BIOSKETCH

I am a Neurologist and a doctoral student at the University of Bologna in Italy with a primary interest in Sleep Medicine. I started attending the laboratory and outpatient clinic of the Sleep Center of Bologna in 2016 under the guidance of Professor Federica Provini. I graduated in June 2018 with full marks with the thesis in Sleep Medicine *"Physiological movements during sleep of young adults: a video-polysomnographic study*". I continued the clinical and research activities at the Sleep Center of Bologna by winning a post-graduate scholarship in March 2018. I started the Neurology residency training in November 2019 and during the last year of residency program, I completed a research stay at the Sleep Unit of the Hospital Clinic of Barcelona where, under the supervision of Professor Alex Iranzo, I developed the research project "*Scoring Sleep in Neurodegenerative Diseases: A Pilot Study in the Synucleinopathies".* The latter evolved into my Neurology thesis, and I successfully graduated in November 2023. In the same months, I was awarded a scholarship to pursue a doctoral research program at the Department of Biomedical and Neuromotor Sciences of the University of Bologna, obtaining the top position in the department's ranking. During these years I had the opportunities to engage in public speaking, conduct literature analysis and actively participate in the realization of research projects. I had major publications in international indexed journals, some of them as the first author, and most in the field of sleep medicine.
I joined the European Restless Legs Syndrome Study Group (EURLSSG), in December 2022 and from the outset, I contributed with oral presentations at the EURLSSG Meetings. Since May 2023, I have been part of the EURLSSG task force working on the development of a common database to collect clinical and instrumental data of RLS patients. In October 2023, I was invited to speak at a symposium at the International RLS Study Group (IRLSSG) Annual Meeting with the title "*Role of Inflammation in RLS".* This topic has become one of my areas of interest following the development of a collaborative project titled "*Biomarkers of Inflammation in Restless Legs Syndrome (iRLS)*" with the Brain Ageing Laboratory at Bellaria Hospital in Bologna. The preliminary results of this project, presented in the abstract "*Metagenomic analysis in Restless Leg Syndrome",* have received two awards at the World Sleep Congress 2023: the "*New Investigator Awards - World Sleep Congress*" from the World Sleep Society and the "*Wayne Hening Young Investigator Awards*" from the IRLSSG Study Group.

PROJECT

Background

The pathophysiological mechanisms underlying RLS are still largely unknown, although the role of inflammation is supported by growing evidence from genetics (*Hennessy, 2014*), proteomics (*Patton, 2013; Bellei, 2018; Shin, 2020; Mondello, 2021; Cederberg, 2023*), and the observation of increased levels of serum/plasma pro-inflammatory cytokines in RLS patients (*Jiménez-Jiménez, 2023*). The association with infectious-inflammatory (*Weinstock, 2012*) and gastrointestinal diseases (*Guo, 2021; Loosen, 2023*), suggests a link between inflammation and the gut microbiota, already underscored in other neurological disorders (*Quigley, 2017*), paving the way for new therapeutic approaches (*Chandra, 2020*). Furthermore, the microbiome-gut-brain axis is well known to contribute to the etiology of sleep disorders (*Wang, 2022*).

Objectives

1. to analyse the gut microbiota in stool samples from patients with RLS compared to control groups of patients with insomnia and of healthy subjects, age and sex-matched. The analysis of the gut microbiome (i.e., the genome of microorganisms that inhabit the human gut) will be performed by amplification and sequencing of the V3-V4 hypervariable region of prokaryotic 16S ribosomal RNA (16S rRNA). The study of 16S rRNA allows a phylogenetic classification into genus and species of the different bacterial populations and an estimation of the latter in terms of abundance and prevalence (*Ghosh, 2020*).
2. to profile short-chain fatty acids in stool samples using Gas Chromatography-Mass Spectrometry (GC-MS). SCFAs are the main metabolites produced in the colon by bacterial fermentation of dietary fiber and have the ability to cross the blood-brain barrier. SCFAs are believed to play a key role in neuro-immunoendocrine regulation and are among the mediators of the neuroinflammatory process in the brain (*Dalile, 2019*).
3. to observe the gut microbiota composition in patients with RLS after 1 year of follow-up and build a comprehensive biological signature of RLS patients, including iron status and systemic biomarkers of inflammation.

Scientific relevance

- to implement knowledge regarding the RLS pathogenesis;
- to identify possible biomarkers supporting diagnosis and therapeutic targets.

Preliminary results and study design

The project "Biomarkers of Inflammation in restless legs syndrome (iRLS)" originates from the collaboration between the Brain Ageing Laboratory and the Sleep Laboratory at the Institute IRCCS Istituto delle Scienze Neurologiche of Bologna. The study protocol has already been approved by the CE-AVEC committee at its meeting on 15/11/2022 (internal code 794-2022-SPER-AUSLBO) and recruitment is currently underway. The metagenomic analysis of the initial samples (30 RLS patients; 29 healthy controls) revealed that, compared to controls, RLS patients showed a significant decrease in the levels of *Lachnoclostridium* genus. The latter had a direct correlation with disease duration ≥15 and clinical phenotype. These results may pave the way for the development of new diagnostic biomarkers and interventions for RLS.

To validate our findings, we need to include:

1. a control group of insomnia patients: given the high prevalence of insomnia in patients with RLS, our aim is to compare individuals with both RLS and insomnia to those with isolated insomnia;
2. metabolomic analysis: this will allow us to assess the production of bacterial metabolites and their relationship with CNS homeostasis in RLS patients (*Lopetuso, 2013; Guo, 2020*);
3. the follow-up of RLS patients: to observe potential changes in the microbiota over time. Furthermore, increasing the number of RLS patients will give us the opportunity to stratify patients according to phenotypes, comorbidities, and iron status.

Key activities

Subjects' recruitment (objectives 1 and 3)
We will consecutively recruit RLS (Allen, 2014) and insomnia patients (International Classification of Sleep Disorders. 3rd ed. TR) referred to the Sleep Center of IRCCS Istituto delle Scienze Neurologiche of Bologna. Healthy controls will be recruited among unrelated patients' caregivers.

Metagenomic analysis (objectives 1 and 3)
The analysis of the gut microbiome will be performed by amplification and sequencing of the V3-V4 region of the 16S rRNA gene on MiSeq platform. The investigation protocol has already been validated at the Brain Aging Laboratory.

Metabolomics analysis (objective 2)
The analysis of SCFAs by GC-MS will be carried out in collaboration with the Proteomics, Metabolomics and Bioanalytical Chemistry Laboratory of the IRCCS Istituto delle Scienze Neurologiche in Bologna according to a protocol already optimised at that laboratory.

Project overview, scientific paper writing, and support

We aim to conclude the "first objective" of sample collection and analysis (RLS baseline, along with both insomnia and healthy controls) by the end of 2024. The differences in the microbiota composition between RLS and the two control groups could be presented at the Annual Congress EAN 2024 and serve as the basis for publications in international indexed journals.
Then, we will focus on the metabolomics analysis ("second objective") and continue recruiting RLS patients and 1-year follow-up ("third objective"). As these data require more time, we plan to present them at upcoming conferences or during the EURLSSG Meeting.
We require funding for plastics and consumables for sample collection and iron status assessment; plastics and consumables for metagenomic and metabolomic analyses (reagents for extraction, sequencing, buffers, and cartridges).

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