UNIVERSITÀ DEGLI STUDI DI MILANO

SELEZIONE PUBBLICA, PER TITOLI ED ESAMI, PER IL RECLUTAMENTO DI N. 1 UNITÀ DI TECNOLOGO DI SECONDO LIVELLO CON RAPPORTO DI LAVORO SUBORDINATO A TEMPO DETERMINATO DELLA DURATA DI 18 MESI, PRESSO L'UNIVERSITÀ DEGLI STUDI DI MILANO - DIPARTIMENTO DI SCIENZE DELLA SALUTE, PER L'ATTUAZIONE DEL PROGETTO DAL TITOLO "D3 4 HEALTH - DIGITAL DRIVEN DIAGNOSTICS, PROGNOSTICS AND THERAPEUTICS FOR SUSTAINABLE HEALTH CARE" (CUP B53C22006080001) - PIANO NAZIONALE COMPLEMENTARE (CODICE PNC0000001), NELL' AMBITO DEL PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) - CODICE 22306

La Commissione giudicatrice della selezione, nominata con Determina Direttoriale n. 12415 del 25/07/2023, composta da:

Prof.ssa Sandra D'Alfonso	Presidente
Prof. Dario Ronchi	Componente
Prof. Filippo Martinelli Boneschi	Componente
Dott. Paolo Zanuttini	Segretario

comunica i quesiti relativi alla prova orale:

TEMA 1

Metodologie di analisi omiche
Methods of omics analysis

Objective: Neuronal damage is the morphological substrate of persisting neurological disability. Neurofilaments (Nf) are cytoskeletal proteins of neurons and their release into cerebrospinal fluid has shown encouraging results as a biomarker for neurodegeneration. This study aimed to validate the quantification of the Nf light chain (NfL) in blood
samples, as a biofluid source easily accessible for longitudinal studies.
Methods: We developed and applied a highly sensitive electrochemiluminescence (ECL) based immunoassay for quantification of NfL in blood and CSF.
Results: Patients with Alzheimer's disease (AD) (30.8 pg/ml, n=20), Guillain-Barré-syndrome (GBS) (79.4 pg/ml, n=19) or amyotrophic lateral sclerosis (ALS) (95.4 pg/ml, n=46) had higher serum NfL values than a control group of neurological patients without evidence of structural CNS damage (control patients, CP) (4.4 pg/ml, n=68, p<0.0001for each comparison, p=0.002 for AD patients) and healthy controls (HC) (3.3 pg/ml, n=67, p<0.0001). Similar differences were seen in corresponding CSF samples. CSF and serum levels correlated in AD (r=0.48, p=0.033), GBS (r=0.79, p<0.0001) and ALS (r=0.70, p<0.0001), but not in CP (r=0.11, p=0.3739). The sensitivity and specificity of serum NfL for separating ALS from healthy controls was 91.3% and 91.0%.
Conclusions: We developed and validated a novel ECL based sandwich immunoassay for the NfL protein in serum (NfLUmea47:3); levels in ALS were more than 20-fold higher than in controls. Our data supports further longitudinal studies of serum NfL in neurodegenerative diseases as a potential biomarker of on-going disease progression, and as a potential surrogate to quantify effects of neuroprotective drugs in clinical trials.

TEMA 2
Modalità di estrazione degli acidi nucleici
Extraction methods of nucleic acids

Introduction
Neurofilaments (Nf) are highly specific major structural proteins of neurons, consisting predominantly of four subunits: Nf light (NfL), Nf medium (NfM) and Nf heavy (NfH) chain and alpha-internexin [1]. Nf are released in significant quantity following axonal damage or neuronal degeneration. Disruption to the axonal membrane releases Nf into the interstitial fluid and eventually into cerebrospinal fluid (CSF) and blood. Therefore, blood

Nf levels could be useful for both predicting and monitoring disease progression and for assessing the efficacy and/or toxicity of future neuroprotective treatment strategies.
Several previous studies have demonstrated the presence of NfH and NfL in CSF, which has been assumed to reflect brain pathology more accurately than the peripheral blood compartment [2–12]. However, obtaining longitudinal CSF samples is considered too invasive outside the clinical trial arena, precluding the broader clinical use of Nf. In contrast to CSF, serial blood samples can readily be collected, hence reliable quantification of NfL in blood would be a major stride towards a biomarker of the course of neurodegeneration.
Several reports have suggested peripheral blood levels of NfH as a potential marker of neurodegeneration [13–22]. In contrast to this, there is only one recent study investigating serum NfL; this paper examined the relationship between serum NfL and neurological outcome following cardiac arrest [23].

TEMA 3

Conservazione e stoccaggio dei materiali biologici
Conservation and storage of biological materials

A highly sensitive method for the detection of a clinically relevant biomarker of neurodegeneration has been developed.
Importantly, our method allows us to make use of readily available longitudinal patient blood samples, instead of being restricted to ethically difficult to obtain CSF samples. One potential clinical application for serum NfL levels is demonstrated by the diagnostic sensitivity of 91.3% for ALS, a rapidly progressive neurodegenerative disease [29,30].
We present the first ECL based solid phase immunoassay for the NfL protein in blood based on two non-competitive, monoclonal antibodies. These antibodies have been widely used and validated in a commercial ELISA for CSF measurements of NfL (NF-light® assay) [5,24,31]. NfL is considered to represent the most abundant and also most soluble Nf subunit [1]. The optimised ECL-NfL assay protocol proved to be highly accurate (intra-assay CV < 6%, inter-assay CV < 24%), sensitive (sensitivity 15.6 pg/ml) and demonstrated linearity and parallelism (Figures 1 and 2) over a wide analytical range (15.6-10,000 pg/ml). In addition we found NfLUmea47:3 to be stable in serum [25]. This is relevant for a potential value to monitor drug effects by serum NfL in ALS where Nf aggregate formation is a key pathological finding [28].

Milano, 29 agosto 2023

La Commissione

Prof.ssa Sandra D'Alfonso Presidente

Prof. Dario Ronchi Componente

Prof. Filippo Martinelli Boneschi Componente

Dott. Paolo Zanuttini Segretario