# 1) **Progetto di Ricerca della Dott.ssa Roberta Maria Antonello**

## Titolo Progetto di Ricerca

**Surveillance and characterization of resistance mechanisms to new β-lactams and β-lactam/β-lactamase inhibitors on clinical and environmental multidrug-resistant *Enterobacterales***


**Abstract**

Antimicrobial resistance (AMR) is a major health threat and multidrug-resistant Gram-negative bacteria, listed as priority bacterial pathogens by the World Health Organization (WHO), are emerging as a global health issue due to their ability to survive in the environment and therefore spreading both within and outside the nosocomial setting. Multidrug-resistant *Enterobacterales* require surveillance for a prompt identification and actions aiming at reducing their spread and preserving the available therapeutic options.

The aim of this project is to describe the activity and mechanisms of resistance to new antibiotics, in particular recently marketed β-lactams and β-lactam/β-lactamase inhibitor combinations (BLICs) in clinically-relevant Gram negatives isolated from clinical and environmental samples.

The isolates will be identified by MALDI-ToF mass spectrometry and antimicrobial susceptibility testing will be performed with reference methods. Isolates exhibiting relevant antimicrobial resistance will be screened for the presence of common carbapenemases, extended-spectrum β-lactamase and acquired class C β-lactamase genes and subsequently will be studied by whole genome sequencing (WGS) analysis to characterize their resistome. Isolates with unknown mechanisms of resistance will be further characterized. Possible outbreaks sustained by clonal expansion will be studied by phylogenetic analysis. Moreover, isolates with relevant AMR patterns will be evaluated to estimate the impact of antimicrobial-induced mutations on bacterial fitness and virulence, performing also *in vivo* studies using appropriate infection models. Finally, the project will focus on the development of new possible rapid diagnostic tests.

The results of this project will be useful to monitor the emergence of resistance to last-line antibiotics, provide an updated epidemiology of carbapenem-resistant *Enterobacterales* in nosocomial and community settings, define the activity of new BLICs and the impact of resistance genes on bacterial fitness and virulence, and will lay a foundation to the development of new diagnostic tests.


**Introduction**

Antimicrobial resistance (AMR) is a global emergency expected to cause up to 10 million deaths per year globally by 2050. Multidrug-resistant Gram-negative bacteria represent a major threat since they are related to worse clinical outcomes and increased cost compared to infections sustained by drug-susceptible isolates. The last report of the World Health Organization (WHO, 2024) listed carbapenem- and third-generation cephalosporin-resistant *Enterobacterales* in the critical group of bacterial priority pathogens. In recent years, these have been isolated from both clinical samples of inpatients and other settings, including community-acquired and long-term care facilities and actually representing a global health problem due to their ability in surviving in the environment.

Among the most promising therapeutic options, new β-lactams and β-lactam/β-lactamase inhibitor combinations (BLICs) have been developed recently. Despite this, AMR also to last-line antibiotics has been observed in both clinical and environmental isolates. Surveillance is a crucial step to promptly identify the emergence of resistance and therefore limiting the spread through appropriate infection control measures and antimicrobial stewardship strategies.

**Aim of the project**

The aim of this project is to describe the activity and mechanisms of resistance to new β-lactams and BLICs in clinical and environmental isolates of *Enterobacterales*.

**Methods**

**1. Sample collection and species identification (months 1-21)**

Clinical (surveillance swabs and blood, urine or lower respiratory tract cultures) and environmental (nosocomial environment swabs, wastewater) samples will be collected. Bacterial strains will be identified by MALDI-ToF mass spectrometry to obtain a collection of relevant *Enterobacterales*.

**2. Antimicrobial susceptibility testing (months 2-24)**

Antimicrobial susceptibility testing (AST) will be performed by reference broth microdilution to detect the minimum inhibitory concentration (MIC) to extended-spectrum cephalosporins, carbapenems, cefiderocol, piperacillin-tazobactam, ceftazidime-avibactam, aztreonam-avibactam, imipenem-relebactam, meropenem-vaborbactam, cefepime-enmetazobactam, cefepime-zidebactam, cefepime-taniborbactam and ceftolozane-tazobactam.

**3. Molecular screening of common carbapenemases and extended-spectrum β-lactamase genes (months 2-24)**

Isolates presenting resistance to carbapenems or extended spectrum cephalosporins will be evaluated by real-time PCR to investigate the presence of acquired carbapenemases, extended-spectrum β-lactamase genes and acquired class C β-lactamase genes (e.g. *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, *bla*$_{CTX-M}$, *bla*$_{PER}$, *bla*$_{GES}$, *bla*$_{VEB}$, *bla*$_{CMY}$, *bla*$_{DHA}$, *bla*$_{FOX}$, *bla*$_{MOX}$).

**4. Whole-genome sequencing (months 6-30)**

Isolates with resistance to new BLICs or cefiderocol will be selected for whole-genome sequencing (WGS). In addition, the phylogenetic analysis will allow the surveillance and evaluation of possible outbreaks sustained by clonal expansion. Isolates with unknown mechanisms of resistance will be further characterized.

**5. Development of new diagnostic tests and new therapeutic strategies (months 15-35)**

Based on the characterization of the mechanisms of resistance, the project will have a focus also on the design and development of new rapid molecular diagnostic tests targeting acquired resistance mechanisms to new BLICs and cefiderocol. In addition, new therapeutic strategies (e.g. drug repurposing) will be evaluated.

**6. Evaluation of the impact of mutations responsible for AMR on bacterial fitness and virulence (months 18-35)**

Isolates with relevant AMR patterns will be evaluated to estimate the impact of antimicrobial-induced mutations on bacterial fitness. Fitness will be evaluated by growth curves and head-to-head competition assays with parental drug-susceptible strains. Bacterial virulence will be evaluated in isolates with relevant AMR patterns using appropriate *in vivo* models of infection (e.g. *Galleria mellonella*) to assess the impact of resistance genes on virulence.

**7. International agreements for co-tutoring doctoral thesis**

The present doctoral project is under evaluation for international agreements for co-tutoring, pending administrative issues. Further details will be provided as soon as possible, and the complete list of co-supervisors will be updated accordingly.

**Milestones:** Presentation of *in itinere* relevant results in national and international congresses. Publication of main results on indexed international journals.

**Expected results**

Difficult-to-treat *Enterobacterales* are priority pathogens that require surveillance and actions to limit their spread and protect the antimicrobial activity. Moreover, due to their ability to persist in the environment, they can cause both nosocomial and, especially in highly endemic settings, community-acquired infections.

The present project is expected to provide valuable insights on this hot topic. These include a prompt identification of emerging mechanisms of resistance in Gram negatives to last-line antibiotics, including possible mechanisms not previously described, and the surveillance of possible outbreaks. Moreover, this project will provide an updated epidemiology of carbapenem-resistant *Enterobacterales* in nosocomial and community settings, define the activity of new β-lactams and BLICs and better understand the impact of resistance genes on bacterial fitness and virulence. Additionally, the possible development of new diagnostic tests could further optimize the detection and therefore the management of these life-threatening pathogens.
The results of this project will provide useful tools to develop prompt responses to face the emergence of AMR with the rapid adoption of advanced infection control measures and antimicrobial stewardship strategies.

## 2) **Progetto di Ricerca del Dott. Marco Campolmi**

## Titolo Progetto di Ricerca

**Use of Artificial lntelligence and Deep Learning Approaches for the ldentification of Predictive Factors of Aneurysm Rupture Following EVAR**

**Abstract**

Endovascular Aneurysm Repaìr (EVAR) is currently the most widely adopted technique for treating infrarenal abdominal aortic aneurysms. Although rare, post-EVAR aneurysm rupture remaìns one of the most feared complications due to the hìgh perìoperatìve morbidity and mortality rates associateci with reintervention. Traditionally, patient fo//ow-up relies on serial cross-sectional imaging with manual measurements performed by physicians, a process subject to significant inter-operator variability. The recent development of artificial intelligence (Al)-driven software has enabled more standardized and operator-independent evaluations of morphometric and volumetrie parameters.

This multicenter, observational, retrospective study aims to leverage Al technologies to identify morphological an volumetrie predictors of aneurysm rupture after EVAR. The analysis will be conducted using the PRAEVAorta p\atform by NUREA, a cloud-based, Al-driven software validated for automated 2D/3D measurement and endolea detection . Eligible patients include those who underwent EVAR and experienced a subsequent rupture, with available preoperative and serial follow-up CT angiographies (CTA), including the scan immediately preceding the rupture.

All CTA images will be anonymized and processed through the PRAEVAorta 2 platform to assess parameters such as sac volume, residuai sac dimensions, endoleak presence, neck angulation, sealing loss, and thrombus distribution. The temporal evolution of these parameters will be analyzed to detect recurring trends associated with impending rupture. Aggregateci data will be used to train and validate predictive models through machine learning and deep learning techniques. Model performance will be evaluated using k-fold cross-validation and ROC curve analysis, and compared with traditional assessments by experienced clinicians.

The study anticipates that Al-driven image analysis will reveal consistent predictive patterns of rupture risk and u tp e r_for c nventional surveillance methods. Findings are expected to contribute to the development of Al-based risk stratification tools, promote early intervention strategies, and support the integration of Al in clinical guidelines to improve outcomes and reduce complications in EVAR patients.

**Introduction**

Currently, EVAR represents the most widely adopted technique tor the treatment of infrarenal abdominal aortic aneurysms. Among the potential complications of these procedures is rupture which, although infrequent, presents considerable technical challenges in the context of reinterventionand is associated with extremely high perioperative morbidity and mortality rates

Over the years, numerous attempts have been made to identify early risk factors or patient categories who might benefit from timely reintervention or prosthetic explantati on3

To date, such patients have undergone serial lifelong surveillance, which has traditionally relied on cross-sectional imaging with manual axial measurements performed by physicians, resulting in significant inter-operator variability.

The recent development of Al-driven software has allowed tor the standardization of such measurements and assessments, thereby reducing operator dependence.

The PRAEVAorta 2 platform by NUREA has been validated by recent stu dies4 8 tor performing both two-dimensional and volumetrie measurements, as well as tor the detection of endoleaks.

The objective of this study is to retrospectively analyze, using the dedicated software, ali CTA scans of patients who underwent EVAR and subsequently experienced aneurysm rupture, starting from the first preoperative CTA, in order to evaluate ali morphological and volumetrie parameters and identify trends potentially predictive of impending rupture, thereby supporting decisionsfar early reinte rvention.

**Methodology**

*Study Design*

This is an observational, retrospective, multicenter study aimed at morphological and volumetrie analysis of CTA images from patients who underwent EVAR and subsequently developed rupture of the infrarenal abdominal aortic aneurysm.

Multiple ltalian and European vascular centers with established expertise in endovascular surgery andaccess _ to digitai PACS archives with complete tollow-up data will be involved. Each center will be responsible tor data collectron and pseudonymization in accordance with GDPR regulations.

*Jnclusion Criterio*

Patients who underwent EVAR tor infrarenal abdominal aortic aneurysm and experienceda documented aneurysm rupture during post-EVAR follow-up will be included. At least one preoperative CTA and two or more follo-wup CTAs (including the one immediately preceding rupture) must be available.

*Exclusion Criterio*

Patients with thoracic or thoracoabdominal anatomies or non-degenerative aneurysms will be excluded. Cases with incomplete follow-up or CTAs with a slice thickness greater than 2.0 mm will also be excluded.

*lmage Analysis*

All CTA images will be exported in DICOM format, anonymized, and uploaded to the cloud-based PRAEVAorta platform by NUREA. The software, validated in prior studies, enables automated and standardized analysis maximum and minimum dlameters along the entlre endograft, total aneurysm volume, residuai sac volum automatic segmentatlon of the lumen and thrombus, automated identification and classification of endoleak curvature and angulation of both proximal and distai neck, and assessment of any proximal and/or distai sealing loss

*Temporal and Predictive Analysis*

For each patient, the tempora/ evolution of morphological parameters will be analyzed from the preoperative pha up to the rupture event. Variation patterns including volumetrie trends, angular changes, onset of endoleaks, a increase in residuai diameter will be identified and correlated with the adverse outcome.

*Statistico/ ond Al Anolysis*

Aggregated data will be used to train predictive models using machine learning and deep learning algorith Demographic and clinica! data will be collected in an anonymized dataset using SPSS software (SPSS 29 .0.2 .0 MacOS, SPSS lnc., Chicago, USA).

Models will be validated using k-fold cross-validation and ROC curve ana/ysis. The predictive accuracy of the models will be compared to that of traditional evaluations performed by human experts.

**Expected Results**

lt is expected that the retrospective ana/ysis of CTA scans using the Al-driven PRAEVAorta 2 p/atform wi// a/low the identification of recurring morphological and volumetrie patterns in patients who experienced rupture following EVAR. Specifically, it is hypothesized that progressive and significant changes in certain parameters, such as increased aneurysmal sac volume, the appearance or worsening of endoleaks, angular changes in the neck, and alterations in thrombotic profile, may represent predictive markers of aneurysmal instability.

The application of supervis d. artificial intelligence models is expected to enable the development ofa predictive lgonthm c pab/e of recognizing early evolutionary trends suggestive of impending rupture, stratifying rupture risk in EVAR pat,ents, and proposing cnt,cal morphometric thresholds for early reintervention.

Furthermore, it is anticipated that comparison between the predictive accuracy of Al models and that of traditio clinical assessment will reveal a reduction in inter- and intra-operator variability, thus demonstrating the superio of the Al-based approach in standardizing and enhancing the reliability of postoperati\/e surveillance.

## 3) **Progetto di Ricerca della Dott.ssa Chiara Cappugi**

## Titolo Progetto di Ricerca

**Platelet-activating anti-PF4 disorders: a new clinical entity. The clinical paradox of thrombocytopenia associated with a thrombotic risk.**

**Summary**

Heparin-induced thrombocytopenia (HIT) can be defined as a rare immune mediated adverse drug reaction occurring after exposure to heparin. In the Covid-era, vaccine-induced immune thrombotic thrombocytopenia (VITT) has emerged as a pathological entity which recognizes the same pathophysiology of HIT. The recent identification and understanding of VITT have focused interest by the scientific community in anti-PF4 disorders, even if large knowledge gaps in pathophysiological and clinical features still persist. The incidence of all these conditions appears to be very low and so are considered rare, even if they're probably often misdiagnosed.

While in classical HIT and VITT a relationship between a specific trigger (heparin or adenoviral vector-based vaccination) is demonstrated, other anti-PF4 disorders seem to be related with a wider range of risk condition, like surgery, infectious diseases, hematological neoplasms and so on, but the specific process of antiPF4 platelet activating antibodies formation in this context is not completely understood. High risk patients seem to be the ones that have concomitant condition associated with increased PF4 levels.

*Introduction*

Platelet factor 4 (PF4) is a highly cationic tetrameric protein [1], normally present at very low concentrations in blood circulation (<10 ng/ml); it is released from α-granules of activated platelets and disappear rapidly from plasma as it transfers to high affinity heparan sulfate on endothelial cells, inhibiting local antithrombin activity and thus promoting coagulation [2]. PF4 has many other biological effects, including maintenance of hematopoietic stem cell quiescence, negative regulation of megakaryopoiesis [3,4]; stimulation of myofibroblast differentiation and collagen synthesis [5]; promotion of inflammatory/immune response by induction of local inflammatory processes at sites of vascular injury and activation of leukocyte [6]. Therefore, PF4 plays an important role in a variety of diseases, including atherosclerosis, fibrotic diseases and aging. Among them, the most important one might be its effect in immune-related thrombotic diseases [7].

PF4 was first found to have a prothrombotic effect due to its electrostatic interaction with heparin, forming multimolecular PF4/heparin complexes [8], which become neoantigens and induce the formation of IgG antibodies against them [9]. Antibodies against PF4 occur often, but only in some cases they are found to be pathological through the activation of platelets by binding to their FcγRIIA receptors, inducing severe prothrombotic disorders with associated thrombocytopenia.

The prototypic anti-PF4 disorder is HIT, a rare immune mediated adverse drug reaction occurring after exposure to heparin, in which anti-PF4/heparin IgG antibodies activate platelets and also monocytes, neutrophils, endothelial cells, triggering thromboinflammation with a high risk for venous and arterial thrombosis [10]. HIT is estimated to occur in up to 1 out of 5,000 hospitalized patients, and it can be considered the most frequent immune adverse drug reaction. A greater incidence is reported in women, post-surgical patients, and those treated with unfractionated heparin (UFH) [11-13]. A meta-analysis of around 7,300 patients has shown a nearly 10-fold lower risk of HIT with prophylactic dose of low molecular weight heparin (LMWH) compared to UFH, but this difference has not been confirmed at therapeutic doses of both drugs [14]. Moreover, LMWH prophylaxis has been shown to be associated with an incidence of HIT of about 0.5% in orthopaedic and 0.8% in medical patients [15].

There is an approximately 50% fall in platelet count and thrombocytopenia occurs in more than 85% of HIT cases, usually of moderate severity (median counts of 50-60×$10^9$/L), typically observed 5-10 days after starting heparin; thromboembolic complications occur in 35-75% of HIT patients and are usually severe [16].

Since 2001, atypical and often more severe forms of HIT have been reported. To date, approximately 40 cases have been described that presented clinical features similar to HIT with the same serological profile in the absence of

proximate exposure to heparin (spontaneous HIT, sHIT), typically in surgical setting (post-orthopedic, especially post-total knee arthroplasty) or post-infectious conditions [17].

Another entity is autoimmune HIT (aHIT), caused by anti-PF4 antibodies with heparin-independent platelet-activating properties, despite an established proximate exposure to heparin [18]. Autoimmune HIT includes delayed-onset HIT (onset or worsening of thrombocytopenia despite stopping heparin), persisting or refractory HIT (thrombocytopenia persistence despite stopping heparin), heparin "flush" HIT (triggered by small amounts of heparin) and fondaparinux-induced HIT. Patients with sHIT or aHIT often show unusual clinical features, such as severe thrombocytopenia (median platelet count nadir approximately $20x10^9$/L) that can persist for weeks, thrombosis frequency extremely high (75-90%), with a predilection for cerebral and splanchnic-portal vasculatures, often accompanied by disseminated intravascular coagulation (DIC) and microvascular thrombosis [19].

In 2021, within the first months of the COVID-19 vaccination campaign, previously healthy recipients who developed severe thrombosis and thrombocytopenia typically between 5 and 20 days after adenoviral vector-based vaccination were identified. Similarities between this syndrome and HIT prompted recognition of the causative role of antiPF4 antibodies (heparin independent). The incidence of that condition called vaccine-induced immune thrombotic thrombocytopenia (VITT) is estimated to be 3-15 cases per million initial vaccinations; over 90% of patients develop cerebral venous and/or splanchnic thrombosis and arterial thrombosis are also frequent. A marked elevation in plasma D-dimers and a reduction in fibrinogen are common, indicative of DIC [20].

Recently, the same VITT-like clinical and laboratory features were reported in some patients without any proximate COVID-19 vaccination or heparin exposure [21]. VITT-like antibodies have been detected in some patients with monoclonal gammopathy [22] or following viral infection [23,24,25]. A recent proteomic analysis demonstrated that VITT and VITT-like antibodies are extremely similar [26].

All these pathological entities are grouped together as platelet activating anti-PF4 disorders, in which can be observed a specific trigger that promotes an immune mediated reaction with the formation of highly pathological antiPF4 antibodies [27]. The formation of immunogenic complexes also depends on the availability of PF4 [28], that is increased in inflammatory or infectious diseases, diabetes, cardiovascular and renal diseases, or after surgery [7]. This suggests an explanation for the common observation that specific patient populations are known to be at an increased risk of developing antiPF4 antibodies [29]. To detect HIT and VITT antibodies automated immunoassays are used and then a platelet activation test must be performed [30]. Antibodies in HIT and VITT are slightly different, in the way they bind to different epitopes on PF4 [31]. VITT antibodies often test negative in the classic functional tests for HIT. These test performances have been overcome by a recently developed rapid chemiluminescence assay, which recognizes VITT antibodies but not most of HIT antibodies, and by modifying the functional test by adding PF4 instead of heparin [32].

*Methods*

Aims of the study

The endpoints of the study are to evaluate

1. the incidence of PF4 antibodies (BOTH heparin dependent or independent) and their platelet activating properties in patients with high probability to have high PF4 levels.
2. the incidence of PF4 antibodies in a group of patients with idiopathic deep venous thrombosis with or without pulmonary embolism and concomitant low platelet count.

Study population

a. 100 patients from the following clinical setting: cardiac surgery; dialysis; surgery with a 4T score more than 3.
b. 100 patients with deep venous thrombosis or pulmonary embolism and a concomitant platelet count lower than 150,000 at the time of the event (or alternatively, if possible, with a reduction of at least 30% of platelet count at the time of the event).

Laboratory analysis

We'll evaluate anti-PF4 antibodies with different methods in order to establish sensitivity and specificity of each method and in order to identify BOTH heparin dependent and heparin independent anti PF4 antibodies; D dimer, fibrinogen, aPTT, PT, antithrombin, thrombin-antithrombin complexes, prothrombin fragment 1+2 (F1+2). We will correlate the results of biomarkers with the clinical phenotype.

In addition, we'll evaluate: the inflammatory pattern by measuring the cytokines levels (both pro- and anti-inflammatory); genomic, metabolomic and proteomic data to identify possible characteristics able to recognize patients at high risk of developing these diseases.

Expected results

We expect to obtain 1) the prevalence of PF4 antibodies in different populations in order to assess the risk to develop a spontaneous PF4 disorder in each clinical context; 2) the risk of developing venous and arterial thrombosis; 3) the risk of developing DIC and bleeding events; 4) the possible role of genomic, proteomic or metabolomic in the clinical risk of developing these diseases.

A possible limitation of the present study could be the sample size, as we have not enough data in the literature to exactly calculate the sample size needed to obtain significant results.

## 4) **Progetto di Ricerca della Dott.ssa Chiara Cappugi**

## Titolo Progetto di Ricerca

### Modulating Cardiomyocyte Dysfunction and Inflammation in Transthyretin Cardiac Amyloidosis: The Role of Tafamidis and SGLT2 Inhibitors

**Brief presentation of the project**

Cardiac amyloidosis, primarily due to misfolded wild-type transthyretin (ATTRwt), is an age-related disorder. Tafamidis is currently the only FDA-approved treatment for ATTRwt cardiomyopathy (ATTRwt-CM), although several other agents are under investigation, including the stabilizer AG10, gene silencers such as eplontersen and vutrisiran, and the monoclonal antibody. Additionally, therapies traditionally used in heart failure, such as sodium-glucose cotransporter 2 inhibitors (SGLT2i), have shown potential benefits in cardiac amyloidosis., though their molecular mechanisms remain unclear.

This PhD project investigates the cellular and molecular effects of dapagliflozin and tafamidis via in vitro studies (patch clamp experiments, mitochondrial function assays) and retrospective **clinical analyses.** The project will correlate electrophysiological, metabolic and inflammatory findings with real-world clinical outcomes, leveraging collaboration with the Inter-University Centre for Molecular Medicine and Applied Biophysics laboratories (Neurofarba Department, Florence) and the Department of Pathological Anatomy at the University of Bologna.

Electrophysiological, metabolic and inflammatory data from myocardial, valvular and blood samples will be correlated with clinical outcomes including six-minute walk test (6MWT), Kansas City Cardiomyopathy Questionnaire (KCCQ), echocardiography and biomarkers such as NT-proBNP and troponin.

By clarifying the intracellular effects of SGLT2i and tafamidis, this research aims to provide new mechanistic insight to support more effective and personalized treatment strategies.

**Background**

Cardiac amyloidosis is a progressive cardiomyopathy caused by extracellular amyloid fibril deposition, leading to myocardial stiffness and impaired ventricular function.[1,2] Once rare, it is now recognized as un underdiagnosed cause of heart failure with preserved ejection fraction (HFpEF)[3]. Of 42 amyloidogenic proteins,[4] AL (light chain) and ATTR (transthyretin) forms account for over 95% of cardiac cases [5]. Misfolded transthyretin (TTR), due to mutations (ATTRv) or aging (ATTRwt), disrupts cardiomyocyte calcium handling, mitochondrial function and promotes oxidative stress and inflammation.[7,8,9] Aging, metabolic dysfunction, and chronic inflammation further exacerbate myocardial hypertrophy, fibrosis and amyloid deposition.[10] Amyloid deposits are patchy in myocardial layers[5], causing wall thickening, restrictive physiology and diastolic dysfunction progressing to restrictive cardiomyopathy.[19]

## Molecular mechanism

Amyloidogenesis involves TTR tetramer destabilization, monomer dissociation and aggregation into β-sheet-rich fibrils.[5,16,17] Deposits often include proteoglycans, glycosaminoglycans and serum amyloid P-component (SAP), which confer proteolytic resistance and structural stability.[18] Tissue-specific deposition may be influenced by local protein concentration, pH and interaction with extracellular matrix components.

## Therapeutic Approaches

Current therapies focus on TTR stabilization, gene silencing and fibril clearance.[20] Tafamidis stabilizes TTR by binding thyroxine sites,[21,22] reducing all-cause mortality, hospitalizations and improving functional capacity and quality of life over 30 months (ATTR-ACT trial).[8] Recent data (Porcari et al., 2024) shows that SGLT2i are well tolerated in ATTR-CM, reducing NT-proBNP, preserving renal function, and lowering cardiovascular events.[23]

## Aim of the study

The study investigates cellular, molecular and anti-inflammatory effects of tafamidis and dapagliflozin in cardiac amyloidosis, integrating patch-clamp electrophysiology (effects on ion current and membrane stability), mitochondrial and inflammation assessment with clinical outcomes (Armadillo RedCap registry analyses).

In vitro analyses on aortic valves and blood samples from patients with amyloidosis red flags will explore drug effects on inflammation. The clinical, electrophysiological, and inflammatory responses will be correlated with individual patient phenotypes.

## Methods

### Study Design: clinical setting

This monocentric, prospective observational cohort study will enroll patients (age ≥ 18 years) with confirmed ATTR-CM diagnosis in therapy with dapagliflozin and/or tafamidis (group 1). Patients with a suspect of amyloidosis undergoing aortic valve replacement will be enrolled to evaluate explanted aortic valve and myocardial portion to confirm diagnosis and studying myocyte physiology and inflammation (Group 2). Patients will be followed for at least 3 years with periodic clinical, biochemical, and instrumental evaluations.

*Group 1 – Clinical Arm (Armadillo Registry)*

| Inclusion criteria | Exclusion criteria |
|---|---|
| Confirmed ATTR-CM | Inability to ensure ≥ 6 months follow-up |
| Ongoing treatment with tafamidis and/or SGLT2i | Ongoing conflicting experimental therapies |
| Availability of complete pre/post-treatment data (NT-proBNP, troponin, echocardiography, 6MWT, KCCQ) | Incomplete biochemical or echocardiographic data before or after treatment |
| | Non-ATTR amyloidosis (e.g., AL, AA) |

*Group 2 – Surgical Arm (Histopathological and Functional Analysis)*

| Inclusion criteria | Exclusion criteria |
|---|---|
| *Severe aortic stenosis scheduled for surgical replacement* | *Aortic stenosis without amyloidosis suspicion* |
| *Presence of clinical red flags (e.g. bilateral carpal tunnel syndrome, biceps tendon rupture, unexplained LV hypertrophy, suggestive ECG findings)* | *Clinical instability precluding biopsy* |
| *Consent for myocardial and valvular tissue collection during surgery* | *AL amyloidosis diagnosis* |

Phase II: Follow up (Armadillo Registry)

- Clinical evaluations. At 6 and 12 months after therapy initiation
- Echocardiography
- Laboratory assessments (High Sensitivity Troponin T or I and NT-proBNP, inflammatory markers).

Phase III: Data analysis

Approximately 20 myocardial samples will be collected. Data from both cohorts will be integrated and compared with non-amyloid LV hypertrophy controls (e.g., hypertrophic cardiomyopathy, aortic stenosis).

Statistical analyses will include t-test/ANOVA for continuous data, chi-square for categorical variables, and multivariate regression to correlate clinical, electrophysiological, metabolic, and inflammatory markers. Longitudinal changes will be assessed by repeated-measures ANOVA. $p<0.05$ significant.

Study Design: Experimental setting

Experimental work will be performed in Florence (Neurofarba) and Bologna (Pathological Anatomy), using myocardial and aortic valve specimens obtained intraoperatively. Myocardial samples obtained via minimal myectomy will be divided: one portion fixed in Karnovsky's solution for histology and amyloid typing; the other preserved in cardioplegic solution for functional in vitro studies. Patch-clamp electrophysiology will assess ion channel function and membrane stability; mitochondrial assays will evaluated membrane potential, oxidative phosphorylation, and ROS production to investigate tafamidis and dapagliflozin's effects. Inflammatory responses will be characterized via immunohistochemistry and molecular profiling on myocardial, valvular and blood samples, exploring immune activation[11] and drug modulation.

Control myocardial tissues from patients with non-amyloid hypertrophy will serve as comparators.

**Expected results**

We expect tafamidis and dapagliflozin to improve both clinical and cellular outcomes. Clinically, we anticipate stabilization or reduction of cardiac biomarkers, preservation of echocardiographic parameters, and improved functional capacity, particularly in patients receiving SGLT2 inhibitors. At the cellular level, improved mitochondrial function, membrane stability, and reduced oxidative stress are predicted. Inflammatory analyses may reveal reduced local immune activation with treatment.

Finally, integrating clinical, histological, and molecular data may allow development of a predictive tool for early detection of cardiac amyloidosis in surgical patients undergoing aortic valve replacement, enabling timely therapeutic intervention.

## 5) **Progetto di Ricerca della Dott.ssa Luisa Fanciullacci**

## Titolo Progetto di Ricerca

**Trajectories of psychological variables in females with endometriosis**

**Abstract**

The present project aims at understanding psychological variables related to endometriosis (ENDO) through

three complementary studies. Study 1 (French cohort in collaboration with the EpiGyn Group, Inserm, longitudinal) will examine trajectories of anxiety, depression, and quality of life in subjects with ENDO in relation to pain. Study 2 (Italian convenient sample in collaboration with the Italian Association for the ENDO Project, longitudinal) will assess trajectories of psychological variables in subjects with ENDO. Study 3 (randomized controlled trial) will test the possible benefits in terms of change of trajectory of selected psychological variables of a psychoeducation program with or without Acceptance and Commitment Therapy (ACT) ingredients in subjects with ENDO recruited in Study 2.

**Introduction**

Endometriosis (ENDO) is a chronic inflammatory disease affecting 4-18% of women, with symptoms such as dyspareunia, dysmenorrhea, dyschezia, low back pain, and chronic pelvic pain (CPP) (WHO, 2019). Meta-analyses showed higher anxiety, depression, and poorer quality of life in women with ENDO compared to

healthy controls (Cano-Herrera et al., 2024). Cross-sectional studies suggested that the psychological burden is linked to pain (Facchin et al., 2015; Pontoppidan et al., 2023) and also to factors such as pain catastrophizing, self-criticism,

psychological inflexibility (PI), maladaptive coping strategies, negative body image, and low psychological flexibility (PF) (Geller et al., 2021; González-Echevarría et al., 2019; Grinberg et al., 2024; Sundström et al., 2023). Unfortunately, the large majority of the literature is based on cross-sectional study design research. Studying trajectories of psychological variables in subjects with ENDO via longitudinal observations is warranted.

Psychological interventions might change the trajectory of psychological variables in subjects with ENDO, thus being of benefit. Evidence supporting the benefits of psychological interventions in ENDO is limited. Psychoeducation (Breton et al., 2025), mindfulness techniques, and cognitive behavioural therapy (CBT) showed promising results in terms of improvement of quality of life, anxiety, and depression (Donatti et al., 2022; Hansen et al., 2023; Samami et al., 2023). However, this literature is sparse, and the methods used are heterogenous, in some cases weak (Del Pino-Sedeño et al., 2024). In addition, besides the growing evidence supporting the effectiveness of Acceptance and Commitment Therapy (ACT) - based interventions for chronic pain (McCracken, 2024), only a couple of studies applied ACT ingredients to ENDO and in combination with mindfulness techniques (Hansen et al., 2023; Maindal et al., 2025). Thus, overall, studying via rigorous methods possible benefits of psychoeducation with or without ACT ingredients seems to be of help in sheding some light on the possible complementary therapies to propose to women with ENDO to modify the trajectories of their psychological burden.

Accordingly, this project will be articulated in three studies, which are described below.

**Study 1** (French cohort in collaboration with the EpiGyn Group, Inserm, longitudinal) will examine trajectories of anxiety, depression, and quality of life in subjects with ENDO in relation to pain.

**Study 2** (Italian convenient sample in collaboration with the Italian Association for the ENDO Project,

longitudinal) will assess trajectories of psychological variables in subjects with ENDO.

**Study 3** (randomized controlled trial) will test the possible benefits of a psychoeducation program with or without ACT-ingredients in subjects with ENDO recruited in Study 2.

**Methodology**

**Study 1** will take advantage of data collected in an ongoing e-cohort managed by the French Epidemiology of

Gynaecological Health (EpiGyn) Group (CESP, Inserm, Paris). This is a large French cohort of women with ENDO who complete an online annual assessment on psychological and pain variables (for more details please visit https://www.ephect.org/tools/) and a monthly assessment on patient-reported outcome measures (PROMs) and experience measures (PREMs) (the ComPaRe-Endometriosis e-cohort). A total of about 11,000 women will be eligible.

For **Study 2**, women with ENDO (n = 210) will be recruited among affiliates to the Italian Association for the ENDO Project (AEP) and asked to complete an online survey assessing clinical data on ENDO, pain, and psychological variables (e.g., anxiety, depression, quality of life). Data will be collected via self-reported standardized tools in at least three different waves.

**Study 3** will be conducted on a subset of subjects enrolled in Study 2 who will be randomly assigned to psychoeducation with or without ACT-ingredients. Psychoeducation will be administered online. Participants will be assessed at baseline, after having received the first half of sessions, at post-treatment, at 3-month follow-up. At baseline and at post-treatment, diagnostic interviews will be administered by properly trained clinical psychologists to verify the occurrence of mental disorders/psychosomatic syndromes. At all assessment points, standardized tools will be used to measure psychological variables.

Study 1 and 2 were approved by an Ethical Committee. Study 3 will be conducted after being approved by the Ethical Committee.

**Statistical analyses**

Study 1. Mixed-effects models will be applied to examine trajectories of anxiety, depression, pain over time

and differences between ENDO patients with and without pain.

Study 2. Multiple regression models will be used to quantify the main and interaction effects of pain and

psychological variables on functioning. Assuming that the interaction between two variables explains a proportion of the deviance equal to 5% of the total, a sample size of 210 will be required to test the interaction at the 5% significance level with at least 90% power.

Study 3. The two groups will be compared for outcome variables over time change.

The statistical models will be adjusted for potential sociodemographic and clinical confounders. All analyses will be conducted with the software R.

**Expected results**

New insights into trajectories of psychological variables of ENDO will be provided via Studies 1 and 2. Study 3 will provide evidence of potential capacity of psychoeducation to modify psychological variables.

## 6) Progetto di Ricerca della Dott.ssa Maria Lucia Gallo

## Titolo Progetto di Ricerca

**Development of a structured robotic functional and reconstructive urology program at Careggi University Hospital**

**Summary**

The application of robotic surgery in functional and reconstructive urology (FRU) is constantly growing. Yet, there is still little evidence regarding the superiority of this minimally invasive approach compared to traditional surgery. Furthermore, despite the burden of diseases potentially requiring surgery, this field faces several challenges, including lack of standardised techniques, poor mentorship programs and complexity of clinical cases, often requiring a multidisciplinary approach. This project aims to fill this gap by acting on three complementary fronts: the creation of a multidisciplinary FRU board at Careggi University Hospital for evidence-based, individualised patients' management; the development of a structured training program in FRU, including the definition of standardized surgical curricula for robotic FRU procedures and implementation of modular training programs based on simulation and mentored surgical sessions; the development of a prospective registry to analyse surgical and functional results of robotic procedures, as a foundation for prospective clinical trials.

The PhD project will allow standardised, equitable care for patients who are candidate for FRU robotic procedures; development of new minimally invasive techniques in robotic FRU; structured, simulation-based training of surgeons in minimally invasive FRU (making Careggi Hospital a referral Centre in the field); finally, prospective multicentre studies evaluating the impact of robotics in RFU.

**Introduction**

Since the introduction of the DaVinci surgical system in 2002, robotic surgery has seen exponential growth throughout the world across several surgical disciplines, effectively marking the beginning of a new era where surgeons can provide patients with the advantages of this minimally invasive approach, while shortening the steep learning curve associated to the traditional laparoscopic surgery. Although urology has been a pioneering discipline in this field, foreseeing the potential advantages of this approach in the deep narrow spaces of the pelvis, robotic surgery has been mainly applied to uro-oncology, in particular for the treatment of localized prostate cancer [1], while the experiences in functional and reconstructive urology (FRU) have remained limited to procedures like sacrocolpopexy [2] and pyeloplasty [3].

Nonetheless, recently there has been a growing interest regarding the application of robotic surgery to several FRU procedures: the feasibility and safety of this approach has been proven for different complex procedures including supra-trigonal cystectomy with augmentation ileocystoplasty [4], continent urinary diversions [5], genitourinary fistula correction [6] and artificial urinary sphincter placement in men and women [7-8]. Although the results currently available in the literature are encouraging and suggest technical and functional advantages deriving from this approach, the evidence is still not solid due to the retrospective nature of most of the available works and the poor diffusion of expert academic centres for the treatment of these pathologies. Furthermore, there is a lack of mentors and structured programs for training the new generations [9], in addition to the absence, in most cases, of multidisciplinary management programs for patients who often have complex histories of previous pelvic oncology surgery.

To fill this gap, leveraging an ongoing collaboration with world-renowned Institutions, the aim of the PhD project is to develop a robotic functional and reconstructive urology program at Careggi University Hospital through a stepwise approach including:

a) definition of a standardized surgical curriculum for robotic functional urology procedures;

b) definition of a mentored and simulation-based structured training program for young surgeons;

c) implementation of a multidisciplinary management pathway for patients who are candidate for surgery (including diagnosis, treatment and follow-up);

d) development of a prospective registry of patients undergoing robotic FRU interventions, aiming to provide an evidence base for clinical audits and prospective clinical trials.

**Methodology**

The PhD project is grounded into the following steps:

**Step 1**:

Building a multidisciplinary FRU board at Careggi University Hospital, allowing a tailored management pathway for patients undergoing robot-assisted FRU procedures, as a first step toward the robotic FRU program. Mirroring multidisciplinary tumour boards, the board will include all the following healthcare figures: urologists, neuro-urologists, gynaecologists, proctologists, dedicated radiologists, physiatrists, specialized nurses and physiotherapists. The path would be structured as follows:

- Establishment of a weekly or biweekly multidisciplinary meeting for the discussion of complex cases and validation of surgical indications, involving the healthcare figures mentioned above and any others (oncologists, nephrologists, neurologists etc.), as needed;

- Implementation of interdisciplinary collaboration through pre-operative assessment by multiple specialists (where necessary) and through joint consultations (e.g. urologists + gynaecologists or urologists + proctologists);

- Scheduling of shared operating sessions for optimal management of complex cases.

The impact of such a multidisciplinary FRU board would be assessed through both clinical quality metrics (e.g., through a comparative analysis of surgical and functional results of the prospective series versus retrospective historical data) and patient-reported outcomes (PROMs), including satisfaction questionnaires administered to the involved patients.

**Step 2**:

Development of a structured training program in FRU, including the definition of a standardized surgical curriculum for all robotic FRU procedures and implementation of modular training programs based on simulation and mentored surgical sessions (PhD theme n° 3).

In particular, robotic FRU interventions will include:

- urinary incontinence and pelvic organ prolapse correction procedures (sacrocolpopexy, artificial urinary sphincter placement, colposuspension);

- upper tract reconstructive procedures (pyeloplasty, ureteric reimplantation);

- bladder reconstruction and urinary diversions (augmentation ileocystoplasty, continent and incontinent urinary diversions for neurogenic patients);

- prostate and bladder neck surgeries (simple prostatectomy, bladder neck reconstruction);

- procedures for the management of retroperitoneal fibrosis (ureterolysis)

- genitourinary fistulas corrections.

The training program will include:

1. the creation of specific modules dedicated to robotic FRU that will be integrated into the didactic activity of trainees, which would also include the illustration of surgical steps and techniques;

2. targeted and scheduled sessions with the DaVinci robotic simulator (where appropriate) as indispensable criteria before performing the steps of the interventions (e.g. completion of training on surgical knots before fixing the mesh of a sacrocolpopexy);

3. lectures and live-surgery sessions with visiting professors, experts in FRU, coming from Italy and Europe, with the aim of developing new techniques (PhD theme n°1) and training young surgeons on the most complex procedures, through the direct execution of the steps of the operation (also thanks to the double console) and the recording of the procedures.

**Step 3** (in parallel with the previous steps):

Development of a prospective registry including patients undergoing robotic FRU interventions. The Registry will be approved by the Ethical Committee of Careggi University Hospital (as an addendum to the already approved observational study OSS 25002) and may include other referral national/international Centres, leveraging ongoing scientific collaborations between Careggi University Hospital and the Italian Society of Urodynamics (SIUD) and European Association of Urology (EAU) Young Academic Urologists (YAU) Functional Urology research networks.

The registry will be grounded into a a priori developed online dataset (using the REDCap platform; https://project-redcap.org) and will prospectively collect the following data:

- pre-operative clinical data;

- pre-operative quality of life (QoL) evaluation through specific validated questionnaires;

- intra- and perioperative outcomes;

- postoperative data and follow up, including surgical complications, functional results, patients' satisfaction and QoL.

The registry will allow robust analyses on the impact of robotics in FRU (PhD theme n°1), providing a foundation for larger multicentre clinical trials in this field even beyond the 3-year PhD project timeframe.

*Expected results*

The expected results of the PhD program encompass 3 domains (Table 1):

1. Realization of a novel pathway where all patients with potential indications for FRU surgery will have the opportunity to be managed by a dedicated multidisciplinary board. The multidisciplinary nature of this approach will lead to improved patient care, standardised indications for surgery (vs conservative management) and more individualised and tailored treatments, that would ultimately improve objective outcomes (clinical, surgical and functional results) and increase the satisfaction of the patients at the end of the treatment (PROMs).

2. Development of a structured training program in FRU aiming to increase the number of surgeons trained in FRU and of minimally invasive FRU procedures offered to patients, making Careggi Hospital a referral Centre. Such a strategy will improve surgical outcomes, patients' satisfaction and future residents' involvement in FRU.

3. The prospective Registry on robotic FRU interventions will be the foundation for congress abstracts/presentations and peer-reviewed publications on the impact of minimally invasive surgery in this field, including surgical metrics and patients' satisfaction and quality of life.

## 7) Progetto di Ricerca del Dott. Mattia Lo Re

## Titolo Progetto di Ricerca

**Phase I/II Clinical Trial of Vibrio alginolyticus Collagenase (CVA) for Peyronie's Disease - Research Protocol**

**Abstract**
Peyronie's Disease (PD) is a fibrotic disorder of the penile tunica albuginea leading to plaque formation, curvature deformity, and sexual dysfunction. The only approved non-surgical therapy is intralesional collagenase from Clostridium histolyticum (CCH), which has shown significant curvature reduction in PD. However, CCH has limitations including low substrate specificity and potential immunogenicity. This protocol describes a Phase I/II multicenter clinical trial evaluating a novel collagenase derived from Vibrio alginolyticus (CVA) as an innovative treatment for PD. Phase I is an open-label dose-escalation to determine a safe dose/regimen; Phase II is an exploratory efficacy phase comparing two different regimen of somministration. Key outcomes include adverse event profile, change in curvature angle, and immunogenicity (antibody formation). We hypothesize that CVA will safely reduce plaque size and curvature with minimal side effects, representing a pivotal game changing technological innovation in urology.

**Introduction**
Peyronie's Disease (PD) is an acquired connective tissue disorder characterized by fibrous plaque formation in the penile tunica albuginea, resulting in penile curvature, pain, and erectile dysfunction. Its prevalence is about 3–9% of adult men and increases with age.

PD plaques consist predominantly of type I and III collagen fibers. Untreated, PD can severely impair sexual function and quality of life.

Current treatments comprehend conservative management (oral or topical agents) with limited efficacy or surgical correction, with possible side effects such as hematoma, loss of length, and erectile dysfunction. Collagenase Clostridium histolyticum (CCH) is the first FDA-approved intralesional therapy for PD, demonstrating significant curvature reduction (~30% on average) and symptom improvement in clinical trials. Standard CCH (marketed as Xiaflex/Xiapex) treatment involves multiple plaque injections combined with penile "modeling" (mechanical plaque deformation) to further reduce curvature. Despite its efficacy, CCH has important drawbacks. It is a heterogeneous enzyme mixture with relatively low specificity – it can degrade non-collagenous proteins like decorin and fibronectin that are vital to normal tissue structure, leading to collateral tissue damage or adverse effects (e.g. hematoma, corporal rupture). Moreover, CCH treatment can elicit anti-drug antibodies in patients, raising concerns about immunogenic reactions and potential loss of efficacy over time. CCH therapy is also costly and, as of 2020, it was withdrawn from the European market, underscoring the need for alternative therapies.

A novel collagenase derived from Vibrio alginolyticus (CVA) has been developed to address these limitations. Collagenases from the Vibrio genus are promising due to their high collagen selectivity and purity. In vitro comparisons have shown that CVA cleaves collagen I and III efficiently while sparing key extracellular matrix proteins such as fibronectin and decorin. Preclinical studies support CVA's potential in fibrosis: Bassetto et al.(2016) demonstrated that Vibrio collagenase effectively degraded collagen in Dupuytren's contracture cords (a hand fibromatosis analogous to PD) in a dose-dependent manner without harming cell viability. Similarly, Riccio et al.(2023) found CVA to be selective for type I/III collagens in fibrotic tissue, minimizing risk to vessels or skin during plaque degradation. These data suggest that CVA could degrade PD plaques (rich in collagen I/III) while preserving surrounding healthy penile tissue. To date, no CVA has been tested clinically for PD, making this a technological innovation in urology.

This PhD research project aims to evaluate the safety and preliminary efficacy of CVA in men with Peyronie's Disease. We hypothesize that CVA will safely reduce penile curvature and plaque size, with improved tolerability over existing collagenase therapy. The study is designed as a Phase I/II trial. The goal is to position CVA as a novel, targeted treatment for PD.

**Methodology**

*Study Design*

This is a Phase I/II, open-label, multicenter clinical trial of CVA collagenase in adult male patients with PD. The trial will be conducted collaboratively by the Urology departments of the University of Florence and IRCCS San Raffaele, with appropriate regulatory and ethics approvals. The study is divided into two sequential phases:

- **Phase I** (Dose-Escalation Safety Phase): An open-label dose-escalation to evaluate safety, tolerability, and determine the recommended Phase II dose. Successive 4 cohorts of 10 patient each will receive escalating doses of CVA injected into their Peyronie's plaque. Dose levels are predefined (0.1 mg, 0.144 mg, 0.2 mg, and 0.3 mg), with escalation proceeding only after review of safety data for the prior cohort.
- **Phase II** (Preliminary Efficacy Phase): An exploratory phase to evaluate the efficacy and safety of the Maximum Safe Dose (MSD) identified in Phase I in two different regimens. Sixty patients will be treated with CVA, divided in two cohorts: regimen A (3 injections at MSD each 4 weeks) or regimen B (4 cycles of 2 injections with half MSD, every 24-72 hours, with 6 weeks of stop after each cycle until reaching 8 injections).

**Study Population and Selection Criteria**

Inclusion Criteria: Adult male patients (≥18 years) with a diagnosis of PD in the stable phase (defined by ≥ 12 months since plaque onset with no significant change in curvature or pain in the past 3 months). Key inclusion criteria include:

- Penile curvature deformity between 30° and 90° attributable to a well-defined PD plaque.
- Palpable plaque in the penile shaft.
- Erectile function sufficient for sexual activity (with or without pharmacological aid).
- Ability to comply with all study procedures and follow-up visits, and provision of written informed
- consent.

**Exclusion Criteria:**

- Active (acute phase) PD, indicated by significant penile pain or rapidly worsening curvature in
- the past 6 months.
- Ventral plaques or complex hourglass deformity.
- Plaque calcification, which could impede injection or enzymatic activity.
- Prior treatment for PD.
- Bleeding disorders or current anticoagulant therapy that cannot be temporarily halted.

- Known hypersensitivity to collagenase or any components of the study medication.
- Uncontrolled comorbidities that would increase risk of complications or interfere with outcomes.

**Intervention and Administration**

**Study Drug**: The investigational product is a lyophilized collagenase enzyme derived from Vibrio alginolyticus, provided in single-use vials. Each vial is reconstituted with a sterile diluent to a specific concentration per dose.

**Injection Procedure**: Under sterile conditions and local anesthesia, CVA collagenase is injected directly into the PD plaque. The goal is to evenly distribute the collagenase throughout the plaque. If the regimen is split-dose, the second injection (containing the remaining half-dose) is administered 1–3 days after the first, into an adjacent location in the same plaque.

**Penile Modeling**: At 1–3 days after the injection, a penile modeling procedure is performed to mechanically reduce the curvature. These exercises aim to maximize plaque elongation and prevent re-contracture as healing occurs.

**Outcome Measures and Assessments**

**Safety and Tolerability**: Safety is the primary endpoint in Phase I and a key endpoint in Phase II. Specific local adverse events (AEs) of interest include penile swelling, bruising, pain, hematoma, and any signs of corporal rupture. A dose-limiting toxicity (DLT) in Phase I is defined as any Grade ≥3 (CTCAE v5.0) treatment-related AE or serious adverse event (SAE) occurring during the first injection cycle. If a DLT occurs, escalation will be halted and the previous lower dose may be declared the maximum tolerated dose.

**Efficacy Endpoints**: The primary efficacy endpoint (in Phase II) is the change in penile curvature angle from baseline to the end of study. Curvature is measured by standardized photography of an erection and image analysis with a goniometer. Secondary efficacy endpoints include change in plaque size as measured by penile ultrasound or in plaque consistency; improvement in the Peyronie's Disease Questionnaire (PDQ) symptom bother score or in sexual function as measured by the International Index of Erectile Function (IIEF-5); any change in stretched penile length from baseline, to detect if treatment preserves length.

**Immunogenicity**: Because CVA is a bacterial enzyme, patients may develop antibodies against it. To monitor immunogenicity, we will collect blood samples for antibody testing. Serum anti-CVA IgG and IgE levels will be measured at baseline (pre-treatment) and at the end of the study (and midway through treatment).

**Follow-Up Assessments**: Participants will be followed for at least 6 months after their last treatment cycle. Follow-up visits are scheduled at approximately 2, 6, and 12 weeks after each cycle, and a final assessment at 6 months post-treatment.

**Expected Results**

- **Safety and Tolerability**: CVA collagenase is expected to be well tolerated at the doses tested. Based on its collagen-selective action, we hypothesize that fewer severe local reactions will occur compared to historical CCH data. We expect most adverse events to be mild to moderate. Importantly, we expect no occurrences of serious complications like penile fracture at the recommended dose, confirming a favorable safety profile. We also anticipate no systemic toxicity. Monitoring for immunogenicity may detect antibody formation, but we expect no anaphylactic reactions or loss of efficacy within this short treatment course.
- **Plaque Degradation and Curvature Improvement**: We expect to observe meaningful reductions in penile curvature in the Phase II cohort. We anticipate curvature improvements on the order of those achieved with CCH therapy (roughly 15°–20° absolute reduction, depending on baseline) or potentially better, given the precise collagen targeting of CVA.
- **Scientific Innovation**: If our hypotheses are correct, this trial will provide the first clinical evidence that a Vibrio-derived collagenase can safely and effectively treat PD. A key innovative result would be demonstrating plaque degradation with minimal damage to surrounding tissues. Additionally, showing that CVA remains effective without excessive immune response will validate its feasibility for repeated use. Positive outcomes from this study will mark a significant advancement in urologic treatment innovation.

# 8) **Progetto di Ricerca del Dott. Edoardo Lo Russo**

## Titolo Progetto di Ricerca

**SAFE -T. Studio attività fisica ed esercizio dopo trapianto**


**Abstract**

Il progetto mira a determinare il protocollo di esercizio fisico più efficace nei soggetti post trapianto di fegato, con l'obiettivo di migliorare la condizione di salute generale. Le variabili analizzate saranno indicatori di flogosi di stress ossidativo, di funzionalità cardio-vascolari e di composizione corporea.


**Razionale scientifico**

Il trapianto di organo solido è una strategia terapeutica nella fase terminale della malattia dell'organo o in casi di emergenza. Negli ultimi anni, le tecniche chirurgiche e sanitarie sono migliorate notevolmente, garantendo una sopravvivenza dopo il trapianto sempre maggiore. Negli ultimi dieci anni in Italia si è registrato un aumento del 26% del numero di trapianti di organi solidi, tra cui il trapianto di fegato ha avuto uno degli incrementi più elevati, raggiungendo il 44% [1].

Lo stile di vita, che preveda una regolare attività fisica, rappresenta una cura per molte malattie croniche [2]. Le malattie cardiovascolari sono una delle principali cause di mortalità dopo il trapianto di fegato [3], ed è stabilito ormai da tempo che sane abitudini riducono i fattori di rischio migliorando i parametri di fitness correlati alla salute (forza muscolare, resistenza aerobica, composizione corporea, equilibrio) nei riceventi di trapianto di organi solidi [4].

L'inattività fisica è un fattore cruciale nella progressione e negli esiti avversi della malattia epatica [5]. In particolare, il ruolo dell'esercizio fisico nella riduzione della mortalità è attualmente dibattuto nei riceventi di trapianto di fegato [6]. Dopo un trapianto di fegato avvenuto con successo, i pazienti mostrano una ridotta capacità di esercizio e un precoce affaticamento per sforzi fisici lievi a causa della diminuzione della tolleranza allo sforzo [7]. Questa affaticabilità è spiegata dal riposo a letto prolungato dopo il trapianto, dai farmaci immunosoppressori, dalle comorbilità associate (obesità, diabete, iperlipidemia, ipertensione e sindrome metabolica) e dalla sarcopenia [8].

In questo contesto, le evidenze scientifiche attuali suggeriscono che i programmi di esercizio adattato in questa specifica popolazione sono:

- sicuri, non si sono registrati effetti collaterali né in acuto né in cronico nei partecipanti che hanno eseguito tali allenamenti [6];
- efficaci, in termini di parametri di fitness fisica relativa alla salute con aumento della forza muscolare, della tolleranza allo sforzo e della riduzione del grasso corporeo [9, 10];
- efficaci in termini di riduzione dei trigliceridi sierici in risposta all'esercizio [11].

Pertanto, l'attività fisica è sempre più raccomandata come approccio terapeutico dopo il trapianto di fegato [12].

Tuttavia, ad oggi risultano ancora numerosi aspetti da approfondire per proporre su larga scala un approccio terapeutico basato sull'esercizio fisico nei pazienti dopo un trapianto di fegato. In particolare la letteratura sottolinea In muncnnzn di 3tudi rigoro3i che 3inno cnrnttcrizznti da:

-rigore metodologico (RCT con le valutazioni effettuate in cieco);

-utilizzo di un maggior numero di outcomes clinici di routine come valutazione di efficacia in seguito alla proposta di allenamento;

- differenziazione dei protocolli di allenamento per stabilire quale tipologia di allenamento sia da raccomandare;

-valutazione delle modificazioni degli aspetti psicologici e della qualità della vita.

**Metod**
**i**

Partecipanti

La proposta di progetto di ricerca è destinata alle persone che hanno ricevuto un trapianto di fegato di entrambi i sessi da almeno un anno ed abbiano una condizione clinica stabile (ad esempio, assenza di complicazioni correlate al fegato nei sei mesi precedenti, inclusi episodi di rigetto acuto e aumento delle transaminasi sieriche). Per evitare fattori confondenti saranno applicati i seguenti criteri di esclusione:

- trapianto combinato,
- re-trapianto di fegato,
- limitazioni fisiche e cardiovascolari che rappresentano controindicazioni assolute all'esercizio fisico,
- disturbi neurologici o psichiatrici che potrebbero compromettere l'adesione al programma di allenamento nel tempo.

Terapia immunosoppressiva e steroidea, comorbidità, come diabete, ipertensione o altre malattie metaboliche, non saranno motivo di esclusione.

Disegno di studio

Nella figura 1 è riportato il diagramma del disegno di studio. Lo studio sarà longitudinale di intervento, randomizzato a 3 bracci:

- esercizio di resistenza aerobica svolto 1 ora a seduta 3 volte alla settimana;
- esercizio di forza muscolare svolto 1 ora a seduta 3 volte alla settimana;
- esercizio di forza e di resistenza svolto 1 ora 3 volte alla settimana;

Le altre tre proposte di allenamento avranno lo stesso volume in modo da stabilire quale modalità sia più efficace.

Ogni allenamento sarà illustrato sotto da un chinesiologo laureato magistrale in Magistrale in Scienze e tecniche dello sport e delle attività motorie preventive e adattate (LM 67/68) e seguendo le linee guida internazionali [13] per l'esercizio nelle malattie croniche.

[candidati ritenuti eleggibili allo studio dal medico epatologo saranno inviati alla medicina dello sport per stabilire ventuali controindicazioni muscolo-scheletriche e cardiovascolari all' esercizio. Da queste due prime visite mediche avremo dati relativi:

- agli esami ematici per valutare lo stato infiammatorio e lo stress ossidativo;
- alla valutazione del consumo di ossigeno di picco come indicatore di capacità aerobica;
- alla valutazione della forza muscolare distrettuale, della flessibilità; alla composizione corporea.

L'intervento di esercizio fisico avrà la durata di un anno con valutazioni che saranno effettuate all'arruolamento (iniziale, TO), dopo sei mesi (intermedia, T6) e al termine (finale, Tl2).

Pertanto gli outcomes di studio saranno suddivisi in primari e secondari. Primari:

numero di eventi avversi durante il periodo di studio (mortalità e infortuni); esami

ematici.

Secondari:

compos121one corporea; forza e

flessibilità muscolare; resistenza

cardiovascolare.

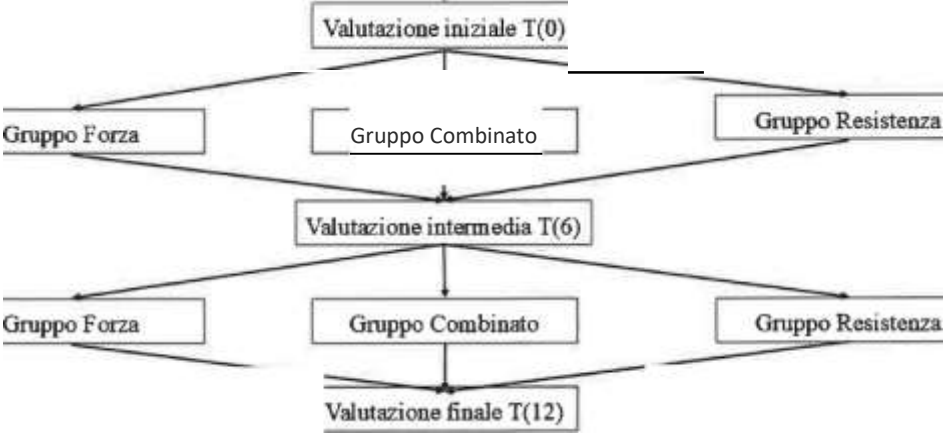


Figura 1 Flow-chart del disegno di studio.

L'analisi statistica prevedrà una valutazione delle differenze tra i valori iniziali, intermedi e finale. In aggiunta, l'analisi multivariata consentirà di stabilire quale sia il programma di allenamento che ha mostrato maggiore efficacia negli outcomes sia primari che secondari.

**Risultati attesi**

Da tale studio, i ricercatori ipotizzano che l'esercizio fisico sia efficace nel miglioramento degli:

l. esami ematici di stress ossidativo e dell'infiammazione;

2. fitness fisica relativa alla salute.

Tuttavia, verrà svolta una valutazione differenziale per i tre bracci di intervento con diverso schema di esercizio fisico. Infatti, si ipotizza che l'allenamento di resistenza abbia un maggiore effetto sui parametri cardiovascolari e di grasso corporeo, mentre quello di forza nel contrastare la sarcopenia e sulla massa muscolare.

Infine, diverse le proposte di allenamento potrebbero avere ricadute differenziate sugli esami ematici, con importanti ricadute sulla pratica di gestione clinica.

## 9) Progetto di Ricerca del Dott. Lorenzo Mainardi

## Titolo Progetto di Ricerca

**Peripheral innervation in intrahepatic cholangiocarcinoma: the role of Schwann cell phenotype switching via Midkine signaling**

**ABSTRACT**

The microenvironment of intrahepatic cholangiocarcinoma (iCCA) is rich in nerve fibers, the significance of which is currently elusive. In other tissues, innervation has been shown to modulate the biological features of cancer. Schwann cells, responsible for nerve support and repair, can exhibit a "repair phenotype" in response to both nerve injury and cancer. While this repair phenotype is crucial for nerve regeneration, it can also be hijacked by cancer cells, contributing to tumor progression and metastasis. This project aims to elucidate the molecular mechanisms driving tumor invasiveness, resistance, and microenvironment remodeling in response to peripheral innervation in iCCA, an aggressive liver cancer with limited treatment options and poor prognosis. Specifically, this study will focus on repair Schwann cells (repair SCs) and Midkine (MK), a neurotrophic factor implicated in tumor proliferation, immune evasion, and chemoresistance, dissecting the molecular and cellular crosstalk between iCCA cells, SCs, and Tumor-Associated Macrophages (TAMs). The investigations will exploit advanced in vitro approaches, including primary and established iCCA cell lines, SCs spheroids, and Patient-Derived Organoids (PDOs). The role of specific molecules will be assessed using CRISPR/Cas9 gene editing, and RNA silencing. The ultimate goal is to identify novel molecular targets and signaling pathways that will be eventually explored by employing an orthotopic in vivo mouse iCCA model, through the injection of MK knockout/silenced iCCA cells, allowing the investigation of a complex biological system where tumor, and neural components interact dynamically in a specific microenvironment.

**RATIONALE**

Intrahepatic cholangiocarcinoma (iCCA) is an aggressive liver cancer originating in the intrahepatic biliary tree, with limited therapeutic options (1). iCCA is characterized by a dense stroma that hinders chemotherapy efficacy, making curative treatments challenging. Currently, surgery combined with chemotherapy remains the primary potential intervention (2). Recent evidence has highlighted the bidirectional interaction between cancer and the peripheral nervous system (3). Approximately 75% of iCCA exhibit perineural invasion, a feature independently predicting a worse prognosis and shorter survival (2). Schwann Cells (SCs) have been found to be closely associated with highly metastatic solid tumors (4). Due to their plasticity and abundance, SCs have been suggested to contribute to shape and maintain a tumor-supportive microenvironment. SCs-conditioned medium enhances migration, invasion, survival, and proliferation of iCCA cells in vitro, inducing a cadherin switch correlated with the Epithelial-Mesenchymal Transition (EMT) process (5). Moreover, SCs could migrate toward cancer cells (5). After peripheral nerve injury, as in the Tumor Microenvironment (TME), SCs detach from axons, undergo major phenotypic and genetic changes and convert into activated repair SCs that can promote invasiveness, migratory capacity and tumor progression (4). The transcription factor c-Jun acts as the master regulator of the SC repair phenotype, suppressing myelin genes and inducing the expression of various factors that may modulate the behavior of different cell types within the TME (6,7). Concurrently, the STAT3 transcription factor sustains survival of repair SCs and is crucial for maintaining their repair capabilities (8). Among factors released by repair SCs affecting both tumor cells and relative TME in a paracrine fashion, Midkine (MK) has gained attention in this context. MK is a neurotrophic factor promoting neurite growth and tumor progression in several solid tumors (9), MK binds to multiple receptors (9,10) and is expressed at high levels in many malignant tumors, including pancreatic cancer (11) and iCCA (12). Notably, it has been shown that in iCCA MK is overexpressed in 40% cases and in vitro promotes gemcitabine resistance by inducing EMT thus facilitating perineural invasion and tumor progression (12,13). According to Single-cell RNAseq data from the "Human Protein Atlas" (https://www.proteinatlas.org/ENSG00000110492-MDK/single+cell#single_cell_type_summary), SCs express MK, raising the hypothesis that they may represent a source of MK within the TME (especially when they adopt a repair-like phenotype). Based on this, the aim of the project is to investigate how MK-mediated paracrine signaling facilitates tumor-nerve interactions and perineural invasion in iCCA, focusing on the complex interplay among iCCA cells, repair SCs, and Tumor-Associated Macrophages (TAMs) within the TME that contributes to the malignancy of this aggressive cancer.

## METHODOLOGY

My Ph.D. project will pursue three main objectives:

- Identification of the role of the repair SCs secretome in iCCA

  SCs with a reparative phenotype will be studied using a 3D model known as spheroids in 10% methylcellulose hydrogel-coated plates, as recently published (14). I will first analyze the secretome composition performing a multiplex ELISA assay for several specific factors (i.e. GDNF, Artemin, BDNF, NT3-4, NGF, NGFR, N-cadherin, TNF-α, IL-1α, IL-1β, LIF, MCP-1, Shh, NRG1, IGF-1 (6,7)), including the protein MK. These data will allow to identify factors potentially implicated in tumor biology. Next, the effects of the repair SCs-derived secretome on iCCA will be evaluated after validation of the specific receptors of the abovementioned factors such as SDC3/4, LRP1, PTPRZ1 ERBB3, IGF1R, LIFR. To this aim, migration (Boyden chambers), invasion (Matrigel® invasion assay), proliferation (bromodeoxyuridine incorporation and MTT assay), viability (crystal violet assay) and chemoresistance will be tested in different iCCA cell lines primary as well as established (CCLP1, HUCCT1, SG231) and in Patient-Derived Organoids (PDOs) through indirect (using conditioned medium) and direct co- culture methods. We will also measure MK tissue levels in CCA specimens and investigate their potential clinical relevance by analyzing correlations with a range of clinicopathological features, including tumor grade and stage, presence of metastases, microvascular invasion, and early recurrence. Additionally, we will assess associations with key mutational subgroups, such as IDH1/2, FGFR2, KRAS, and HER2, to determine whether MK expression may serve as a marker of biological aggressiveness or be linked to specific molecular profiles.

- Exploring the contribution of MK in iCCA biology and in long-term maintenance of the SC repair phenotype

Particular attention will be dedicated to the role of MK, as a factor implicated in neuro-oncology in different solid tumors. The expression of MK and its multiple receptors in iCCA cell lines will be determined by real time PCR and Western blotting analysis. After assessing the presence of MK, cocultures will be performed together with pharmacological or genetical inhibition of MK expression to evaluate the effects on iCCA. We will also investigate the

role of MK in mediating resistance to second-line treatments in iCCA. According to current literature (1), the standard second-line regimen for iCCA is FOLFOX, a combination of folinic acid, 5-fluorouracil, and oxaliplatin administered in patients who progress after or are intolerant to first-line cisplatin-gemcitabine therapy. To model this, iCCA cell lines and iCCA PDOs, which mimic the original tumor's molecular characteristics and patient-specific drug responses (15), will be used to assess the effects of FOLFOX and chemoresistance mechanisms possibly mediated by MK. Co-culture experiments between iCCA and SCs will be conducted through MK silencing, as well as in the presence of the potent and selective MK inhibitor, HBS-101. In parallel, recombinant human MK will be applied to iCCA cells cultured in soluble factor-depleted media, to evaluate its direct biological effects, such as the ability to induce resistance to FOLFOX. The specificity of these effects will then be confirmed using HBS-101. To further validate these findings in a physiologically relevant setting, an in vivo model will be developed in an orthotopic mouse model through transplantation of MK-knockout or silenced iCCA cells, thus providing a preclinical platform to assess the functional relevance of MK signaling and the MK-dependent chemoresistance to abovementioned drugs. Concurrently, the effects of MK-mediated signaling on the functional features of repair SCs will be evaluated by employing CRISPR/Cas9 gene editing to generate MK knockout, short hairpin RNA (shRNA) to silence the MK gene, or HBS-101. To this end, primary human SCs will be used to evaluate whether MK signaling contributes to the long-term maintenance of their repair phenotype. MK may act on SCs through its release into the TME and subsequent binding to specific receptors expressed on SCs, such as LRP1, PTPRZ1, NOTCH2, and SDC3/4 (receptors indicated in previous reviews (9,10)).

3. Decoding the interplay between TAMs, repair SCs and iCCA

TAMs in the iCCA TME often exhibit an M2-like phenotype that promotes tumor progression and immune evasion (16). Therefore, understanding how this cell type interacts with other cellular components of the TME, such as repair SCs and cancer cells, is crucial. SCs spheroids-derived CM has a marked propensity to recruit macrophages in vitro (14). This relationship will be explored by performing indirect and direct co-culture methods firstly, employing buffy coat-derived monocytes from human donors treated with repair SCs CMs with and without MK inhibitor. The effects on iCCA cells, including invasion, proliferation, and drug resistance will then be assessed. Data will be next validated using TAMs isolated from tumor tissue or monocytes derived from iCCA patients. These experimental strategies will enable the assessment of a potential enrichment of the pro-tumoral M2 macrophage phenotype, particularly in response to soluble factors released by repair SCs within iCCA TME.

## EXPECTED RESULTS

My research project is expected to clarify the impact of the repair SCs secretome within the iCCA TME, decoding the entangled interplay between repair SCs, iCCA and TAMs in terms of molecular and cellular mechanisms. Particularly, the functional role of protein MK will be defined, among other factors, in the iCCA context, exploring its expected pro-tumoral effects, including the capacity of long-term maintenance of SCs repair phenotype, induction of chemoresistance and the establishment of an iCCA-permissive microenvironment. The project has the potential to pave the way toward the discovery of innovative targets for more effective iCCA treatments.

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## 10) Progetto di Ricerca della Dott.ssa Margherita Parrini

## Titolo Progetto di Ricerca

**Characterization of STS-derived exosomes through the integration of liquid biopsy and a multi-omics approach: novel molecular signatures as metastasis biomarkers**

### 1. Abstract


Soft tissue sarcoma (STSs) are rare tumors (1-2% of all adult cancers) arising from mesenchymal tissues anywhere in the body, with a high rate of lung metastasis. Exosome-based liquid biopsy represents a promising approach as a non-invasive method to provide a comprehensive molecular profiling of STSs, offering deeper insights into tumor biology and progression. This project aims to identify proteomic, lipidomic and transcriptomic signatures linked to metastasis in exosomes from STS cell lines. Additionally, by integrating multi-omics analysis and validating findings in patient-derived exosomes, it seeks to develop a minimally invasive liquid biopsy approach for detection of metastasis biomarkers.


### 2. Introduction

Soft tissue sarcoma (STSs) are a rare and heterogeneous group of neoplasms of mesenchymal origin that include more than 100 histological subtypes. Their rarity, associated with their vast histological diversity, poses a great challenge in STS diagnosis and monitoring [1]. As of today, surgery and adjuvant radiation therapy are the standard treatment [2]. On one hand, local treatment offers good outcomes, on the other, almost 40% of patients develop metastasis after therapy [3], most of which occur in the lungs [4], with an overall survival of about 18 months [1]. Liquid biopsy allows the detection, analysis and monitoring of STS through biological fluids with a minimally invasive approach, for example by exosomes isolation. Exosomes are nanosized extracellular vesicles (30-150 nm in diameter) secreted in the bloodstream by several different cells, including tumor cells [5]. Exosomes cargo ability of mirroring the tumor cell of origin, and its vast array of molecules, represents a promising tool for tumor monitoring and prognostic prediction, as they provide an insight into tumor-specific transcriptomics and proteomic signatures [6]. The role of exosomes as early tumor biomarkers has been reported in numerous cancers and, more recently, in sarcomas [7]. However, it has not yet been translated into standard clinical practice. 1 Focusing on exosomes, they have a pivotal role in intercellular communication, both in adjacent cells and at a great distance in the body, where their cargo uptake could lead to an alteration of the pathophysiological conditions of the recipient cell [8]. Specifically, exosomes play a role in the formation of a pre-metastatic niche (PMN) and overall, STSs metastasis formation. In recent years, an increasing number of studies focused on the role of exosomes in sarcoma metastasis, stressing how there is still a need of probing more detailed mechanisms and additional biomarkers in this process [9,10]. In fact, while exosomes are known to influence metastasis in epithelial cancers [11], their in-depth role in STS metastasis promotion is still being unraveled. STS cells actively secrete exosomes, thus making them, and their cargo, promising candidates as novel biomarkers for sarcoma metastasis.

### 3. Methodology

Exosomes isolation and characterization will be performed on STS-patient derived exosomes and on a panel of cell cultures, specifically, a normal human cell line (HEK ATCC #CRL-1573), one STS-derived cell line with low metastatic potential and one with high metastatic potential.

- **Cell line-derived exosomes isolation**

Cell lines will be cultured with complete cell culture media until 70% of confluence is reached. After a 48h incubation with media without FBS, cells supernatant will be collected. Exosome isolation will be performed with serial steps of ultracentrifugation and filtration (0,22 µm).

- **Cell line-derived exosomes characterization: investigating metastasis signatures**

*Nanoparticle Tracking Analysis (NTA)*: after ultracentrifugation, exosomes diluted 1:100 in cold filtered PBS (0,22 µm) will be evaluated at the Nanosight N300 to confirm that the size of isolated molecules fall into the expected exosomes range and to assess their distribution. For each sample 5 videos of 60 seconds will be recorded.

*Western Blot (WB):* exosomes will be lysed using a radioimmunoprecipitation assay (RIPA) and supplemented with protease and phosphatase inhibitors. Protein lysates will be separated with 10% SDS-PAGE, transferred to a PVDF membrane and blocked with 2 non-fat milk for 1h. The membrane will be incubated overnight at 4°C with the following primary antibodies: anti-CD9, anti-CD63, anti-CD81 used as exosomes markers. Calnexin will be used as a negative control. Additionally, a panel of known molecules associated with tumor metastasis has been selected based on literature: anti-TGF-β1, anti-CD44 and anti-CXCR4 [12,13,14,15]. The membrane will then be incubated with the corresponding secondary antibodies. LI-COR Odyssey will be used to visualize the bands and ImageJ software will be used for quantification, comparing STS cell lines with high and low metastatic potential.

*Flow cytometry*: high sensitivity flow cytometry (FC) (CytoFLEX S System) will be used to evaluate the presence, on exosomes membranes, of up to three markers previously investigated with WB. Exosomes will be incubated with antibodies against CD9, CD63 and CD81 (as exosomes markers) and anti-TGF-β1, anti-CD44 and anti-CXCR4 (as metastasis markers). FC analysis will be performed following the MISEV2023 guidelines to identify any differences between high and low metastatic potential cell lines.

*Super resolution microscopy*: single-particle characterization will be performed using the Oxford Nanoimaging (ONI) Nanoimager platform. 4x1010 exosomes, will be captured on the ONI image slides using the Tetraspanin Trio (TetTrio) panel. This will allow the detection and quantification of tetraspanins CD9, CD63 and CD81, at single-exosome level. As for FC, exosomes will be also analyzed for TGF-β1, CD44 and CXCR4. Exosomes will be immunolabeled overnight at 4°C with the various antibody cocktails. After incubation, serial wash steps will be performed to remove unbound antibodies and allow frames collection at 640, 561 and 488 nm to identify subpopulations that express one or more markers on their membrane when comparing high and low metastatic potential cell lines.

*Multi-omics analysis*: a multi-omics approach will then be used to further characterize exosomes. Nuclear Magnetic Resonance (NMR) spectroscopy will be used for metabolomic analysis, while Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) for lipidomic ones. Exosome lipidomic and metabolomic profiles will give insights on biomarkers involved in tumor development and progression as it has been seen in other types of cancers [16,17,18]. Transcriptomic analysis will be carried out after total RNA extraction from exosomes, their mRNA cargo will be analyzed using the 3 NanoString nCounter platform, providing an insight on mRNA targets associated with tumor-progression mechanisms.

- **Patients-derived exosomes isolation**

Blood samples were previously collected from diagnosed STS patients, after informed consent and preserved in a biobank. The chosen cohort will be composed of STS patients that did not metastasize (15) and patients that developed metastasis within the first 1 year (15). Also, a group of samples from healthy controls (15) is needed. Plasma will be separated from whole blood via centrifugation, exosomes will be isolated from 2 mL of diluted plasma with serial steps of ultracentrifugation and filtration (0,22 µm) as described above.

- **Patients-derived exosomes characterization: investigating metastasis signatures**

Based on the results obtained from the comparison of exosomes derived from high and low metastatic potential cell lines, the same protocol will be used for patients-derived exosomes. Briefly, exosomes isolation will be confirmed using NTA and subsequently the presence of exosomes markers (CD9, CD63 and CD81) will be investigated along with a panel of molecules associated with metastasization (TGF-β1, CD44 and CXCR4). First these markers will be investigated with WB and FC analysis and then at single-particle level with ONI, identifying subgroups based on marker expression. A further multi-omics characterization will be performed with NMR for metabolomic analysis, LC-MS/MS for lipidomic analysis and finally exosomes mRNA cargo will be investigated as transcriptomic analysis.

- **Data analysis**

Data obtained will be investigated using Gene Ontology and KEGG analysis. Also, a recently developed graphical pathway-based platform, PathLay, will be used. Specifically, PathLay will allow to integrate up to 6 -omics analysis and associate them to their respective targets. Variations observed from the identified pathways will be compared to patient clinical information to match and identify possible metastasis biomarkers.

## 4. Expected results

The first aim of this project is to identify, in STS cell lines-derived exosomes, subgroups based on metastasis correlated marker expression. This will be integrated with specific proteomic, lipidomic and transcriptomic signatures associated with metastasization when comparing high and low metastatic potential. Taking it to the next level, the integration of multi-omics analysis aspires to identify molecules, at protein and mRNA levels, pinpointing strong metastasis biomarkers. Once this has been achieved, the same 5 signatures will be sought in STS patients-derived exosomes (comparing non-metastatic, metastatic and healthy subjects). The ultimate goal is to develop a minimally invasive liquid biopsy approach, uncovering potential non-invasive biomarkers for clinical application.

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## 11) **Progetto di Ricerca del Dott. Lorenzo Pelagatti**

## Titolo Progetto di Ricerca

**Diagnosi eziologica precoce della sepsi e conseguente ottimizzazione della terapia antimicrobica: applicazione dei pannelli molecolari sindromici in Pronto Soccorso nei pazienti settici**


**ABSTRACT**

**Introduzione:** La sepsi è una grave disfunzione d'organo causata da una risposta disregolata alle infezioni, con alta mortalità globale. L'aumento di patogeni multiresistenti rende essenziale una diagnosi precoce e accurata per ottimizzare la terapia antibiotica. La rapidità nella diagnosi e nel trattamento antimicrobico è cruciale per ridurre la mortalità, ma le metodiche microbiologiche tradizionali sono lente e poco sensibili. I pannelli molecolari sindromici (PMS), consentono un'identificazione rapida di patogeni e geni di resistenza, potenzialmente migliorando la gestione clinica in Pronto Soccorso (PS), che ad ora rimane un setting inesplorato.

**Metodologia:** Studio di coorte prospettico con disegno pre/post, della durata prevista di 3 anni, condotto in PS presso AOU Careggi. Si arruoleranno pazienti adulti con sepsi o shock settico, in due periodi successivi: nel primo verranno utilizzate le indagini tradizionali, mentre durante il secondo le indagini tradizionali saranno integrate con i PMS su campioni biologici. La terapia antibiotica sarà ottimizzata in tempo reale in base ai risultati dei PMS ed alla stewardship infettivologica. L'outcome primario sarà la mortalità a 14, 30 e 90 giorni; gli outcome secondari includeranno l'appropriatezza terapeutica, la durata degenza, l'escalation della terapia e l'analisi costo-efficacia.

**Risultati attesi:** Si ipotizza che l'uso precoce dei PMS in PS migliori l'appropriatezza della terapia antibiotica e l'inserimento tempestivo di una terapia mirata, migliorando gli esiti clinici, la mortalità e gli outcome secondari. Inoltre, lo studio fornirà dati aggiornati sulla prevalenza locale di patogeni e resistenze, supportando strategie di antimicrobial stewardship più efficaci.


**INTRODUZIONE**

La sepsi è una disfunzione d'organo potenzialmente letale causata da una risposta disregolata dell'ospite alle infezioni (1). La sepsi e lo shock settico sono gravi problemi sanitari, che colpiscono milioni di persone in tutto il mondo ogni anno e portano al decesso di una persona ogni 3-6 (2–4). L'identificazione precoce ed un trattamento appropriato nelle prime ore successive allo sviluppo della sepsi sembrano migliorare gli esiti clinici.

Le fonti più frequenti sono le **Infezioni delle vie respiratorie** (il 35-50%), le **Infezioni urinarie** (10-30%) e quelle **addominali** (5-20%) (5-7).

Come evidenziato da Kumar et al. e da successive analisi (14), un ritardo nella somministrazione della terapia antibiotica nei pazienti con sepsi si associa ad un incremento della mortalità, con un impatto particolarmente rilevante nelle prime ore. La somministrazione precoce di antimicrobici appropriati è uno degli interventi più efficaci per ridurre la mortalità nei pazienti con sepsi (11-13). Tuttavia, nella maggior parte dei casi, il trattamento iniziale è empirico e basato su presupposti epidemiologici più che su dati microbiologici, a causa dei tempi tecnici e della bassa sensibilità delle metodiche colturali tradizionali.

Le metodiche colturali convenzionali, pur rimanendo il gold standard per la definizione dell'antibiogramma, richiedono tempi lunghi (48-72 ore) e presentano una sensibilità limitata. Negli ultimi anni, la diagnostica molecolare, in particolare attraverso pannelli sindromici basati su PCR multiplex (PMS), ha rivoluzionato l'approccio alle numerose sindromi infettive acute, consentendo l'identificazione rapida (in poche ore) di molteplici patogeni e di geni di resistenza agli antibiotici. Questa tecnologia ha già dimostrato di essere

efficace nei reparti di terapia intensiva, ma l'impatto clinico del suo utilizzo precoce nei dipartimenti di emergenza è ancora scarsamente esplorato.

La crescente prevalenza di patogeni multiresistenti (MDR) – come *Klebsiella pneumoniae* resistente ai carbapenemi, *Pseudomonas aeruginosa* MDR, *Acinetobacter baumannii*, e *Staphylococcus aureus* meticillino-resistente (MRSA) – rappresenta una minaccia concreta alla gestione empirica della sepsi (16). In questi casi, una diagnosi eziologica rapida ed affidabile diventa ancora più essenziale per orientare l'antibioticoterapia ed evitare sia il sottotrattamento sia l'uso inappropriato di antibiotici ad ampio spettro (15).

Nel contesto del PS, l'impiego precoce dei PMS su campioni biologici idonei – sangue, campioni respiratori, feci – potrebbe rappresentare un nuovo modello diagnostico-terapeutico per migliorare la gestione clinica dei pazienti settici.

Il presente studio si propone di valutare l'impatto clinico dell'introduzione dei pannelli molecolari sindromici nella gestione precoce della sepsi e dello shock settico in Pronto Soccorso. Tramite un disegno di coorte prospettico con analisi pre/post, si intende misurare l'effetto della metodica sulla mortalità a 14, 30 e 90 giorni (outcome primario) e su outcome secondari quali appropriatezza terapeutica, durata della degenza, necessità di escalation antibiotica ed impatto economico. Lo studio fornirà inoltre un aggiornamento del profilo microbiologico e di resistenza degli agenti eziologici della sepsi in PS, contribuendo allo sviluppo di percorsi assistenziali più efficaci e sostenibili.

**METODOLOGIA**

Studio di coorte prospettico con disegno **pre/post**, condotto in due fasi temporali consecutive, per valutare l'impatto dell'introduzione dei pannelli molecolari sindromici nella diagnosi eziologica precoce della sepsi in Pronto Soccorso rispetto alle metodiche microbiologiche standard.

È attesa una durata di 3 anni. In allegato 1 sono sintetizzate le fasi dello studio. È previsto di reclutare nello studio pazienti adulti consecutivi affetti da sepsi e/o shock settico afferenti al Pronto Soccorso dell'AOU Careggi e all'Osservazione ad Alta Intensità Soccorso dell'AOU Careggi entro 24h dal primo contatto sanitario ospedaliero del paziente (Triage). Saranno considerati affetti da sepsi tutti quei pazienti che abbiano una disfunzione d'organo che minaccia la vita causata da una risposta infiammatoria disordinata dell'ospite all'infezione in accordo con la definizione Sepsis-3 [8]. Saranno considerati affetti da shock settico: pazienti con **sepsi confermata** (ovvero disfunzione d'organo acuta con SOFA score ≥2 in risposta a infezione), **necessità di vasopressori per mantenere una pressione arteriosa media (MAP) ≥65 mmHg** e **lattato sierico >2 mmol/L (18 mg/dL)**.

Durante la prima fase verranno arruolati i pazienti del braccio di controllo: in tutti i pazienti settici saranno inviate le emocolture. Inoltre, in base al possibile focus infettivo identificato mediante valutazione clinica, laboratoristica (e.g. esame urine) e radiologiche (eg. TC cranio, RX/TC/ecografia torace, TC/ecografia addome) saranno eseguite le colture su campioni respiratori, feci, urine come da buon pratica clinica. In caso di crescita alle colture del patogeno questi campioni potranno essere processati con PMS come da pratica clinica corrente. In questi sarà impostata una terapia antibiotica empirica che potrà essere ottimizzata non appena disponibile l'esito dei colturali.

Durante la seconda fase verranno arruolati i pazienti del gruppo di trattamento: in tutti i pazienti settici saranno inviate le emocolture, che saranno analizzate anche con i PMS precocemente. Inoltre, anche in questo caso saranno eseguite le colture su campioni respiratori, feci, urine come da buon pratica clinica; anche questi campioni verranno processati con PMS, ove possibile.

I campioni saranno processati con pannelli molecolari sindromici validati per uso diagnostico (di seguito alcuni esempi, che potrebbero essere non esclusivi di altri sistemi che si rendano successivamente disponibili):

- Sangue: Pilot Gene Digital PCR system e/o the InfectID-BSI assay
- Campioni respiratori: BioFire FilmArray® Pneumonia Panel® Plus
- Feci: BIOFIRE® FILMARRAY® Gastrointestinal (GI) Panel

La terapia antibiotica empirica sarà modificata dopo poche ore dalla raccolta del campione in base al patogeno e ad i geni di resistenza identificati; è prevista una discussione tra il clinico di riferimento e l'infettivologo in stewardship per l'ottimizzazione precoce della terapia empirica (entro 2 ore dal risultato PMS); una successiva ottimizzazione potrà avvenire non appena disponibili i risultati delle indagini colturali. In caso non venga rilevato alcun patogeno il paziente verrà trattato con la terapia antibiotica empirica indicata in base al focus infettivo dalle linee guida locali.

Inoltre, si creerà un database dettagliato volto a raccogliere la prevalenza e la natura delle resistenze antimicrobiche locali nei pazienti con sepsi , così da mettere in pratica quanto richiesto dalle linee guida internazionali.

I criteri di inclusione ed esclusione sono indicati nell'allegato 2.

Trattandosi di uno studio con due coorti di pazienti, e volendo analizzare l'impatto dei PMS sulla mortalità (outcome dicotomico), vista una mortalità dei pazienti con sepsi a 14 gg del 20-22%, a 30 gg del 25% circa, ed a 90 gg del 30% [9], aspettando una riduzione a tutti e tre i tempi (14-30-90 gg) dei pazienti trattati con PMS di almeno il 7% [10], essendo la potenza 80% e $\alpha$=0.05, è necessario arruolare 1246 pazienti (623 pazienti in ciascuna coorte). In caso di uno scenario migliore, con una riduzione della mortalità del 10% sarebbe sufficiente una popolazione di 600 pazienti.

Da dati riportati in letteratura, i pazienti con sepsi sono 4-17/1.000 accessi in PS; l'invio di esami colturali avviene in circa l'80% dei pazienti (17-19). Da stime retrospettive eseguite presso il Pronto Soccorso della nostra azienda ospedaliera, si stima che vengano diagnosticate circa 2500 sepsi all'anno con un tasso di invio delle emocolture sovrapponibile a quello riportato in letteratura, dimostrando la fattibilità dello studio in termini numerici.

Come outcome secondari si valuteranno la durata dell'ospedalizzazione, la necessità di escalation della terapia di supporto (ricovero in degenza a maggiore intensità di cura, necessità di inotropi, necessità di ventilazione invasiva o non invasiva), la percentuale di modifica della terapia rispetto alle linee guida (escalation e de-escalation), il tasso di riospedalizzazione a 30 gg. Come outcome di sicurezza si valuterà il tempo dalla raccolta del campione alla modifica della terapia antibiotica. Inoltre verrà effettuata una analisi costo-efficacia in base agli outcome clinici rilevati.

**RISULTATI ATTESI**

La creazione di questo nuovo percorso diagnostico-terapeutico in Emergenza-Urgenza volto all'identificazione precoce dell'agente eziopatogenetico della sepsi e dei suoi geni di resistenza potrebbe consentire di indirizzare e mirare precocemente la terapia antibiotica. L'outcome primario sarà rappresentato dalla differenza di mortalità nei due gruppi. Si valuterà, inoltre, l'impatto sulla durata della degenza, necessità di escalation della terapia antibiotica e di supporto, impatto economico, e qual è l'impatto che i PMS hanno nella gestione della sepsi anche in relazione al focus d'origine ed alla presenza di fattori di rischio per MDR.

Ad ora non esistono prove di efficacia, per quanto riguarda gli outcome clinici, che supportino l'utilizzo dei PMS nella pratica clinica quotidiana; gli studi condotti esistenti, oltre a dimostrare l'efficacia nei PMS nell'indirizzare la terapia, hanno fallito nel dimostrare al momento la loro efficacia. Tuttavia, tali studi sono condotti o in rianimazione o in setting multipli. Ciò che ci si aspetta di dimostrare dal presente studio è che i PMS impiegati precocemente, vale a dire in dipartimento di emergenza, possano indirizzare tempestivamente la terapia antibiotica in maniera mirata, migliorando gli outcome clinici dei pazienti.

Inoltre, l'identificazione dei geni di resistenza permetterà di elaborare una mappa locale della loro prevalenza e dei profili di resistenza ai singoli antibiotici, differenziata per i vari foci settici, configurandosi come un'azione strategica di antimicrobial stewardship.

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Allegato 1

| Fase | Attività | Timeline |
|---|---|---|
| **Fase 0** | Preparazione e approvazione del comitato etico (presentazione , formazione, logistica) | Mesi 1–3 |
| **Fase 1** | Arruolamento della coorte di controllo; raccolta di dati clinici e microbiologici | Mesi 4–18 |
| **Fase 2** | Arruolamento della coorte di intervento; raccolta di dati clinici e microbiologici | Mesi 19–33 |
| **Fase 3** | Analisi preliminari e subanalisi caso-controllo | Mesi 8–34 |
| **Fase 4** | Analisi finale dei dati e diffusione dei risultati | Mesi 34–36 |

**APPENDICE 2**

**Criteri di eleggibilità**

Saranno arruolati tutti i pazienti adulti consecutivi che si presentano con sepsi e/o shock settico, secondo la definizione dei criteri Sepsis-3 [14], entro 24 ore dal primo contatto ospedaliero (triage) che accedono al Pronto Soccorso (ED) o all'Unità di Osservazione ad Alta Intensità del Pronto Soccorso dell'Ospedale Universitario Careggi.

**Criteri di inclusione**

- Età ≥ 18 e < 90 anni
- Modified Rankin Score ≤ 5

- SOFA score ≥ 2
- Campioni microbiologici raccolti entro 24 ore dall'accesso in Pronto Soccorso
- Aspettativa di vita stimata > 3 mesi

**Criteri di esclusione**

- Rifiuto a fornire il consenso informato
- Condizione di fine vita o cure palliative attive;
- Campioni inadeguati o impossibilità di raccogliere campioni microbiologici appropriati
- Gravidanza
- Età < 18 o ≥ 90 anni
- Aspettativa di vita stimata < 3 mesi
- Modified Rankin Score ≥ 5

## 12) Progetto di Ricerca della Dott.ssa Francesca Petrini

## Titolo Progetto di Ricerca

**"Riparazione e sostituzione della Valvola Aortica: analisi comparativa delle differenti alternative disponibili tramite valutazione della performance emodinamica, del fol/ow-up a lungo termine, dei risultati clinici e del costo- beneficio in casi isolati e complessi ed in approccio mininvasivo"**

**Summary of proposal**

The project aims to analyze and compare various aortic valve treatment modalities (repair and replacement) through a retrospective and prospective observational study at AOU Gareggi.
Aortic valve disease is the most prevalent valvular heart disorder in developed countries, particularly affecting elderly populations. The two primary conditions, aortic stenosis and aortic regurgitation, require surgical intervention in symptomatic or severe cases.
The study includes all patients undergoing AVR, excluding borderline or unclear cases. For repair, only techniques without device implantation (e.g., valve- sparing root replacement) are excluded.

This comprehensive study will provide valuable insights into the long-term management of aortic valve disease, integrating clinica! performance, safety, patient recovery, and healthcare resource utilization. lt will support a tailored and evidence-based approach for both surgical and percutaneous treatments, helping clinicians to select the most appropriate treatment strategy tor each patient across all levels of complexity.

**lntroduction**

Aortic valve disease represents the most common valvular heart pathology in developed countries with increased incidence in elderly patients1. There are two main disorders. Aortic valve stenosis, leading to a reduced orifice area and rise in pressure gradients across the valve itself, and aortic valve insufficiency due to inadequate leaflets closure during diastole2• While aortic valve stenosis develops over many years caused by progressive endothelial damage with increasing calcification in tricuspid or bicuspid aortic valves, aortic regurgitation can occur in acute or chronic settings1•3.

Acute aortic insufficiency can mainly be caused by infective endocarditis damaging the valve leaflets or type A aortic dissection extending to the valve while less common etiologies including complications of percutaneous procedures such as valvuloplasties and transcatheter aortic valve replacement: (TAVR)4 The most prevalent etiology far chronic aortic regurgitation in developing countries is rheumatic heart disease which often creates a mixed condition known as steno-insufficiency typical of mitral valve disease 5•

Both conditions can be treated either through repair or replacement6•

Aortic valve repair often represents a difficult task to achieve even far experienced surgeons due to the complex anatomy and physiological functionality of the aortic root. Several surgical techniques, such as annuloplasty; sub-commissural plication; cusps resection and valve-sparing aortic root replacement are now available far treating aortic regurgitation based on its etiology and functional status7•

Hecently, Hankin and colleagues introduced the use of an internal geometrie annuloplasty ring - hemispherical aortic annuloplasty reconstructive technolog) (HAART) (BioStable Science and Engineering, lnc., Austin, TX) far regurgitant bicuspid and tricuspid aortic valves to restare and preserve the natural elliptica shape of aortic annulus8•

Despite the diffusion of TAVR, surgical replacement stili represents the most effective treatment tor aortic valve stenosis 9•

Starting from Harken and colleagues who performed the first successful aortic valve replacement (AVR) in 196210, surgical techniques and valve prosthesis used changed aver the years.

Aortic bioprostheses have progressively and constantly seen an increasing in usage during the last decades due to many factors such as the possibility to perform transcatheter valve-in-valve encouraging surgeons to implant bioprostheses in younger patients orto avoid long-life anticoagulation11•12•

Nowadays minimally invasive cardiac surgery, defined in 2008 by American Heart Association as a small chest wall incision that does not include the convent1ona1 ru11 sternotomy"", represents tne surg1ca1 approacn or cno1ce ror isolated AVR.

Partial upper mini-sternotomy followed by right anterior mini-thoracotomy are the most common surgical approaches used in cardiac surgery units14•

In this context sutureless and rapid deployment valve technology has more recently been developed for SAVR patients to reduce operation times and therefore complications associated with cardiopulmonary bypass and cardioplegic arrest15•

**Materiai and methods**

This project is designed as a retrospective and prospective analysis aimed to collect and analyze data for evaluation of aortic disease treatment with aortic valve replacement or repair at our facility.

Far AVR no particular exclusion criteria will be followed. lsolated and combined procedures will be analyzed.

Borderline cases will be excluded to reduce bias impact.

For aortic valve repair surgical techniques that don't require aortic valve devices implantation (e.g: Aortic valve sparing techniques, sub-commissural plication) will be excluded.

We will focus on different key points:

- Collect the clinica! and echocardiographic follow-up of biologica! AVR patients (2016-2028) to perform comparative analysis among different types of prostheses (e.g. stented vs sutureless; mechanical vs biologica!); propensity match comparison can also be performed to reduce bias in case of statistically significant differences among the study groups.
- Monitor and document rates of bioprosthetic valve degeneration, according to VARC 3 definition subanalysis by valve model.
- Analyze clinica! and echocardiographic follow-up of biologica! AVR patients:

a. by age group

b. by gender

c. by risk score

d. by surgical approach (full sternotomy vs minimally invasive)

e. by type of surgery (lsolated AVR and combined procedures) Statistica! matching will be held tor the different analysis groups.
4. The above-mentioned sub-analyses will be conducted also by valve models.

5. Analyze clinica! and echocardiographic follow-up of biologica! AVR patients in combined procedures and isolated ones. Specific sub-analysis on multiple valves procedures will also be performed.
6. Compare surgical AVR with the Gareggi TAVI experience; propensity match to check for paravalvular leak and permanent pacemaker implantation will be done.
7. Evaluation of economie and ERAS project impact analysis in biologica! AVR and repair.
8. Collect the clinical and echocardiographic follow-up of patients with aortic valve repair, with a focus on the use of aortic annuloplasty devices such as HAART.
9. Propensity match comparison for surgical AVR and aortic repair for early and late outcomes with hemodynamic differences evaluation.

Primary endpoints:

- Ali-cause mortality analysis

- Stroke onset due to cardio-embolic events

- Degeneration ratio for different prostheses and incidence of reintervention

- lncidence of infectious endocarditis for different prostheses

- Rate of rehospitalization at 12 months

Secondary endpoints

1. Safety endpoints
- Need for reintervention for bleeding

- Renai replacement therapy at 30days

- New permanent pacemaker implantation at 30 days
- Re-hospitalization for cardiac reasons at 30 days and at 12 months
- Mortality and morbidity at long term

2. Effectiveness endpoints
- NYHA class status at 30days and 12 post-operative months
- ICU and hospital stay with its economie impact
- Echocardiographic valve analysis according to VARC-3 criteria at 30 days, 1 year and long-term follow-up.

Statistica! analysis will include basic descriptive statistics, t-tests and chi- square testing, Kaplan-Meier survival analysis, uni/multivariate linear and logistic regression, Cox-proportional hazards analyses and
propensity score matching.

Expected outcomes

With more than 400 aortic valve procedures (lsolated, combined or percutaneous) performed in AOU Gareggi cardiac surgery unit we expect to identify new sets of possible application for aortic valve bioprostheses.
We expect to improve surgical outcomes by identification of risk factors and possible predictors of early structural valve degeneration for different type of bioprostheses to optimize surgical strategies.

We expect to improve knowledge of correct prostheses suggestion for each patients.
We expect to develop tailored based surgical approches for AVR and aortic valve repair for both aortic valve pathologies, improving overall patients' management and outcomes. We will focus upon understanding the correct clinica! and therapeutic approach for elderly patients comparing surgical and percutaneous treatments.
We expect to identify and suggest !ife-time management perspective particularly in terms of initial intervention and future interventions in possible hybrid procedures.
We expect to identify possible new correlation between ERAS PDTA and long- term outcomes for different prosthesis implanted especially with new generation bovine pericardial valves.

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## 13) Progetto di Ricerca del Dott. Michele Piazzini

## Titolo Progetto di Ricerca

**" Stratified model far Al-supported Telerehabili t at ion to Personalize Exercise PathwaYs in Osteoarthritis - PREPARE"**


**Abstract**

Osteoart hritis (OA) is o n e of the most prevalent chronic musculoskeletal conditions worldwide and a major cause of pain and disabi lity in ag in g populat ions [1]. PREPARE p r o p os es the development of an Al-supported telerehabil it at ion {TR) m o d el capabl e of dynam icall y tailoring exercise pathways for OA patients with.in community and home settings. By integrat ing wear ab le sensors, a modular digitai platf or m, and adaptive therapeutic contents, t he system will enable dat a-driven pat i ent strat ifi cat ion and real-t im e in divi dual ized care. A part icipat or y design pr ocess will guide all ph ases- co-creat i on, system development, and pilot evaluation-to ensure cli nica! feasibilit y, t echnological r obustness, and high usability. PREPARE aims to enhance accessibility, sustain adh erence, and increase the persona li za ti on of rehabili tat ion in osteoarthritis.


**Introduction**

Ost eoart hrit is (OA ) aff ects m ore than 250 million people globally and is the most prevalent joint disorder in adults aver 60 years of age [1]. Part icu larly in the knee and hip, OA leads to progressive pain, joint stiffness, and loss of function, signlficantly compromising aut onomy and quality of li fe. The burden of OA is projected to rise due to aging populat ions and increased obesi ty prevalence [1] . In ltal y, rural and economlcally disadvantaged communities often experience reduced access to rehabili t at ion service s, exacerbating disease progression and inequali t i es in care [ 2].

Th er ap eutic exercise is widely recognized as a cornerst on e of OA t r eat men t, with strong evidence supporting its role in alleviat ing symptoms, m aint aini ng joint functio n, and reducing disabilit y [3]. However, adherence to exercise program s r emains subop tim al, p art icular ly in unsup ervised or home-based sett ings [4).

Tradi tional r ehabi li t at ion models are predominan t ly based on in-person sessions, which are oft en limited by provider avail ab ili ty, long w ait ing list s, and geograph i c or logi st ic a! barri ers. Telerehabili tation (TR) ha s emerged as an eff ective alt ernative delivery m od el, sh o wing clini ca! outcomes compar ab le to conventional care across mul t iple muscul oskelet al condit ions, including OA [S] .

Despite promising evidence, real-world adoption of TR faces chall eng es: li mited digitai skills in older populations, insu ff icient traini ng for healthcare professionals, and lack of system-level int egrat ion [ 6]. Moreover, many digitai rehabil it at ion t oo ls r ely on st an dar di zed, static prot oco ls that fail to account for the complex and evolving needs of individuals with OA.

Recent technological developments offer new solutions. Wearable sensors can provide continuous monitoring of joint movement, gait dynamics, and daily activity patt erns. Artificial intelligence (Al) algorithms can analyze these data st ream s t oget her wìt h clinica! informat ion to generat e individualize d exercise reco mmendat ions in real time. This dynam i c, responsive approach has the pot ential to signif icantly improve patient engagement, clinica! relevance, an d outcomes in OA rehabi li t at ion [7,8].

**Methodology**

**Phase 1 - Co-creation and cont extual analysis**

This ini t ial p hase will involve:

- Analyzing local digitai read in ess by mapping access to int ernet infrastr uctur e, device availability, and digit ai li t eracy in the target population.

  - Engagi ng r elevant stakeholders (patients with OA, healt hcare professio nal s, caregivers, and generai practitìoners) using qualit ative int erviews and focus groups to i dent ify bar riers, expectations, and usability needs in TR services.
  - Defining clinica!, fun ctional, and t echnical requirements necessary to design an inclusive, responsive, and equitable telerehabil it at ion platform. In addition, stakeholder input will be used to co-design novel

educat ional and rehabili t at ive contents that are clinically relevant, culturally appropriate, and al ìgned with patient preferences. These mat er ials will be integrateci into the digitai pl atf orm and tailored to the functional p ro fil es identified in Ph ase 2.

  - Definition of the eHealt h coach, a healt hcar e professional who will be trained in the final version of the TR system to support part i cipan t s in navigating, engaging, and troubleshooting technical issues dur ing the pilot phase. .

Coll ect ed qualit at ive data will be an alyzed using thematic content analysis. Outputs will inform syst em architect ur e and user interface design.

**Phase 2 - System development**

Key technological modules in clude:

- **Wearable sensor integration:** Usin g in ert ial m easurement units (IM Us) to measu r e join t r ange of mot ion, cadence, postur e, and functional m obility during exercise an d d aily act iviti es.
- **M odular digitai platform:** Th e syst em will include an app for users an d a cli nica! d ash board for monitoring. The exercise content w il l be cat egorize d by ty pe (st rengt h, f lexibili t y, aerobics) and struct ur ed accor ding to international OA guideli nes [3]. Incorporat eci in t o t h e p lat form will also be the ed ucational and therapeutic materi al s co-design ed during Phase 1, ensuring alignment with user pr ef er ence s, cu lt ur a! relevance, and cli nica! ap pli cability .
- Sel f -assessm ent t ools: Users will com plete w eekly reports on pain, function, and perceived exert ion using validat ed scales embedded within the syst em.

Al algorithm functionality:

  - Patient stratificati on: Based on cli nica! assessm en ts (e.g., WOMAC, HOOS/ KOOS, NRS}, users will be grouped int o funct ional profiles with recommended baseli n e intensity leve ls.
  - Exer cise per sonali zation: Al m od els wil l analyze adherence t rends, sensorderived kinematic parameters,

and progress data to suggest the next best exercise in sequence, progressively adapting the therapeutic pat h.

16) Feedback and support : The syst em will generat e actionabl e feedback to users and offer decision-support information to healthcar e professionals moni t ori ng remote session s.

Al m od el development will use supervised machine learning methods. Aut oM L p la t forms will be used to optimize model accuracy and interpret abili ty. Dat a privacy and pr ot ection will comply with GDPR standards. Al! data will be anonymìzed and stored securely on encrypted servers.

Clinician lnvolvement

Healthcare professionals will participat e in the init ial calibr ation of exercise param et ers and will have access to a

dashboard en abling real-time supervision. Their feedback will be used to iterative ly r eti ne algorithm logie and user interaction [9].

**Architecture and lnteroperability**

The TR pl atf orm will be structur ed on a multi-lay er architect ure:

- Fr ont -end (m ob il e/ web app for users),
- Middleware (real-time dat a pro cessing, Al decision engine),
- Back- end (secure dat a storage, model deployment), with planned interoperability with electronic health reco rd (EHR) systems t hr ough FHIR standards [10].

Th e system architecture will follow a design appr oach similar to existing Al-assi sted platforms used inhea l t hcar e, incorporat ing mod ul arity, scalabili ty, and secure integrati on - without explicitly referencing any specific
commerci ai solut ion.

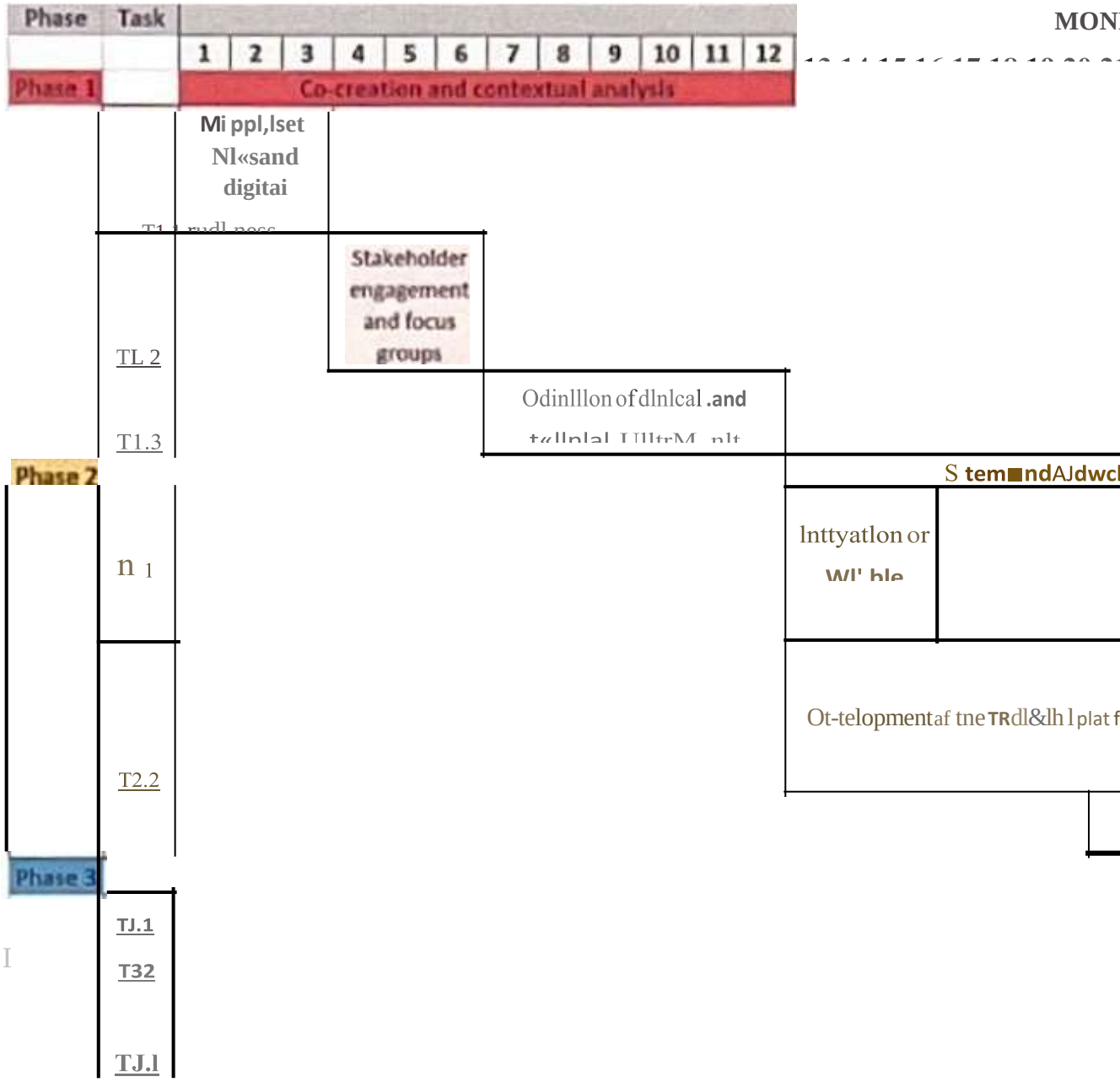
**Phase 3 - Pilot study**

A feasibility pilot wìll be conducted with 30 particìpants dìagnosed with hip or knee OA. The intervention will consist of:

- Mu l t i d im ens io nal assessment and int erdiscipl ìnary defìnition of the individuai telerehabilitationpr oj ec t
- D elivery of a pe rsonalized 8-week remote telerehabilitation program, *.via* the 1-'K t l-'A K t: system.
- Weekly asynchronous m on it or ing by health care professionals.
- Par t ici pants w il l also receive support from an eHealth coach-a healthcare professional trained in the use of the PREPARE p latf orm- who will assist them ìn navigating the system, solving t echnical issues, and
- optimizing engag ement throughout the intervention.

- Assessm en ts at TO (b aseline), Tl (po st-ln t ervention), and T2 (2-month followup). Participants will interact with both standard and co-cr eat ed content, including tailored educational reso urces and rehabilitative exercisesdevelo ped with stak eholder input.
-
- Primary outcomes:
-
- Pain intensit y (Numerica! Rating Sca le),
- Functio nal stat us (WOMAC, HOOS/ KOOS),
- Par ticipat io n (CIQ-R),
- Exe rcise adher ence and progressi on ,
- System usability (SUS),
- Patient satisfaction and engagement exp erience (Ad-hoc questio nn aire).
-
- **Expected Results**
-
- An op erational telerehabi lit at ion syst em with Al-d r iven personali zat i on and wearable integration.
- lmproved access to rehabilitat ion services for OA patients with mobility limitations or living in underserved areas.
- lncreased adh erence to therapeut ic exercise t hrough real-time feedback, adap tiv ity, and patient empowerment.
- Training and support resou r ces for clinicians an d users to fost er digitai confidence and acceptance.
- Open-access m at erials and clinica! protocols shared with academic and institutional stakeholder•s
- Demonstrated feasibi li ty and add ed value of a human-suppo rt raie (eHealth coach) to increase digitai confidence and user adherence in remote rehabilitat io n contexts.

- **Impact**
- Cli ni caI: Optimized rehabilitat io n outcomes and continuity of care in OA, reducing strain on specialized outpat ient services.
- Organi zation: Strengthened locaI and primary care networks through scalable, remotely deliverable rehabilitat i on interventions.
- Technological: Real- world implementation of explainable Al systems in the field of physical rehabilitation.
- So cia!: Reduced health inequalities through improved accessibility and digitai inclusio n for elderly or rural populat ion s. The involvement of an eHealth coach aims to mitigate challenges relateci to limited digitai confidence and promote equitable participation in TR interventions.
- **Scientific:** Adva ncement of reproducible methods in Al-base d personalization and generation of open,
- validateci dat aset s for musculoskeletal resear ch.

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