DARE

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Spoke 3 Deliverable

S3.D4.1 Concept and relevant design of the models

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1.0	30/12/2023	IOR, UNIBA, UNIROMA2	Final draft

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Publishable Summary

Work Package 4 of the DARE project titled "Digitally-enabled Biomarker Discovery" is a part of Spoke 3 "Digitally-enabled secondary and tertiary prevention".

It comprises seven Pilot Studies focused on identification and validation of specific biomarkers by the integration of different digital technologies, machine learning approaches and digitalized medicine supported by Information and Communication Technology (ICT) infrastructures. The major aim for all these pilots is the integration of ICT tools into clinical protocols capable of identifying biomarkers in a number of different diseases, from orthopaedic, to diabetes, to oncology, to brain diseases.

The pilots incorporate a spectrum of tools, spanning from wearable devices to algorithms supporting clinical decision-making processes. The principal hurdle hindering the maximization of ICT tools lies in the absence of their integration into systemic interventions—this is precisely the challenge that the Spoke 3 pilots strive to surmount.

These seven pilots employ diverse studies, ranging from observational approaches to randomized study designs. They encompass a wide array of patient groups, including those with diabetes, oncological conditions, transplant recipients, psychiatric and cognitive disorders, as well as adults with and without chronic diseases. The overarching focus of these studies revolves around various cross-cutting issues related to identifying biomarkers for early disease diagnosis and prognostication. The efficacy of these methodologies stands as a pivotal determinant for the success of the pilot studies.

Throughout the initial 12 months of the project, December 2022 to December 2023, the pilot concepts were meticulously formulated also in collaboration with Spoke 1 experts. Simultaneously, a comprehensive list of indicators was developed to gauge the proposal's impact.

1. Introduction

1.1. WP4 overview and aims

The Work Package 4 of the DARE project, titled "Digitally-enabled Biomarker Discovery", is part of the Spoke 3 "Digitally-enabled secondary and tertiary prevention" and gathers seven Pilot Studies focused on the identification and validation of specific biomarkers useful for secondary and tertiary prevention intervention supported by ICT- infrastructures.

WP4 encompasses research on different patient's populations, aiming to uncover methodologies that enhance the well-being of patients while equipping clinicians with increased, precise, and easily manageable information. The realization of such improvements is contingent upon the integration of ICT. These investigations might lay the groundwork for advancing ICT applications in both preventive measures and treatment procedures, pending the demonstration of their effectiveness through reliable indicators.

The primary focus of the upcoming WP4 pilots is to investigate how new ICT developments can be adapted to identify biomarkers useful into different clinical and public health fields. In particular, WP4 is committed to introducing technology to identify specific biomarkers, and thus to improve diagnosis and prevention outcomes for the populations here addressed. By strategically integrating various ICT tools and systems into different pilot programs, physicians and researchers will be empowered to create models for prediction and monitoring of risks across a variety of clinical and health-care scenarios. The overarching goal is to fully leverage the potential of ICT in the realms of prevention and healthcare, by the identification and monitoring of specific biomarkers. The diverse applications highlight the flexibility of ICT as a general infrastructure platform suitable for various situations.

Specifically, the adoption of digital eHealth/mHealth platforms for home/remote monitoring, IoT and wearable devices, decision support systems, and data integration is expected eventually to:

- Enhance prevention, to limit or to delay critical diseases progression (such as diabetes, cancer, neurological disorders), and to relevant reduce health-care costs.

- Provide accessible and easy-to-use tools and instruments for the assessment of specific diseases.
- Support decision-making processes for personalised preventive treatments to each patient's specific need and disease progression.
- Develop specific tests, based on specific disease-biomarkers, useful to decrease the incidence and seriousness of complications and improve the survival rates of patients with specific diseases such as cancer.
- Assess whether the implementation of AI in decision processes can improve the outcome prediction, and to validate the role of specific biomarkers in this scenario.
- Improve knowledge on the pathogenetic mechanisms sustaining diseases onset and progression.

1.2. Project status at month 12

Employing multidisciplinary approaches, all pilots within the DARE Spoke 3 program must be at the stage of conceptualizing and designing the model. Extensive discussions at different program levels have contributed to this progress. Ethical approval has been obtained for some pilots, while others are awaiting approval, or preparing the necessary documentation. Additionally, academic and technical positions have been filled to enhance the multidisciplinary composition of the research groups.

1.3. Deliverable outline

In Section 2, a detailed account is provided at the individual task level, offering insights into the objectives and hurdles faced by the WP. This section also includes information on the study design, anticipated outcomes, and the advancements achieved at the 12th month of the project. The study design implemented during these initial 12 months is documented, followed by a description of the expected results. Moving to Section 3, general conclusions are presented, along with a list outlining key steps and challenges expected in the upcoming period.

2. Research concept and design

2.1. Task 4.1 - Accessible measurements of mobility and deformity as biomarkers for orthopedic treatments (Task Leader: Paolo Caravaggi, IOR)

2.1.1. Overview

Osteogenesis Imperfecta (prevalence 6-7:100.000; ORPHA:666) and Multiple Osteochondromas (prevalence 1-2:100.000; ORPHA:321) are rare genetic bone disorders (RBDs) which significantly decrease the quality of life from an early age by altering joints mobility and posture due to deformities, increased bone fragility and alterations in plate cartilage growth. RBDs are characterised by a wide clinical variability and by progressive degeneration of the overall health conditions. However, only few highly specialized centres treat RBDs thus restraining accessibility to patients suffering from these pathologies. There is need for wider monitoring and evaluation of the disease progression across the territory.

In order to improve the monitoring of deformity and mobility of the musculoskeletal system of RBDs patients, we propose the assessment and validation of novel, accessible and easy-to-use instruments and relevant tools such as Inertial Measurement Units (IMUs), plantar pressure measurement systems and 3D body scanners. These tools should be made available in small clinics, community hospitals and at patients' homes for the continuous monitoring of the disease to support the clinicians in the diagnosis and in the prescription of corrective strategies.

2.1.2. Objectives

General objective

To provide accessible and easy-to-use tools and instruments for the assessment of musculoskeletal alterations in RBDs patients for:

1) Biomechanical characterization of Osteogenesis Imperfecta and Multiple Osteochondromas;

Ultimately supporting decision-making regarding preventive treatments personalised to each patient's specific need and disease progression.

Specific Objectives

1) Validation of novel devices and tools to measure joint mobility and bone deformity in RBDs patients;
2) Identification of postural and functional "biomarkers" (i.e. biomechanical parameters) capable to discriminate non-physiological alterations in patients with bone fragility and/or growth plate defects;
3) Providing information and data relevant to the design of custom prostheses and orthoses, to improve the quality of life of RBDs patients and to properly assess the timing for surgical treatments.

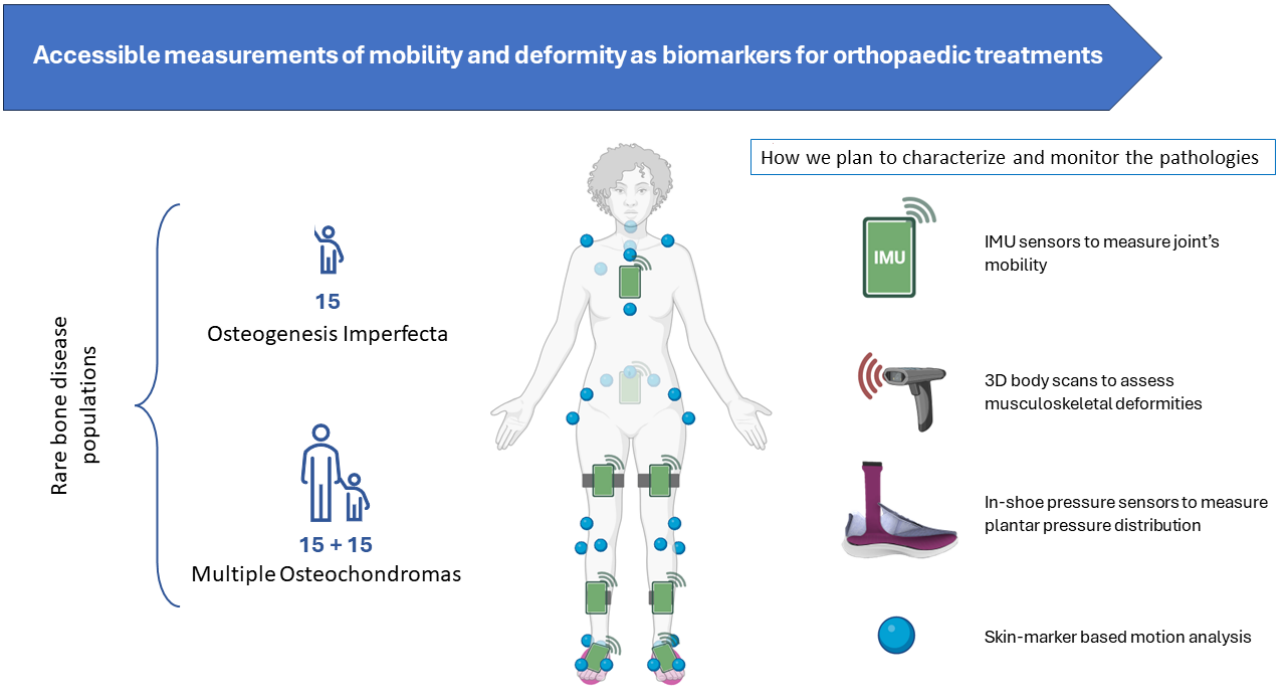

Figure 1: Graphical abstract task 4.1

2.1.3. Study design

Study characteristics:

This pilot is designed as a prospective observational study without drugs and medical devices. The study concerns with secondary prevention strategies for pathological alterations of the musculoskeletal system.

Ethical Committee authorization status:

Submission of the project proposal to the local ethical committee for approval is expected in April 2024.

2.1.4. Expected results

The expected results from this study are:

1) Novel, easy-to-use and accessible tools to assess and measure alterations in gait patterns, joints' mobility and morphology;
2) Identification of biomechanical "biomarkers" for the early-stage diagnosis, characterization and monitoring of the progression of the disease.

2.1.5. Project progress

An ongoing review of the literature on Osteogenesis Imperfecta and Multiple Osteochondromas is being performed to better understand the biomechanical alterations in RBDs patients and the existing clinical evaluation tools.

A multidisciplinary team comprised of engineers and clinicians has identified two RBDs patients' groups (6-13 years and 18-40 years) that will be recruited in the study. A thorough analysis of the instruments and tools available in the market addressing the aims of the project is also undergoing.

2.2. Task 4.2 - Biomechanical features for early detection of diabetic foot complications (Task Leader: Alberto Leardini, IOR)

2.2.1. Overview

Diabetes is a global epidemic affecting about 530 million people worldwide, and thus it is definitely a heavy burden on all health-care systems. In particular, foot functions and locomotion are severely affected, and the costs for associated treatments are huge. In the worst scenario, partial or complete foot amputations are necessary (worldwide, one each 20 seconds!). Clinical assessments are performed carefully and routinely but additional biomarkers shall be searched to prevent as much has possible complications at the foot. Therefore, established techniques for identification of circulating biomarkers and for multi-instrumental biomechanical measurements (plantar pressure, kinematics, kinetics, clinical data, original medical imaging, etc.) are meant to be exploited in this Pilot as modern combined biomarkers to predict ulcerations and other foot ailments. This is expected to support prevention and to facilitate early standard personalized care.

The partners and the actors involved are now:

- Uberto Pagotto and Giulia Casadei, Scienze Mediche e Chirurgiche UNIBO, Settore MED/13 ENDOCRINOLOGIA, Sant'Orsola Bologna – IRCCS AOU BO
- Lisa Berti, Dipartimento di Scienze Biomediche e Neuromotorie, Settore scientifico disciplinare MED/34; and IRCCS Istituto Ortopedico Rizzoli Bologna - Medicina Fisica e Riabilitativa
- Alberto Leardini and Claudio Belvedere, Movement Analysis Laboratory IRCCS Istituto Ortopedico Rizzoli Bologna
- Elena Tremoli and Luca Dalla Paola, Maria Cecilia Hospital – Gruppo Villa Maria, Cotignola (RA)

2.2.2. Objectives

General objective

To enhance prevention, to limit or to delay critical foot and function conditions as effects of the diabetes, and thus to reduce the present huge health-care costs.

Specific Objectives

In particular, we want to correlate standard clinical data and other known predisposing factors, with advanced state-of-the-art functional and imaging measures, together with circulating biomarkers with modern laboratory analyses. These would detect biological and biomechanical markers, for better tracing of the progression of the pathology, and to identify thoroughly, quantitatively and promptly possible alarming signs.

This eventually shall promote and facilitate access of proper standard physical, pharmacological and orthotic-based (offloading, etc.) interventions, and thus to ameliorate patient mobility and quality of life. Finally, the project will propose those modern measures (instruments, protocols, analyses, etc.) and relevant thresholds for the scope.

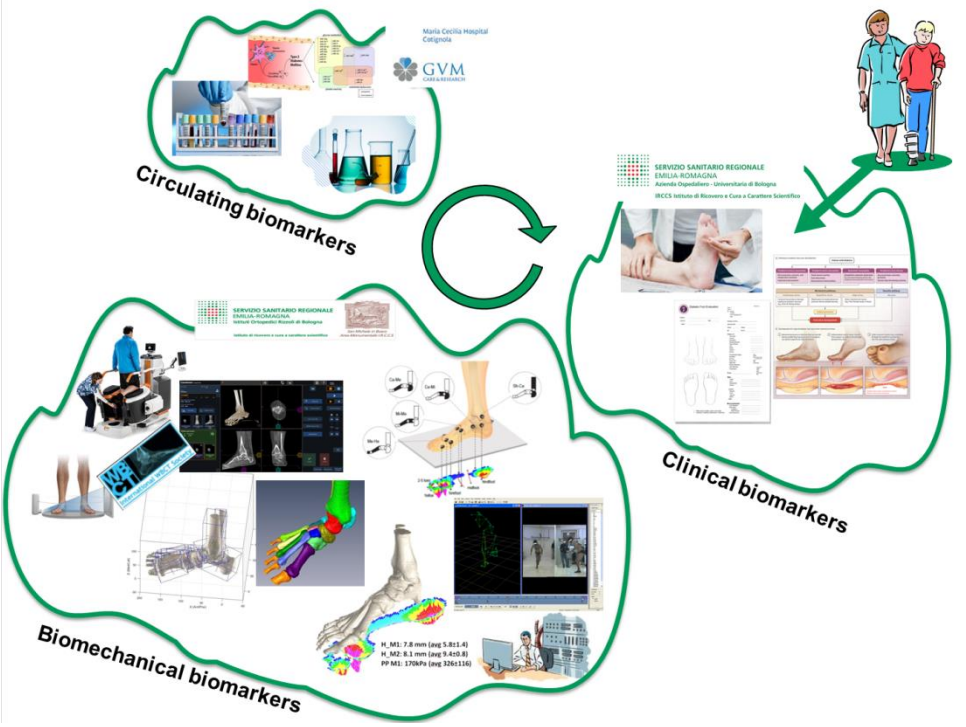

Figure 2. Graphical overview of Task 4.2

2.2.3. Study design

Study characteristics: The three analyses mentioned above, i.e. traditional clinical assessments - circulating biomarkers - multi-instrumental biomechanical measures, are established techniques, and have demonstrated already their value in isolation. By using measures and analyses in combination, particularly with novel sophisticated instruments, foot ulcer appearance can be predicted earlier. From these, those simpler, less invasive, and possibly cheaper measures shall also be selected to make this prediction feasible in many clinical and research contexts.

Ethical Committee authorization status: Preparation of the documentation, particularly difficult because of the perspective multicentre study, which implies a sort of hub centre and two spoke centres, but each with a specific authorization from the local Ethical Committee.

2.2.4. Expected results

It is expected that those instrumental tests and relevant experimental protocols shall be identified, thus to add these to the traditional clinical assessments, for a better identification of those patients at higher risk of foot ailments.

2.2.5. Project progress

Several meetings have been arranged among the three centres involved, with participation of the major actors, to clarify more specifically the necessary measures and analyses, and to work synergically. Other major issues have been addressed, such as the clinical population (type 1 and/or 2, inclusion / exclusion criteria, age & gender, etc.), the collection and exchange of measurements, etc. At the moment, the major protocol for the clinical study is under review, to be distributed then and configured specifically to each single centre. Meanwhile each partner is refining the techniques to be exploited during the project.

2.3. Task 4.3a - Single oncological marker detection for early diagnosis (Task Leader: Luisa Torsi, UNIBA)

2.3.1. Overview

More than 95% of Cervical cancer (CC) is caused by human papillomaviruses (HPV), and 604,127 new CC cases are diagnosed annually worldwide with about 342,000 deaths each year (estimations for 2020). The pilot study T4.3a aims at developing a point-of-care (POC) highly reliable and sensitive assay of HPV infections' markers/viruses. The study will engage the Single-Molecule with a large Transistor (SiMoT) POC platform, based on a bio-electronic reader coupled with a disposable cartridge, for the early detection of HPV. The sensing measurements will be processed using machine learning approaches to reach a reliable fully digital output, with false-positive and false-negative rates within 1-5%. The single-molecule identification of the marker in a patient sample (saliva, brushing specimens, etc.) will be validated, and the performance level will be benchmarked against the gold-standard PCR-based techniques.

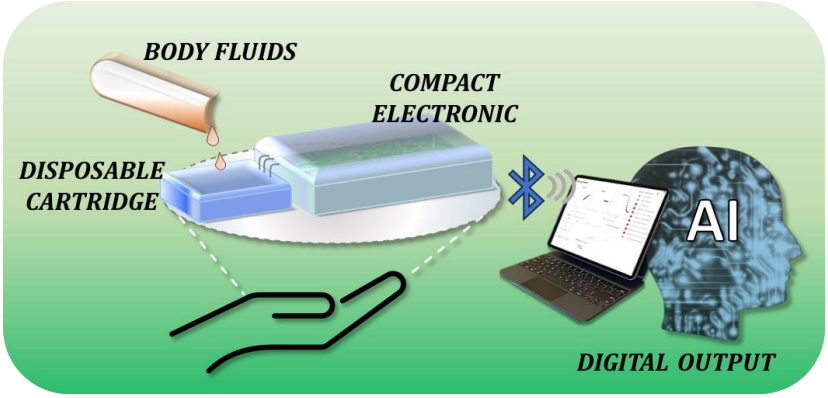

Figure 3. Graphical overview of pilot study Task 4.3a, "Single oncological marker detection for early diagnosis"

2.3.2. Objectives

General objective: Development of a reliable and single-molecule sensitive POC test for HPV infections (including cervical, vulvar and head-neck cancers) with the SiMoT device that detects a single virus or a marker in 0.1 ml of brushing specimens and body fluids with false positives and false negatives below 1-5%.

Specific Objectives:

- To improve the survival rates of oncological patients (subjects at risk or in the very early stages of cervical, vulvar and head-neck cancers);
- To decrease the incidence and seriousness of complications related to HPV infection.

2.3.3. Study design

Study characteristics: The SiMoT disposable cartridge will be modified with capturing antibodies or probes for the selective HPV assay. The SiMoT optimization will be accomplished by assaying reference fluids spiked with known concentrations of the biomarker. Furthermore, real body fluid, such as saliva, and brushing specimens, will be analyzed to validate the analytical performance of the SiMoT platform. To provide a fully digital output for the bio-electronic assay a machine-learning approach will be developed.

Ethical Committee authorization status: The study has been submitted to the IRCCS GPII-BA Ethical Committee and approved on 23/6/2023. The pilot activities have started accordingly.

2.3.4. Expected results

The excellent selectivity of the bio-electronic sensor, along with its high sensitivity guarantees false positives and false negatives to be below 1-5%.

Reference fluids spiked with known concentrations of the virus/markers will be used as the first proof-of-concept, to prove the applicability of the SiMoT platform for the detection of the target species at the physical limit. Then, the assay in real patients' samples will be demonstrated following the optimized experimental conditions.

2.3.5. Project progress

The recruitment campaign has started in the IRCCS GPII-BA infrastructures, under the supervision of the IRCCS GPII-BA partners involved in the pilot. For each patient involved in the study, the collection of saliva, plasma, and vaginal swab is required. The samples are collected and treated according to the protocols approved by the Ethical Committee.

A first set of probes/markers for the SiMoT electrode modification has been selected. The knowhow on the SiMoT platform, already employed for the detection of clinically relevant biomarkers, served to select a commercially available recombinant anti-HPV16 L1 antibody (Abcam-product: https://www.abcam.com/en-it/products/primary-antibodies/anti-hpv16-l1-antibody-hpv16-2058r-ab234305), which has been tested by the vendors with Immunohistochemistry (IHC-P), against the recognition of HPV16-L1 capsid protein (Abcam-product https://www.abcam.com/en-it/products/proteins-peptides/recombinant-hpv16-l1-protein-ab119880). The selected antibody/antigen pair will be used as the initial test protocol in the SiMoT platform. The selection of further probes or equivalent anti-HPV16 L1 antibody/ HPV16-L1 capsid protein from different vendors is ongoing.

2.4. Task 4.3b - Liquid-Biopsy and Liquid-based Cytology Biomarkers for early diagnosis and recurrence risk assessment of HPV positive and negative Gynaecological Cancers (Task Leader: Amalia Azzariti, IRCCS GPII BA)

2.4.1. Overview

Even today, gynaecological cancers, as well as other oncological diseases, are detected at an advanced stage, with limited treatment options and often poor prognosis at that time. The

possible capacity to detect them early can substantially improve survival because cancers early are more treatable. Additionally, predicting cancer recurrence allows doctors to make effective treatment decisions improving patient survival.

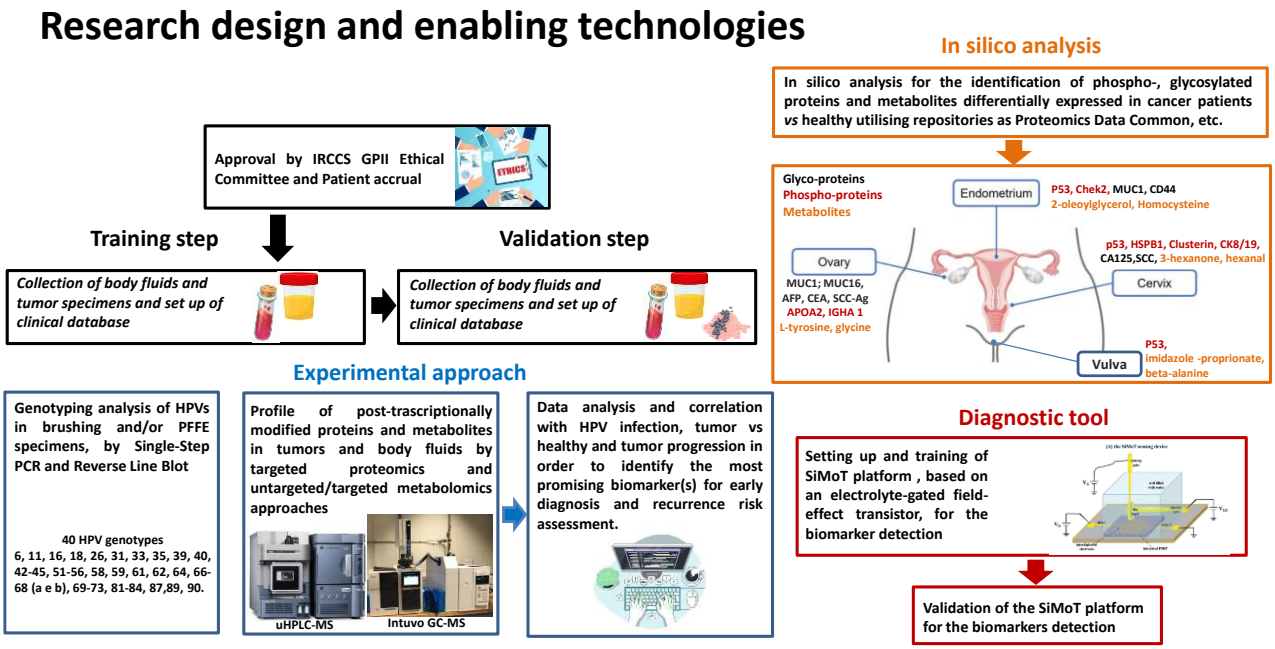

Figure 4. Graphical overview of the pilot-study

2.4.2. Objectives

General objective

In the pilot-study we plan to identify two panels of biomarkers for the early diagnosis of gynaecological cancers, such as ovarian, cervical, endometrial and vulvar cancers and for the recurrence risk assessment. Post-translational modified proteins (PTM proteins), differentially expressed in tumors compared to healthy ones, and metabolites, again differentially expressed in the two populations, patients and controls, will be evaluated. Furthermore the Single-Molecule with a large Transistor (SiMoT) platform will be the innovative device to early detect PTM proteins.

Specific Objectives:

1. Genotyping of HPV positive patients with cervical and vulvar cancer enrolled in the training and the validation cohorts

2. Analyses of the differential expression levels of selected phosphoproteins, glycoproteins and metabolites in patients' body fluids as respect those of healthy donors
3. Proof of the panels of PTM proteins and metabolites as biomarkers of early diagnosis and recurrence risk assessment in a validation cohort of patients
4. Development and training of SiMoT platform for the detection of selected PTM proteins
5. Validation of the SiMoT platform for early diagnosis and recurrence risk assessment in gynaecological cancers

2.4.3. Study design

Study characteristics:

- Patient cohort: Enrollment of 200 patients with gynecological cancers (ovarian, cervical, endometrial and vulvar cancers) and 50 healthy donors.
- Collection of plasma and clinical data from each subject .
- Correlation between PTM proteins and metabolites and HPV infection (in cervical and vulvar cancer) and patient clinical data.

Ethical Committee authorization status: IRCCS GPII Ethical Committee approved the study (Prot. n. 1240/CE – Del. 529 del 23-06-23)

2.4.4. Expected results

• Identification of two panels of PTM proteins and metabolites by targeted proteomics and of metabolomics or other methods in samples of the training cohort

• Validation of these as biomarkers for early diagnosis and prediction of the onset of recurrences

• Development and validation of the SiMoT platform

2.4.5. Project progress

1. A preliminary analysis in silico was performed using Endometrial and Ovarian Cancer datasets of CPTAC repository. In particular, a tumor/normal comparison was performed using cptac()?? python package to detect significantly deregulated

phoshoproteins. The analysis allowed selecting 20 phospho-proteins that are differentially expressed in endometrial and ovarian tumors compared to healthy ones. Of these proteins, caveolin 1(Y14), HSPB6(S16) and LRP1(S4523) were identified with a strong scientific rationale such as to be a tumor suppressor gene in human ovarian epithelium, to decline in high-grade squamous intra-epithelial lesions and cervical carcinoma cells and to be involved in the formation of endometrial carcinoma, respectively. So, we plan to test them as possible factors in early diagnosis.

2. Until December 2023, we collected:

Pathology	Enrolled patients (n.)	Urine	Plasma	brushing specimens	PFFE specimens
Ovarian cancer	41	✓	✓		
Vulvar cancer	6	✓	✓	✓	✓
Endometrial cancer	30	✓	✓		
Cervical cancer	7	✓	✓	✓	✓

Table 1. Samples collected by pilot study in Task 4.3b

3. We have already set up the HPV DNA detection and genotyping system, by Single-Step PCR and Reverse Line Blot (AMPLIQUALITY HPV-TYPE EXPRESS v3.0-AB Analitica).

4. We began the characterization of PTM proteins by analyzing p-AKT and p-STAT3 with Western Blot in 4 cervical cancer patients and 4 healthy patients.

2.5. Task 4.4a Bringing Medicine Digitalization into the Italian Solid Organ Transplant Network (Task Leader: Patrizia Burra, UNIPD)

2.5.1. Overview

Solid organ transplantation represents an effective therapeutic option for patients with end-stage chronic disease. Liver transplantation (LT) provides significant survival benefit for patients with end-stage liver disease or hepatocellular carcinoma within defined criteria. Similarly, kidney transplantation (KT) achieves superior medical outcomes in terms of

survival and quality of life as compared to dialysis in end-stage renal disease. Predicting post-transplant graft function, as well as forecasting the development of post-operative complications (both graft-related and unrelated) still represents a significant goal in such setting. Donor and recipient factors, and a proper matching thereof (e.g., balancing donor and recipients features at time of organ allocation in order to maximize organ utilization, whilst still protecting individual patient interests), play a major role in combination with intraoperative factors and graft quality. As in other fields of Medicine, Artificial Intelligence (AI) has been proposed as a valuable tool to increase the predictive accuracy of post-operative patient and graft survival after solid organ transplantation. Some studies have already applied AI in the complex scenario of organ allocation, assessing the post-transplant outcome through a comprehensive evaluation of donor and recipient features, even if its application on donor histological features is still scant.

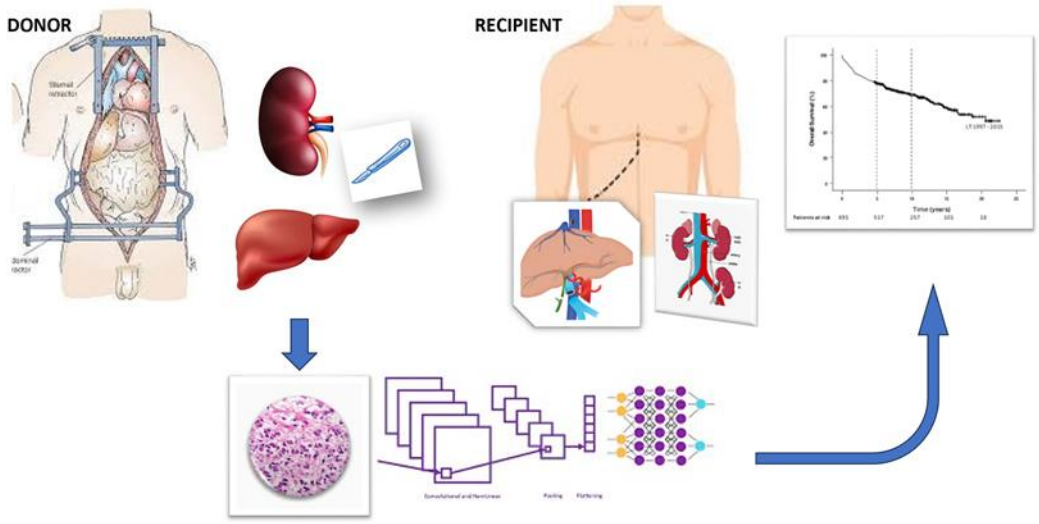

Figure 5. Graphical overview of Task 4.4. Development of a machine learning model able to predict post- liver or kidney transplant graft and patient survival, using histological features of donor grafts.

2.5.2. Objectives

General objective

The present study aims at assessing whether the implementation of AI in donor-recipient matching can improve the outcome prediction in liver and kidney transplant recipients.

Specific Objectives

To evaluate, according a novel machine learning model, donor morphological features (after digitalization of pre-transplant graft biopsies), that will predict post-transplant patient and graft survival in patients who underwent liver or kidney transplantation at a single centre.

2.5.3. Study design

Study characteristics: Retrospective observational study

Ethical Committee authorization status: waiting approval (due to Ethical issues).

Expected results: This project will set the technological background to develop a machine-learning model to improve donor-recipient matching, which will ultimately lead to a more individualized management of complex clinical situations. The model will be disseminated at national and international levels for further validation and implementation in clinical practice.

2.5.4. Project progress:

- Pending Ethical Committee authorization.
- Order for digital pathology scanner.

2.6. Task 4.4b - Use of machine-learning algorithms and biomarkers to personalize medical management of heart and liver transplant recipients (Task Leader: Luciano Potena, IRCCS AOU Bologna)

2.6.1. Overview

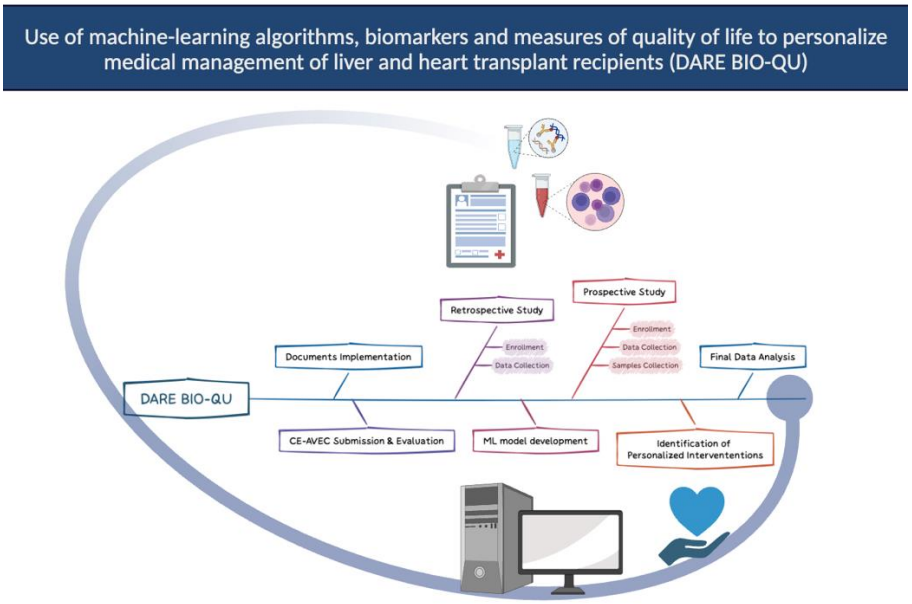

Figure 6. Graphical overview of Task 4.4b.

2.6.2. Objective

General objective

The primary aim of this study is to predict the risk of infections, cardiovascular events, new onset malignancies and chronic graft dysfunction at 3, 5 and 10 years after transplantation of solid organs by applying AI methods.

To achieve this aim we will review clinical charts of all patients who survived at least 6 months after transplantation, have received transplant between 2008 and 2020, and have been followed by the liver and heart transplant clinic at IRCCS AOUBO. This cohort consists of about 1000 patients and it will be randomly divided into a training cohort and a testing cohort.

Specific Objectives

The secondary aim of the study is to test the hypothesis that relevant biomarkers and scores of frailty, quality of life and cognitive ability may correlate with the AI-developed algorithms and therefore these may represent early markers of subsequent clinical events.

2.6.3. Study design

Study characteristics:

This is an observational, low risk tissue-based, non-pharmacological, retrospective-prospective study that will include adult patients undergone or that will undergo heart and liver transplant followed or that will follow at:

- SSD Insufficienza Cardiaca e Trapianti - Director Dr. Luciano Potena;
- U.O. di Medicina Interna per il Trattamento delle Gravi Insufficienze d'Organo – Director Dr. Maria Cristina Morelli.
- IRCCS Azienda Ospedaliero-Universitaria di Bologna (IRCCS AOUBO).

In order to achieve the objectives of this study, collaboration with the following groups is also planned:

- SS Servizio di Psicologia Clinica Ospedaliera sotto la Direzione Generale (IRCCS AOUBO) - Contact Person: Dr. Lucia Golfieri;
- Programma Dipartimentale "Terapie cellulari avanzate" (IRCCS AOUBO) - Director: Dr. Francesca Bonifazi;
- Dipartimento di Ingegneria dell'Energia Elettrica e dell'Informazione "Guglielmo Marconi" - Contact Person: Dr. Sabato Mellone;
- Dipartimento di Scienze Mediche e Chirurgiche (DIMEC) dell'Università di Bologna, UO Genetica Medica - Contact Person: Prof. Elena Bonora.

No patient will be subject to any experimental treatment or device, and clinical decision making will not be influenced be study-specific procedures. These will consist in additional blood sampling for biomarkers analyses, interviews for quality of life and cognitive impairment assessment, and frailty score determination. No patient will be exposed to any additional risk in relation to study procedures.

Ethical Committee authorization status:

Submitted to IRB Central Emilia Wide Area Ethical Committee of the Emilia-Romagna Region (CE-AVEC). Protocol n° 682/2023/Oss/AOUBO assessed on 19/10/2023, with request for additions.

2.6.4. Expected results

1. The results of the study will provide the clinical framework to improve post-transplant care in the outpatients setting, therefore improving clinical outcomes and patients' quality of life, while reducing the need for hospitalizations and costly therapeutic interventions.
2. This project will provide the technological background to develop AI-based approaches to customize therapeutic management in patients with complex disease mechanisms.
3. Finally, given the peculiar characteristics of transplant recipients representing a multimorbid frail population at high risk of cardiovascular, neoplastic and infectious events, the information gained from this project, in the context of global risk assessment, may serve as a hypothesis-generating for future studies aiming to assess profiles for cardiovascular and neoplastic risk in the general population.

2.6.5. Project progress

Under evaluation by IRB.

2.7. Task 4.4c - Non-invasive biomarkers for early diagnosis of delayed graft function and acute rejection after solid organ transplantation (Task Leader: Giuseppe Tisone, UNIROMA2)

2.7.1. Overview

Solid organ transplant recipients represent a very frail population with high risk of morbidity and mortality at short term after transplantation. The current project aims to development of a new non-invasive biomarker for the diagnosis of delayed graft function (DGF) and acute rejection (AR) after kidney and liver transplantation, which represents the major complications after solid organ transplantation, in order to create an artificial

intelligence and machine learning tool providing guidance in the clinical management of complications and personalized therapy after solid organ transplantation.

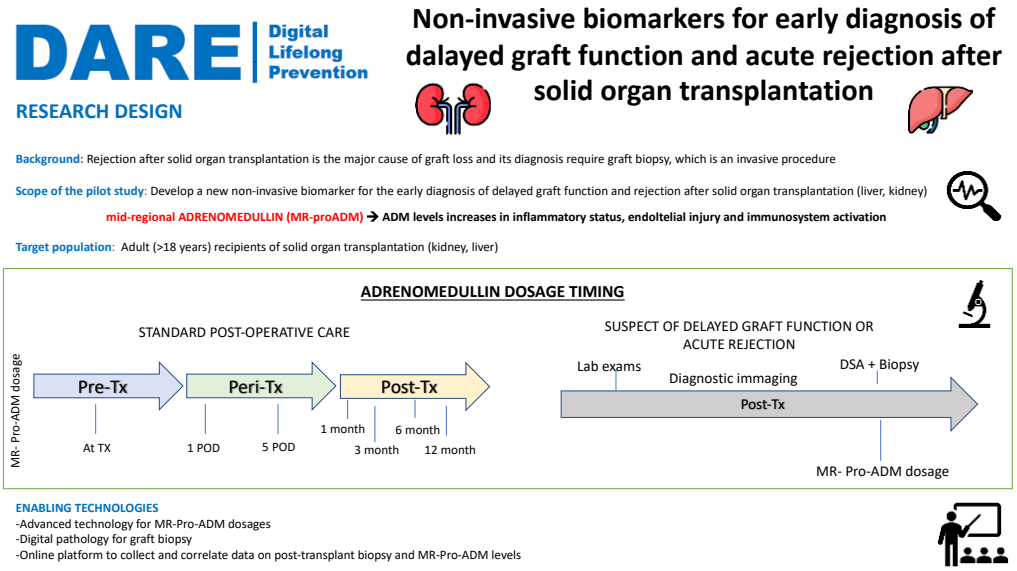

Figure 7. Graphical abstract.

2.7.2. Objectives

General objective:

Assessment of the role of a new biomarker, the Mid-regional pro-adrenomedullin (MR-proADM), in predicting the risk of post-transplant complications such as early allograft dysfunction and acute rejection after kidney and liver transplantation, which might guide physicians to personalize therapies and prevention.

Specific Objectives:

-To define the sensibility and the specificity of increased levels of MR-proADM for early, non-invasive, diagnosis of AR and DGF after kidney and liver transplantation;

- Creating a predictive model of complications after kidney and liver transplantation based on the pre-operative and post-operative levels of MR-proADM by machine learning process Study design.

2.7.3. Study characteristics:

Mid-regional pro-adrenomedullin (MR-proADM) is a peptide with a variety of physiological functions such as immunomodulatory, direct bactericidal, and has a function in maintaining renal homeostasis and vasodilator activity. Elevated MR-proADM levels have been associated with endothelial damage, clinically identified by end-organ disease, and have been primarily associated with sepsis. MD-proADM has also recently been associated with the risk of specific organ failure such as acute renal and hepatic injury, acute respiratory distress syndrome, and acute cardiac injury. Elevated levels of MR-proADM have been demonstrated in patients with chronic renal failure, both on and off dialysis.

Preliminary data have shown that MD-proADM levels tend to increase in case of liver damage and in cirrhotic patients. However, few data have been reported in kidney and lung transplantation, while none in liver transplantation.

The hypothesis of the study is that MR-proADM may increase in the early stages of post-transplant organ dysfunction (delayed graft function/early graft dysfunction) and acute rejection, both after kidney and liver transplantation, as a response to endothelial damage and immune activation.

The research activity consists in a prospective pilot study based on the dosage of MR-proADM from peripheral blood sampling in kidney and liver transplant recipients. Peripheral blood analysis will allow the serum assay of MR-proADM via the time-resolved amplified cryptate emission assay. The sampling will be performed together with the routine samplings in ordinary hospitalization and outpatient settings according to the common clinical follow-up practice of post-operative transplant patients. The examination will be performed on the day of the transplant, on the 1st and 5th post-operative day, 1-3-6-12 months after the transplant, and in case of suspicion of acute rejection and post-operative complications. The study will use advanced technology for MR-Pro-ADM dosage and digital pathology for graft biopsy and will develop an online platform to collect and correlate data on post-transplant biopsy and MR-Pro-ADM levels.

Ethical Committee authorization status:

The project has been submitted to the Local Ethical Committee of the Policlinco Tor Vergata on May, 22 2023 and approved on June, 5th 2023 (protocol number 96/22).

2.7.4. Expected results

Solid organ transplant recipients have high risk of post-transplant complications, mainly represented by delayed graft function (DGF) and early acute rejection (AR). Therefore, development of new non-invasive practical tools for the prevention and early diagnosis of the major post-transplant complications are needed.

From the current study we expect to 1) Identify non-invasive molecular biomarker (MR-proADM) that can increase the predictive ability of early diagnosis of post-operative complications and its correlation with the current available tools; 2) Creating a predictive model/algorithm of complications after kidney and liver transplantation based on the pre-operative and post-operative levels of MR-proADM by machine learning process

2.7.5. Project progress

1) Submission and approval to the Local Ethical Committee (May-June 2023)
2) Positive Evaluation of the Final Project by the Technical Project Manager (University of Bologna) – 25 July 2023
3) Definition of the logistics of samples analysis and storing with Local Labs (Policlinico Tor Vergata) – August 2023
4) Start of enrolling patients: Kidney→ September 1st, 2023 (enrolled 17 patients), Liver→October 1st, 2023 (enrolled 6 patients)- update December 13, 2023
5) Start of retrospective analysis in Kidney Transplant recipients (40 patients) – December 2023

2.8. Task 4.5 - Digital biomarkers for Parkinson and Alzheimer diseases in subjects with psychiatric and cognitive disorders or with Down Syndrome

2.8.1. Overview

Neurodegenerative diseases (ND) are a leading cause of death and disability worldwide and their prevalence is continuously increasing, mainly as a consequence of aging population. The two most frequent ND are Alzheimer's disease (AD) and Parkinson's

disease (PD). Several evidences suggest that for both the diseases the neurodegenerative process starts several years before the manifestation of the clinical symptoms characteristic of overt ND. The diagnosis and characterization of this prodromal phase is of utmost interest, as it represents a critical window where therapeutic interventions are expected to be most effective. Despite recent advances in the field, several challenges remain in our understanding of prodromal conditions and in the ability to translate it to clinical practice. Although some prodromal features are very specific for ND, others are highly prevalent in the population and it is difficult to interpret them taken individually. The biological alterations underpinning prodromal conditions is poorly understood. Most importantly, markers to predict if, when, and how a prodromal patient will convert to overt ND are not available.

In the present project we will focus on cohorts of prodromal patients with a high conversion rate to overt ND: patients with subjective cognitive decline (SCD) and mild cognitive impairment (MCI) who are at risk to convert to AD; patients with REM behaviour sleep disorder (RBD) who are at risk to convert to PD; and patients with Down syndrome (DS) as they are at high risk of developing AD. We will integrate clinical/neuropsychological, imaging, biochemical and omic data from these cohorts of patients in order to identify markers for the early diagnosis of ND, potentially applicable to the general population.

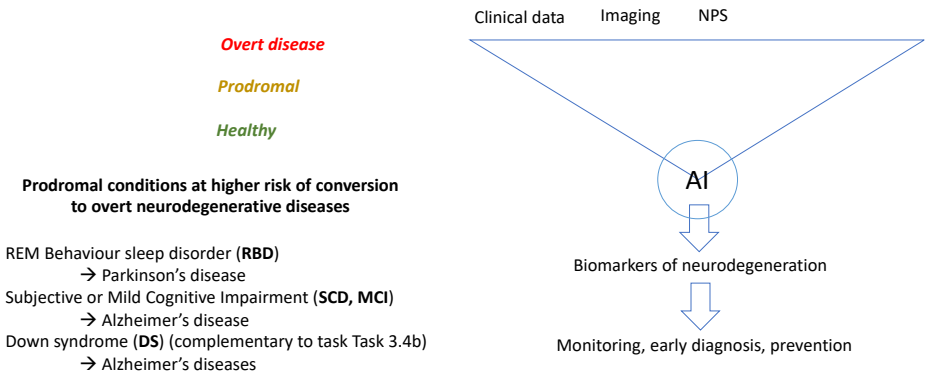

Figure 8. Graphical overview of Task 4.5

2.8.2. Objectives

General objective

To develop markers to predict conversion from prodromal conditions to overt ND.

Specific Objectives

- To develop a comprehensive framework to collect multimodal data from prodromal patients.
- To collect data from RBD patients, in part longitudinally followed for conversion to PD or other degenerative parkinsonisms (MSA).
- To collect data from SCD and MCI patients, in part longitudinally followed for conversion to AD.
- To collect data from DS patients, in part longitudinally followed for conversion to AD.
- To integrate multimodal data in order to develop markers of conversion and disease progression rate.
- To improve knowledge on the pathogenetic mechanisms sustaining neurodegeneration onset/progression.

2.8.3. Study design

Study characteristics:

The study will include both existing cohorts (at least 45 patients for each clinical group) and newly recruited patients (20-80 patients, depending on the clinical group).

Several potential predictors of phenoconversion from prodromal phase to overt ND have been proposed so far and will be considered within the project. Some of these predictors derive from clinical practice (clinical measurements, scales, instrumental assessments, biochemical data) and are routinely collected by physicians of ISNB. During the course of the project, electronic records containing these data will be extracted and harmonized. Additional potential biomarkers of phenoconversion, including molecular and omic measurements in peripheral tissues and biological fluids, will be selected on the basis of existing scientific literature or of preliminary data from ISNB researchers and will be specifically generated during the course of the project. Finally, ISNB researchers are currently evaluating the possibility of using of questionnaires to evaluate patients' socio-economic status and of using wearable devices to monitor physiological and movement parameters.

Existing and newly generated data will be comprehensively analysed through machine learning and AI approaches.

Of note, a synergy with Task 3.4b is envisaged for what concerns the cohorts of adults with DS.

Ethical Committee authorization status:

ISNB is currently preparing the documents to be submitted to the local Ethical Committee.

2.8.4. Expected results

The results of the project will improve the clinical management of SCD, MCI, RBD and DS patients through the development of predictors of phenoconversion to overt ND. This will allow to apply early therapeutic interventions when necessary, and at the same time to improve the quality of life of the patients and caregivers given the uncertainty related to their prodromal condition.

2.8.5. Project progress

The harmonization of existing data and the definition of experimental approaches to be applied to biological samples are currently ongoing.

2.9. Task 4.6 - A ML-based approach to establish an effective and accurate indicator of the eubiosis/dysbiosis status through DNA metabarcoding microbiome assessment (Task Leader: Bruno Fosso, UNIBA)

2.9.1. Overview:

The human body is a complex ecosystem that houses trillions of microorganisms, collectively known as the human microbiome, which plays a critical role in maintaining our overall health and wellbeing. In particular, the physiologic balance among the gut microbiome and the host (**eubiosis**) is characterized by high taxonomic diversity, whose perturbation (**dysbiosis**) is often coupled to reduced diversity and pro-inflammatory microenvironment. The advent of metagenomics allowed to fully access the complexity of microbial communities. Among the others, DNA-metabarcoding represented the most applied approach. Indeed, it is fast and cheap both in terms of experimental procedure and bioinformatic analysis. It relies in the selective amplification and sequencing of a barcode and its taxonomic classification to profile the microbiota composition. The main features of a barcode are ubiquitousness in the target taxonomic range of interest, suitable variability

needed for the classification specificity, and the possibility to use "universal" primers for its amplification thanks to the conserved regions on both sides. Finally, the overall length of the barcode should be compatible with technical limitation of sequencing platforms. A remarkable example of barcode is the prokaryotic 16S rRNA gene. It is characterized by 9 hyper-variable regions suitable for taxonomic classification flanked by constant ones, useful for primer design. These features allowed researchers to target one or more 16S rRNA hyper-variable regions and ensure an amplicon length compatible with NGS technical limitations. In the current state of the art, an operational and objective definition of a healthy microbiome is lacking, likely because of many confounding factors particularly gender and age. In 2020 Gupta et al., introduced the Gut Microbiome Health Index (GMHI), a statistical approach to predict the likelihood of eubiosis/dysbiosis by using shotgun metagenomics data available in public repositories. Within this framework, machine learning (ML) approaches emerge as potentially powerful tools for forecasting the state of eubiosis or dysbiosis, circumventing the limitations inherent in traditional statistical methodologies. The overall goal of this proposal is to define a precise and accurate model able to predict eubiosis/dysbiosis.

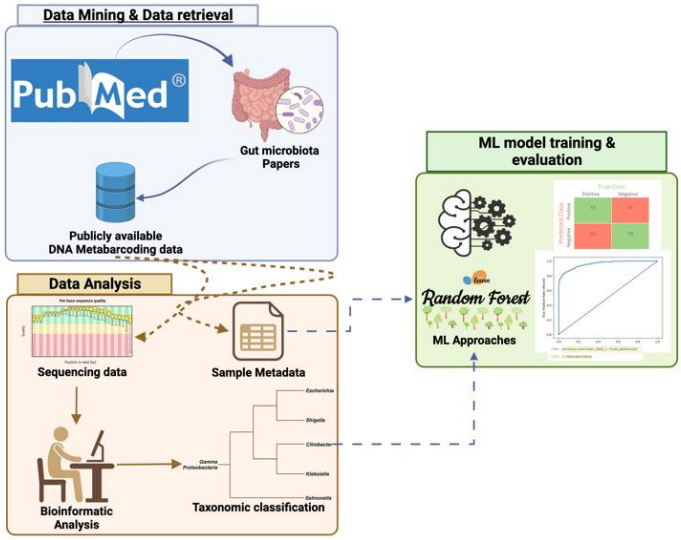

Figure 9: Graphical overview of Task 4.6. The Pilot Project tasks can be enclosed in three main subtasks: i) Data Mining and Data retrieval; ii) Data Analysis; iii) ML model training and evaluation.

2.9.2. Objectives

General objective

Overall, the research activities are structured in three main tasks (Figure): i) Data Mining and Data Retrieval; ii) Data Analysis and iii) ML model training and evaluation; which are propaedeutic to a iv) pilot case study. The data mining steps is actually the most relevant one, considering it will generate the data collection we plan to download and process. In order to carefully define it, a thoroughly mining of scientific literature has been performed so far, allowing to select 39 comparative studies. Considering we aim to develop a classification model avoiding overfitting on pathologies already known to be associated to microbiome perturbation, we considered all available comparative studies. A key feature of our proposal is the application of the same bioinformatic framework to all the selected data. This approach will limit the confounding effects related to bioinformatic procedures. To this aim we will take advantage of the activities that will be carried out in the PNRR project ELIXIRNextGenIT. Through a comprehensive data analysis of the available dataset and fine tuning the ML approaches also taking advantage of a combination of different algorithms, we plan to generate an effective and accurate metrics to assess eubiosis and dysbiosis. Finally, we plan to validate the new methodological approach taking into account the cohort enrolled by the Pilot Project proposed by Prof. Pontrelli (Spoke 3 WP5 Task 5.2).

2.9.3. Study design

Study characteristics: This pilot project could be considered as a retrospective survey. It relies on the analysis of data and contextual metadata, already analyzed for different purpose and aims to leverage new knowledge about health determinants.

Ethical Committee authorization status: This pilot project does not require the approval of an ethical committee, because the model development relies on both publicly available data and data shared through a DTA (Data Transfer Agreement). For the validation of the obtained model, we plan to use an external cohort enrolled in the Pilot Project exploited in DARE W4 task 5.2, under the supervision of Prof. Paola Pontrelli.

2.9.4. Expected results

The aim of the Pilot Project is to obtain a classification model among healthy and dysbiotic microbiome by using DNA-metabarcoding data able to reach at least an overall accuracy above 80%.

2.9.5. Project progress

According to the proposed GANNT, we have carried out the DNA metabarcondig dataset and metadata selection from publicly available repositories. Actually, we have selected and downloaded about 10,081 datasets and contextual metadata. Moreover, we have completed in July the bureaucratic process to access the 100IDB cohort metabarcoding data and we were granted to access the data and metadata for about 300 samples. As proof of concept, on a small cohort of 1,300 samples spanning 13 phenotypes we have trained a classification model. In particular, we combined a Random Forest classifier with a Permutation based feature selection process to discriminate among healthy and non-healthy samples. The obtained model reached a 76% overall accuracy.

2.10. Task 4.7 - Neurotransmission enriched connectivity as a biomarker of healthy and accelerated ageing in human brain (Task Leader: Mattia Veronese, UNIPD).

2.10.1. Overview

Age-related diseases are becoming increasingly important in developed countries due to their association with increasing life expectancy. This project will impact a significant portion of the global population. The World Health Organization predicts that by 2040, neurodegenerative conditions such as Alzheimer's Disease and Parkinson's Disease will become major leading causes of death in western countries (World Health Organisation, World Health Statistics 2022, https://www.who.int). Currently, more than 55 million people worldwide live with dementia, and there are nearly 10 million new cases every year. Therefore, this project will significantly influence the quality of life for millions of people.

The aim of this research project is to develop a neuroimaging method capable of modelling healthy aging at an individual level and providing biologically informed early predictions of neurodegeneration and cognitive decline. This idea is based on the fact that individuals

on fast-aging trajectories have been associated with cognitive impairment and other brain disorders. The project will span four years, beginning with the definition of the model on healthy controls and concluding with the implementation of the method on patients' data. It will leverage the use of functional Magnetic Resonance Imaging to measure both normal and abnormal brain connectivity.

2.10.2. Objectives

General objective

The goal of the project is to develop and validate a neuroimaging method capable to model healthy and accelerated brain ageing trajectories at individual level and to provide biologically informed early prediction of neurodegeneration and cognitive decline.

Specific Objectives

The specific objectives are the following:

- to identify a clinically viable neuroimaging biomarker to calculate neurotransmission enriched brain connectivity measures;

- to use the neuroimaging measures to model healthy brain ageing trajectory across adulthood lifespan [18 to 90 years old];

- to apply this class of biomarkers to identify altered ageing trajectories in clinical models of neurodegenerative diseases;

- to explore potential relationships between enrichment maps and other factors (genetics, electrophysiological signals like EEG, ECG, etc.).

2.10.3. Study design

Study characteristics:

Neurotransmission-enriched connectivity measures aim to quantify brain functional connectivity through analysis, such as that derived from resting-state functional MRI, informed by neurotransmitter distribution densities. The rationale behind this approach is to enhance the biological specificity of MRI measures by incorporating the spatial distribution of molecular pathways. This aims to illuminate the pathophysiological mechanisms of diseases and suggest potential pharmaceutical targets.

The work of this project will commence with the re-engineering and extension of the Receptor-Enriched Analysis of Functional Connectivity (REACT) method, which was recently proposed by O. Dipasquale et al. in 2019. REACT utilizes molecular information regarding drug target distribution provided by PET to enrich rs-fMRI analysis. It involves a two-level multivariate regression analysis, resulting in an estimation of spatial maps reflecting whole-brain connectivity related to each target included in the model. In the initial step, the PET target distribution maps are employed as a set of spatial regressors to estimate target-relevant dominant fluctuations in rs-fMRI. In the second step, the obtained fluctuations are inputted into a general linear model to estimate subject-specific target-related spatial maps (informed FC maps). This method is later going to be validated with correlation tests with well-known ageing changes associated with brain structures and cerebral perfusion, using Arterial Spin Labelling images.

Ethical Committee authorization status:

The initial activities of the task do not require any ethical approval, as they are based in reanalysis of open-access neuroimaging data, already authorized for re-use. Ethical approval will be required for the last milestone of the task, which will consist in MRI data acquisition from healthy controls. We have started to approach local ethical committee in order to obtain information on the process.

2.10.4. **Expected results**

We shall identify a clinically viable neuroimaging-based biomarker to assess functional brain ageing in patients at early stages of neurodegeneration to facilitate early intervention and monitor cognitive decline thought out time.

Understanding and modelling how the brain ages remains a challenge, primarily due to its susceptibility to a variety of complex genetic and environmental factors. If we can accurately assess brain age, it could serve as a biomarker for age- or disease-related pathologies, leading to the development of better treatments and an enhanced overall quality of aging. The ability to slow down neurodegenerative processes in the initial stages of the disease would not only improve the quality of life for these patients but also result in a significant reduction in both direct and indirect healthcare costs.

2.10.5. Project progress

From 1.10.2023 (start date of the task): 1) Review of existing neuroimaging databases containing healthy controls across the life-span and selection of the most relevant ones in term of completeness of information and type of biomarkers included; 2) application submission for main datasets including Cam-CAN (approved) and UK Biobank (pending); 3) Literature review for Normative Modelling applications to resting state functional MRI applications in the context of ageing

3. Conclusion and next steps

In conclusion, the architecture of the WP4 at 12 months seems to meet the original scope of testing new procedures to integrate ICT technologies into clinical and prevention pathways, from the collaboration between Spoke 3 and Spoke 1, in particular with WP1 of Spoke 1, as valuable support to pilots design in terms of legal, technological and statistical aspects other than definition of ethical aspects for ethical committee approval.

At Month 12, the development of the pilot studies of WP4 of this Spoke 3 is in line with the timing of the whole DARE project. The objectives of the pilot studies are now more clearly defined and appropriate to the scope of the overall DARE programs.