>
<td>1. Cells, which can be easily isolated and promote neovascularization</td>
</tr>
<tr>
<td>2. Clonal expansion</td>
<td>2. Functional integration into the tissue (thrombing, migration, invasion)</td>
</tr>
<tr>
<td>3. Stemness characteristics (not yet clearly defined; may include proliferation and resistance to stress)</td>
<td></td>
</tr>
</tbody>
</table>

Urich et al. 2004

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(Morancho et al. 2015)
**Endothelial Progenitor Cells**

Blood, bone-marrow, spleen

*Navarro-Sobrino. (Microvasc Res, 2010)*

**Flow Citometry**

Cell culture

Specific coating and endothelial media

"Early" EPCs (day 10)

Expanded OECs (P1)

No single marker for EPCs

**Coronary Artery Disease, EPCs & Cardiovascular events**

**Stroke & TIA patients with diagnosed intracranial atherosclerotic disease**

<table>
<thead>
<tr>
<th>Variable (n)</th>
<th>NO</th>
<th>YES</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (42)</td>
<td>309 ± 371</td>
<td>251 ± 273</td>
<td>0.480</td>
</tr>
<tr>
<td>Gender (Men: 32)</td>
<td>260 ± 257</td>
<td>248 ± 252</td>
<td>0.950</td>
</tr>
<tr>
<td>Age (&gt;66 years: 30)</td>
<td>322 ± 314</td>
<td>223 ± 214</td>
<td>0.296</td>
</tr>
<tr>
<td>Current smoking (4)</td>
<td>303 ± 297</td>
<td>111 ±  101</td>
<td>0.212</td>
</tr>
<tr>
<td>Hypertension (36)</td>
<td>168 ± 482</td>
<td>216 ± 429</td>
<td>0.315</td>
</tr>
<tr>
<td>Diabetes mellitus (20)</td>
<td>105 ± 263</td>
<td>190 ± 254</td>
<td>0.154</td>
</tr>
<tr>
<td>Hyperlipidemia (31)</td>
<td>263 ± 271</td>
<td>246 ± 278</td>
<td>0.842</td>
</tr>
<tr>
<td>Coronary artery disease (7)</td>
<td>266 ± 297</td>
<td>208 ± 259</td>
<td>0.031</td>
</tr>
<tr>
<td>Intermittent claudication (3)</td>
<td>245 ± 244</td>
<td>304 ± 318</td>
<td>0.474</td>
</tr>
<tr>
<td>Number of CVRFs &gt;2 (27)</td>
<td>368 ± 337</td>
<td>186 ± 211</td>
<td>0.032</td>
</tr>
<tr>
<td>Aspirin (15)</td>
<td>258 ± 286</td>
<td>234 ± 254</td>
<td>0.204</td>
</tr>
<tr>
<td>Clopidogrel (34)</td>
<td>466 ± 394</td>
<td>200 ± 214</td>
<td>0.102</td>
</tr>
<tr>
<td>Statins (36)</td>
<td>312 ± 309</td>
<td>241 ± 270</td>
<td>0.544</td>
</tr>
<tr>
<td>Rifampin (18)</td>
<td>230 ± 256</td>
<td>341 ± 348</td>
<td>0.159</td>
</tr>
<tr>
<td>AMI (13)</td>
<td>268 ± 287</td>
<td>208 ± 252</td>
<td>0.527</td>
</tr>
<tr>
<td>Severe intracranial stenoses (14)</td>
<td>256 ± 272</td>
<td>241 ± 285</td>
<td>0.871</td>
</tr>
<tr>
<td>ICAD associated with &gt;50% ICA stenoses (5)</td>
<td>267 ± 159</td>
<td>177 ± 169</td>
<td>0.180</td>
</tr>
<tr>
<td>Number of intracranial stenoses &gt;2 (%)</td>
<td>579 ± 154</td>
<td>193 ± 130</td>
<td>0.208</td>
</tr>
</tbody>
</table>

Schmid-Lucke et al 2005

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Increased EPC differentiation in blood from subacute patients

Cells obtained in the subacute phase presented better function, in vitro.

(Navarro-Sobrino, 2010)

Permanent MCAO in mouse

(Morancho, et al. 2014)
Neurorehabilitation & angiogenesis

From pre-clinical Models we know that physical exercise improves recovery from stroke related to enhanced angiogenesis and tissue remodelling EPC mobilization.

Number of EPCs in mice doing voluntary exercise (running well) vs. No exercise

SMARTS Study
(Studying Markers of Angiogenesis during Rehabilitation Therapy after Stroke)

Are angiogenic markers (molecular and cellular) related to motor and functional recovery during rehabilitation?
Protocolo de estudio SMARTS

Centrifugar los tubos a 1500 g durante 15 minutos a 4°C.

Resultados Preliminares (n=17/grupo)

EPCs estudio en SMARTS

CD45+, KDR+, y CD34+.

7° SYMPOSIUM KUOPIO STROKE
Battery of Neurological (motor and function) Scales during follow-up

- Chedoke Arm and Hand Activity Inventory (CAHAI).
- Fugl-Meyer Motor Assessment (FMA).
- Medical Research Council Scale (MRC)
- Functional Ambulation Classification de Holden (FAC).
- 10M Walking test
- Barthel Index (BI)
- Escala Rankin
- NIHSS

Baseline characteristics of the control and stroke cohorts

<table>
<thead>
<tr>
<th></th>
<th>Control (n=15)</th>
<th>Strokes (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.5±7.6</td>
<td>53.9±10.6</td>
<td>0.008</td>
</tr>
<tr>
<td>Gender, male</td>
<td>46.7 (7)</td>
<td>80.0 (12)</td>
<td>0.058</td>
</tr>
<tr>
<td>Alcohol</td>
<td>53.3 (8)</td>
<td>20.0 (3)</td>
<td>0.058</td>
</tr>
<tr>
<td>Tobacco</td>
<td>13.3 (2)</td>
<td>33.3 (5)</td>
<td>0.195</td>
</tr>
<tr>
<td>Hypertension</td>
<td>80.0 (12)</td>
<td>66.7 (10)</td>
<td>0.409</td>
</tr>
<tr>
<td>Dyslipidemia</td>
<td>60.0 (9)</td>
<td>53.3 (8)</td>
<td>0.712</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>26.7 (4)</td>
<td>20.0 (3)</td>
<td>0.665</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>0 (0)</td>
<td>13.3 (2)</td>
<td>0.143</td>
</tr>
<tr>
<td>Obesity</td>
<td>60.0 (9)</td>
<td>33.3 (5)</td>
<td>0.143</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.9±3.6</td>
<td>23.0±3.8</td>
<td>0.046</td>
</tr>
<tr>
<td>Comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteoarticular disorders</td>
<td>26.7 (4)</td>
<td>13.3 (2)</td>
<td>0.361</td>
</tr>
<tr>
<td>Ischemic cardiopathy</td>
<td>0 (0)</td>
<td>6.7 (1)</td>
<td>0.309</td>
</tr>
<tr>
<td>Psychiatric disorders</td>
<td>26.7 (4)</td>
<td>20.0 (3)</td>
<td>0.665</td>
</tr>
<tr>
<td>Previous Exercise</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Physical activity</td>
<td>73.3 (11)</td>
<td>50.0 (7)</td>
<td>0.196</td>
</tr>
<tr>
<td>Physical activity (hours)</td>
<td>7.0±10</td>
<td>7.0±10</td>
<td>0.288</td>
</tr>
<tr>
<td>Previous Medication</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiplatelets</td>
<td>26.7 (4)</td>
<td>20.0 (3)</td>
<td>0.666</td>
</tr>
<tr>
<td>Anticoagulants</td>
<td>0 (0)</td>
<td>6.7 (1)</td>
<td>0.309</td>
</tr>
<tr>
<td>Statins</td>
<td>33.3 (5)</td>
<td>33.3 (5)</td>
<td>1</td>
</tr>
<tr>
<td>Antihypertensives</td>
<td>73.3 (11)</td>
<td>46.7 (7)</td>
<td>0.136</td>
</tr>
<tr>
<td>Antidiabetic</td>
<td>20 (3)</td>
<td>13.3 (2)</td>
<td>0.624</td>
</tr>
</tbody>
</table>
Clinical characteristics of stroke patients

<table>
<thead>
<tr>
<th>Measure</th>
<th>Baseline</th>
<th>1 months</th>
<th>3 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>NIHSS score at admission</td>
<td>8 (5-12)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS motor score at admission</td>
<td>4 (2-7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS score after 3-4 days</td>
<td>5 (3-9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>NIHSS motor score after 3-4 days</td>
<td>3 (1-5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Early neurological outcome                   | Improvement: 33.3 (5)  
Stability: 53.4 (8)  
Worsening: 13.3 (2)  
Lacunar: 13.3 (2)  
Cardioembolic: 33.3 (5)  
Atherothrombotic: 13.3 (2)  
Others: 13.3 (2)  
Unknown: 26.8 (4)  |
| Location, Territory                         |          |          |          |
| Carotid:                                     | 73.3 (11) |          |          |
| Vertebrobasilar                              | 26.7 (4)  |          |          |
| Laterality                                   | Right: 53.3 (8)  
Left: 46.7 (7)  |
| OCSP classification                          | TACI: 46.7 (7)  
LACI: 20.0 (3)  
PACI: 12.3 (2)  
POCI: 20.0 (3)  |
| Thrombolytic therapy                        | Yes: 26.7 (4)  
No: 73.3 (11)  |
| Hemorrhagic transformation                   | Yes: 20 (3)  
No: 80 (12)  |
| Time stroke-intensive rehabilitation (days)  | 11.4±4.4  |

Outcome measures

- FMA (0-6)
  - Baseline: 38 (0-52)  
  - 1 month: 55 (4-51.5)  
  - 3 months: 60 (16-63)  
- Modified RANKIN (0-6)
  - Baseline: 4 (3-4)  
  - 1 month: 2 (1-5)  
  - 3 months: 1.5 (0.3)  
- Barthel index (4-100)
  - Baseline: 57 (31-63)  
  - 1 month: 94 (64-100)  
  - 3 months: 100 (88.6-100)  
- FAC (0-5)
  - Baseline: 1 (0-3)  
  - 1 month: 4 (1.5-5)  
  - 3 months: 5 (7-8)  
- CAVAI (13-91)
  - Baseline: 15 (13-71)  
  - 1 month: 72 (13-85)  
  - 3 months: 82 (13-90)  
- 10 meters walk test (m/s)
  - Baseline: 0.5 (0-1)  
  - 1 month: 0.9 (0.3-1.4)  
  - 3 months: 1 (0.8-1.3)  
- MRC Superior-Proximal (0-5)
  - Baseline: 4 (0-4)  
  - 1 month: 4 (2.5-5)  
  - 3 months: 5 (2-5)  
- MRC Superior-Distal (0-5)
  - Baseline: 4 (0-4)  
  - 1 month: 4 (0-5)  
  - 3 months: 5 (0-5)  
- MRC Inferior-Proximal (0-5)
  - Baseline: 4 (0-4)  
  - 1 month: 5 (4-5)  
  - 3 months: 5 (5-5)  
- MRC Inferior-Distal (0-5)
  - Baseline: 4 (0-5)  
  - 1 month: 5 (2-5)  
  - 3 months: 5 (4-5)  

Patients improved motor function and functional scores, mostly during the first month.

And MMP levels remained stable (at the studied time-points), However....
Association of MMP levels and baseline stroke severity and extension.
MMP3 was not related to baseline injury

Increased MMP12 or MMP13 levels in patients with a worse motor recovery during rehabilitation
Patients showing a larger improvement on motor and functional recovery presented higher plasma levels of MMP3 during the study period.

EPCs & Angiogenin as Biomarkers during Rehabilitation

Could cells and Growth Factors help to monitor rehabilitation and individual patients' response?

7th KUOPIO STROKE SYMPOSIUM
Biomarkers can be used to monitor Stroke patients and help to take personalized decisions.

- Look for biomarkers beyond proteins.
- But… Not ready to use stroke-biomarkers by next week.
- Inflammation and brain injury do matter as much as neurorepair and recovery.
- Now we need:
  - Verification and validation of good candidate biomarkers
  - Replication in well designed multicentric and international studies
  - Available technology for accurate and fast biomarker measures

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Take away Message

- Biomarkers can be used to monitor Stroke patients and help to take personalized decisions.
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