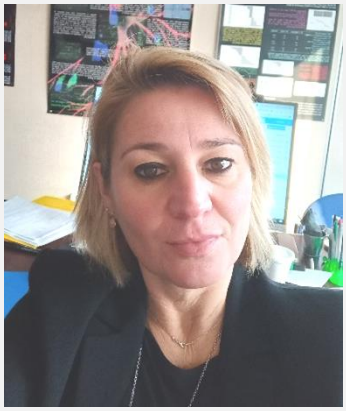
ImmunoTools *FlowISiAM* Award 2024



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Searching for new targets for Alzheimer's disease

Background

Alzheimer's disease (AD), a progressive neurodegenerative disorder, poses a significant global health challenge, affecting millions of individuals and their families. Characterized by cognitive decline, memory loss, and impaired daily functioning, Alzheimer's gradually erodes one's ability to think, reason, and remember. With an aging population, understanding the complexities of Alzheimer's becomes crucial for developing effective interventions, promoting early detection, and advancing research to unlock the mysteries of this devastating condition.

Early diagnosis of Alzheimer's could facilitate early intervention, enabling better management of symptoms, improved quality of life, and greater support for caregivers.

To this end, new predictive and/or diagnostic markers need to be found and analyzed.

Mitochondria are highly dynamic organelles that undergo continuous fission and fusion. Disruption of the delicate balance between mitochondrial fission and fusion may lead to abnormal morphology, impaired function of mitochondria and may contribute to neuronal degeneration.

The expression of the mitochondrial fission proteins dynamin-related protein 1 (Drp1), S-nitrosylated Drp1 (SNO-Drp1) and Fis1 was found to be altered in brain tissue and skin fibroblasts of AD patients and, more recently, also in lymphocytes[1].

The results obtained in lymphocytes showed that the altered mitochondrial fission proteins Drp1, SNO-Drp1 and Fis1 are relatively sensitive and specific in identifying AD patients and could serve as biomarkers in the diagnostic procedure. Information on their expression on monocytes is not yet available. It has been reported that treatment with Aβ1-42 leads monocytes to secrete pro-inflammatory cytokines such as IL-1β, IL-6, IL10 and TNF-α, which in turn support the pro-inflammatory environment that characterizes AD[2].

Interest in monocytes is increased by the evidence that these cells, being endowed with phagocytic activity, can act as sensors of the presence of pathology, and thus several results can be combined from their analysis.

The *FlowISiAM* assay is a state-of-the-art technique that revolutionizes the study of monocytes, a crucial component of the immune system. Monocytes are endowed with phagocytic capacity and, in line with this, the analysis of their content can provide useful information on the presence of proteins closely related to the onset of specific diseases.

While the evaluation of a single marker may provide unclear results on the presence/progression of a disease, the simultaneous evaluation of a panel of molecules could greatly enhance the diagnostic significance of the analysis. Considering that the *FlowISiAM* assay offers multiplexing capabilities, allowing researchers to simultaneously analyze multiple parameters within individual monocytes, the identification of new specific molecules becomes paramount.

Objectives

Primary objective

Evaluation of potential biomarkers for AD diagnosis using *FlowISiAM* technology: Analysis of the expression of Drp1, SNO-Drp1 and Fis1 in monocytes isolated from the blood of AD patients (AD-Mon) and healthy controls, under resting conditions (C-Mon) and after treatment with Aβ1-42 (Aβ-Mon) to mimic the pathological environment of AD.

Secondary objective

To test the expression of other potential biomarkers for AD diagnosis using *FlowISiAM* technology, such as:

- CD33: CD33 has been reported to link genetic susceptibility, altered myeloid function and amyloid pathology to an early role in the pathogenesis of AD; furthermore, the rs3865444C risk allele is known to be associated with increased cell surface expression of CD33 in monocytes[3].

- FK506 Binding Protein (FKBP51): interferes with tau degradation and promotes the formation of tau oligomers. Higher levels of FKBP51 have been associated with AD progression. Its expression on monocytes has so far been evaluated in depression as a condition of prolonged stress[4].

- amyloid precursor protein cleavage enzyme 1 (Bace1): APP cleaving enzyme 1 is required for the generation of Aβ42, which aggregates and initiates toxicity in AD. Bace1 concentrations and activity rates are increased in AD brain and body fluids, supporting the hypothesis that Bace1 plays a critical role in the pathophysiology of AD[5].

- Macrophage Scavenger Receptor I (MSRI): it is involved in the phagocytosis of Aβ-peptides and plaques and its expression is reduced in monocytes from AD patients[6].

Experimental design

Our study aims at analyzing the *FlowISiAM* assay:

1. to evaluate the basal expression of Drp1, SNO-Drp1 and Fis1 in C-Mon, as well as their secretion of IL-1β, IL-6, IL-10 and TNF-α;
2. to evaluate changes in marker expression in Aβ-Mon at different concentrations/times, as well as their secretion of IL-1β, IL-6, IL-10 and TNF-α;
3. To evaluate the basal expression of Drp1, SNO-Drp1 and Fis1 in AD-Mon, as well as their secretion of IL-1β, IL-6, IL-10 and TNF-α.
4. To assess the expression of CD33, FKBP51 and Bace1 in all conditions described above.

Methods

1. Blood samples from healthy controls and AD patients will be collected and used for the isolation of monocytes by means of Ficoll density gradient centrifugation. For the collection of blood samples, the proposer has already obtained the approval of the Ethics Committee of the Università Politecnica delle Marche (23-09-2021; no. 2021-218) for this type of sample; it would therefore be sufficient to provide for the placement of a new investigator at the neurological clinic (the hospital is adjacent to the university campus where the research will take place), thus having immediate access to patient enrolment.

2. C-Mon will be stimulated with Aβ1-42 at different concentrations/times.

3. C-Mon, Aβ-Mon and AD-Mon will be used to assess the intracellular staining of IL-1β, IL-6, IL-10 and TNF-α using antibodies by ImmunoTools. This step will allow us to assess whether treatment with Aβ1-42 is able to mimic the pathological environment of AD and its effect on monocytes; by varying the concentration/time of treatment, we could recreate different AD settings.

4. Supernatants of C-Mon, Aβ-Mon and AD-Mon will be used to assess the secretion of IL-1β, IL-6, IL-10 and TNF-α by ImmunoTools ELISA TEST.

5. *FlowISiAM* analysis of Drp1, SNO-Drp1 and Fis1 in C-Mon, Aβ-Mon and AD-Mon.

6. *FlowISiAM* analysis of CD33, FKBP51 and Bace1 in C-Mon, Aβ-Mon and AD-Mon.

Impact

This project may help to elucidate certain molecular mechanisms, such as the involvement of mitochondria dynamics, in the development of AD; the project aims to consider a set of molecules whose single expression is not altered enough to suggest it as a marker for diagnosis but which together may form a panel that can be assessed by the *FlowISiAM* assay, providing a simple, rapid and valid tool for the diagnosis of AD.

Cooperation partner

Prof. Dr. Orciani's group will collaborate with ImmunoTools to optimise the experimental and instrumental set-up to conduct *FlowISiAM*. In addition, ImmunoTools will provide antibodies for cytometric examination and ELISA tests or intracellular cytokine detection to assess cytokine secretion by C-Mon, Aβ-Mon and AD-Mon.

After an initial general evaluation of the data obtained with the already commercially available antibodies against Drp1, SNO-Drp1, Fis1, CD33, FKBP51 and Bace1, ImmunoTools' SME partner, INVIGATE, will take on the task of developing specific monoclonal antibodies against the best performing target(s), which are truly sensitive and specific in the identification of AD patients and available as biomarker in the diagnostic procedure, and will support the initial evaluation. In addition, INVIGATE will provide other AD-related monoclonal antibodies from its pipeline (pTau181; Aß1-42; modified Aß peptide motifs and synaptic break down) for experimental evaluation within *FlowISiAM* in control and AD blood samples.

Optionally, ImmunoTools and its partner INVIGATE will be asked to develop a multicolour flow cytometry kit to simultaneously detect the expression of selected biomarkers. Prof. Dr. Orciani and Dr. Sebastian Krause (INVIGATE) intend to develop and permanently improve *FlowISiAM* -based strategies for the prediction of AD disease progression and early diagnosis of AD.

They intend to create good prerequisites for a joint research grant application.

References

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2. doi:10.3390/jcm9092887
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ImmunoTools *FlowISiAM* AWARD for **Monia Orciani** includes

antibodies for *FlowISiAM*, know how transfer and protocol, support regarding selection of specific antibodies against specific biomarkers from INVIGATE, expert assistance in evaluating the results obtained, and integration into the ImmunoTools *FlowISiAM* network.