Universitat Autònoma de Barcelona

# Biomarkers Of Alzheimer's Disease: In Search Of A Non-Invasive Diagnosis

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## Introduction

Alzheimer's Disease (AD) is the most common type of dementia in the elderly, being a **severe neurodegenerative disorder** characterized by cognitive impairment and behaviour changes.

Pathological hallmarks of AD include **synaptic and neuronal loss**, **astrocytosis**, intraneuronal inclusions of hyperphosphorilated tau protein in **neurofibrillary tangles** (NFT) and extracellular deposits of amyloid β (Aβ), also called **senile plaques**. Aβ is synthesized from β -amyloid precursor protein (APP), which can be cleaved by two proteolytic pathways: the non-amyloidogenic pathway and the amyloidogenic pathway (which generates Aβ peptides) (Fig. 1). Disruptions in Aβ clearance and/or production results in their accumulation in brain and blood vessels.

There are two main types of AD: **early-onset** (or familial), which develops before 65 years and is a rare autosomal dominant disease caused by mutations in APP or presenillin genes; and **late-onset** (or sporadic), that occurs late in life and is a predominant multifactorial and heterogeneous disease.

Definitive AD diagnosis is restricted to ***postmortem* evaluation** of senile plaques and neurofibrillary tangles in the brain

Currently, AD diagnosis is restricted to **neuropsycological tests and brain imaging**

The **identification of potential biomarkers** is required to achieve an early and specific diagnosis.

Clinical diagnosis of AD is **unreliable**, particularly:
- During early stages of the disease
- When it presents with other dementias

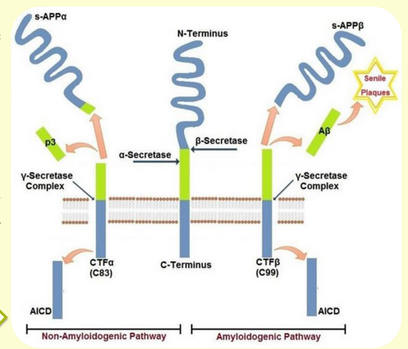



Fig 1. APP cab be cleaved by β-γ- secretases, releasing Aβ peptides or by α-γ-secretases. From Canobbio et al (2015)

## Objectives

- Analyse **current diagnosis** of the disease: emphasize the need of less invasive biomarkers.
- Establish **what is a biomarker** and how should be an **ideal biomarker for AD**
- Describe biomarkers studied in **blood cells** and **skin fibroblasts**
- Summarize biomarkers found in **plasma**.
- Mention the use of **miRNAs** as potential biomarkers for AD

## Materials & Methods

The project has been made as a scientific review using the bibliography obtained from Pubmed Database and Scopus. At first, the search was focused on reviews containing the keywords *biomarkers*, *Alzheimer's disease*, *peripheral*, *blood, fibroblast*, *miRNA*, among others. Then, the information provided by these papers allowed further searches in order to find more specifical original articles. Papers used have been published mainly between 2010 and 2015 in journals classified in Q1 or Q2.

## Biomarkers of Alzheimer's Disease From Peripheral Tissue

A **biomarker** is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention. An ideal AD biomarker should:
1) Detect fundamental neuropathological hallmarks
2) Differentiate AD from non-AD dementias
3) Recognize early stages and distinguish the progression of AD
4) Be easy to perform and inexpensive
5) Use minimally invasive sample collection
6) Be highly reliable, with high levels of sensitivity and specificity

**Cerebrospinal fluid biomarkers (CSF)**
- Aβ 1-42
- Total tau
- Phosphorylated tau 181

**Neuroimaging approaches**
- Magnetic ressonance imaging
- Amyloid tracer imaging
- 18C-fluorodeoxyglucose uptake

✓ Stablished and validated
✗ Too expensive
✗ CSF collection implies an invasive procedure

→ Impractical for initial diagnosis or routine screenings

### Blood-based biomarkers

**Plasma-based biomarkers**

**Proteomics**

Plasma protein profiles or single candidate proteins, including homocysteine, C reactive protein, cytokines, chemokines, apolipoproteins and growth factors.

✗ Lack of replication and standardization
✗ Variability and complexity of blood proteome

**Lipidomics**

Alterations in lipid pathways related to AD**.** Some of the most studied are:
- Ceramide/sphingomyelin ratio
- Abnormal glycerophospholipids
- Abnormal lipid peroxidation
- Lower desmosterol /cholesterol ratio

**Plasma Aβ species**

Results obtained are not clear, probably by reason of:
- Plasma Aβ = brain Aβ + peripheral tissues Aβ
- Blood brain barrier can be altered in AD
- Aβ tends to bind to plasma proteins and test tube walls

**Blood cell-based biomarkers**

✓ Molecular signaling abnormalities arise at early stages
✓ Easy and minimally invasive collection
✗ Processing of blood cells is more complex and time-consuming

**Platelets**

Are the first peripheral source of APP, containing secretase proteolytic machinery.

sAPPα>>Aβ: ↑↑ α-secretase activity

stored in α granules and released upon stimulation → Regulation of hemostasis (Fig. 2)

**Erythrocytes**
- Alteration of PKC conformation

**Leucocytes**
- ↑ GSK-3
- Alteration of PKC conformation (lymphocytes)

**Altered APP ratio**

$\downarrow \frac{120-130kDa\ APP}{110kDa\ APP}$ correlated with AD progression, including early stages.

**Altered secretase activity**

↓ α-secretase + ↑ β-secretase = ↑ Aβ secretion

↑ clot formation
↓ fibrinolysis
↑ clotting factors

**Enhanced platelet activation**

↑ coated platelets
- ✓ early stages
- ✓ correlated with AD progress

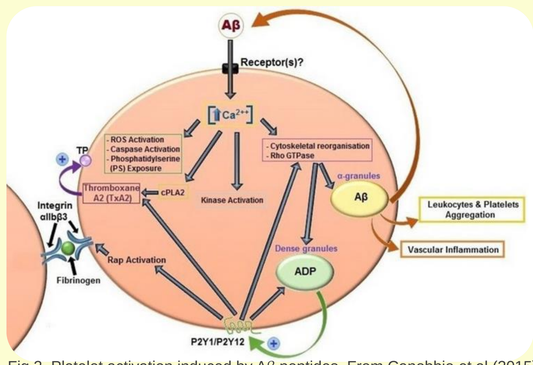



Fig 2. Platelet activation induced by Aβ peptides. From Canobbio et al (2015)

**Challenges of Blood-based biomarkers**

Currently there are no fully validated blood-based biomarkers, owing to…
- Failure to replicate findings due to the high variability between studies
- Interferences with the multiple conditions that affect elderly people

### Skin Fibroblast-based biomarkers

✓ Simple and inexpensive
✓ Easy to culture fibroblasts without contamination
✓ It's possible to repeat experiments
✓ Detect signaling disruptions → Early diagnosis
✗ Slow growth in culture → Biopsy ⟷ Test results

**Erk 1/2 phosphorilation in response to bradykinin** (Fig. 3)

$$\text{AD index} = \left[\frac{P-Erk1}{P-Erk2}\right]^{BK+} - \left[\frac{P-Erk1}{P-Erk2}\right]^{BK-}$$

→ Positive value in AD patients

✓ Could distinguish:
- AD/controls
- AD/ non-AD dementias

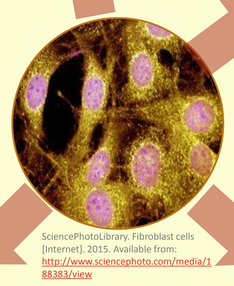
✓ Inversely correlated with AD progress

→ No significant breakthroughs done in recent years

**Fibroblasts aggregation rate**

Elevated aggregation rate and increasing cell density in AD fibroblasts

→ Novel approach that has to be replicated in larger studies



SciencePhotoLibrary. Fibroblast cells [Internet]. 2015. Available from: http://www.sciencephoto.com/media/188383/view

**PKCε deficit**

PKCε
- ↓ PKCε in the brains of AD patients
- Aβ peptides cause ↓PKCε
- Enhanced PKCε activity ↓Aβ levels
- PKCε activation prevents synaptic loss and memory deficits in AD mice
- PKCε ↓ in skin fibroblast from AD patients in correlation with disease progression

→ Promising but very recent: further research is needed

**$Ca^{2+}$ imaging**

Calcium dysregulation ⟷ Disruption in amyloid β and tau production → Observed in AD brain and skin fibroblasts

Huge variability between studies of $Ca^{2+}$-based bioassays
✗ Cytoplasmic ionic $Ca^{2+}$ levels
✗ TEA ($K^+$ channel blocker)
✗ Bradykinin (Fig. 3)

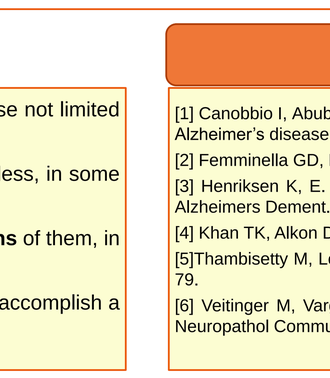



Fig 3. An.overview of PKCε and Erk1/2 signaling cascade. Adapted from Thomson Reuters

### miRNA as AD biomarkers

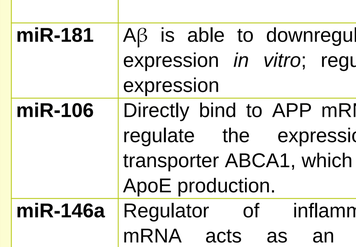



Fig 4. Mechanism of action of miRNAs.From Mpietrangelo.com. 2009 . Available from: http://www.mpietrangelo.com/hbio/unit/8_genetics/Chapter_11/B_Jpegs_of_Art_and_Photos/11_Labeled_Art_and_Photos/11_07microRNAs-L.jpg

miRNAs (microRNAs) are small coding RNAs that act as regulatory modulators of gene expression by two main mechanisms of action (Fig 4)

Specific miRNAs are expressed in the CNS → neuronal differentiation, synaptic plasticity, neurite outgrowth

miRNA dysregulation seems to be involved in neurodegenerative processes, such as AD(Table 1)

Blood → ✓ suitable for clinical diagnosis
CSF, Brain → ✗ Impractical

**Table 1. Some of the most common miRNA related to AD.** Adapted from Femminella et al (2015)

| miRNA | Role in AD pathophysiology | Evidence in AD patients |
|---|---|---|
| **miR-9** | Involved in neurogenesis and cell survival during brain development. | Downregulated in serum of AD patients. |
| **miR-107** | Negative correlation with BACE1 and neuritic plaque density; targets BACE1, CDK5 and ADAM10 | Downregulated in temporal cortex and serum of AD patients |
| **miR-29** | Inversely correlated with BACE1; it has been shown to increase amyloid production *in vitro* | Downregulation in human AD temporal cortex, cerebellum and serum. |
| **miR-34** | Regulates the expression of p53 | Higher expression in the hippocampus of patients with AD |
| **miR-181** | Aβ is able to downregulate miR-181 expression *in vitro*; regulates SIRT1 expression | Downregulated in human temporal cortex and patient serum |
| **miR-106** | Directly bind to APP mRNA; can also regulate the expression of the transporter ABCA1, which is involved in ApoE production. | Downregulated in temporal cortex of AD patients |
| **miR-146a** | Regulator of inflammation-related mRNA acts as an inflammatory response repressor in the CNS | "Selective" upregulation in brain regions affected by AD pathology, such as temporal cortex and hippocampus. |
| **miR-155** | Appears to have specific effects on complement factor H (ICFH) down-regulation in neurodegenerative brain | Upregulated in Down's syndrome (AD-like neuropathology with age) |
| **miR223*** | Seems to have a neuroprotective effect | Downregulated in serum of AD patients. Suggested as a neurodegenerative biomarker. |
| **miR-125b*** | Brain-enriched miRNA abundantly expressed in AD neocortex. | Downregulated in serum from patients with AD. |

* Data from Galimberti et al (2014)

**Will miRNA be used in the future for AD diagnosis?**
- Extensive validation and follow-up studies are required, in order to ensure their potentiality.
- Circulating miRNAs may be the next generation of promising biomarkers for AD, alone or in combination with other biomarkers

## Conclusions

- The present situation points to a **promising future** for this field: novel approaches are emerging continuously. The view of AD as a disease not limited to the brain has made easier the detection of potential biomarkers in peripheral tissues such as blood or skin fibroblasts.
- Results obtained until now show a **wide range of abnormalities related to AD** that would serve as biomarkers of the disease. Nevertheless, in some cases there is controversy between studies and in other cases **replication and validation is needed**.
- In fact, as none of the individual markers is powerful enough to be applied in routine AD diagnosis, it may be useful to employ **combinations** of them, in order to achieve high levels of sensitivity and specificity.
- Henceforth it is essential to reach a **harmonization of protocols**, incorporate **methods of validation** and start **larger studies** in order to accomplish a non-invasive AD diagnosis.

## References

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