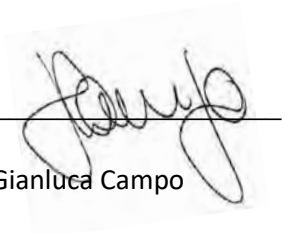
Effectiveness of an early, tailored, Physical activity Intervention in ELderly patients with myocardial INfarction: the PIpELINe randomized clinical trial.

Version number 1 of April 26th, 2019

Signature page, approval of Study Protocol

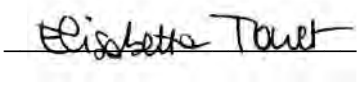
I, the undersigned, have read this protocol and agree that it contains all necessary information required to conduct the study. I agree to conduct this study in full accordance with Food and Drug Administration Regulations, Institutional Review Board/Ethic Committee Regulations, and ICH Guidelines for Good Clinical Practices.

Study Principal Investigator

_____ 

Date: 26/APR/2019 Gianluca Campo

Study Principal Investigator

_____ 

Date: 26/APR/2019 Elisabetta Tonet

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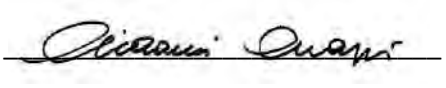
_____ 

Date: 26/APR/2019 Giovanni Grazzi

Investigator Statement

By signing below, I agree that:

I have read this protocol. I approve this document and I agree that it contains all necessary details for carrying out the study as described. I will conduct this study in accordance with the design and specific provision of this protocol and will make a reasonable effort to complete the study within the time designated. I agree to conduct this study in full accordance with Food and Drug Administration Regulations, Institutional Review Board/Ethic Committee Regulations, and ICH Guidelines for Good Clinical Practices.

Site: _____

Address: _____

Principal Investigator: _____

_____ _____

Date: Signature

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1 STUDY SYNOPSIS

Study Title:	Effectiveness of an early, tailored, Physical activity Intervention in ELderly patients with myocardial INfarction: the PIpELINe randomized clinical trial.
Protocol version:	V1
clinicaltrials.gov	NCT04183465
Date:	April 26, 2019
Study Sponsor:	Azienda Ospedaliero Universitaria di Ferrara
Study Principal Investigator:	Gianluca Campo, MD

2 STUDY ORGANIZATION

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3 BACKGROUND

3.1 Incidence and prevalence of myocardial infarction in older adults

In the last years, the average age of patients admitted to hospital for myocardial infarction (MI) is significantly increased: more than half of patients admitted to hospital for MI are aged 70 years and more (1). Older adults with MI are at higher risk for recurrences and mortality. Taking into account the ageing of the population, MI prevalence and impact will further boost. Actual prevalence and incidence of MI in elderly patients (≥75 years) in Western countries range between 15% and 20% (2).

Epidemiology of Myocardial Infarction in Older Patients From the Cardiovascular Health Study

	Age Range (yrs)	Overall	Men	Women
MI†				
Prevalence				
	75-79	13.4	19.2	10.1
	80-84	14.4	21.1	10.2
	85-89	16.5	22.5	12.9
Incidence				
	75-79	12.9	18.3	9.6
	80-84	17.1	24.5	12.5
	85-89	19.5	26.9	14.9

3.2 Physical performance in older adults with myocardial infarction

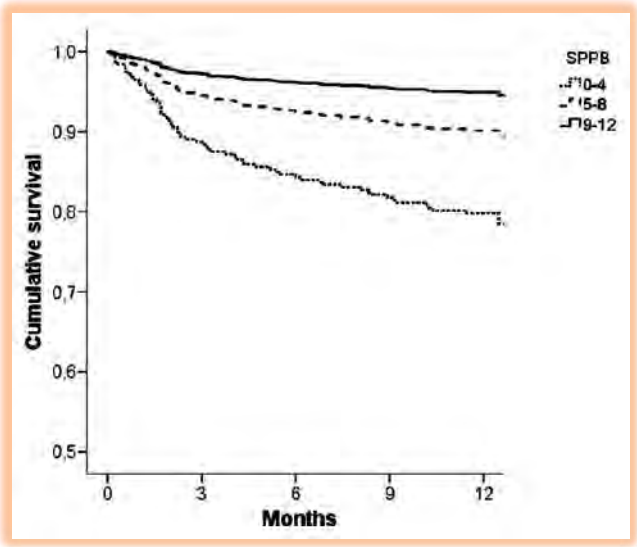
Mobility limitation, sedentary behavior and physical inactivity are considered risk factors for a bad prognosis in MI patients. Older adults are the least fit and active group and cardiovascular diseases accelerate physical deconditioning [3]. Occurrence of disability following MI, heart failure or cardiac surgery ranges between 45% and 75% in older patients [4,5]. Taking into account the high rate of cardiovascular events in older adults and the above-mentioned high rate of disability following each event, assessing and preserving physical performance in older adults represent a clinical and public health challenge. Even though the assessment of functional capacity in older patients with cardiovascular disease is strongly recommended [6], the assessment of physical performance of these patients is not included in routine clinical practice of cardiologists, mainly because of lacking time and of the absence of trained personnel in performing physical performance scales.

3.3 Scales for the assessment of the physical performance

Different scales and tools have been previously described with the aim to evaluate physical performance. The most important methods are described as follows:

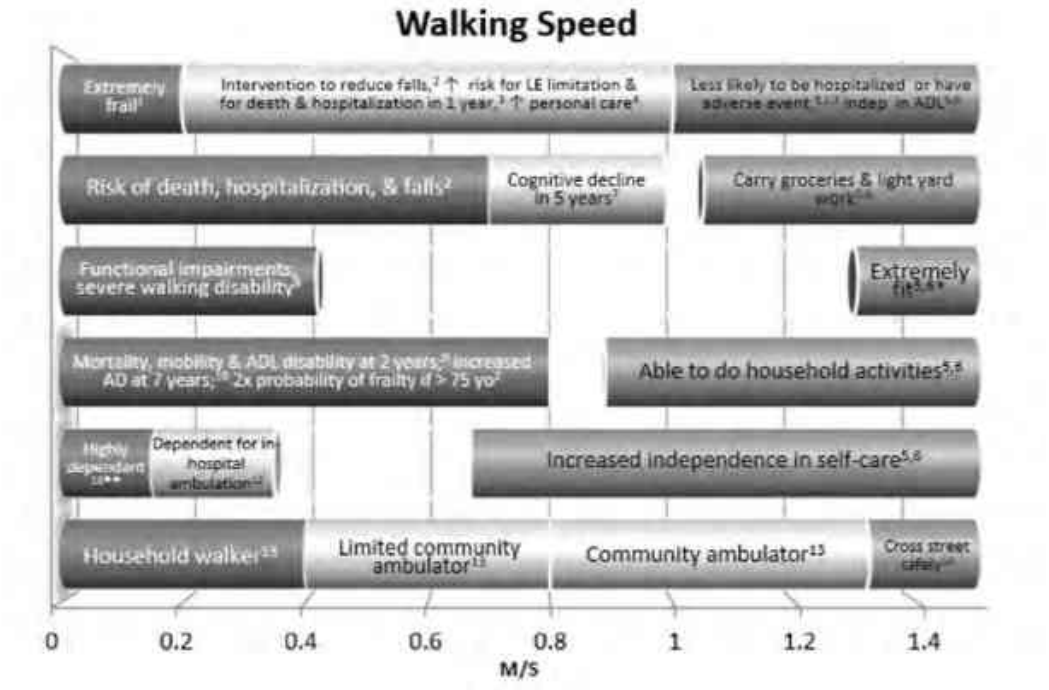
- **Short Physical Performance Battery (SPPB)**: this battery is made up of 3 different sections designed to assess lower limb function. The final score derives from the sum of the three sections. The total SPPB score ranges from 0 (worst performance) to 12 (best performance) [7]. A score between 4 and 9 shows seniors still independent, but with reduced physical performance, which can therefore be regarded as frail and at high risk of poor outcome, deserving special attention and specific interventions in order to reduce the risk of adverse events. Data from literature confirm that SPPB correlates with the risk of developing disability regardless of age, sex and presence of certain chronic diseases. The SPPB score emerged as a powerful predictor of disability and mortality in the elderlies with cardiovascular diseases such as heart failure and acute coronary syndrome [8].

SPPB score and outcome



- **Gait speed**: this is a valid, reliable, sensitive measure appropriate for assessing and monitoring functional status and overall health in a wide range of populations. Thanks to its easy evaluation, it may replace more complex physical performance tests. Recently, gait speed has been proposed as a new useful "vital sign". Previous studies demonstrated that usual gait speed is indicative of current functional status and of several health outcomes. Decreased gait speed is associated with increased all-cause mortality, especially cardiovascular mortality in older adults [9].

Depiction of walking speeds and the associated outcomes



- **Handgrip strength**: this test measures the force of contraction of the flexor muscles of the fingers and forearm through the use of a dynamometer. From literature it is known as such index correlates with the eventual malnutrition of the subject, with its capacity of recovery and with functional recovery post-surgery, as well as to be in relation with age [10]. This test is not laborious, virtually cost-free, well-administered in all patients admitted for MI or aortic valve disease; as such, it is readily identifiable as a method of coarse screening in older patients without known diagnosis of "frail older adults", but at risk of being such. A recent metanalysis demonstrated that in older adults with cardiac disorders, grip strength predicted cardiac death, all-cause death and hospital admission for heart failure [11].

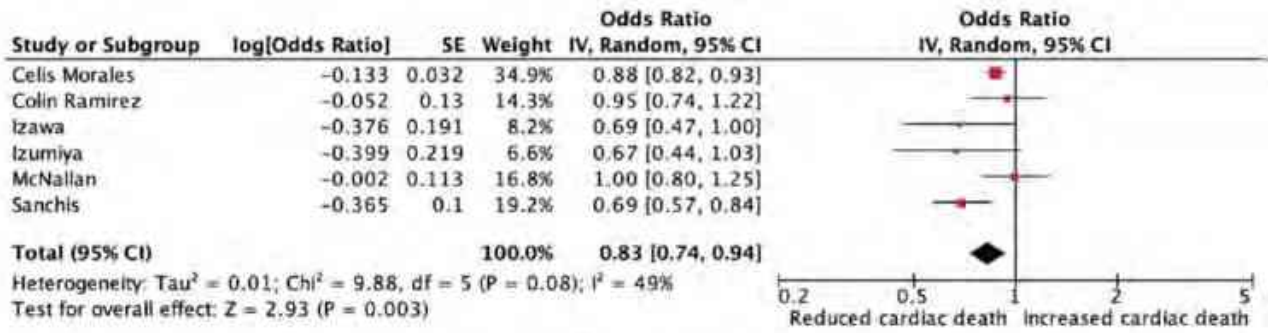


Figure 2 Forest plot of the relationship between grip strength (5× kg) and cardiac death. Data are displayed as OR (95% CI). CV, cardiovascular; IV, inverse variance.

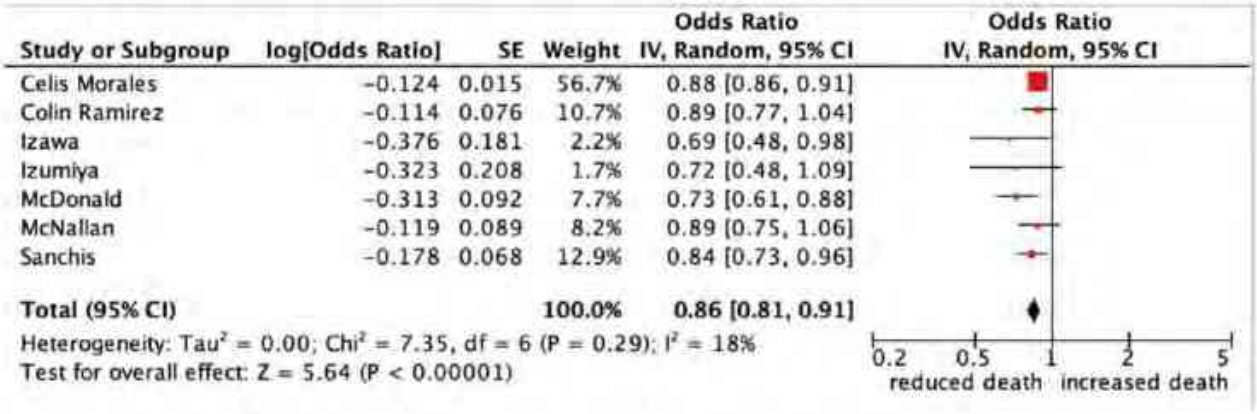


Figure 3 Forest plot of the relationship between grip strength (5× kg) and all-cause death. Data are displayed as OR (95% CI). IV, inverse variance.

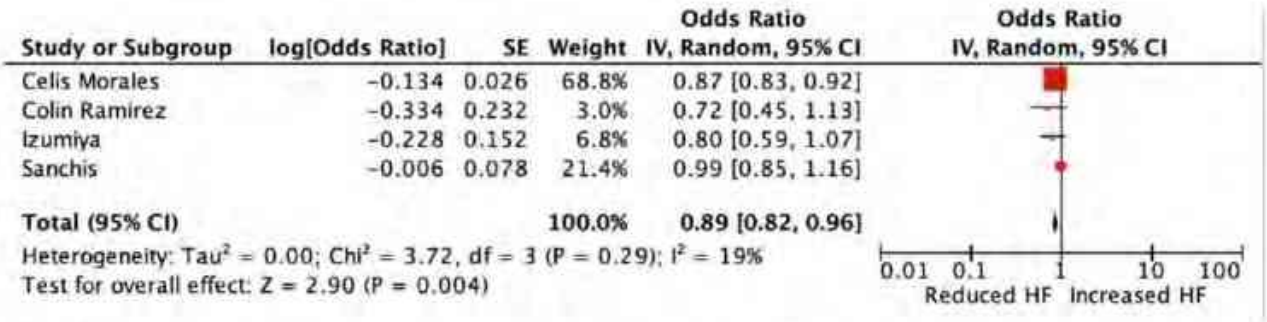


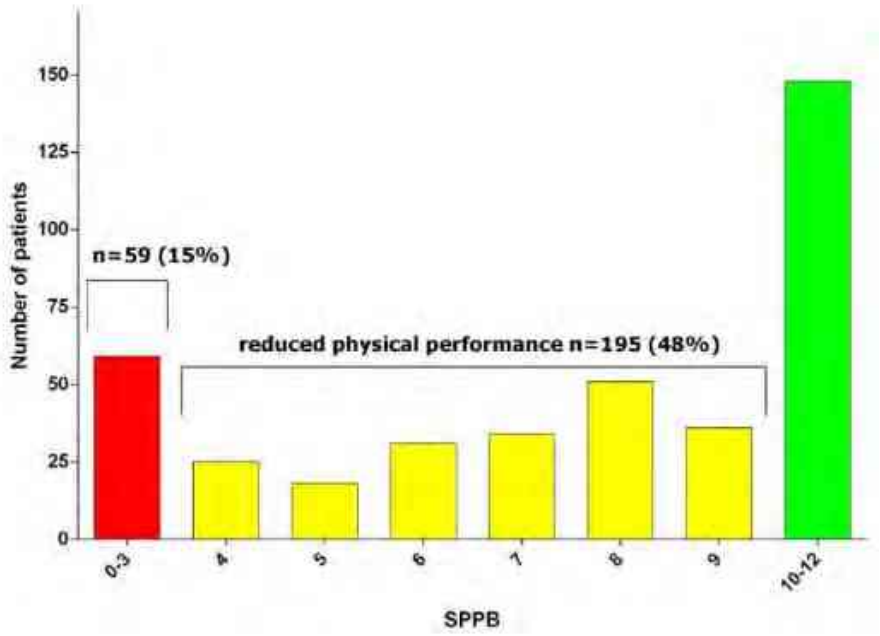
Figure 4 Forest plot of the relationship between grip strength (5× kg) and hospital admission for heart failure (HF). Data are displayed as OR (95% CI). IV, inverse variance.

3.4 Preliminary findings from the FRASER study (NCT02386124)

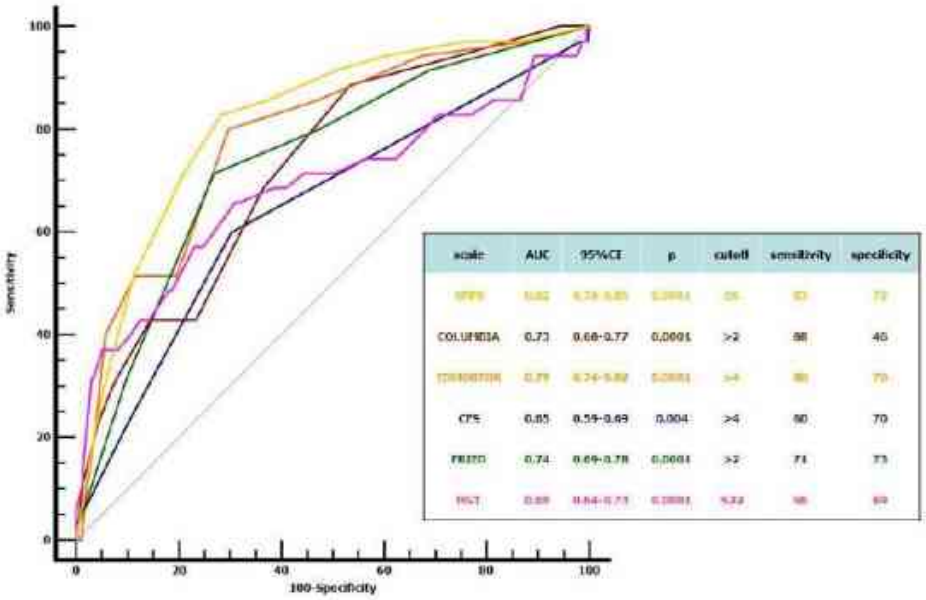
Recently we conducted the "Frailty in elderly patients receiving cardiac interventional procedures" (FRASER) study [12]. It assessed frailty and physical performance in older patients admitted to hospital because of MI. Main results showed that:

- many older patients admitted to hospital for MI have an impaired physical performance at the time of the discharge
- the commonly available scales of frailty and physical performance predict adverse events in older adults admitted to hospital for MI, and SPPB demonstrates to have the best predictive value.
- SPPB shows the strongest association with adverse events, contributing the greatest incremental value when added to a model containing baseline clinical and laboratory variables.
- SPPB shows a significant incremental value when added to validated risk scores such as GRACE score and TIMI risk score.

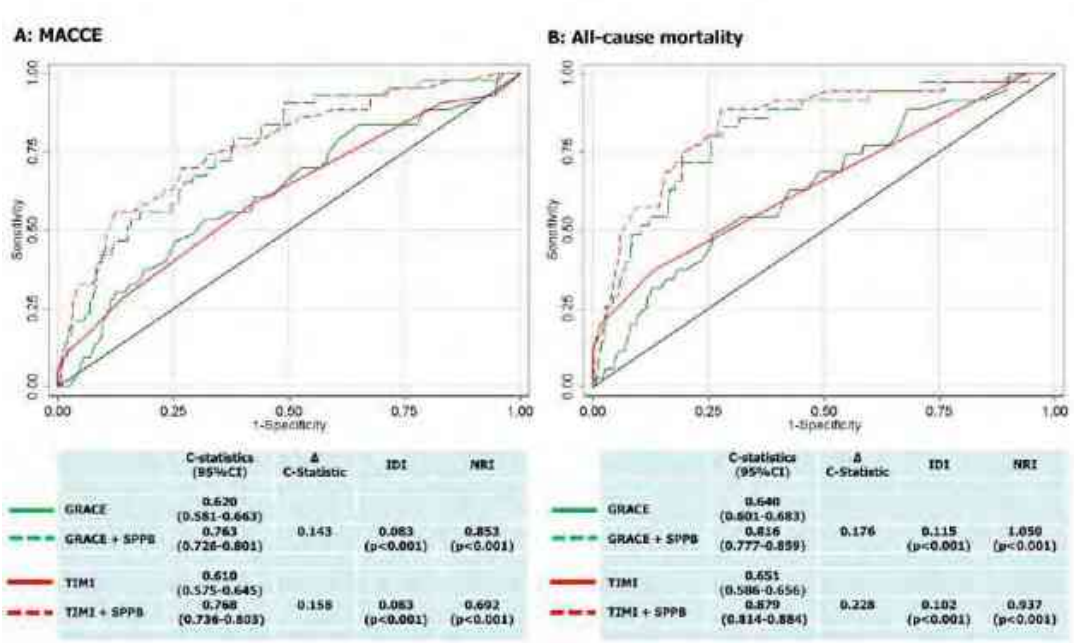
Distribution of SPPB value in the FRASER population



Predictive value of the different frailty scales



Incremental value of SPPB to GRACE and TIMI risk score



3.5 Relationship between physical performance and quality of life

Mobility - the ability to walk without assistance - is a critical characteristic for functioning independently.

Mobility critically impacts on older adult's wellbeing and quality of life [13]. There is evidence that low

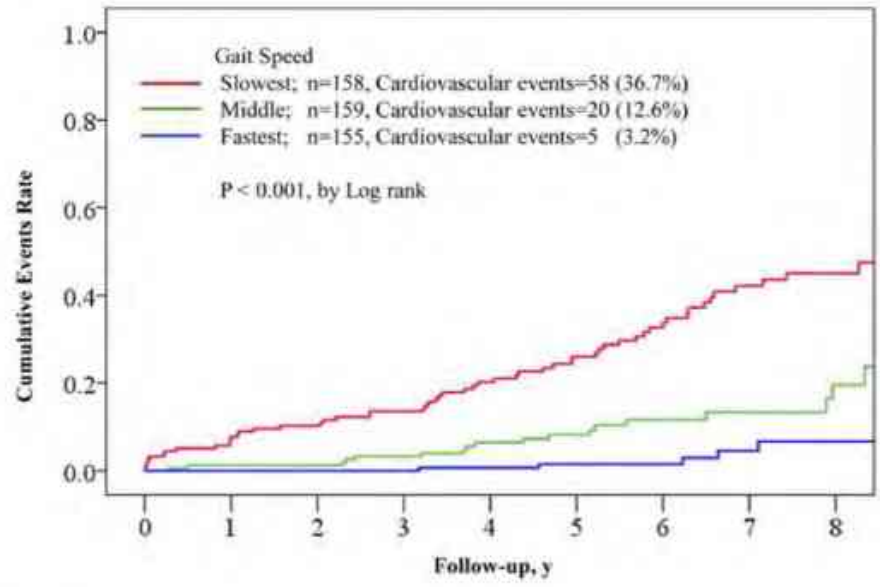
physical performance in daily activities is associated with poor quality of life in older adults. Furthermore, previous studies demonstrated that muscle strength plays an important role regarding quality of life. In particular, lower extremity muscle mass, strength, power, and physical performance are critical determinants of independent functioning in later life, a central tenet of quality of life. Data from literature demonstrated that decreased muscle mass and physical performance were independently associated with increased fear of falls and declined physical quality of life. These findings underline the importance of preserving skeletal muscle health with advancing age [14].

Spearman correlation between physical performance assessed by SPPB and quality of life evaluated by EQ5D *p<0,05

Instrument	Short Physical Performance Battery
EQ-5D Individual Domains	
Mobility	−0.2577*
Self-Care	−0.1295*
Usual activities	−0.1679*
Pain	−0.1400*
Depression	−0.1230

3.6 Relationship between physical performance and mortality and hospitalizations

Low physical performance is common among elderly patients and it is related to higher rates of morbidity, hospitalization and mortality [15]. Slow gait speed is strongly related to further cardiovascular events in MI patients who underwent successful percutaneous revascularization [15]. Data from SWEDEHEART registry demonstrated in 22227 MI patients that low physical activity level after MI is related to increased mortality over a 4-year follow-up. In older adults, any hospital admission for acute cardiac events further reduces the overall physical performance with potential prognostic worsening in terms of death and re-hospitalization [16].



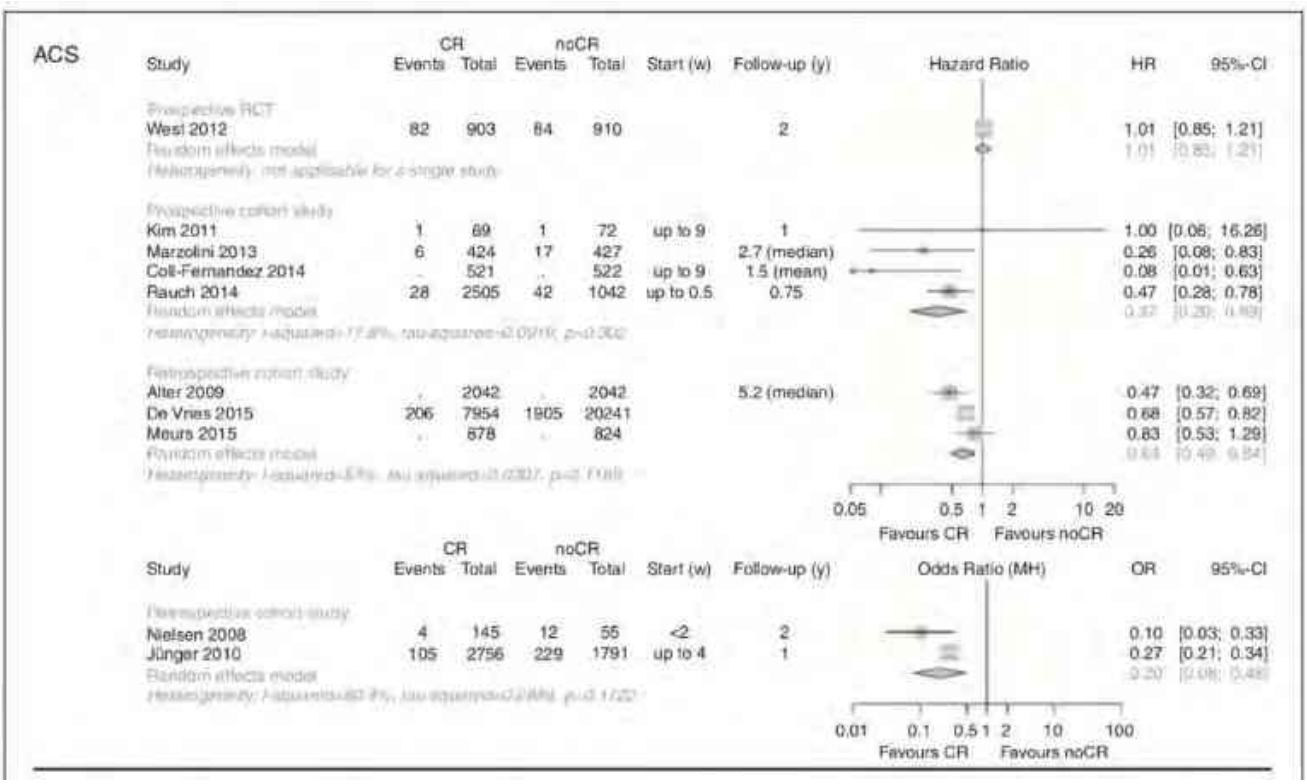
No. at Risk

Slowest	158	143	136	128	100	84	63	43	28
Middle	159	157	155	131	113	89	67	42	25
Fastest	155	155	155	154	136	106	77	51	18

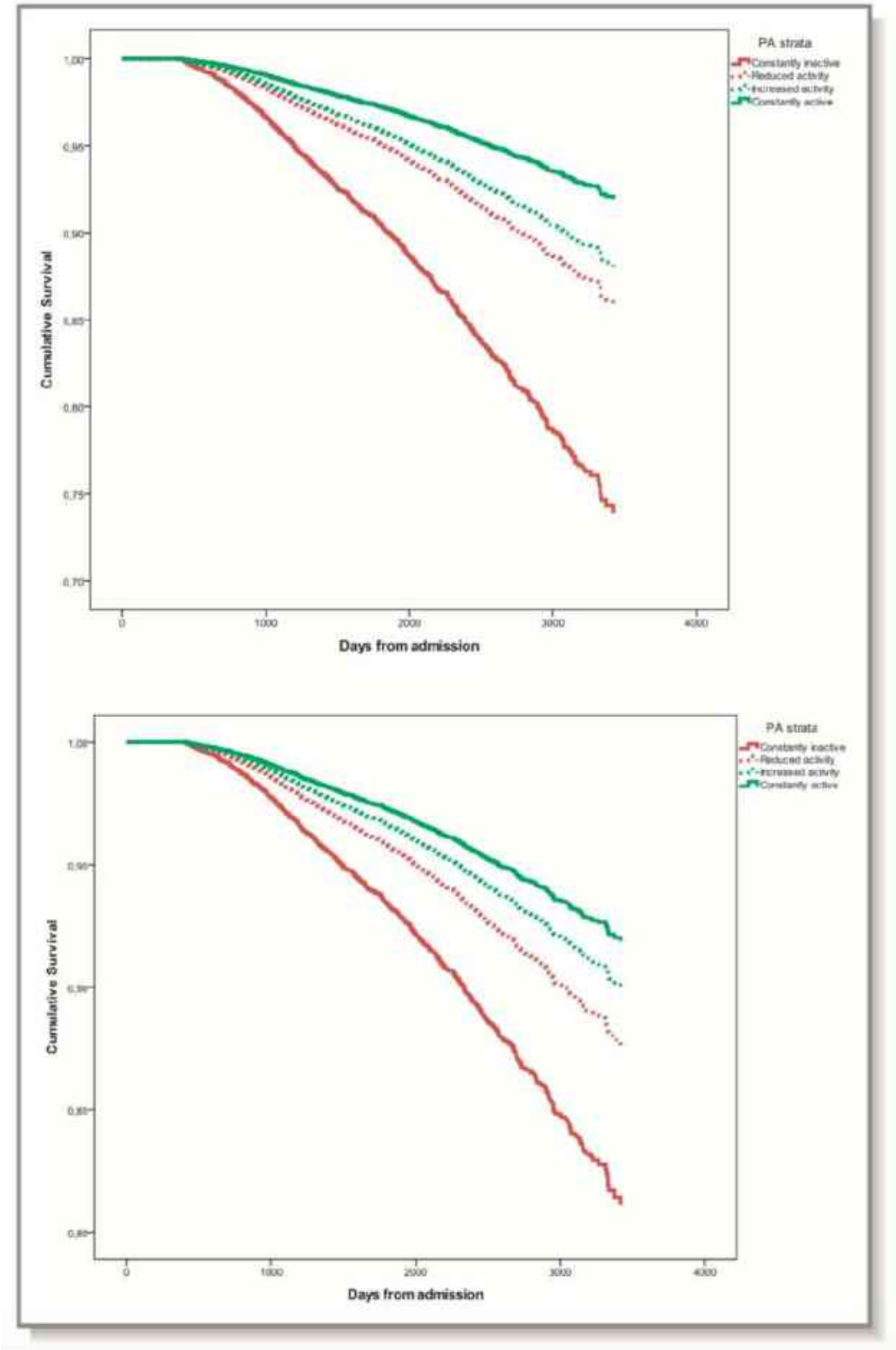
3.7 Cardiac rehabilitation and traditional centre-based secondary prevention programs

Benefits of cardiac rehabilitation are widely demonstrated. While at the beginning cardiac rehabilitation had been only provided for patients undergoing cardiac surgery, more recent evidence underlined the importance of rehabilitation programs also in patients treated with percutaneous intervention [17]. As a matter of fact, cardiac rehabilitation after ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI) is a Class IA recommendation from the American Heart Association/American College of Cardiology and the European Society of Cardiology guidelines [18, 19]. In a large meta-analysis, exercise training as a part of cardiac rehabilitation program was associated with a 22% reduction in cardiac mortality rate [20]. The benefit of cardiac rehabilitation appears to be through direct physiological effects of exercise training and through cardiac rehabilitation effects on risk factors control and lifestyle behaviors. The

results of the BLITZ-4 study reinforce the evidence that cardiac rehabilitation should be the gold standard for reaching the goals of secondary prevention after MI [20]. In addition, registries' data showed that cardiac rehabilitation in MI patients also reduces hospital readmission [21].



Relationship between physical activity level and survival after MI.



3.8 Drawbacks of cardiac rehabilitation and centre-based secondary prevention programs

Despite the benefits of cardiac rehabilitation, previous studies demonstrated that there is a high rate of early withdrawal from cardiac rehabilitation programs; drop-out almost doubles the risk to die or to have a recurrent cardiovascular event [22]. Furthermore, previous studies about cardiac rehabilitation only considered a certain kind of population with MI, such as young patients. Some features of exercise rehabilitation programs have been denounced as limits of cardiac rehabilitation program provided till now [23]:

- Low enrollment of older MI patients, who however represent the majority of MI subjects
- High number of sessions, which is related to low adherence, early withdrawal and high costs. This concept is particularly important in older adults, who are not able to attend all rehabilitation sessions because of logistic problems and dependency for transportation. Data from previous studies revealed that monthly contacts should be successful [24].
- Not early beginning of rehabilitation sessions after MI, that lead to the leak of capturing patients when they are more receptive to change lifestyle. Indeed, previous studies demonstrated that rehabilitation programs should start 3-4 weeks after the cardiovascular event, because in the subacute phase MI patients are open to accept guidance about physical exercise to avoid future cardiac events. Furthermore, retrospective studies suggested that for every day of delay between hospital discharge and cardiac rehabilitation, there is an associated 1% decrease in participation [25]. Pack et al. found a significant improvement in attendance in the group of patients with early appointment to cardiac rehabilitation centers [26].
- Standardized cardiac rehabilitation activities cannot be generalized to all types of patients. It appears to be necessary a better stratification of patients in term of physical performance in order to make up a tailored physical activity program.

- Long-term maintenance of healthy lifestyle and physical activity is lacking. In meta-analyses it has been demonstrated that these programs effectively reduce the 1-year incidence of total mortality, cardiovascular mortality and nonfatal MI [26]. However, these initial beneficial results were not maintained during longer-term follow-up. The lifestyle changes adopted during the rehabilitation period were probably not incorporated into daily routine. It could be due to several factors such as absence of a home-based program, difficult physical exercises, fear of falls and low motivation.

Main reasons of patients' withdrawal.

	Patients who did not participate in outpatient programme (n = 54), n (%)
Problem with transport to the rehabilitation centre	21 (39)
Incompatibility between work and rehabilitation	7 (13)
Health problems	5 (9)
Necessity of care of a family member	3 (6)
Expressed intention to participate, but did not attend any session	6 (11)
No reason was given	12 (22)

Brovold et al. compared two groups of older patients undergoing physical activity, one group attending supervised physical exercises sessions, the other one following a home-based prescription. The results showed that supervised sessions determine a greater improvement in term of lower limb function, but there were no significant differences among other outcomes such as physical functioning, mental health, social functioning and general health. This data suggests that combining a home-based program and exercise sessions could be the best option [27].

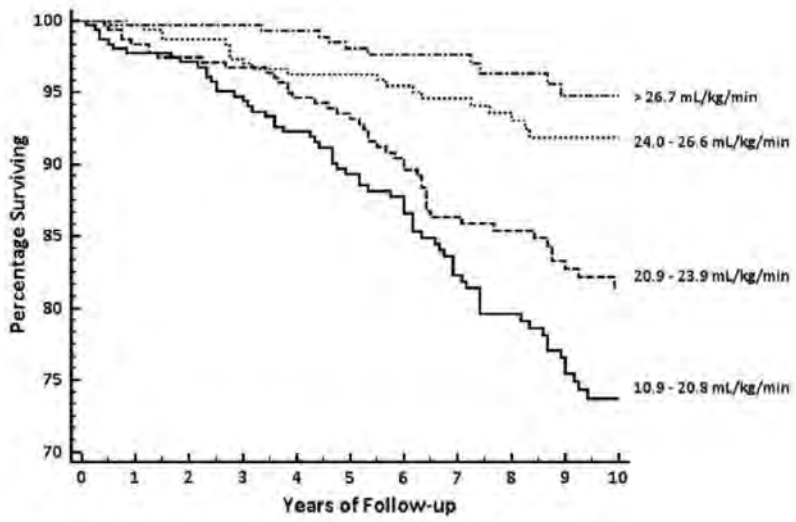
Additionally, it has to be noted that last evidence highlighted the importance of a multi-domain approach including dietary suggestions and physical activity sessions. Kaleta et al demonstrated that cardiac rehabilitation programs improved frequency, duration and intensity of physical activity but nutrients intake

did not meet the recommended levels so that they highlighted the importance of paying attention to dietary habits in rehabilitation programs [28]. Guidelines about cardiovascular prevention published in 2016 underlined the importance of physical activity, diet and cardiovascular risk factors management suggesting a multiparametric assessment of each patient [18].

3.9 Tailored physical activity intervention

Previous studies by Grazzi et al. and Chiaranda et al. provided a protocol of physical activity tailored on physical performance of patients with cardiovascular diseases. It consists of a moderate self-paced 1-kilometre treadmill test (1-k-TWT): the treadmill test is tailored taking into account patients' physical performance and the perceived exertion referred by each patient. This test allows the estimation of cardiorespiratory fitness (i.e. VO2 peak, mL/min/kg) in patients with cardiovascular disease. The VO2 peak determined with this moderate and perceptually-regulated treadmill-walk has been demonstrated to be inversely associated with all-cause mortality in a large cohort of outpatients with cardiovascular disease over a 10-year follow up [29,30].

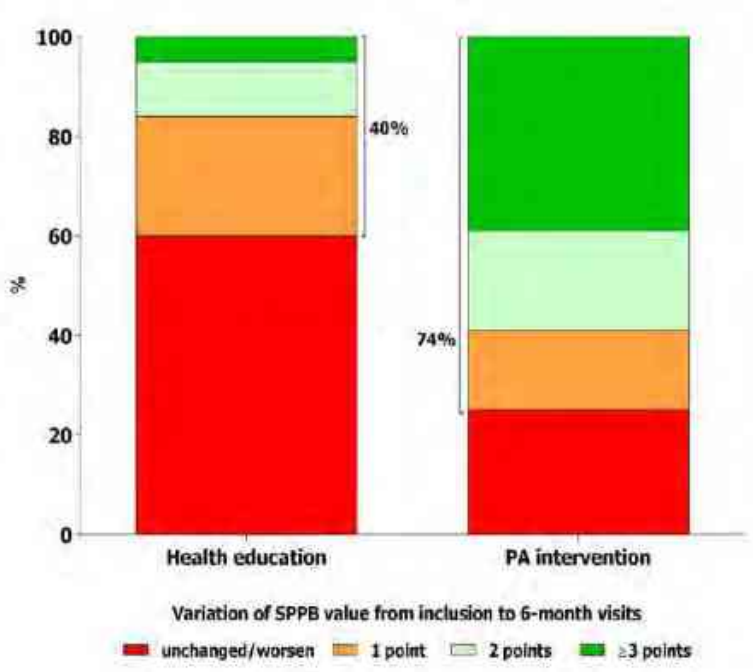
Relationship between VO2 peak estimated by 1-k TWT and survival



3.10 Preliminary findings from the HULK study (NCT03021044)

Last year the "Physical activity intervention for elderly patients with reduced physical performance after acute coronary syndrome" (HULK study) was conducted in order to evaluate feasibility and effectiveness of an early, tailored, low-cost physical activity program in older patients with impaired physical performance discharged after MI. It was a multicenter, investigator-driven, randomized trial enrolling older MI patients with a moderate impairment of physical performance assessed by SPPB. Patients were randomized to physical activity intervention (experimental group) or health education (standard of care group). Physical activity intervention included 4 sessions of physical activity and a home-based program with a series of calisthenic exercises. The primary endpoint was SPPB value 6 months after MI; secondary endpoints included quality of life and performance in some daily activities. Preliminary findings showed that:

- The physical activity intervention was feasible in older MI patients with a high rate of attendance (72% [95%CI 64%-80%])
- The physical activity intervention significantly improved physical performance assessed by gait speed and SPPB when compared to health education alone
- The physical activity intervention significantly improved quality of life when compared to health education alone [31]


100
80
60
40
20
0
%
40%
74%
Health education
PA intervention
Variation of SPPB value from inclusion to 6-month visits
unchanged/worsen
1 point
2 points
≥3 points

4 RATIONALE

Elderly patients presenting with MI are the highest risk population with the worst prognosis. No trial has ever been designed to optimize their outcome through a systematic improvement of their physical performance. Cardiac rehabilitation demonstrated to improve prognosis of patients after MI. However, real-life data shows that older patients are not referred to rehabilitation centers or they have low rate of attendance because of the high number of rehabilitation sessions and of logistic problems. So, data about effectiveness of rehabilitation programs in older MI patients is lacking.

The HULK pilot study enrolled older MI patients, and it demonstrated the feasibility and effectiveness of an early, tailored and low-cost physical activity intervention in terms of physical performance assessed by SPPB score, that is strongly related to prognosis. Thus, our hypothesis for the PIpELINe trial is that an early, tailored and low-cost multi-domain intervention may improve prognosis of older MI patients compared to health education alone. The primary outcome is a composite of 1-year cardiovascular death and hospital readmission for cardiovascular cause.

5 STUDY OBJECTIVES

5.1 Primary objective

- 1-year composite of cardiovascular death or hospital readmission for cardiovascular cause

To assess the superiority of the early and tailored physical activity intervention over health education alone in terms of 1-year composite endpoint of cardiovascular death plus hospital readmission for cardiovascular cause.

5.2 Main Secondary objectives

- 1- and 3-year all-cause death

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 1- and 3-year all-cause death.

- 1- and 3-year cardiovascular death

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 1- and 3-year cardiovascular death

- 1- and 3-year hospital readmission for cardiovascular cause

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 1- and 3-year hospital readmission for cardiovascular cause

- 3-year cardiovascular death or hospital readmission for cardiovascular cause

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 3-year cardiovascular death or hospital readmission for cardiovascular cause

- 1- and 3-year hospital readmission for heart failure

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 3-year cardiovascular death or hospital readmission for cardiovascular cause

- 1- and 3-year myocardial infarction

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 1- and 3-year hospital readmission for myocardial infarction

- 1- and 3-year hospital readmission for coronary revascularization

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 1- and 3-year hospital readmission for myocardial infarction

- 1- and 3-year cerebrovascular accident

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 1- and 3-year cerebrovascular accident

- 1- and 3-year hospital readmission for any cause

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 1- and 3-year hospital readmission for any cause

- 1- and 3-year hospital readmission for any non-cardiovascular cause

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 1- and 3-year hospital readmission for any cause

- 1- and 3-year bleeding adverse events

To test the superiority of the early and tailored physical activity intervention over the health education alone in terms of 1- and 3-year bleeding adverse events.

5.3 Other objectives

- 6-month, 1- and 3-year physical performance.

To assess the effect of the early and tailored physical activity intervention on physical performance (SPPB, gait speed, handgrip strength) at 6-month, 1- and 3-year follow-up.

- 1- and 3-year quality of life

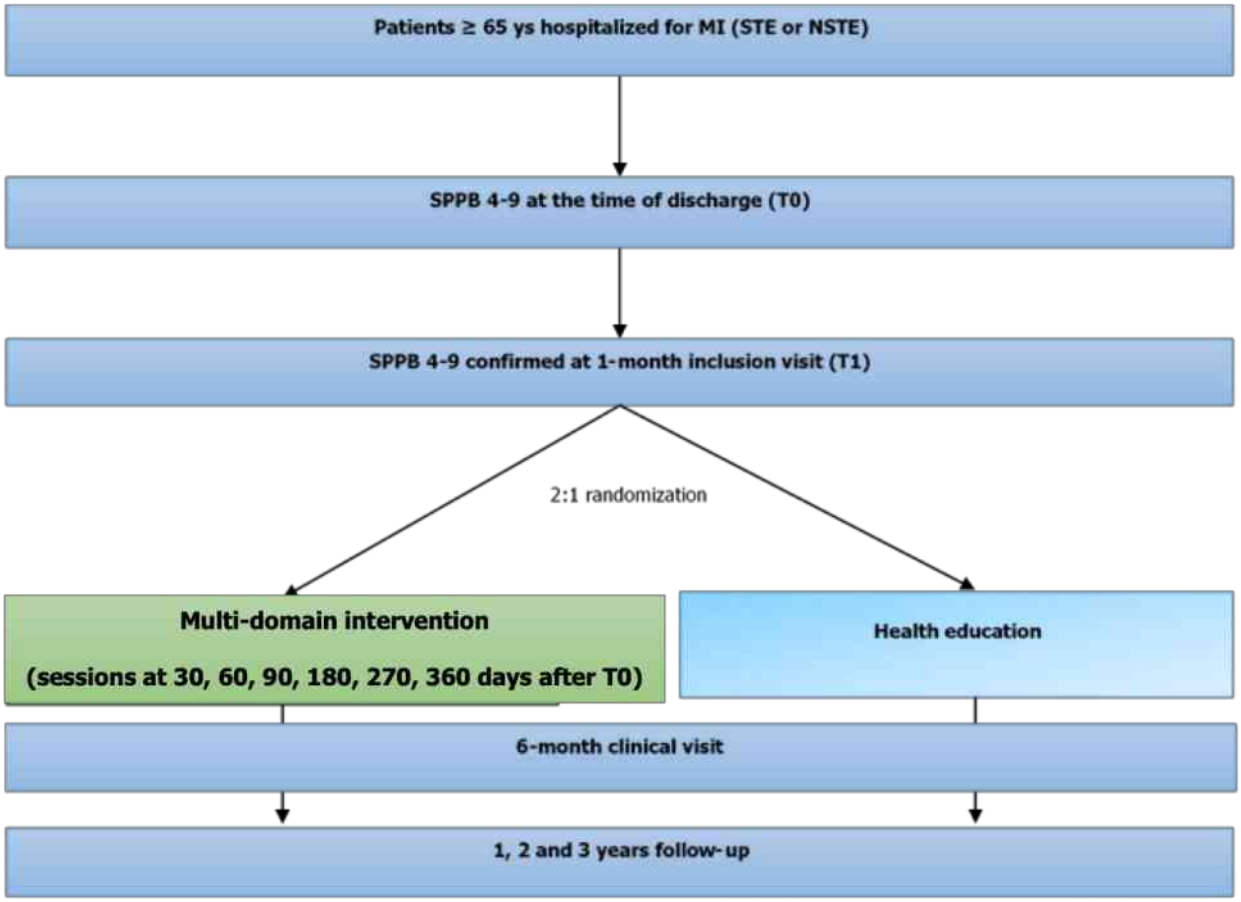
To assess the relationship between physical activity intervention and quality of life at 1- and 3-year follow-up.

6 STUDY DESIGN, SCREENING AND RANDOMIZATION

6.1 Study design

Prospective, randomized, multicenter trial with blinded adjudicated evaluation of outcomes (PROBE).

6.2 Study flow chart



6.3 Screening

All patients aged 65 years and older undergoing coronary angiography because of MI must be screened for eligibility. The patient's eligibility must be assessed after percutaneous revascularization of all lesions considered susceptible of treatment. After verifying inclusion and exclusion criteria and after eligibility is confirmed, written informed consent must be obtained prior to randomization. At the time of the discharge (T0) SPPB test will be performed; in case of a score between 4 and 9, the patient will be evaluated 1-month after discharge at the inclusion visit (T1). If SPPB value is confirmed to be between 4 and 9, randomization will be performed. Key baseline patient characteristics (i.e., inclusion/exclusion criteria, demographics, medical history, details of cardiovascular anatomy and of revascularization, ECG and laboratory test results, echocardiographic data during the index hospitalization) will be recorded on the electronic Case Report Forms (eCRF). All angiographic and echocardiographic data will be collected and forwarded to a core lab for further assessment.

6.4 Randomization

Randomization will be performed during the inclusion visit (T1), 30 days after discharge. Randomization will be performed centrally using an internet-based system. The patient identification number (Patient ID) and the treatment allocation will be assigned by the central randomization system. Patients will be randomized to physical activity group or health education group by a 2:1 allocation. Treatment allocation will be assigned according to a computer-generated randomization list stratified by center. Randomization will also be stratified by sex, clinical presentation (STE vs NSTE-MI), and SPPB value (4-6 vs 7-9). All randomized patients are irrevocably in the study, whether or not they are subsequently found to be eligible, or actually receiving the allocated treatment. Therefore, all patients must be followed until the pre-specified study end date.

7 STUDY POPULATION

7.1 Inclusion criteria

1. Patients ≥65 years **AND**
2. MI (STE or NSTE-MI)
3. Invasive management during index hospitalization including coronary artery angiography (± percutaneous coronary revascularization) **AND**
4. SPPB value 4-9 at 1-month visit after hospital discharge **AND**
5. Informed consent

7.2 Exclusion criteria

1. Multivessel coronary artery disease or left main coronary artery disease candidate to surgical revascularization
2. Planned staged PCI
3. Non-cardiovascular co-morbidity reducing life expectancy to < 1 year
4. Any factor precluding 1-year follow-up
5. Severe aortic or mitral disease
6. Ejection fraction <30%
7. Chronic heart failure NYHA III-IV
8. Severe cognitive impairment (SPMSQ <4)
9. Impossibility to do physical activity due to physical impairment

8 STUDY PROCEDURES

8.1 Management Strategies

After coronary angiography and mobilization of patients, they will be checked for inclusion and exclusion criteria. This information must be reported in eCRF.

8.1.1 General information about hospital discharge

At the time of discharge, a study investigator will explain to all patients aged 65 years and older the study protocol, aims, risks and benefits. If the patient accepts the participation in the study, he/she will sign the informed consent and he/she will be evaluated with SPPB test for the screening evaluation (T0). Patients with SPPB score <4 or >9 will be excluded and will be followed up with exclusively clinical visit every year. Patients with SPPB score 4-9 will be enrolled and data will be collected in the study CRF. A study brochure will be given to patients (Appendix A). These patients will be re-evaluated at the inclusion visit 30 days later (T1); if SPPB score will be confirmed to be between 4 and 9, patients will be randomized to physical activity intervention (experimental group) or to the health education group (control group) with a 2:1 allocation. The randomization will be stratified according to:

- gender M vs. gender F
- admission for STEMI vs. NSTEMI

8.1.2 Health education group

All patients randomized to health education during the inclusion visit are managed according to current guidelines and as follows. Quality of life, functional capacity and home physical activity are assessed by proper tools.

Inclusion visit (T1)

- CHAMPS questionnaire
- EQ5D
- Gait speed evaluation

Study investigators perform a 20-min speech with patient and relatives about the major issues related to a heart-healthy lifestyle with some suggestions as follows:

- **Healthy food choices**
    1. including vegetables, fruit, cereals and water
    2. avoiding foods containing saturated fat, foods and beverages with added sugars and salty foods.
    3. replacing saturated fat with monounsaturated and polyunsaturated fats from vegetable (oleic acid as in olive oil and rapeseed oil) and marine sources
- **Weight control** in order to achieve and maintain healthy BMI (18.5 - 24.9kg/m2)
- **Smoking cessation** and referral to special programs if necessary
- **Stress management** technique encouraging help from relatives and care givers
- **Aerobic physical activity**
    1. Aerobic physical activity such as walking, and cycling should be privileged
    2. At least 20 minutes on 5 days/week should be engaged in physical activity, emphasizing that sedentary lifestyle is a risk factor
    3. Graduation of the physical activity plan
    4. Variation of activities by changing the type of performance or the way to do it
    5. Alternation of physical activity with adequate rest breaks
    6. Wearing the accelerometry while performing physical activity (Accelerometry is provided)
    7. Association of some calisthenic exercises, calibrating breathes and movements (description and teaching of 3 types of exercises)
- **Some suggestions to make healthier patent's daily routine:**
    1. Stairs rather than lift should be preferred
    2. Getting off the bus one stop earlier and walking for the rest of the way
    3. Going out for a walk with friends
    4. For short trips, walking should be preferred

8.1.3 Multi-domain Intervention

All patients randomized to multi-domain lifestyle intervention will receive dietary counselling, strict management of CV and metabolic risk factors and exercise training. They will start the program with counselling and supervised physical activity (PA) session immediately after the inclusion visit. Quality of life, functional capacity and daily activities will be assessed by proper tools. Abou dietary counselling, it will be provided by a nutrition professional with an individual face-to-face interview (60-90 min), where personal dietary and patient's daily diet are agreed. During follow-up sessions, new diet habits and diet compliance will be assessed.

Nutritional interview with a professional	**Dietary counselling**	**Following sessions (60±10, 90±10, 180±10, 270±10 and 360±10 days after T0)**
Weight, height, BMI Abdominal circumference, plicometry Bioimpedentiometry by a libra Allergies and medical therapy assessment Blood values evaluation (heamoglobin, kidney function, glicemic and cholesterol levels etc) Patient's dietary habit: - How many meals/day? - Breakfast: sweet or savoury - Preference for meat or fish - Alcohol consumption	Mediterranean diet Recommendation about preference for: -olive oil -at least 2 seasonal, fresh vegetable servings a day -moderate consumption of fresh fruit -fish at least 2 times per week -legumes instead to meat -limiting red meat -limiting sugar and salt -stop smoking	Weight, height, BMI Abdominal circumference, plicometry Bioimpedentiometry by a libra Medical therapy assessment Diet objectives discussion Patient's new diet habit and compliance to suggested diet evaluation

Agreement on nutritional objectives (weight loss, number of meals/day etc)		

The program provides 6 supervised PA sessions (30, 60, 90, 180, 270 and 360 days after T0).

At the end of each supervised session, calisthenics exercises derived from Otago Exercise Program are prescribed.

Inclusion visit (T1)	**Home-based program**	**Following activity sessions (60±10, 90±10, 180±10, 270±10 and 360±10 days after T0)**
Pre-test: • CHAMPS questionnaire • measure of blood pressure • positioning RS100 Polar heart rate monitor to constantly evaluate heart rate • Exercises derived from Otago Exercise Program • Gait speed evaluation **Start**: walking on the level at 2.0 km/h or lower according to perceived exertion **Every 30 seconds**: increases of 0.3 km/h up to a walking speed corresponding to a perceived	• At least 20 min of continuous moderate walking a day (including 5 min warm up and 5 min cool down), preferably 7 days a week, wearing accelerometry. Patients are instructed to follow the same RPE goals. The home-based program is individualized but in agreement with current international guidelines	**Pre-test**: • CHAMPS questionnaire • Evaluation of data recorded by accelerometry. • Measure of blood pressure • Positioning RS100 Polar heart rate monitor to constantly evaluate heart rate. • Exercises derived from Otago Exercise Program **Start**: walking at an updated intensity compared to the previous activity session **Every 30 seconds**: increases of 0.3 km/h up to a walking speed

exertion of 11–13 on the Borg scale for 1 km *. **Every 2 minutes:** rate of perceived exertion (RPE) recording **Post-test**: • Measure of blood pressure. • Discussion of test results: patients are educated about the clinical meaning of cardiorespiratory fitness obtained by the 1k-TWT, expressed as "cardiorespiratory index", that is the VO2peak expressed as percent of the age-predicted value. Patients are advised that the index will be used to monitor efficacy of the training program. • Counselling on physical activity: participants are instructed to follow the same RPE goals for the home-based exercise program • Counselling on daily activities, such as gardening, or household work encouraging to gradually perform these activities. • Distribution of home accelerometry	• Exercises derived from Otago Exercise Program	corresponding to a perceived exertion of 11–13 on the Borg scale for 1 km *. **Every 2 minutes:** rate of perceived exertion (RPE) recording **Post-test**: • Measure of blood pressure • Discussion of obtained results • Counselling on physical activity and daily activities, such as gardening, or household work. • Distribution of home accelerometry

***:** Subjects walking at a perceived moderate speed < 3.0 km/h will perform the test over the distance of 500-m. At the end of the test the averaged walking speed will be calculated.

8.1.4 Optimal Medical Therapy (OMT)

All patients regardless of randomization group receive OMT. Unless there is an absolute contraindication, all patients receive complete revascularization (including culprit lesion and non-culprit lesions, the

discrimination of the non-culprit lesions needing PCI can be based on angiography, coronary physiology or intracoronary imaging) and standard secondary prevention with:

1. Aspirin 75-100 mg po daily
2. Ticagrelor 180 mg loading dose then 90 mg po BID or Clopidogrel 600 mg loading dose then 75 mg od or Prasugrel 60 mg loading dose then 10 mg od. The choice of P2Y12 inhibitor is at Physicians' discretion. Duration of DAPT will be prescribed according to DAPT guidelines, taking into account the risk profile of each single patient (1 month for high bleeding risk patients).
3. An ACE inhibitor or ARB appropriately titrated to target a blood pressure of < 130/85. If the patient continues to experience hypertension with BP > 130/85, additional blood pressure lowering treatments should be added. The goal of antihypertensive therapy is to achieve and maintain a target blood pressure (BP) of < 130/85 mm Hg.
4. Statin therapy: Atorvastatin 40-80 mg daily or rosuvastatin 20-40 mg daily or simvastatin 40 mg daily. An LDL-C goal of < 1.8 mmol/L (70 mg/dL) or a reduction of at least 50% if the baseline LDL-C is between 1.8–3.5 mmol/L (70–135 mg/dL) is recommended. If this goal is not achieved additional lipid lowering therapy should be added (e.g: ezetimibe, PCSK9).
5. If the patient is diabetic, the goal is to maintain fasting blood glucose levels between 80-135 mg/dl and hemoglobin A1c levels <7.0%, in accordance with published recommendations of International Guidelines.
6. If the patient has significant LV dysfunction, diuretics, aldosterone antagonist or sacubitril/valsartan should be considered according to symptoms and following current International Guidelines.

8.1.5 Follow up

After hospital discharge, routine clinical follow-up occurs at 1 month (T1, inclusion visit), 6 months (clinical visit), 1 year and yearly clinic visits thereafter up to 3 years. At each visit, clinical outcomes (death, re-hospitalization), compliance with medical therapy and smoking cessation, will be assessed. LDL, blood pressure and glycemic targets, echocardiographic data, quality of life (EQ-5D), physical activity at home

(CHAMPS), gait speed, handgrip and physical performance (SPBB) will be assessed at the 6-month, 1, 2 and 3 year-follow-up visits. Echocardiography will be performed at the time of the discharge and during the 1-year follow-up visit. The following echocardiographic data will be collected:

- Morfological and functional measures of systolic and diastolic function of the left ventricle
- Morfological and functional measures of systolic and diastolic function of the right ventricle
- Aortic root diameters
- Left ventricle Global Longitudinal Strain (GLS) and GLS of the free wall of the right ventricle.
- Valvulopathy evaluation
- Estimation of mean and systolic pulmonary arterial pressure
- Estimation of the left and right ventricle stroke volume

8.1.6 Subject discontinuation

Each enrolled subject will remain in the study until completion of the required follow-up period, however, a subject's participation in any clinical study is voluntary and the subject has the right to withdraw at any time without penalty or loss of benefit. Conceivable reasons for discontinuation may include, but not be limited to, the following:

- Subject death
- Subject voluntary withdrawal
- Subject lost-to follow-up

A patient randomized to physical activity intervention may decide to stop the supervised physical activity sessions, remaining in the protocol. The reason for subject discontinuation (to study or physical activity sessions) must be documented on the eCRF and source documents.

8.1.7 Study completion

The study is considered complete for the subject if:

- The subject is considered lost to follow-up
- The subject voluntarily withdraws from the study
- The Investigator withdraws the subject from the study
- Upon study completion (3 years from enrolment).

9 STUDY DEFINITIONS

9.1 Myocardial Infarction (MI)

MI will be defined according to the 4th universal definition of myocardial infarction (32).

Universal definitions of myocardial injury and myocardial infarction

Criteria for myocardial injury

The term myocardial injury should be used when there is evidence of elevated cardiac troponin values (cTn) with at least one value above the 99th percentile upper reference limit (URL). The myocardial injury is considered acute if there is a rise and/or fall of cTn values.

Criteria for acute myocardial infarction (types 1, 2 and 3 MI)

The term acute myocardial infarction should be used when there is acute myocardial injury with clinical evidence of acute myocardial ischaemia and with detection of a rise and/or fall of cTn values with at least one value above the 99th percentile URL and at least one of the following:

- Symptoms of myocardial ischaemia;
- New ischaemic ECG changes;
- Development of pathological Q waves;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischaemic aetiology;
- Identification of a coronary thrombus by angiography or autopsy (not for types 2 or 3 MIs).

Post-mortem demonstration of acute athero-thrombosis in the artery supplying the infarcted myocardium meets criteria for *type 1 MI*.
Evidence of an imbalance between myocardial oxygen supply and demand unrelated to acute athero-thrombosis meets criteria for *type 2 MI*.
Cardiac death in patients with symptoms suggestive of myocardial ischaemia and presumed new ischaemic ECG changes before cTn values become available or abnormal meets criteria for *type 3 MI*.

Criteria for coronary procedure-related myocardial infarction (types 4 and 5 MI)

Percutaneous coronary intervention (PCI) related MI is termed *type 4a MI*.
Coronary artery bypass grafting (CABG) related MI is termed *type 5 MI*.
Coronary procedure-related MI ≤ 48 hours after the index procedure is arbitrarily defined by an elevation of cTn values > 5 times for *type 4a MI* and > 10 times for *type 5 MI* of the 99th percentile URL in patients with normal baseline values. Patients with elevated pre-procedural cTn values, in whom the pre-procedural cTn level are stable (≤ 20% variation) or falling, must meet the criteria for a > 5 or > 10 fold increase and manifest a change from the baseline value of > 20%. In addition with at least one of the following:

- New ischaemic ECG changes (this criterion is related to *type 4a MI* only);
- Development of new pathological Q waves;
- Imaging evidence of loss of viable myocardium that is presumed to be new and in a pattern consistent with an ischaemic aetiology;
- Angiographic findings consistent with a procedural flow-limiting complication such as coronary dissection, occlusion of a major epicardial artery or graft, side-branch occlusion-thrombus, disruption of collateral flow or distal embolization.

Isolated development of new pathological Q waves meets the *type 4a MI* or *type 5 MI* criteria with either revascularization procedure if cTn values are elevated and rising but less than the pre-specified thresholds for PCI and CABG.
Other types of 4 MI include *type 4b MI* stent thrombosis and *type 4c MI* restenosis that both meet *type 1 MI* criteria.
Post-mortem demonstration of a procedure-related thrombus meets the *type 4a MI* criteria or *type 4b MI* criteria if associated with a stent.

Criteria for prior or silent/unrecognized myocardial infarction

Any one of the following criteria meets the diagnosis for prior or silent/unrecognized MI:

- Abnormal Q waves with or without symptoms in the absence of non-ischaemic causes.
- Imaging evidence of loss of viable myocardium in a pattern consistent with ischaemic aetiology.
- Patho-anatomical findings of a prior MI.

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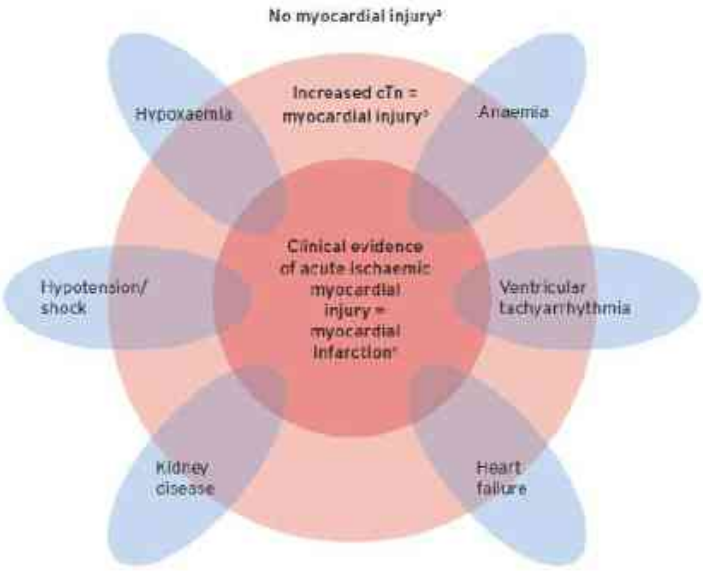
The leading symptom that initiates the diagnostic and therapeutic cascade in patients with suspected MI is chest pain. Based on the electrocardiogram (ECG), two groups of patients should be differentiated: (1) Patients with acute chest pain and persistent (>20 min) ST-segment elevation. This condition is termed ST-elevation ACS and generally reflects an acute total coronary occlusion. Most patients will ultimately

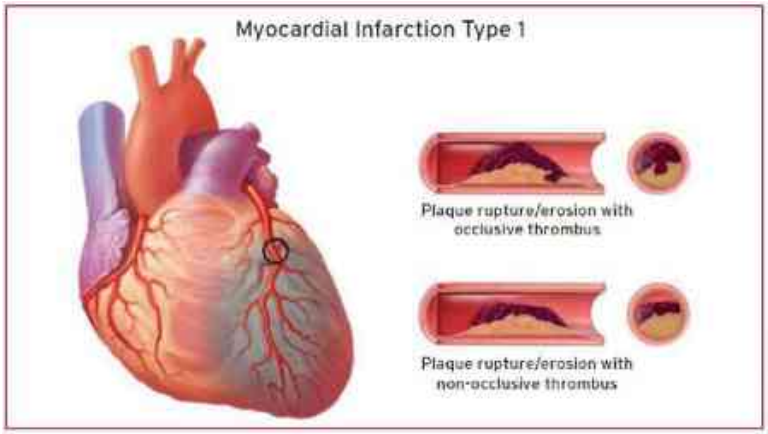
develop a STEMI. The mainstay of treatment in these patients is immediate reperfusion by primary angioplasty or fibrinolytic therapy. (2) Patients with acute chest pain but no persistent ST-segment elevation. ECG changes may include transient ST-segment elevation, persistent or transient ST-segment depression, T-wave inversion, flat T waves or pseudo-normalization of T waves or the ECG may be normal. The clinical spectrum of NSTEMI may range from patients free of symptoms at presentation to individuals with ongoing ischemia, electrical or hemodynamic instability or cardiac arrest. The pathological correlate at the myocardial level is cardiomyocyte necrosis. A small proportion of patients may present with ongoing myocardial ischemia, characterized by one or more of the following: recurrent or ongoing chest pain, marked ST depression on 12-lead ECG, heart failure and hemodynamic or electrical instability. Due to the amount of myocardium in jeopardy and the risk of malignant ventricular arrhythmias, immediate coronary angiography and, if appropriate, revascularization are indicated.



Criteria for type 1 MI

Detection of a rise and/or fall of cTn values with at least one value above the 99th percentile URL and with at least one of the following:

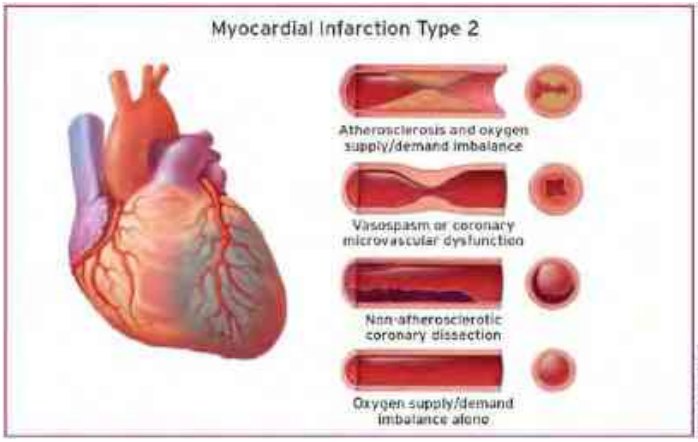
- Symptoms of acute myocardial ischaemia;
- New ischaemic ECG changes;
- Development of pathological Q waves;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischaemic aetiology;
- Identification of a coronary thrombus by angiography including intracoronary imaging or by autopsy.[a]



Criteria for type 2 MI

Detection of a rise and/or fall of cTn values with at least one value above the 99th percentile URL, and evidence of an imbalance between myocardial oxygen supply and demand unrelated to coronary thrombosis, requiring at least one of the following:

- Symptoms of acute myocardial ischaemia;
- New ischaemic ECG changes;
- Development of pathological Q waves;
- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormality in a pattern consistent with an ischaemic aetiology.



Criteria for type 3 MI

Patients who suffer cardiac death, with symptoms suggestive of myocardial ischaemia accompanied by presumed new ischaemic ECG changes or ventricular fibrillation, but die before blood samples for biomarkers can be obtained, or before increases in cardiac biomarkers can be identified, or MI is detected by autopsy examination.

9.2 Death

Deaths will be classified as cardiovascular, non-cardiovascular or undetermined cause according to ARC-2 criteria. Deaths of undetermined cause will default to cardiovascular (but will also be reported as a separate subcategory of cardiovascular death to denote this uncertainty). Deaths related to the procedure or complications of the procedure (or concomitant treatment) will be classified as cardiovascular. Within cardiovascular deaths, hemorrhagic deaths will be clearly identified. Only deaths due to a documented non-cardiovascular cause (e.g., cancer) will be classified as non-cardiovascular [33].

ARC-2 classification of death:

Type of Death	Definition
Cardiovascular death	Cardiovascular death is defined as death resulting from cardiovascular causes. The following categories may be collected: 1. Death caused by acute MI 2. Death caused by sudden cardiac, including unwitnessed, death 3. Death resulting from heart failure 4. Death caused by stroke 5. Death caused by cardiovascular procedures 6. Death resulting from cardiovascular hemorrhage 7. Death resulting from other cardiovascular cause
Noncardiovascular death	Noncardiovascular death is defined as any death that is not thought to be the result of a cardiovascular cause. The following categories may be collected: 1. Death resulting from malignancy 2. Death resulting from pulmonary causes 3. Death caused by infection (includes sepsis) 4. Death resulting from gastrointestinal causes 5. Death resulting from accident/trauma 6. Death caused by other noncardiovascular organ failure 7. Death resulting from other noncardiovascular cause
Undetermined	Undetermined cause of death is defined as a death not attributable to any other category because of the absence of any relevant source documents. Such deaths will be classified as cardiovascular for end point determination.

MI indicates myocardial infarction.

9.3 Cerebrovascular accident

Acute episode of a focal or global neurological deficit with at least one of the following: change in the level of consciousness, hemiplegia, hemiparesis, numbness, or sensory loss affecting one side of the body, dysphasia or aphasia, hemianopia, amaurosis fugax, or other neurological signs or symptoms consistent with stroke or transient ischaemic attack (TIA). Stroke and TIA are defined as follows:

- Stroke: duration of a focal or global neurological deficit ≥24 h; OR <24 h if available neuroimaging documents a new haemorrhage or infarct; OR the neurological deficit results in death
- TIA: duration of a focal or global neurological deficit <24 h, any variable neuroimaging does not demonstrate a new haemorrhage or infarct [34]

9.4 Hospital readmission

It is defined as every not elective admission to hospital after discharge and during study follow up. If patients are admitted more than once, every admission will be collected as a single event. It could be a readmission for any cause or a cardiovascular readmission, if it is due to cardiac problems such as heart failure, arrhythmias, acute coronary syndrome, effort angina requiring hospitalization [35].

- Heart failure defined as hospitalization with clinical symptoms and objective signs of heart failure including pulmonary edema, hypoperfusion or documented volume overload and necessitating a medical intervention i.e. administration of IV diuresis or inotropic therapy, performance of aortic valvuloplasty, institution of mechanical support (IABP or ventilation for pulmonary edema) or hemodialysis for volume overload. Administration of IV therapies in clinic without admission will not qualify as hospitalization events.
- Conduction disturbances and arrhythmias: any reported Arrhythmia: an irregular heart rate or abnormal rhythm resulting in symptoms or requiring medical intervention. This includes (but is not limited to) sinus bradycardia, premature atrial contractions, atrial tachycardia, supraventricular tachycardia, atrial fibrillation, atrial flutter, AV nodal reentrant tachycardia, junctional rhythm, premature ventricular contractions, ventricular fibrillation, ventricular tachycardia, and torsade de

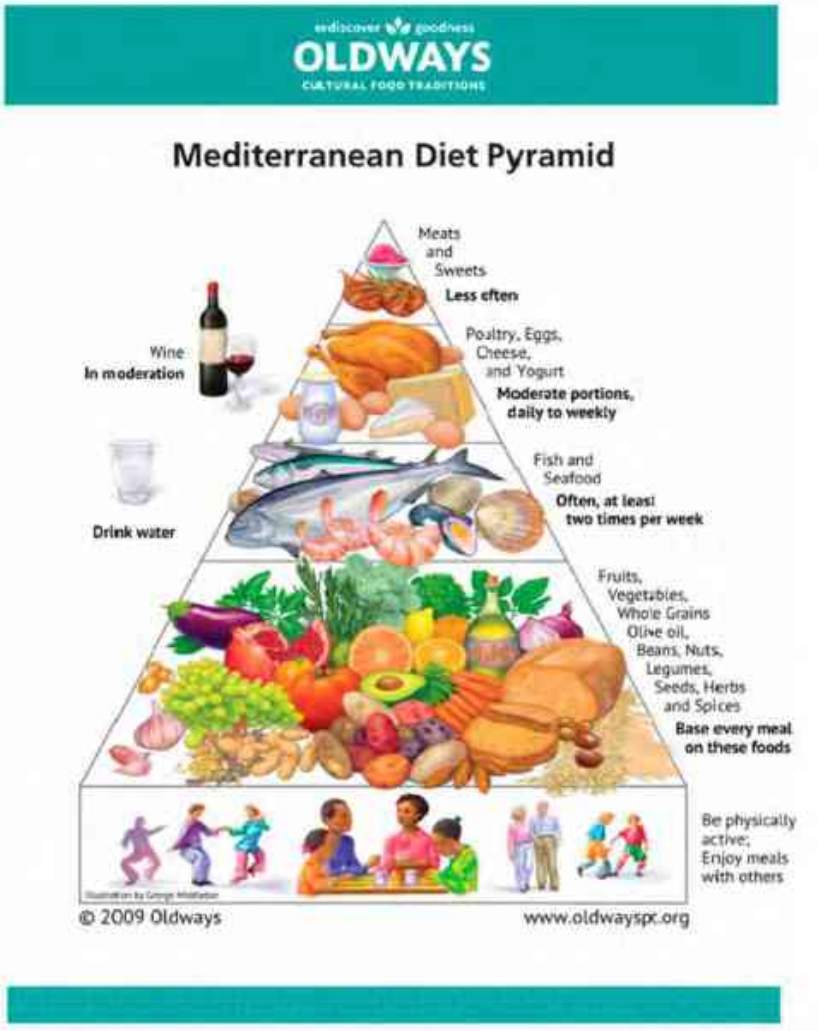
pointes. Conduction system defect requiring hospitalization: 2nd degree AV block (Mobitz I, Mobitz II), 3rd degree (complete) AV block. Interventions that may be required include (but are not limited to) permanent pacemaker, temporary pacemaker, electrical cardioversion, electrical cautery/ablation, medical cardioversion, and new medication (oral anticoagulation, rhythm or rate controlling therapy).

- Acute coronary syndrome: it includes myocardial infarction (see definition above), unstable angina defined as chest pain at rest or during minimal exertion without ECG signs of ischemia or abnormal cardiac biomarkers.
- Stable coronary artery disease: it is defined by effort angina or evidence of new ischemia during stress testing with indications to coronary angiography.

9.5 Detailed description of recommended diet, scales used in the study, calisthenic exercises and of the procedures performed during physical activity sessions.

Appendix B includes a summary table about timing of study procedures.

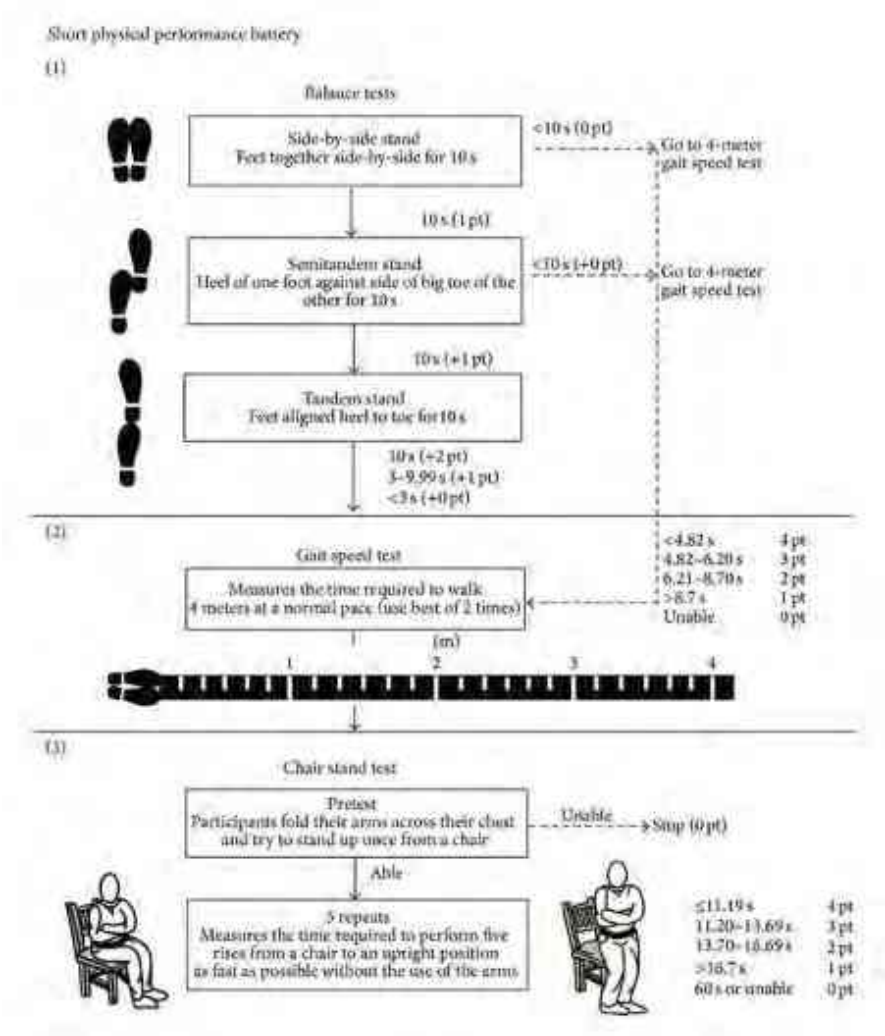
The Mediterranean diet was inspired by the eating habits of countries that border the Mediterranean sea (ie, Spain, Italy, and Southern Greece) and it is rich in whole-grains, leafy green vegetables, fruits, legumes, unsalted nuts, herbs, spices, and extra virgin olive oil. It provides a moderate intake of lean proteins such as fish and poultry, moderate alcohol consumption, and limited intake of red meat and sweets. By its very nature, the Mediterranean diet limits or eliminates saturated fat, highly processed refined grains and sugars, and red meat, lowering the risk of cardiovascular diseases. The Mediterranean diet provides anti- oxidants, reduces inflammation of the vascular wall, modulates pro-atherogenic genes, alters gut microbiome, and improves lipid panels by decreasing low density lipoprotein cholesterol (LDL-C) and raising high density lipoprotein cholesterol (HDL-C) [36].



In order to assess physical performance the following scales are performed:

- **SPPB:** this battery is made up of 3 different sections designed to assess lower limb function by 3 tests: balance, walking speed and chair sit to stand. Balance test includes 3 parts: the maintenance of the position with the feet together for 10 seconds, then the semi-tandem and finally tandem position again for 10 seconds. The score of this section varies from a minimum of 0 if the patient is unable to maintain position with feet together for at least 10 seconds to a maximum of 4 if the patient can accomplish all the three tests. The second section of the test is intended to evaluate the progress for 4 linear meters with usual pace; the score ranges from 0 if unable to 4 points if the patient can accomplish the task in less than 4.80 sec. The third section investigates the ability to run for 5 consecutive times, getting up

from a chair without using the arms. Also in this case, the score ranges from 0 if unable or if the performance has a duration greater than 60 seconds, to a maximum of 4 points if such performance is carried out in less than 11.20 seconds. The total SPPB score ranges from 0 (worst performance) to 12 (best performance). A score between 4 and 9 shows seniors still independent, but with reduced physical performance [7]. SPPB is performed at the time of the discharge as a screening value, during the inclusion visit prior the randomization, at 6 months, 1, 2 and 3 years.



- **Gait speed:** A variety of testing protocols are available for assessing gait speed. Although a standardized protocol has not been adopted, there is evidence available to help guide walking speed test selection. In particular, a test of 10 meter distance is considered more clinically feasible than

longer walkways. Patients are instructed to walk at usual pace for 10 meters. Time employed to perform the test is recorder by a stepwatch [9]. It is performed during the inclusion visit as a basal value and at 6 months, 1, 2 and 3 years.

- **Handgrip strength test (HGT):** it involves the use of a dynamometer to measure the force of contraction of the flexor muscles of the fingers, of muscles of the wrist and forearm. It is associated with nutrition status of the subject, and with functional recovery post-surgery [10]. Each patient is asked to squeeze the dynamometer three different times and the highest of the three is used to reflect HGT. The measurement is performed with the patient seated, with the dominant hand and the elbow flexed 90° [10]. All study staff is educated regarding correct HGT screening. In all centers a Jamar hydraulic hand dynamometer (Patterson Medical, Warrenville, Il, USA) is used. The results are expressed in Kilograms force. It is performed during the inclusion visit as a basal value and at 6 months, 1, 2 and 3 years.

In order to assess quality of life, the following test is performed.

- **EuroQol-5D (EQ5D)**: it is a simple instrument to evaluate health status and quality of life [37, 38]. It has been previously validated in patients with cardiovascular disorders, including participants in rehabilitation programs [39,40]. The EQ-5D instrument measures health status in 5 dimensions: mobility, self-care, usual activities, pain/discomfort, and anxiety/depression. Each dimension is rated according to the following levels: i) no problems; ii) some problems; iii) extreme problems. The questionnaire employs a Visual Analogue Scale (VAS) to quantify perceived health. There is also an algorithm to collect all data in a single score [40]. It is administered during the inclusion visit as a

basal value and at 6 months, 1, 2 and 3 years. A copy of the questionnaire can be found in the Appendix C.

In order to evaluate home physical activity a questionnaire and an accelerometry are provided:

- **Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire**: it consists of 41 questions about sport, leisure time activities, social activities, gardening and household working. Time spent in the above-mentioned activities is required. It investigates self-reported activities performed during the 4 weeks before (see Appendix D). It is one of the first physical activity questionnaires for older adults designed specifically for use in evaluating interventions that primarily aim to increase levels of physical activity in older adults. Its sensitivity confirms the appropriateness of this questionnaire for use in evaluating outcomes of physical activity interventions for older adults [42]. It is a validated questionnaire for older adults and its usefulness was confirmed by LIFE study [43]. It is administered during the inclusion visit as a basal value, 60±10, 90±10, 180±10, 270±10 and 360±10 days after T0, during the 6-month visit and at 1, 2 and 3 years.

Example of CHAMPS question.

In a typical week during the past 4 weeks, did you…							
1. Visit with friends or family (other than those you live with)? ☒ YES How many TIMES a week? 2 → ☐ NO	How many TOTAL hours a week did you usually do it? →	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours

- **Calisthenic exercises**

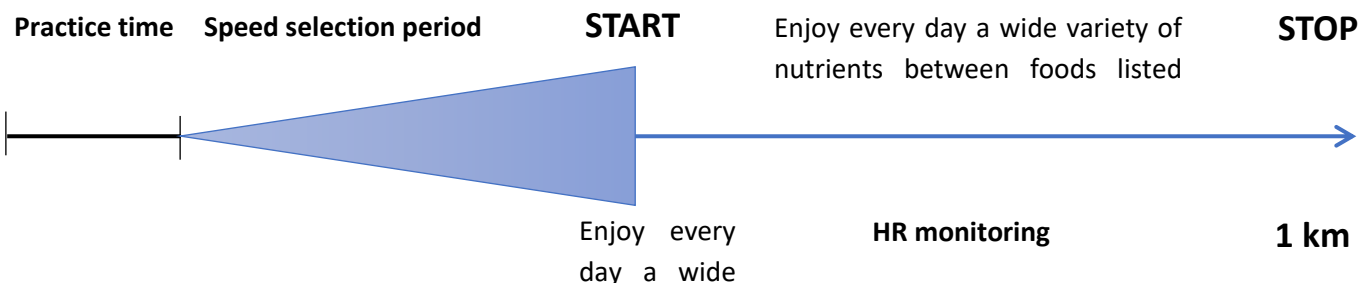
A series of calisthenic exercises derived from Otago Exercise Program are prescribed to physical activity intervention group making up the home-based program [44]. Three types of exercises are also prescribed to health education group: they are selected according to physician decision. A detailed description of calisthenic exercises is reported in the Appendix E.

Physical activity intervention group performs the following protocol:

During the inclusion visit and during the following physical activity sessions each patient performs calisthenics exercises for ≈5 min, followed by the 1k-TWT. This protocol has been demonstrated to be a valid and simple tool for cardiorespiratory fitness estimation. It has been shown to predict survival and hospitalization in outpatients with CVD, with and without preserved left ventricular ejection fraction. During the first session (inclusion visit) subjects are instructed about the proper walking technique on a motorized treadmill (Excite Med, Technogym, Cesena, Italy). Subjects are also instructed to select a pace that they can maintain for 10 to 20 min at a moderate perceived exercise intensity (11–13 on the 6–20 Borg scale). Patients continue their usual medications during exercise sessions to simulate the hemodynamic responses during exercise training at home. Practice time (≈5 min) is allowed to enable subjects to walk comfortably at a light to moderate intensity without support. Tight gripping of the handrails is not allowed. Patients are instructed to lightly rest a finger or two from one or both hands on the handrails for balance only. Following this preliminary practice and warm-up phase, patients begin the walk on the level at a walking speed of 2.0 km/h with subsequent increase of 0.3 km/h each 30 seconds up to the target moderate perceived walking intensity. If the starting exercise intensity is perceived as more than moderate (i.e. ≥14 on the 6-20 Borg scale), walking speed is decreased according to perceived exertion referred by patients. RPE is recorded every ≈2 min adjusting walking speed if necessary in order to maintain the moderate perceived exertion intensity. The time to complete either 1000-m is recorded and average walking speed calculated accordingly. Heart rate (HR) is monitored continuously by using Polar RS 100 HR monitor (Polar Electro, Kempele, Finland). Walking HR is averaged every 5 seconds and mean and maximal values during the 1k-TWT are determined. Based on the results of the treadmill-walking practices, patients are encouraged to replicate similar walking sessions at home and outdoors, in autonomy. As such, the training program is individualized but following international recommendations with 30 to 60 minutes of moderate-intensity aerobic activity (11-13 on the 6-20 Borg Scale), such as brisk walking, at least 5 days and preferably 7 days per week. Less fit or less motivated patients are encouraged to accumulate the daily

recommended volume in bouts of at least 10 min. The home program is adjusted regularly during the subsequent visits that are scheduled at 60±10, 90±10, 180±10, 270±10 and 360±10 days after discharge. Follow-up sessions are created to motivate patients and promote further participation in physically active lifestyle. History of changes in physical activity or symptoms, response to treatments and adverse events, and assessment of walking capacity by the 1k-TWT are assessed at any follow-up examinations. During these "booster" visits, the subject's individualized goals (i.e. achievement of recommended level of physical activity) are discussed and are used as a basis for adjustment of the exercise prescription. Benefits associated with improved walking speed are explained, emphasizing the health consequences of being able to walk faster and longer at a given RPE or heart rate. Patients who report advance toward goal attainment receive praise and are encouraged to attribute accomplishments to their own abilities. Patients who report to be less than ideally active are supported in order to overcome the limiting factors. Barriers to completing the home program are discussed and solutions proposed, even during the 1k-TWT, as such functioning as an informal "problem-solving" activity, integrated within the testing session. After the initial exercise prescription, the intensity of walking is adjusted based on the patient tolerance with the aim of achieving and maintaining rating of perceived exertion of 11 to 14 on the 6-20 Borg scale, which is from light to somewhat hard. After each examination, patients are advised to gradually progress frequency and duration of unsupervised home sessions up to 1-hour daily walks. For a more effective positive behavior change, sports physicians, clinical exercise physiologists, and nurses involved in the program, share with patients the personal goals to achieve and maintain the benefits. To this end, patients are encouraged to find how and when they can realistically include regular walks in their daily routines. Less fit or less motivated patients are encouraged to accumulate the recommended duration for walking training in bouts of at least 10 min. Patients with muscular-skeletal or neurological conditions limiting the walking ability, or simply for those who did not like to walk, are encouraged to find other pleasant aerobic activity more likely to be sustainable. Particularly, patients are educated to associate RPE, walking speed, and heart rate during the 1k-TWT, and comparison between prior sessions were made. Commonly, having increased weekly PA as recommended, corresponds to higher walking speed at a given RPE, heart rate and drugs. On the contrary,

if for any reason patients reduce weekly PA in respect to previous examinations, a reduced walking speed is observed at a given RPE, heart rate and drugs. As such, patients become progressively aware of the relevance of regular physical activity [29,30].



9.6 Adverse event monitoring (and reporting to EC and DSMB)

The Investigator will monitor the occurrence of adverse events for each subject, during the course of the study. All adverse events (AEs) reported by the subject, observed by the Investigator, or documented in medical records will be recorded on the adverse event CRF, whether believed by the Investigator to be related or unrelated to the investigational treatment. All adverse events will be monitored until they are adequately resolved or stabilized. Serious adverse events will be periodically reported to Ethic Committee.

9.7 Adjudication of clinical events

A committee consisting of clinicians who are blinded to treatment allocation will adjudicate primary and secondary outcomes. Adjudication results will be binding for the final analysis.

9.8 Safety monitoring by Data Safety Monitoring Board (DSMB)

All adverse events will be reported to the DSMB and reviewed on an on-going basis throughout the subject enrolment and follow-up period. The DSMB may request additional information as needed.

10 STATISTICAL ANALYSIS PLAN

All statistical analyses will be performed by the Centro di Ricerca Clinica ed Epidemiologica of the University of Ferrara under the supervision of Professor Stefano Volpato. Analysis will be performed on an Intention to Treat (ITT) set, defined as all intentionally randomized patients, by randomization treatment. Supportive per-protocol analyses will be performed on the primary and key secondary endpoints. A detailed statistical analysis plan will be completed before the end of the study. In brief, continuous variables will be tested for normal distribution with the Kolmogorov-Smirnov test and with visual estimate of Q-Q plot. Normally distributed variables will be presented as mean±SD and compared by t test and 1-way ANOVA. Otherwise, median [inter-quartile range], Mann-Whitney U and Kruskal-Wallis tests will be used. Categorical variables will be summarized in terms of absolute and relative frequencies (percentages) and compared by using $\chi 2$ test. One-sided tests will be done in order to check the superiority of physical activity. Statistical significance will be set at $\alpha=0.05$ level. Formal type-I error control will be ensured for the primary and the key secondary endpoint by a sequential procedure where significance for the key secondary endpoint is accepted only if the primary endpoint is positive. Kaplan-Meyer curves will be plot to describe survival free from adverse events, and difference between groups will be test with log-rank test. The primary analysis will be based on events of all follow-up time of each patient at the time of the database lock. Any confounding factor will be tested by Cox regression models. Variables with a p-value <0.1 at univariate analysis will be entered into a multivariable analysis to identify the independent predictors. When appropriate, 95% CI will be calculated. All analyses will be performed with STATA 13 (StataCorp, College Station, TX).

10.1 Determination of the sample size

Considering previous studies and our experience (HULK study, NCT03021044), we may suppose a 1-year primary outcome rate around 25%. Previous studies showed a benefit from physical intervention ranging from 35% to 50%. Although the data should be considered with caution, we may suppose a reduction in the primary outcome around 40%, that is an expected rate around 15%. Thus, based on alpha=0.05 and

beta=80%, a total sample size of 435 patients is required. Estimating a possible loss of patients of 5% (N=21), the final study population should be of at least 456 patients, 152 in control group and 304 in intervention group.

11 SUB-STUDIES

The protocol includes 3 pre-specified substudies. The possibility to participate in the substudy is left to patient's decision and doesn't preclude the procedures of the main protocol. The first substudy, namely anxiety and depression will involve all participating centers. On the contrary, the other two substudies will be only performed in the Ferrara center. The analysis and reporting of these sub-studies will be totally separate from the main study.

- **Anxiety and depression**: previous studies reported an association between cardiovascular events and a subsequent appearance of mood disorders which could determine a lack in following secondary prevention recommendations. No data is available about these disorders in older adults. In order to assess depression after MI in older patients and the effect of physical activity intervention on mood disorders, this sub-study will be performed. For a detailed description of this sub-study see the Appendix F.
- **Mitochondrial function**: in both study groups the mitochondrial functional are investigated starting from blood samples and skin biopsy. Skin biopsy is performed to obtain fibroblasts. A summary of the skin biopsy procedure is reported in the appendix I. In-vitro assessment of the mitochondrial function is done on the fibroblasts of patients. The parameters obtained are related with the effectiveness of physical intervention and with the benefit obtained. A total of at least 30 patients is required. For a detailed description of the sub-study see the Appendix G.
- **Lymphocyte and miRNA activity**: taking into account the immunosenescence and the benefits of physical activity on the immune system, this sub-study aims to obtain data regarding T-lymphocyte function in older adults and to assess the effect of physical activity intervention on the function of the different groups of T-lymphocyte. Analyses of physical activity effects on micro ribonucleic acids (miRNAs) are also performed. For a detailed description of this sub-study see the Appendix H.

12 DATA MANAGEMENT

Data will be collected on electronic case report forms (eCRFs). The CRF must be signed by the investigator or by other appropriate individuals who are authorized by the investigator. Signing of the 'Study completion – Investigator's statement CRF' must be done by the investigator and is considered to be the final authorization of the CRFs.

13 ETHICAL AND REGULATORY STANDARDS

13.1 Good Clinical Practice

The procedures set out in this protocol are designed to ensure that the investigator abide by the principles of the Declaration of Helsinki and Good Clinical Practice Guidelines (ICH-GCP) in the latest version, in conduct, evaluation and documentation of the study. A copy of these documents will be provided to each centre. The study will be carried out in keeping with local legal requirements and International regulations.

13.2 Informed Consent of the Patient

Patients who meet all inclusion criteria and none of the exclusion criteria are deemed eligible. Before being enrolled into the clinical study, the patient must provide written consent to participate in the study after the nature, scope and possible consequences of the clinical study have been explained both orally and in writing. All patients who signed informed consent must be listed on the Screening Log.

13.3 Approval of the Study Protocol

Before the start of the study, the study protocol and the informed consent form used at the site and other appropriate documents must be submitted to and approved by the local Ethics Committee or Institutional Review Board and the appropriate regulatory authorities in accordance with local legal requirements. Documentation of Ethics Committee/IRB approvals will be required before sites are activated to randomize.

13.4 Maintenance of Records

The Investigator agrees to obtain a correctly completed informed consent form for each patient included in the study. The investigator will maintain a personal list of patient numbers and patient names to enable records to be found at a later date. The Investigator must maintain all study records, patient files and other source data for the maximum period of time permitted by the hospital, institution or private practice. However national regulations should be taken into account, the longest time having to be considered. For trials performed in the European Community, the Investigator is required to arrange for the retention of the patient identification codes for at least 15 years after the completion or discontinuation of the trial.

13.5 Confidentiality

All patient names will be kept confidential. Patients will be identified throughout documentation and evaluation by the number allotted to them by the study. The patients will be assured that all findings will be stored on computer and handled in the strictest confidence. The Investigator agrees to maintain the confidentiality of the study protocol.

14 ADMINISTRATIVE RULES

14.1 Curriculum vitae

An updated copy of the curriculum vitae for each Investigator and co-Investigator will be provided prior to the beginning of the study.

14.2 Confidentiality agreement

All goods, materials, information (oral or written) and unpublished documentation provided to the Investigators (or any company acting on their behalf), inclusive of this protocol and the patient case report forms are the exclusive property of the Cardiovascular Department of the University of Ferrara. They may not be given or disclosed by the Investigator or by any person within his authority either in part or in totality to any unauthorized person without the prior written formal consent. The Ethics Committee members having the same obligation of confidentiality. The Investigator shall consider as confidential and shall take all necessary measures to ensure that there is no breach of confidentiality in respect of all information accumulated, acquired or deduced in the course of the trial, other than that information to be disclosed by law.

14.3 Record retention in investigating centre(s)

The Investigator must maintain all study records, patient files and other source data for the maximum period of time permitted by the hospital, institution or private practice. National regulations, however, should be taken into account, the longest time having to be considered. For trials performed in the European Community, the Investigator is required to arrange for the retention of the patient identification codes for at least 15 years after the completion or discontinuation of the trial.

15 OWNERSHIP OF DATA AND USE OF THE STUDY RESULTS

The Promoter (Azienda Ospedaliero- Universitaria di Ferrara) has ownership of all data and results collected during this study. Full publication rights of the study data solely reside with the Study Principal Investigator. The Study Principal Investigator must discuss all the study findings with the Steering committee before their publications.

16 PUBLICATIONS

All study presentations and/or publication of the results will be based on clean, checked and validated data in order to ensure the accuracy of the results. Publication of the main findings of this study will be authored based on the contributions of the individuals to the overall study. All the trial participants (Investigators and committee members) make a prior delegation of responsibility for primary presentation and/or primary publication of the results to the Study Principal Investigator.

17 STUDY ADMINISTRATIVE INFORMATION

17.1 Address list

17.1.1 Sponsor

Azienda Ospedaliero Universitaria di Ferrara, Italy

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17.1.2 Principal Investigator

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17.2 INSURANCE

Subjects who participate in this study will be insured against study related injury according to local regulatory requirements. A copy of the certificate is filed in each investigator site file and in the trial master file.

17.3 FUNDING AND SUPPORT

The project was presented for the call of Ricerca Finalizzata 2018 and obtained the grant by Ministero della Salute (GR-2018-12367114). During the study, to achieve the scientific goal of the study and to permit the coverage of the costs, the Principal Investigator can integrate the study budget with further fund raising from private and public companies. The potential achievements will be immediately communicated to Promoter and Regulatory Agencies (including Ethic Committee).

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19 APPENDIX A

Study brochure

A healthy lifestyle includes proper nutrition

Enjoy every day a wide variety of nutrients between foods listed below:

- Plenty of vegetables of different types and colours, and legumes/beans
- Fruit
- Grain (cereal) foods, mostly wholegrain and/or high cereal fibre varieties, such as breads, cereals, rice, pasta, noodles, polenta, couscous, oats, quinoa and barley
- Lean meats and poultry, fish, eggs, tofu, nuts and seeds
- Milk, yoghurt, cheese with reduced fat

...and drink water

Limit intake of foods containing saturated fat, added salt, added sugars and alcohol such as biscuits, cakes, pastries, pies, processed meats, commercial burgers, pizza, fried foods, potato chips, crisps and other savoury snacks.

Healthy eating

PIpELINe

And now that I come home?

Practical indications for patients discharged after acute coronary syndrome

If you are reading this brochure it means you have been admitted for acute coronary syndrome. This has happened because in the vessels that carry blood to your heart a fatty plaque has broken, obstructing the vessel. Even if the guilty plaque has been settled, you remain at risk of developing other plaques, which may break, causing serious consequences. For this reason, you will need to accurately take the prescribed medications and perform periodic outpatients visits... *But it is not over here!* Drugs are not enough to fight fatty plaques, therefore you need to adopt a healthy lifestyle, based on proper nutrition and physical activity.

Firstly, here below some tips on resuming daily activities after hospitalization are reported:

- *Stop Smoking*

- After hospital discharge, you should stay home in the first 2-3 days, then you are allowed to go out for a short walk in the plain, starting with 15-30 minutes a day, gradually increasing the duration in the following days
- Cigarette smoking **IS ABSOLUTELY NOT RECOMMENDED**
- Sexual activity can be resumed a few days after dischargeYou can return to work 4 weeks after discharge, provided it is not too physically demanding
- Driving can be resumed 3-4 weeks after the heart attack, as long as it does not involve long or challenging travel
- You can go on holiday, but in case of a long journey take breaks as needed. Be sure to carry an adequate supply of medication and clinical documentation. Avoid altitudes above 1500m, scuba diving, and flights if you are afraid of flying.

Physical activity

1) Make physical activity a regular habit
2) Gradually increase your physical activity plan to avoid fatigue and discomfort
3) Vary activities by changing the type of performance or the way to do it
4) Wear your accelerometer when you perform physical activity
5) Alternate physical activity with adequate breaks to regain strength and renew the motivation

Do at least 30 minutes of physical activity on 5 days each week

Moving more and sitting less!

Little suggestions:

- For short trips, walk or cycle and leave the car at home
- For longer trips, walk or cycle part of the way
- Use the stairs rather than the lift
- Get off the bus one stop earlier and walk the rest of the way
- Park further away from your destination and walk.
- Catch up with friends for a walk, rather than sitting to chat
- Plan outdoor activities, like bike riding or walking.
- Don't let the weather stop you. Try indoor activities!

Stop Smoking

Moving more and sitting less!

20 APPENDIX B

Table main study and sub-studies procedures

	Hospital Admission	Screening (T0)	Inclusion (T1)	60, 90, 180, 270, 360 days after T1	6-month clinical visit after T0	1-year clinical visit after T0	2- and 3-year clinical visit after T0
Cardiovascular history	x						
Routine Blood examinations	x	x			x	x	x
Blood samples for substudies		x	x		x x	x x	
Skin biopsy		x				x	
Physical examination	x	x	x		x	x	x
EKG	x	x	x		x	x	x
Medical therapy optimization	x	x	x		x	x	x
Handgrip test			x		x	x	x
SPPB		x	x		x	x	x
Dietary counselling *Only pts experimental arm			x				

Physical activity session *Only pts experimental arm			**x**	**x**			
Strict monitoring CV risk factors and compliance with dietary counselling and exercise training *Only pts experimental arm			**x**	**x**			
CHAMPS questionnaire			**x**	**x**	**x**	**x**	**x**
EuroQoL-5D			**x**			**x**	**x**
SPMSQ		**x**					
Gait speed			**x**		**x**	**x**	**x**
Echocardiography		**x**				**x**	
Beck Depression Inventory			**x**			**x**	

Black: PIPELINE main study

Blue: Depression substudy

Red: Mitochondrial substudy

Green: Lymphocytes and miRNA substudy

21 APPENDIX C

EuroQol-5D questionnaire

Health Questionnaire

English version for the UK

Under each heading, please tick the ONE box that best describes your health TODAY.

MOBILITY

- I have no problems in walking about ☐
- I have slight problems in walking about ☐
- I have moderate problems in walking about ☐
- I have severe problems in walking about ☐
- I am unable to walk about ☐

SELF-CARE

- I have no problems washing or dressing myself ☐
- I have slight problems washing or dressing myself ☐
- I have moderate problems washing or dressing myself ☐
- I have severe problems washing or dressing myself ☐
- I am unable to wash or dress myself ☐

USUAL ACTIVITIES *(e.g. work, study, housework, family or leisure activities)*

- I have no problems doing my usual activities ☐
- I have slight problems doing my usual activities ☐
- I have moderate problems doing my usual activities ☐
- I have severe problems doing my usual activities ☐
- I am unable to do my usual activities ☐

PAIN / DISCOMFORT

- I have no pain or discomfort ☐
- I have slight pain or discomfort ☐
- I have moderate pain or discomfort ☐
- I have severe pain or discomfort ☐
- I have extreme pain or discomfort ☐

ANXIETY / DEPRESSION

- I am not anxious or depressed ☐
- I am slightly anxious or depressed ☐
- I am moderately anxious or depressed ☐
- I am severely anxious or depressed ☐
- I am extremely anxious or depressed ☐

- We would like to know how good or bad your health is TODAY.
- This scale is numbered from 0 to 100.
- 100 means the best health you can imagine.
  0 means the worst health you can imagine.
- Mark an X on the scale to indicate how your health is TODAY.
- Now, please write the number you marked on the scale in the box below.

YOUR HEALTH TODAY = ☐

22 APPENDIX D

Community Healthy Activities Model Program for Seniors (CHAMPS) questionnaire

CHAMPS Activities Questionnaire for Older Adults

This questionnaire is about activities that you may have done in the past 4 weeks. The questions on the following pages are similar to the example shown below.

INSTRUCTIONS

If you DID the activity in the past 4 weeks:

Step #1 **Check the YES box.**

Step #2 **Think about how many TIMES a week you usually did it, and write your response in the space provided.**

Step #3 **Circle how many TOTAL HOURS in a typical week you did the activity.**

Here is an example of how Mrs. Jones would answer question #1: Mrs. Jones usually visits her friends Maria and Olga twice a week. She usually spends one hour on Monday with Maria and two hours on Wednesday with Olga. Therefore, the total hours a week that she visits with friends is 3 hours a week.

In a typical week during the past 4 weeks, did you…							
1. Visit with friends or family (other than those you live with)? ☒ **YES** How many TIMES a week? 2 → ☐ **NO**	How many TOTAL hours a week did you usually do it? →	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours

If you DID NOT do the activity:

- **Check the NO box and move to the next question**

In a typical week during the past 4 weeks, did you …							
1. Visit with friends or family (other than those you live with)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
2. Go to the senior center? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
3. Do volunteer work? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
4. Attend church or take part in church activities? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
5. Attend other club or group meetings? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
6. Use a computer? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours

In a typical week during the past 4 weeks, did you …							
7. Dance (such as square, folk, line, ballroom) (do not count aerobic dance here)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
8. Do woodworking, needlework, drawing, or other arts or crafts? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
9. Play golf, carrying or pulling your equipment (count walking time only)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
10. Play golf, riding a cart (count walking time only)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
11. Attend a concert, movie, lecture, or sport event? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
12. Play cards, bingo, or board games with other people? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours

In a typical week during the past 4 weeks, did you …							
13. Shoot pool or billiards? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
14. Play singles tennis (do not count doubles)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
15. Play doubles tennis (do not count singles)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
16. Skate (ice, roller, in-line)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
17. Play a musical instrument? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
18. Read? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
19. Do heavy work around the house (such as washing windows, cleaning gutters)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours

In a typical week during the past 4 weeks, did you …							
20. Do light work around the house (such as sweeping or vacuuming)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
21. Do heavy gardening (such as spading, raking)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
22. Do light gardening (such as watering plants)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
23. Work on your car, truck, lawn mower, or other machinery? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
****Please note: For the following questions about running and walking, include use of a treadmill.**							
24. Jog or run? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
25. Walk uphill or hike uphill (count only uphill part)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours

In a typical week during the past 4 weeks, did you …							
26. Walk fast or briskly for exercise (do not count walking leisurely or uphill)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
27. Walk to do errands (such as to/from a store or to take children to school (count walk time only)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
28. Walk leisurely for exercise or pleasure? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
29. Ride a bicycle or stationary cycle? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
30. Do other aerobic machines such as rowing, or step machines (do not count treadmill or stationary cycle)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
31. Do water exercises (do not count other swimming)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours

In a typical week during the past 4 weeks, did you …							
32. Swim moderately or fast? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
33. Swim gently? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
34. Do stretching or flexibility exercises (do not count yoga or Tai-chi)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
35. Do yoga or Tai-chi? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
36. Do aerobics or aerobic dancing? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
37. Do moderate to heavy strength training (such as hand-held weights of more than 5 lbs., weight machines, or push-ups)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours

In a typical week during the past 4 weeks, did you …							
38. Do light strength training (such as hand-held weights of 5 lbs. or less or elastic bands)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
39. Do general conditioning exercises, such as light calisthenics or chair exercises (do not count strength training)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
40. Play basketball, soccer, or racquetball (do not count time on sidelines)? ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours
41. Do other types of physical activity not previously mentioned (please specify)? _____ ☐ **YES** How many TIMES a week?_____ ➔ ☐ **NO**	How many TOTAL hours a week did you usually do it? ➔	Less than 1 hour	1-2½ hours	3-4½ hours	5-6½ hours	7-8½ hours	9 or more hours

Thank You

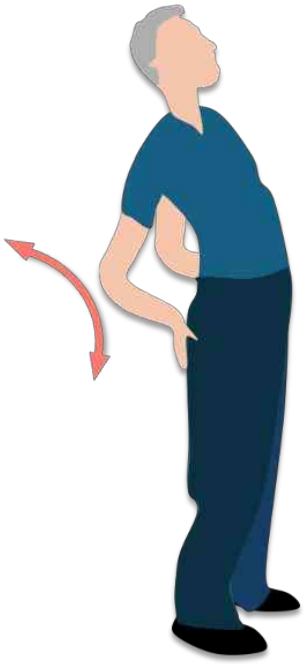
23 APPENDIX E

Exercises for muscle strengthening and mobility

WARM-UP

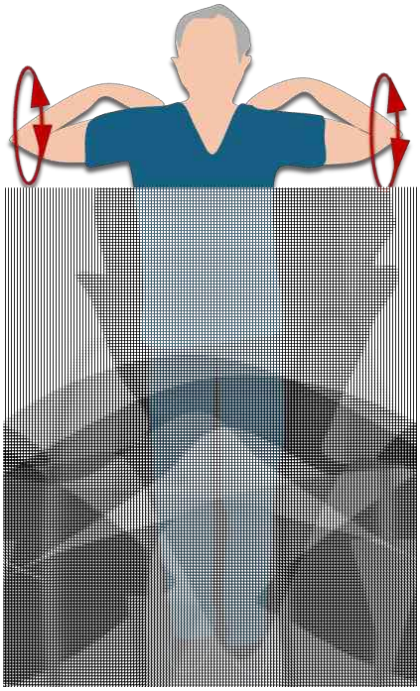
BACK EXTENSION

- Standing, keeping your back straight and your hands on your hips
- Gently arch your back 10 times, avoiding looking at the ceiling



SHOULDER MOBILITY

- Standing, keeping your back straight
- Perform 10 shoulder rotations forward and 10 backwards

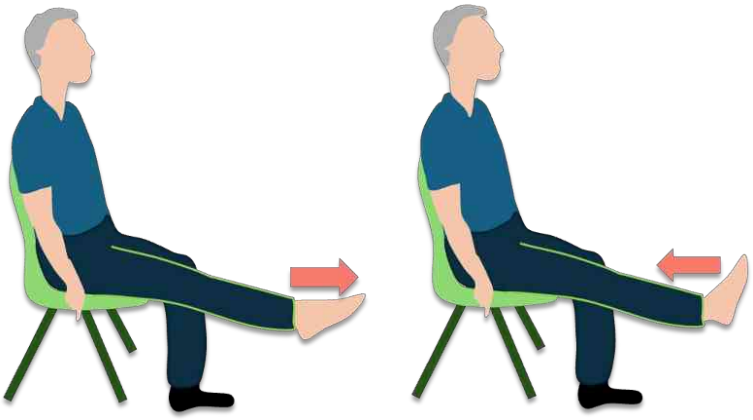


ANKLE MOBILITY

• Sitting, keeping your back straight

• Extend one leg s that the foot is kept off the floor and maintain this position by bridging the toes first forward and then backward

- Perform 10 movements per side



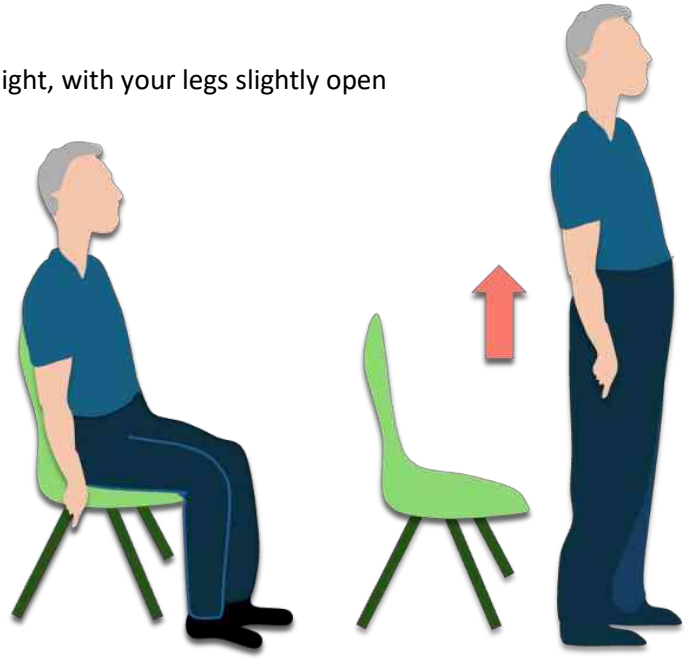
STRENGTH EXERCISES

LEG TRAINING

• Starting from a sitting position, keeping your back straight, with your legs slightly open

• Get up from your chair ___ times trying not to use your hands as support

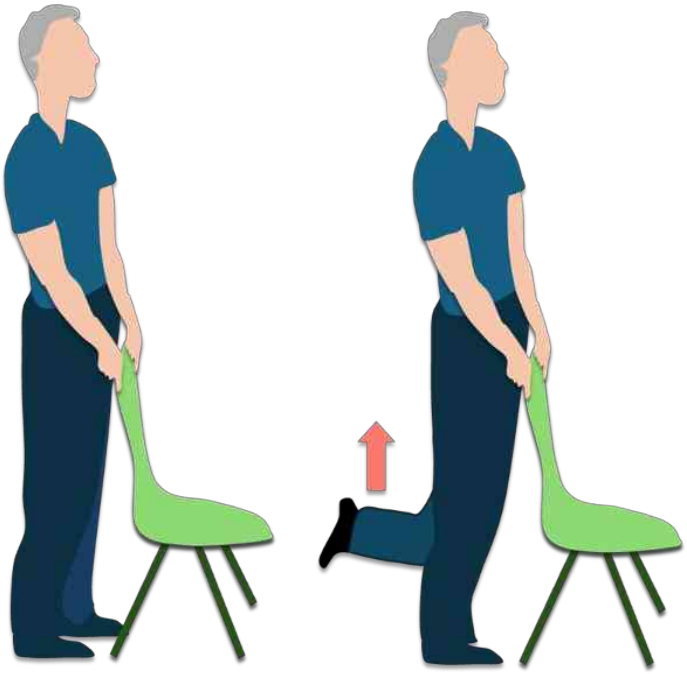
• Repeat the exercise 3 times



• Standing, keeping your back straight, with your hands resting on a stable support

• Raise your leg back 10 times in a slow and controlled movement, then repeat with the other leg

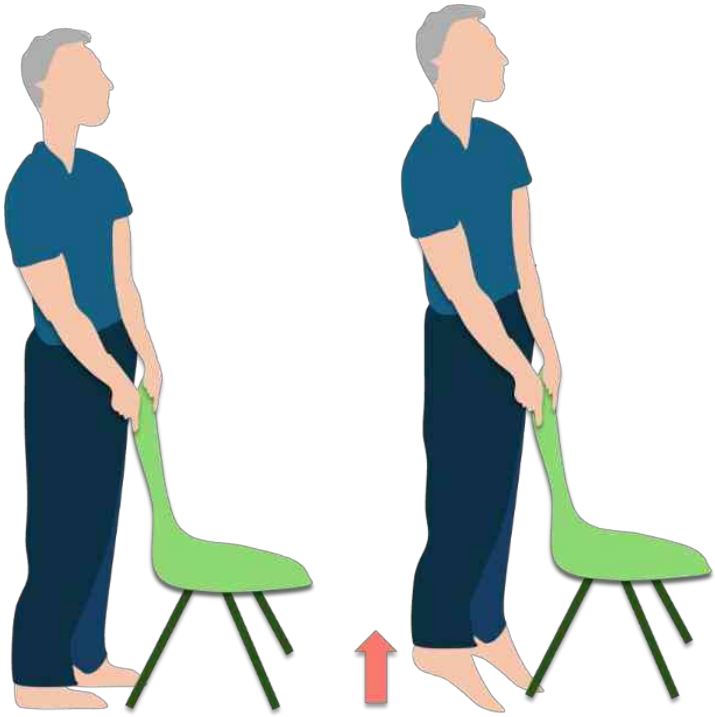
• Repeat the exercise 3 times



• Standing, keeping your back straight, with your hands resting on a stable support

• Lift your heels off the ground 10 times in a slow and controlled movement

• Repeat the exercise 3 times

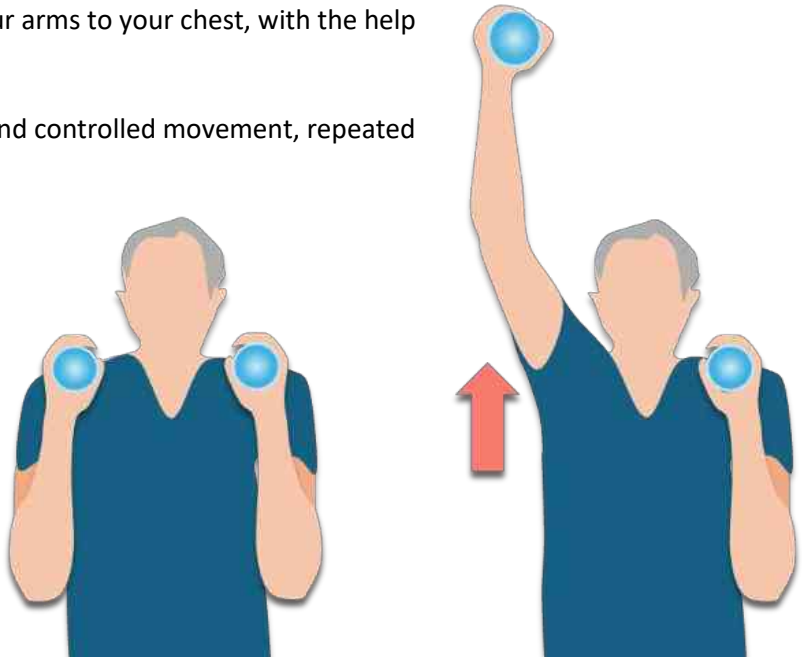


SHOULDER WORKOUT

• Standing, keeping your back straight and your arms to your chest, with the help of two weights (for example: little bottles)

• Bring your arms above your head in a slow and controlled movement, repeated 10 times per side

• Repeat the exercise 3 times



24 APPENDIX F

Effect of Physical activity Intervention on Depression in ELderly after myocardial Infarction- a sub-study of the PIpELINe randomized clinical trial.

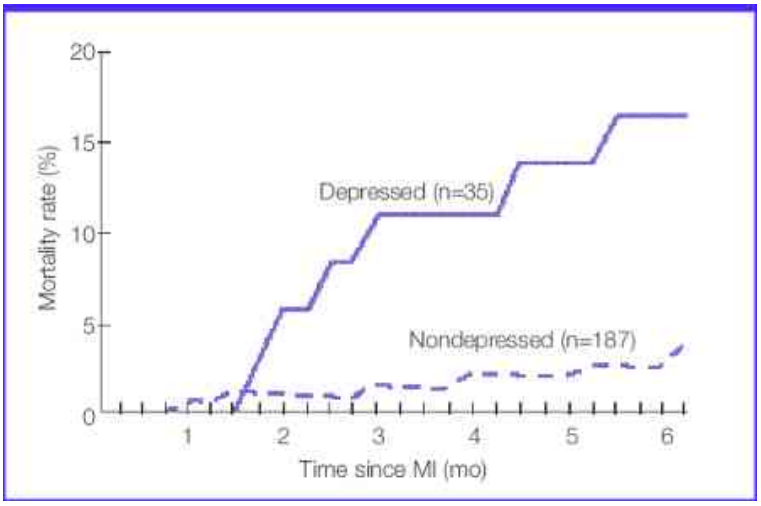
24.1 BACKGROUND

24.1.1 Epidemiology and prognostic value of depression in older adults after myocardial infarction

Depression is common after acute myocardial infarction (MI) and is associated with adverse outcomes, including mortality [1]. It is 3 times more common in patients after MI than in the general community. About 20% of patients experience major depression after MI [1]. Milder depressive symptoms are even more common [2]. These data are particularly evident in patients aged 65 years old and over. Major depression and elevated depressive symptoms are associated with worse prognosis in patients with ischaemic heart disease [3]. In fact, most studies that have examined the relationship between increasing depression severity and cardiac events have shown a dose-response relationship, with more severe depression associated with earlier and more severe cardiac events [3]. Both biological and behavioural mechanisms have been proposed to explain the link between depression and coronary diseases. In comparison with non-depressed individuals, depressed patients with ischaemic heart disease frequently have higher levels of biomarkers found to predict cardiac events or promote atherosclerosis. Although not always consistent, several studies in depressed patients with coronary artery disease have reported reduced heart rate variability (suggesting increased sympathetic activity and/or reduced vagal activity), evidence of hypothalamic-pituitary-adrenal axis dysfunction, enhanced platelet activation), impaired vascular function, and increased C-reactive protein, interleukin-6, intercellular adhesion molecule-1, and fibrinogen levels (suggesting increased innate inflammatory response). Depression can be highly correlated with other mental disorders (eg, anxiety disorders) that have also been associated with adverse cardiac outcomes. Certain behaviors and social characteristics in depressed patients may also contribute to the development and progression of coronary disease. These include diet, exercise, medication adherence, and tobacco use, as well as social isolation and

chronic life stress [4,5]. Although the specific behavioral and biological processes remain unclear, the alteration of these processes is associated with depressive symptoms, consistently in a direction that increases cardiovascular risk.

Mortality rate after MI in patients with and without depression



24.1.2 Depression and compliance to secondary prevention recommendations

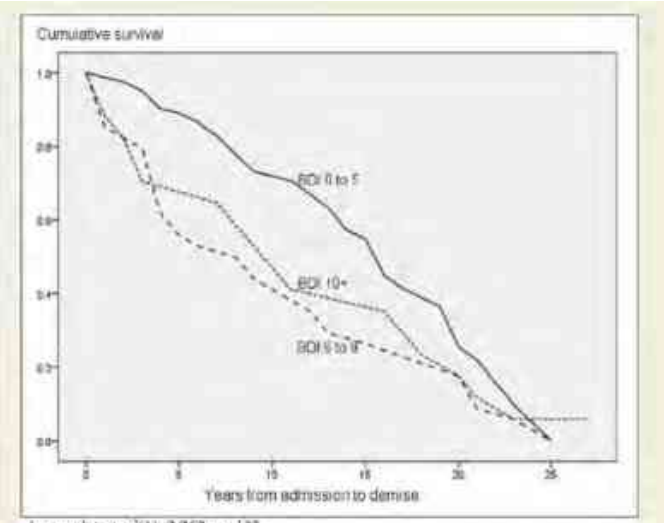
Beyond what many believe to be its pathophysiological effect on the heart, depression is associated with decreased adherence to medications and triple the risk of noncompliance with medical treatment regimens [6]. Depression reduces the chances of successful modifications of other cardiac risk factors and participation in cardiac rehabilitation and is associated with higher healthcare utilization and costs and, not surprisingly, greatly reduced quality of life [7]. Thus whether depression affects cardiac outcomes directly or indirectly, the need to screen and treat depression is imperative.

24.1.3 Scale for the assessment of depression

The Beck Depression Inventory (BDI) has commonly been used in studies of depression after MI [8]. This tool is made up of a series of questions about mood, hobbies and feelings of the 2 weeks before. The final score can range between 0 to 39: most studies used a BDI cut-point of 10 to classify patients with clinically

significant symptoms of depression corresponding to mild depression [9]. A recent study demonstrated that depression after MI assessed by BDI is related to long-term adverse clinical outcomes [10].

Relationship between BDI score and outcomes

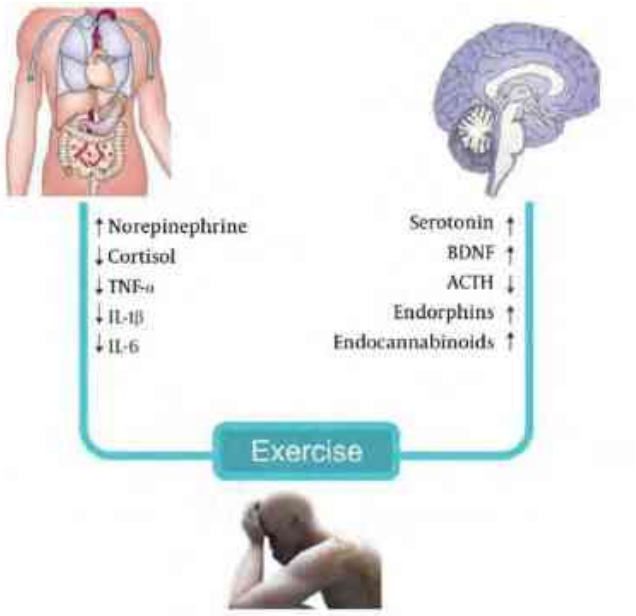


	0	5	10	15	20	25
Dead	0	29	51	72	103	132
Alive	185	156	134	113	82	53
Alive by BDI group						
Low	113	104	91	76	52	31
Mild	46	31	26	21	18	12
Severe	26	21	17	16	12	10

24.1.4 Physical activity and depression after myocardial infarction

Depression may be a barrier to participation in cardiac rehabilitation and exercise programs. Aerobic exercise and cardiac rehabilitation can reduce depressive symptoms in addition to improving cardiovascular fitness. The prescription of exercise would need to be individually assessed based on cardiac status and exercise capacity of the individual [1]. Different mechanisms have been proposed to be responsible of benefits of physical activity on depression: molecular mechanisms such as endorphins and neurotransmitters productions, encouragement in social activities, emotional support [1].

Biological effects of exercise in patients with depression.



24.1.5 Preliminary findings of the HULK study (NCT03021044)

Recently we conducted the "Physical activity intervention for elderly patients with reduced physical performance after acute coronary syndrome" (HULK study) in order to evaluate feasibility and effectiveness of an early, tailored, low-cost physical activity program in older patients with impaired physical performance discharged after MI. It was a multicenter, investigator-driven, randomized trial enrolling older MI patients with a moderate impairment of physical performance assessed by the Short Physical Performance Battery (SPPB). Patients were randomized to physical activity intervention (experimental group) or health education (standard of care group). Physical activity intervention included 4 sessions of physical activity and a home-based program with a series of calisthenic exercises. Primary endpoint was SPPB value 6 months after MI; secondary endpoints included quality of life and performance in some daily activities. Preliminary findings about quality of life showed that the physical activity intervention significantly improved quality of life assessed by EuroQoL 5D; in particular there was a significant improvement in term of anxiety and depression.

24.2 RATIONALE

Depression is common after MI, especially in older ACS patients. Depression is associated to adverse clinical outcome and decreased compliance to therapy. Physical activity program could have beneficial effects on depression. No previous studies compared effects of a physical activity intervention on depression with health education alone in older MI patients. Thus, the hypothesis of this sub-study is to evaluate effects of a physical activity program on depression in older MI patients, compared to health education alone.

24.3 STUDY OBJECTIVES

24.3.1 Primary exploratory objective

- To test the effect of physical activity intervention on depression compared with health education alone in older patients 1, 2 and 3 years after MI

24.3.2 Main Secondary exploratory objectives

- To assess incidence and prevalence of depression after myocardial infarction in older MI patients
- To test if physical activity has an additional effect on depression in older MI patients with a previous diagnosis of depression being treated with drugs
- To assess transtheoretical model of behavior change in order to understand behavior change over time in the two different groups
- To assess if physical activity intervention increases compliance to therapy in older patients with depression after MI, compared with health education alone

24.4 STUDY DESIGN, SCREENING AND RANDOMIZATION

24.4.1 Study design

This is a sub-study of the PIpELINe randomized trial which enrolled older MI patients with moderately impaired physical performance assessed by SPPB at the moment of discharge and confirmed 1-month later during the inclusion visit; the main study provides a randomization of enrolled patients to physical activity intervention (6 supervised sessions and a home-based program) or health education alone in order to evaluate effects of physical exercise on 1, 2 and 3-year clinical outcomes. The present sub-study investigates incidence and prevalence of depression in older MI patients and the effect of physical activity on depression assessed by Beck Depression Inventory. In addition, transtheoretical model of behavior change is assessed. Patients of this sub-study are enrolled in all centers participating in the PIpELINe trial.

24.4.2 Screening

At least 200 patients (100 of the Physical Activity group and 100 of the Health Education group) enrolled in the PIpELINe trail are also enrolled in this sub-study. The present sub-study doesn't require any other specific inclusion/exclusion criterium, except of the proper written informed consent.

24.5 STUDY POPULATION

24.5.1 Inclusion criteria

1. Patients ≥65 years **AND**
2. MI (STE or NSTE-MI) with indication to invasive management **AND**
3. SPPB 4-9 1-month after hospital discharge **AND**
4. Informed consent

24.5.2 Exclusion criteria

1. Multivessel coronary artery disease or left main coronary artery disease candidate to surgical revascularization
2. Planned staged PCI
3. Non-cardiovascular co-morbidity reducing life expectancy to < 1 year
4. Any factor precluding 1-year follow-up
5. Severe aortic or mitral valvulopathy
6. Ejection fraction <30%
7. Chronic heart failure NYHA III-IV
8. Severe cognitive impairment (SPMSQ <4)
9. Impossibility to do physical activity due to physical impairment

24.6 STUDY PROCEDURES

24.6.1 Management Strategies

During the inclusion visit after randomization and at 1-year follow-up visits, in addition to the procedures provided by PIpELINe trial, the Beck Depression Inventory (BDI) is performed. Furthermore, transtheoretical model of behaviour change is assessed.

24.6.2 Privacy and Data sharing

Any data will first be fully anonymised. Data will not be made available for sharing until after publication of the main results of the study. Thereafter, anonymised individual patient data will be made available for secondary research, conditional on assurance from the secondary researcher that the proposed use of the data is compliant with scientific quality, ethical requirements and value for money. A minimum requirement with respect to scientific quality will be a publicly available pre-specified protocol describing the purpose, methods and analysis of the secondary research, e.g. a protocol for a Cochrane systematic review.

24.6.3 Follow up

In addition to procedures provided by PIpELINe trial, BDI is performed at 1-year-follow-up visit. All data are collected in the proper eCRF.

24.7 STUDY DEFINITIONS

24.7.1 Depression assessment

The following scale is used in order to assess depression in enrolled older MI patients.

Beck Depression Inventory: this questionnaire was created by Aaron T. Beck. It is a 21-question multiple-choice self-report inventory and it is one of the most widely used psychometric tests for measuring the severity of depression. Its development marked a shift among mental health professionals, who had until then, viewed depression from a psychodynamic perspective, instead of it being rooted in the patient's own thoughts. The original BDI, first published in 1961, consisted of twenty-one questions about how the subject has been feeling in the last week. Each question had a set of at least four possible responses, ranging in intensity. For example:

- (0) I do not feel sad.
- (1) I feel sad.
- (2) I am sad all the time and I can't snap out of it.
- (3) I am so sad or unhappy that I can't stand it.

When the test is scored, a value of 0 to 3 is assigned for each answer and then the total score is compared to a key to determine the depression's severity. The standard cut-off scores were as follows:

- 0–9: indicates minimal depression
- 10–19: indicates mild depression
- 20–29: indicates moderate depression
- ≥30: indicates severe depression.

Higher total scores indicate more severe depressive symptoms.

See below for the provided BDI.

Beck's Depression Inventory

This depression inventory can be self-scored. The scoring scale is at the end of the questionnaire.

1.
- 0 I do not feel sad.
- 1 I feel sad
- 2 I am sad all the time and I can't snap out of it.
- 3 I am so sad and unhappy that I can't stand it.

2.
- 0 I am not particularly discouraged about the future.
- 1 I feel discouraged about the future.
- 2 I feel I have nothing to look forward to.
- 3 I feel the future is hopeless and that things cannot improve.

3.
- 0 I do not feel like a failure.
- 1 I feel I have failed more than the average person.
- 2 As I look back on my life, all I can see is a lot of failures.
- 3 I feel I am a complete failure as a person.

4.
- 0 I get as much satisfaction out of things as I used to.
- 1 I don't enjoy things the way I used to.
- 2 I don't get real satisfaction out of anything anymore.
- 3 I am dissatisfied or bored with everything.

5.
- 0 I don't feel particularly guilty
- 1 I feel guilty a good part of the time.
- 2 I feel quite guilty most of the time.
- 3 I feel guilty all of the time.

6.
- 0 I don't feel I am being punished.
- 1 I feel I may be punished.
- 2 I expect to be punished.
- 3 I feel I am being punished.

7.
- 0 I don't feel disappointed in myself.
- 1 I am disappointed in myself.
- 2 I am disgusted with myself.
- 3 I hate myself.

8.
- 0 I don't feel I am any worse than anybody else.
- 1 I am critical of myself for my weaknesses or mistakes.
- 2 I blame myself all the time for my faults.
- 3 I blame myself for everything bad that happens.

9.
- 0 I don't have any thoughts of killing myself.
- 1 I have thoughts of killing myself, but I would not carry them out.
- 2 I would like to kill myself.
- 3 I would kill myself if I had the chance.

10.
- 0 I don't cry any more than usual.
- 1 I cry more now than I used to.
- 2 I cry all the time now.
- 3 I used to be able to cry, but now I can't cry even though I want to.

11.

0 I am no more irritated by things than I ever was.
1 I am slightly more irritated now than usual.
2 I am quite annoyed or irritated a good deal of the time.
3 I feel irritated all the time.

12.

0 I have not lost interest in other people.
1 I am less interested in other people than I used to be.
2 I have lost most of my interest in other people.
3 I have lost all of my interest in other people.

13.

0 I make decisions about as well as I ever could.
1 I put off making decisions more than I used to.
2 I have greater difficulty in making decisions more than I used to.
3 I can't make decisions at all anymore.

14.

0 I don't feel that I look any worse than I used to.
1 I am worried that I am looking old or unattractive.
2 I feel there are permanent changes in my appearance that make me look unattractive
3 I believe that I look ugly.

15.

0 I can work about as well as before.
1 It takes an extra effort to get started at doing something.
2 I have to push myself very hard to do anything.
3 I can't do any work at all.

16.

0 I can sleep as well as usual.
1 I don't sleep as well as I used to.
2 I wake up 1-2 hours earlier than usual and find it hard to get back to sleep.
3 I wake up several hours earlier than I used to and cannot get back to sleep.

17.

0 I don't get more tired than usual.
1 I get tired more easily than I used to.
2 I get tired from doing almost anything.
3 I am too tired to do anything.

18.

0 My appetite is no worse than usual.
1 My appetite is not as good as it used to be.
2 My appetite is much worse now.
3 I have no appetite at all anymore.

19.

0 I haven't lost much weight, if any, lately.
1 I have lost more than five pounds.
2 I have lost more than ten pounds.
3 I have lost more than fifteen pounds.

20.

0	I am no more worried about my health than usual.
1	I am worried about physical problems like aches, pains, upset stomach, or constipation.
2	I am very worried about physical problems and it's hard to think of much else.
3	I am so worried about my physical problems that I cannot think of anything else.

21.

0	I have not noticed any recent change in my interest in sex.
1	I am less interested in sex than I used to be.
2	I have almost no interest in sex.
3	I have lost interest in sex completely.

INTERPRETING THE BECK DEPRESSION INVENTORY

Now that you have completed the questionnaire, add up the score for each of the twenty-one questions by counting the number to the right of each question you marked. The highest possible total for the whole test would be sixty-three. This would mean you circled number three on all twenty-one questions. Since the lowest possible score for each question is zero, the lowest possible score for the test would be zero. This would mean you circles zero on each question. You can evaluate your depression according to the Table below.

Total Score	Levels of Depression
1-10	These ups and downs are considered normal
11-16	Mild mood disturbance
17-20	Borderline clinical depression
21-30	Moderate depression
31-40	Severe depression
over 40	Extreme depression

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25 APPENDIX G

Effect of Physical activity Intervention on mitochondrial function in ELderly after myocardial Infarction- a sub-study of the PIpELINe randomized clinical trial.

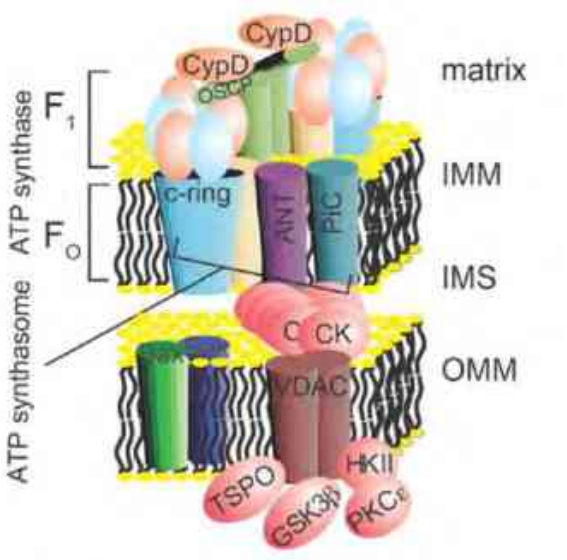
25.1 BACKGROUND

25.1.1 Mitochondrial function in myocardial infarction

Ischaemic heart disease represents the main cause of death in Western countries. With the aim of improving the outcome of patients with myocardial infarction (MI), the attention of numerous new studies is focused on the role of the cellular mediators. In particular, mitochondrial function is investigated in order to better understand mechanisms involved in reperfusion injury and recovery after MI. After myocardial reperfusion, an increase in myocardiocytes' intracellular calcium occurs, resulting in high level of contraction of the cells; this phenomenon is due to the damage produced by oxidative stress involving sarcolemma and the sarcoplasmic reticulum. The cytoplasmic calcium overload leads to a cascade of signals that culminates in the genesis of a variation of the mitochondrial membrane potential, which is generated at the opening of a protein complex between the inner and outer mitochondrial membrane, known as mitochondrial permeability transition pore (mPTP). The prolonged opening of this channel protein determines a sudden change in the mitochondrial membrane potential that is responsible of some crucial changes in the metabolism of the cells starting from a dysfunction of the electron transport chain, followed by an increased production of reactive oxygen species, an increased consumption of ATP and finally the activation of intracellular signals [1] that lead to the activation of caspases and the death of myocardiocyte. MPTP consists of various protein subunits, but only some of these have been identified. Piot et al. investigated the role of cyclophilin D demonstrating how the administration of ciclosporin at the time of reperfusion of a myocardial infarction inhibits this component of mPTP reducing infarct size [2]. Pinton et al. demonstrated that the inhibition of the C-subunit of FoF1ATPase is able to inhibit the formation of a mitochondrial transmembrane potential, probably since the opening of the mPTP is inhibited [3]. FoF1ATPase C subunit is detectable in serum from patients with MI. High FoF1ATPase C subunit serum levels in the early after MI correlate to

several myocardial reperfusion markers (including ST-segment resolution) and outcome in terms of death and heart failure [3]. Furthermore, the mitochondrial calcium uniporter (MCU), which is a calcium channel protein located at the level of the inner mitochondrial membrane, represents another key molecula involved in this complex process of intracellar signaling: it is able to regulate the influx of calcium into the mitochondria for the hydrogenation and ATP production [4]. It would seem that the alteration of the balance that regulates the intracellular signals generated between these proteins is responsible for ischemia-reperfusion damage which leads to poor recovery after MI.

mPTP structure.

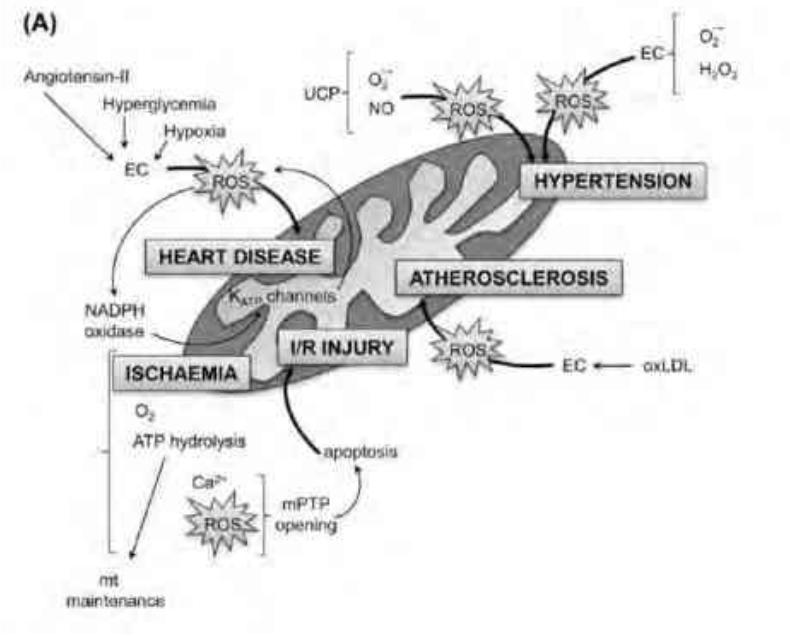


25.1.2 Mitochondrial function in older adults with cardiovascular diseases

Ageing is characterized by mitochondrial dysfunction and consequent increased reactive oxygen species (ROS) production which leads to cellular damage and death. The reactive oxygen-derived molecules can interact with cellular components, causing cumulative oxidative damage that may thus reduces life span. Oxidative damage to DNA genomes, proteins, and lipids has been associated with elevated ROS production, mitochondrial function impairment, and ultimately cell senescence or death. Aging has been also associated with a decline of antioxidant activity efficiency, which together with increased ROS production significantly contributes to an oxidative stress state. ROS can lead to the activation of pathways that control cell differentiation and apoptosis, which are mechanisms of particular relevance to cardiovascular diseases.

Indeed, mitochondrial oxidative damage has been implicated in a range of degenerative conditions that include cardiovascular diseases such atherosclerosis, hypertension, heart failure, and ischemia/ reperfusion (I/R) injury [5]. Age-related mitochondrial abnormalities are related to poor response to myocardial damage and a consequent poor recovery.

ROS, mitochondrial function and cardiovascular diseases in older adults



25.1.3 Physical activity and mitochondrial function

Physical activity increases antioxidant capacity in the heart by augmenting ROS scavenging enzymes such as catalase, superoxide dismutase, and glutathione peroxidase [6]. Heat- shock proteins (HSPs) play important role in cellular defense against oxidative stress. It was shown that physical training induced HSP70 and HSP27 expression in trained old rats compared with sedentary old and young rats partially counterbalanced the heart age-related effects in the antioxidant system without altering peroxidation levels [7]. The anti- oxidant action of exercise seems to be dependent on exercise protocol. While endurance exercise exacerbates oxidative stress and cardiac remodeling, acute or moderate aerobic exercise training preserves cardiac health and prevents remodeling by maintaining myocardial defense system [7]. These pre-clinical data need to be

confirmed by analyses in humans. Furthermore, no data are available about how mitochondrial function varies after MI in older patients participating in physical activity protocols.

25.2 RATIONALE

Mitochondrial proteins are involved in myocardial damage after MI that is related to poor recovery. Older MI adults could be more susceptible to this damage, because of increased mitochondrial dysfunction due to ageing. Physical activity demonstrated to be involved in a cardioprotective mechanism in animal models. No data are available about human models. No studies compared mitochondrial function in older MI adults participating or not to physical activity program. Thus, the hypothesis of this sub-study is to evaluate effects of a physical activity program on mitochondrial function in older MI patients, compared to health education alone.

25.3 STUDY OBJECTIVES

25.3.1 Primary exploratory objective

- To assess effects of physical activity on some gene involved in mitochondrial function (MCU, MICU1, c-subunit, FoF1ATPasi, ANT, VADC, Pic, Cyclophilin D) in older MI patients at 1 year follow up.

25.3.2 Main Secondary exploratory objectives

- To assess the levels of MCU, MICU1, C-subunit, FoF1ATPasi, ANT, VADC, Pic, Cyclophilin D
- To assess levels of C-subunit and FoF1ATPasi in serum and plasma of older MI patients
- To assess mitochondrial function analyzing fibroblasts obtained by skin biopsy.

25.4 STUDY DESIGN AND SCREENING

25.4.1 Study design

This is a sub-study of the PIpELINe randomized trial which enrolled older MI patients with moderately impaired physical performance assessed by Short Physical Performance Battery (SPPB) at the moment of discharge and confirmed 1-month later during the inclusion visit; the main study provides a randomization of enrolled patients to physical activity intervention (6 supervised sessions and a home-based program) or health education alone in order to evaluate effects of physical exercise on 1, 2 and 3-year clinical outcomes. The present sub-study investigates mitochondrial function in older MI patients and the effects of physical activity on mitochondrial function in these subjects. Patients of this sub-study are only enrolled in the center of Ferrara participating to PIpELINe trial, Cardiology Unit Ospedale S. Anna of Ferrara.

25.4.2 Screening

At least 30 patients (15 of the Physical Activity group and 15 of the Health Education group) enrolled in the PIpELINe trail are also enrolled in this sub-study. The present sub-study provides the PIpELINe inclusion criteria and the proper written informed consent, but it is characterized by some additional exclusion criteria as reported below. Baseline blood samples and skin biopsy are collected at the moment of discharge in older MI patients screened for the main study. Samples of patients actually included in the main study during the inclusion visit are finally analyzed. Blood samples are also collected during the 6-month clinical visit and at 1 year follow up visit. Skin biopsy is also collected at 1-year follow-up visit. Samples are analyzed by the Signal Transduction Laboratory (CUBO) of Ferrara.

25.5 STUDY POPULATION

25.5.1 Inclusion criteria

1. Patients ≥65 years **AND**
2. MI (STE or NSTE-MI) with indication to invasive management **AND**
3. SPPB 4-9 1-month after hospital discharge **AND**
4. Informed consent

25.5.2 Exclusion criteria

1. Multivessel coronary artery disease or left main coronary artery disease candidate to surgical revascularization
2. Planned staged PCI
3. Non-cardiovascular co-morbidity reducing life expectancy to < 1 year
4. Any factor precluding 1-year follow-up
5. Severe aortic or mitral valvulopathy
6. Ejection fraction <30%
7. Chronic heart failure NYHA III-IV
8. Severe cognitive impairment (SPMSQ <4)
9. Impossibility to do physical activity due to physical impairment
10. Any mitochondrial disease
11. History of tumors in the last 5 years
12. History of chemiotherapy in the last 5 years
13. Active malignancies

25.6 STUDY PROCEDURES

25.6.1 Management Strategies

In addition to the procedures provided by PIpELINe trial, at discharge a skin biopsy is performed and blood samples are collected: 20 ml of blood are collected in order to obtain serum and plasma for the analysis of C-subunit of FoF1ATP synthetasi. During the 6-month clinical visit, blood samples are collected. At 1-year follow-up visit, skin biopsy and 20 ml blood samples are collected.

25.6.2 Blood samples

Blood samples are collected from an antecubital vein using a 21-gauge needle. The first 2 to 4 mL of blood is discarded. The remaining blood is collected in empty tubes and, after 45 min, centrifuged at 1700 g at 4 °C for 15 min. The serum obtained is stored at −20 °C. Csub is measured in serum from patients with ELISA, using the human ATP synthase lipid-binding protein, mitochondrial (ATP5G1) ELISA kit (Cusabio Biotech, China). The ELISA is performed according to the manufacturer's instructions using strips of microtiter wells with a capture antibody, specific for Csub coated onto the wells of microplates. Because albumin and globulin are the most abundant proteins in human serum, which masked the expression of low molecular weight (LMW) proteins, we selectively remove them to enrich for proteins of lower abundance (among these, Csub at 8 kDa). So, serum samples are first handled to obtain a concentrated solution of LMW proteins by using Amicon Ultra-4 10K Centrifugal Filter Devices at 3000 g for 40 min (+4 °C) and then diluted 1:5 in phosphate-buffered saline (PBS) as kindly suggested by the supplier. In the details, ELISA assay is performed as follows. One hundred microliter samples are placed in the wells. After 2 h of incubation at 37 °C, the liquid is removed from each well. The strips are again incubated for 1 h at 37 °C after adding 100 µl biotin-antibody 1× to each well. The wells are then washed three times with wash buffer and after the last one 100 µl HRP-avidin 1× is added to each well and incubated for 1 h at 37 °C. The wells are washed five times as in the previous step and 90 µl TMB substrate is added. If the Csub protein is present, a blue colour develops between 15 and 30 min. The blue colour changes to yellow with the addition of 50 µl phosphoric acid, which is used to stop the reaction. The result can be read with the aid of a spectrophotometer and compared with the negative control

and the calibration curve supplied with the test kit. A spectrophotometer (450 nm) is used to obtain protein concentration duplicate readings. A computer software (MARS) capable of generating a four parameter logistic (4-PL) curve-fit is used to obtain concentrations as pg/ml protein. To standardized Csub values, pg/ml concentrations are converted into percentages on the basis of the highest value that the calibration curve is able to read. Intra- and inter-assay CV% were b8% and b10%, respectively. All analyses are performed by Signal Transduction Laboratory (CUBO) of Ferrara.

25.6.3 Skin biopsy

Skin biopsy is obtained at the moment of discharge and during 1-year follow-up visit from the forearm of enrolled patients by trained personnel. The frustule of tissue is introduced in a sterile container, pre-filled with an adequate quantity of preservative solution, following the most scrupulous aseptic technique, that is taking it from the clamp and making it fall inside it with the help of a sterile needle. Fibroblasts are isolated from the small frustule of tissue taken from the epidermis, dermis and hypodermis. All the analyses are performed by Signal Transduction Laboratory (CUBO) of Ferrara. Detailed procedure is explained in the Appendix I.

25.6.4 Privacy and Data sharing

Any data will first be fully anonymised. Data will not be made available for sharing until after publication of the main results of the study. Thereafter, anonymised individual patient data will be made available for secondary research, conditional on assurance from the secondary researcher that the proposed use of the data is compliant with scientific quality, ethical requirements and value for money. A minimum requirement with respect to scientific quality will be a publicly available pre-specified protocol describing the purpose, methods and analysis of the secondary research, e.g. a protocol for a Cochrane systematic review.

25.6.5 Follow up

In addition to procedures provided by PIpELINe trial, blood samples are collected at 6-month and 1-year-follow-up visit. Samples are analyzed by Signal Transduction Laboratory (CUBO) of Ferrara.

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26 APPENDIX H

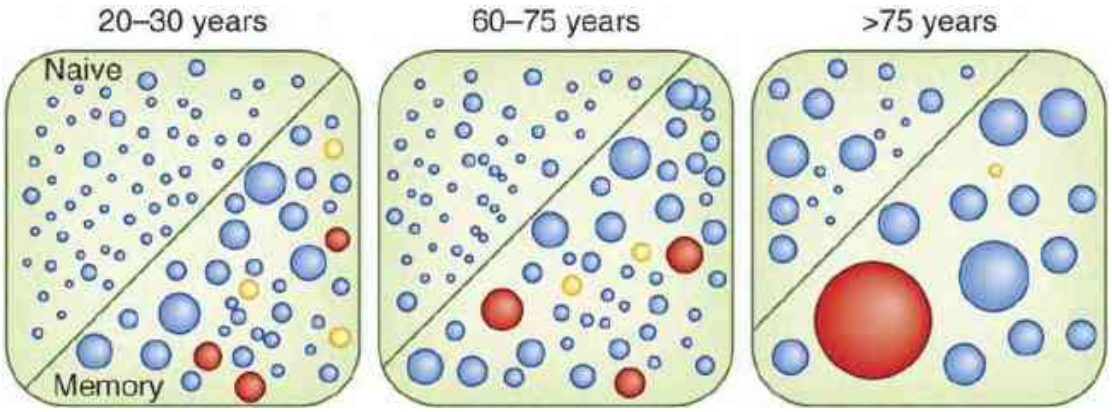
Effect of Physical Activity on T-Lymphocyte and miRNA function – a Physical activity Intervention in ELderly after myocardial INfarction (PIpELINe) sub-study

26.1 BACKGROUND

26.1.1 Immunosenescence

Immunosenescence is the loss of function of the main components of the immune system that occurs with age [1]. Previous studies highlighted the poor effectiveness of vaccines in older adults and the poor ability of older adults to control new infections and tumors [2]. On these bases a low reactivity of naïve T-lymphocyte subpopulation has been suspected [3]. Naïve T-lymphocytes are stimulated by neo-antigens and are able to generate long-term responses in order to defend from future infections. Several studies underlined that there is a dysfunction of naive T-lymphocyte in older adults [3]. The molecular mechanisms that explain this dysfunction are almost unknown. Data from literature demonstrated a straight relationship between lymphocyte function and cellular metabolic properties, which could be altered by ageing [4,5]. With this background, the identification of the fundamental metabolic pathways for lymphocyte activation is of paramount importance in order to find some means for the recovery of immune dysfunctions of older adults.

Immunosenescence and T-lymphocytes.



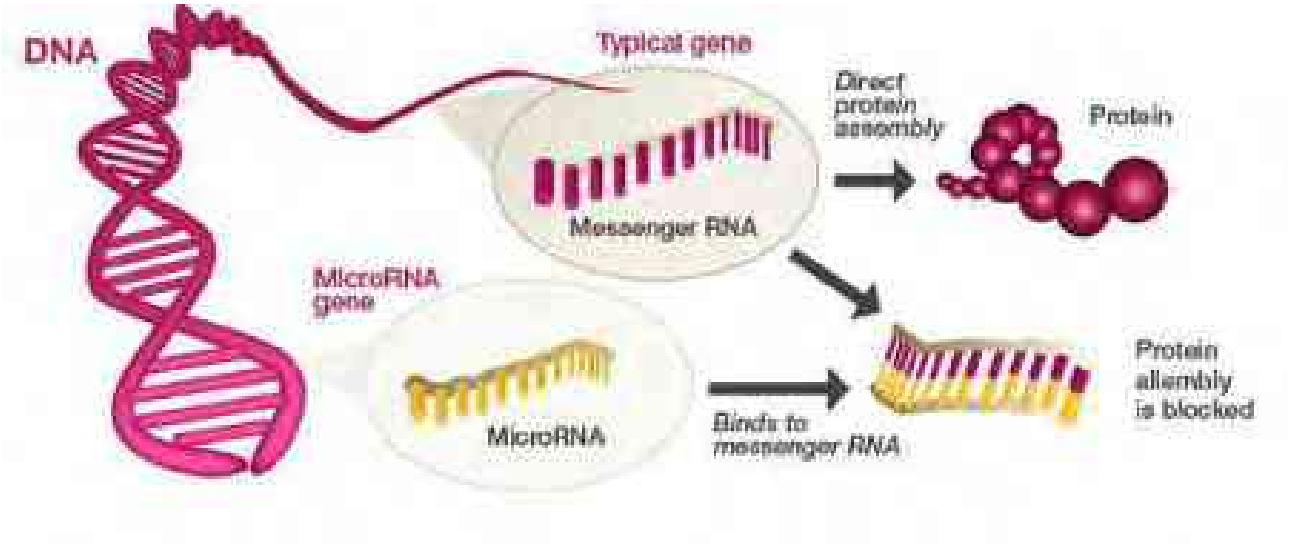
26.1.2 Physical activity and immunosenescence

Benefits of physical activity on the responsiveness of immune system have been largely demonstrated [6]. Previous studies showed that physical activity positively influences prevention, development and the course of inflammatory diseases and immune system degeneration. There are strong indications that physical exercise in older adults may prevent the age-related decline in immune response without side effects. Consequently, exercise is being considered as a safe means of intervention to reduce immunosenescence [6].

26.1.3 Micro ribonucleic acids (miRNAs) and myocardial infarction

Micro ribonucleic acids (miRNAs) are short ribonucleic acid sequences which do not encode a protein, but instead regulate the expression of several proteins. These molecula have been hailed as powerful novel biomarkers for a number of diseases, including coronary artery disease. Indeed, many studies have reported altered plasma levels of mRNAs in patients with established coronary artery disease. Endogenous miRNAs are crucial in regulating various aspects of cardiomyocyte biology, ranging from contractility to hypertrophy. Modulation of this miRNA network stands as an innovative therapeutic strategy in patients with myocardial infarction [7].

miRNA function



26.1.4 Physical activity and miRNA

Previous studies demonstrated that aerobic and resistence training determine substantial miRNA profile changes in patients with diabetes [8]. Other studies underlined that physical activity induced changes in the levels of miRNAs involved in lipid metabolism [9]. Mayr et al.demonstrated that there is a different expression of some miRNAs in patients with coronary artery disease at rest and also after exercise [10]. Data about physical activity and mRNA function in older patients with a recent myocardial infarction are lacking.

26.2 RATIONALE

Aging affects negatively the immune system, defined as immunosenescence, which increases the susceptibility of older adults to infection, autoimmune disease and cancer. Physical activity may prevent immune system disfunction and immunosenescence. However, there are no data about the effects of exercise on markers of cellular immunosenescence in older adults; in addition, no data are available about the comparison between immune system of older adults attenders of physical activity program and older adults that don't participate to physical activity intervention.

Effect of physical activity on mRNA function after myocardial infarction are still unknown.

Thus, this sub-study aims to evaluate immunosenescence and its metabolic pathways and presence and function of miRNAs in older adults and to assess the effects of physical activity on immune system and miRNAs compared to a group of older patients that do not attend physical activity intervention. In particular, the present sub-study is focused on two elements:

-T-lymphocyte function and cellular metabolic pathways which could be responsible of immune system decline

-miRNAs function which could be influenced by physical activity after myocardial infarction

26.3 STUDY OBJECTIVES

26.3.1 Primary objective

- To evaluate the impact of physical activity intervention on metabolic pathways and T-lymphocyte function and miRNAs in older MI patients

26.3.2 Main Secondary objectives

- To assess the metabolic properties (glucose up-take, fatty acids up-take, mitochondrial potential) and the function of the different subpopulations (naïve, central memory, effector memory) of T-lymphocytes (CD8+ and CD4+) in older MI patients
- To test the lymphocyte response to stimulation in terms of release of cytockines and markers' expression
- To test the CD8+ response to neo-antigens in subjects HLA-A2+ using the antigen of melanoma HLA-A2 restricted
- To test the CD8+ response to neo-antigens in subjects HLA-A2+ using the antigens of EBV, CMV and HSV expressed by HLA-A2
- To quantify different types of miRNAs before and after a program of physical activity

26.4 STUDY DESIGN AND SCREENING

26.4.1 Study design

This is a sub-study of the PIpELINe randomized trial which enrolled older MI patients with moderately impaired physical performance assessed by Short Physical Performance Battery (SPPB) at the moment of discharge and confirmed 1-month later during the inclusion visit; the main study provides a randomization of enrolled patients to physical activity intervention (6 supervised sessions and a home-based program) or health education alone in order to evaluate effects of physical exercise on 1, 2 and 3-year clinical outcomes. The present sub-study investigates two cellular aspects:

-cellular metabolic pathways on immunosenescence and the effect of physical activity on immune system function, focusing on T-lymphocytes;

-presence and activity of miRNA in the blood of older patients with a recent MI and the effect of physical activity on these molecula.

Patients of this sub-study are enrolled in the only main participating center, Cardiology Unit of Ospedale S. Anna of Ferrara.

26.4.2 Screening

At least 50 patients (25 of the Physical Activity group and 25 of the Health Education group) enrolled in the PIpELINe trail are also enrolled in this sub-study. The present sub-study doesn't require any other specific inclusion criterium, except of the proper written informed consent. It requires some other exclusion criteria, as shown below.

26.5 STUDY POPULATION

26.5.1 Inclusion criteria

1. Patients ≥65 years **AND**
2. MI (STE or NSTE-MI) with indication to invasive management **AND**
3. SPPB 4-9 1-month after hospital discharge **AND**
4. Informed consent

26.5.2 Exclusion criteria

1. Multivessel coronary artery disease or left main coronary artery disease candidate to surgical revascularization
2. Planned staged PCI
3. Non-cardiovascular co-morbidity reducing life expectancy to < 1 year
4. Any factor precluding 1-year follow-up
5. Severe aortic or mitral valvulopathy
6. Ejection fraction <30%
7. Chronic heart failure NYHA III-IV
8. Severe cognitive impairment (SPMSQ <4)
9. Impossibility to do physical activity due to physical impairment
10. Diseases of hematopoietic system
11. Malignancies
12. Metabolic diseases

26.6 STUDY PROCEDURES

26.6.1 Management Strategies

During the inclusion visit after randomization, at 6-month and 1-year follow-up visits, in addition to the procedures provided by PIpELINe trial, 3 venous blood samples are collected in all patients enrolled in this sub-study Two blood samples are used to obtain purified T-lymphocyte and assess their expression of HLA-A2; the samples are anonymized and stored in the laboratory of Dipartimento di Scienze Chimiche e Farmaceutiche dell'Università di Ferrara. The other 1 blood sample is used to evaluate presence and function of miRNA and they are anonymized, analysed and and stored in the Laboratorio di Biologia Cellulare e Genetica Molecolare of Dipartimento di Morfologia, Chirurgia e Medicina Sperimentale of Ferrara.

26.6.2 Blood samples for the analyses of T-lymphocytes

Blood samples are obtained from an antecubital vein using a 21-gauge needle. The first 2–4 mL of blood are discarded. The remaining is analysed in order to evaluate T-lymphocytes. The cells are obtained by standardized protocols of purification with Ficoll-Pacque gradient. Then, the cells are frozen in a solution containing for a 90% fetal bovine serum (FBS) and for a 10% dimetilsofosside (DMSO) as a preservative component. Finally, they are stored in liquid nitrogen containers.

26.6.3 Blood samples for the analyses of miRNA

Blood samples are obtained from an antecubital vein using a 21-gauge needle. The first 2-4 mL of blood are discarded. The remaining is analyzed in order to quantify miRNAs and evaluate their function. Patients don't have to perform physical activity in the 72 hours before the collection of blood samples. The samples are centrifugated at room temperature; the supernatant is then aliquoted in fractions of 500u uL, stored in little tubes free from RNAsi and finally analyzed.

26.6.4 Privacy and Data sharing

Any data will first be fully anonymized. Data will not be made available for sharing until after publication of the main results of the study. Thereafter, anonymized individual patient data will be made available for secondary research, conditional on assurance from the secondary researcher that the proposed use of the data is compliant with scientific quality, ethical requirements and value for money. A minimum requirement with respect to scientific quality will be a publicly available pre-specified protocol describing the purpose, methods and analysis of the secondary research, e.g. a protocol for a Cochrane systematic review.

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27 APPENDIX I

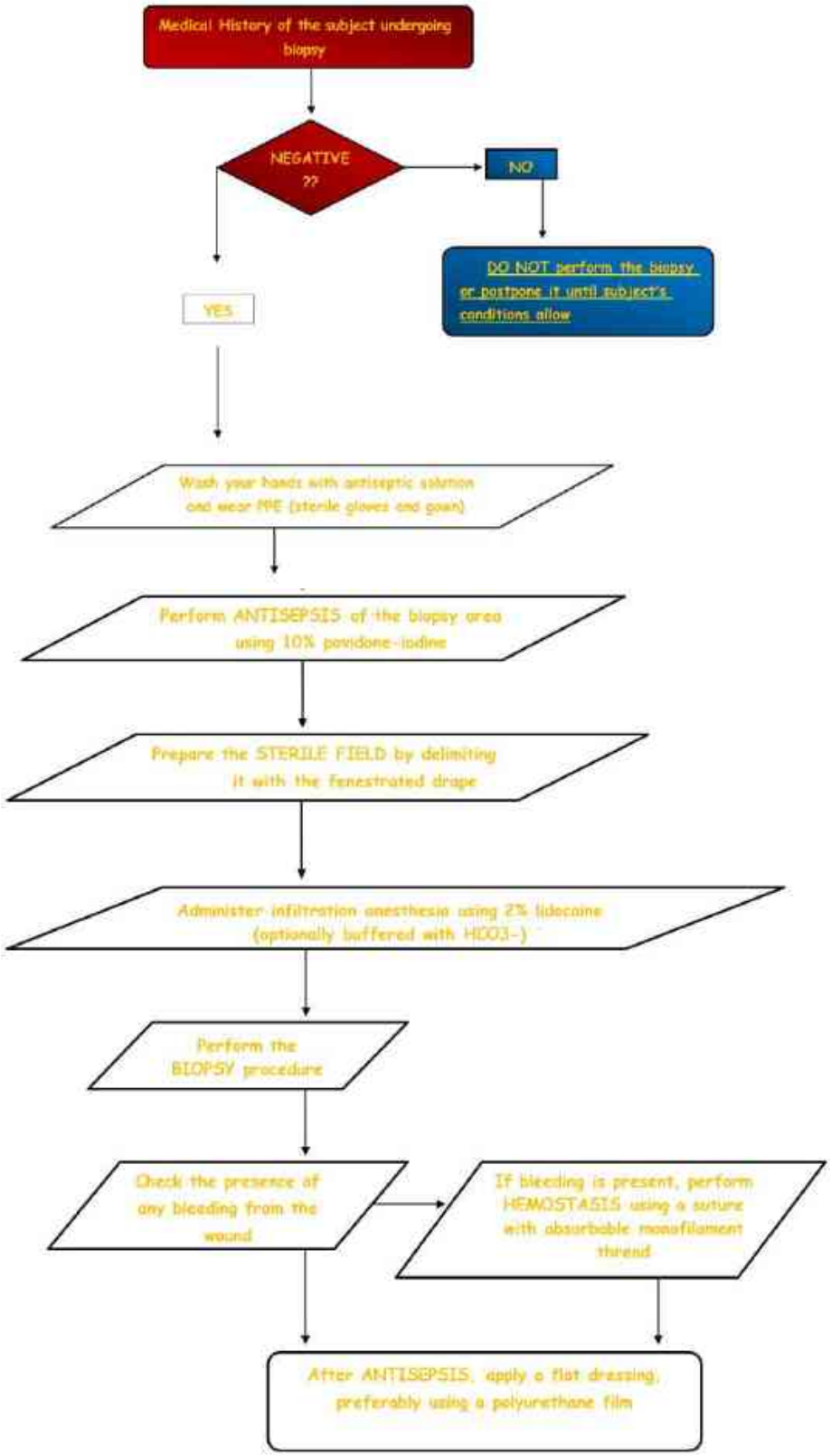
Standards for cutaneous biopsy

27.1 DESCRIPTION

Since this is a surgical procedure involving the use of a local anesthetic, operators performing the biopsy must have immediate access to all necessary resources to manage potential adverse reactions in the subject, such as lipothymia, syncope, or anaphylactic/anaphylactoid reactions. Before commencing the procedure, the operator must take appropriate precautions to minimize the risk of adverse events, such as anaphylaxis and infections, for the subject undergoing the biopsy. To reduce the likelihood of anaphylaxis, it is essential for the operator to conduct a thorough medical history review. This should include an assessment of any underlying conditions affecting the volunteer, the use of medications for their treatment, and the administration of local anesthetics during past or recent dental procedures or minor surgeries, which could suggest allergic reactions or sensitization. The study underscores the critical importance of vigilance and preparedness to ensure the safety of participants and the success of the procedure. After performing a thorough antiseptic hand wash, the operator dons the necessary personal protective equipment (PPE) for both their own protection and that of the patient (sterile gloves and gown). The operator then performs adequate antisepsis of the area to be biopsied using 10% povidone-iodine and delimits the sterile field with a sterile disposable fenestrated surgical drape. Using a syringe with a hypodermic needle, the operator administers local anesthesia via infiltration with 2% lidocaine (potentially buffered with bicarbonate) and proceeds to the actual biopsy using a sterile disposable punch. The tissue sample obtained in this manner should be placed in the sterile container, pre-filled with an appropriate amount of preservation solution, following the strictest aseptic technique. Specifically, the tissue should be carefully transferred from the forceps and allowed to fall into the container with the assistance of a sterile needle. At the end of the procedure, after performing adequate post-surgical antisepsis on the biopsy site, the operator covers the area with a flat dressing, preferably using a transparent polyurethane film if available. The follow-up on the healing of the surgical wound, which is expected to heal within

approximately 10 days, is the responsibility of the personnel who performed the biopsy. They will monitor the wound's condition over time and, in the event of any infectious or inflammatory complications, will recommend the most appropriate local or systemic medication, as well as any additional measures that may be necessary.

27.2 FLOW CHART



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