Title	**Effects of Shift Work on Health: Assessment of Sleep Quality, Motor Control and Cardiovascular Risk**
Sponsor	**IRCCS Orthopedic Institute Galeazzi di Milano**
Principal investigator (PI)	**Prof. Fabio Esposito**
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INTRODUCTION AND RATIONALE OF THE STUDY

Shift work can exert numerous effects on the temporal and behavioral organization of the individuals. Working hours of shift workers, which are outside the normal daily social program, lead to a circadian desynchronization due to a temporary misalignment between working time and physiological/behavioral functions, similar to what is observed in the jet lag syndrome. This may lead to deterioration in the sleep quality, to a reduction in the working ability during waking hours, with the occurrence of sleepiness and reduction in the vigilance state.

Over the years, the impact of shift work has invested many areas and its effects have been investigated at the cardiovascular (coronary artery disease, hypertension), metabolic (diabetes and obesity) and immunological level. Several works provided evidence of the shift work-induced negative results on health, including carcinogenic effects. Consequently, the scientific community paid to these problems great attention.

In analyzing the health effects of shift work, the workers circadian typology (chronotype) is very important. In fact, the circadian rhythms represent a dimension of the human personality that should not be underestimated. The human being presents a temporal organization, determined by the interaction of endogenous and environmental factors, and organizes most of the biological and behavioral activities according to a twenty-four hour period and in sync with the light-dark cycle. We must also bear in mind that the biological rhythms in man present interindividual differences that determine precisely the chronotype, which is the tendency to express preferences toward morning or in the evening activities. Within the population, it is possible to recognize individuals that can be traced to three circadian types: (i) morning-types subjects (M-Types) that tend to be more active and efficient in the first part of the day, (ii) evening-types subjects (E-Types) who find it difficult to get up in the morning and require more time to reach the optimal level of physical and mental efficiency, and (iii) intermediate subjects (Neither-Types, N-Types) that have intermediate characteristics between the previous two.

Previous studies suggested that the eveningness could determine an easier adaptability to the changes determined by shift work. However, the role of the chronotype on this aspect is yet to be related to the type of shift: on one side the E-Types tend to have more sleep disorders induced by a day shift, on the other side the M-Types tend to adapt worst to a night shift. In any case, shift work determines a growing sleep debt that can have a not negligible impact on the wellbeing and health of the individual.

The association between shift work and cardiovascular risk is very interesting. Sleep at night, in fact, can have important effects on blood pressure; some studies have shown that a good sleep quality may have potential effects in the prevention of hypertension. Arterial pressure decreases by an average of 10-20% during nighttime hours, so sleep debt could lead to higher average blood pressure over the course of twenty-four hours. In addition, by modifying the circadian rhythms, the shift may lead to an alteration of the autonomic nervous system regulation with hypertensive consequences.

AIMS

Primary objective: In relation to the participants' chronotype, the objective will be to evaluate the effect of irregular working hours on the quality of sleep, on the circadian rhythm of activity levels and on motor control, to identify the type of shift with less impact on the state of health of the hospital staff.

Secondary objective: Evaluations of heart rate variability, circadian rhythm of activity levels, sleep quality and motor control for the definition of alterations related to the effect of the shift.

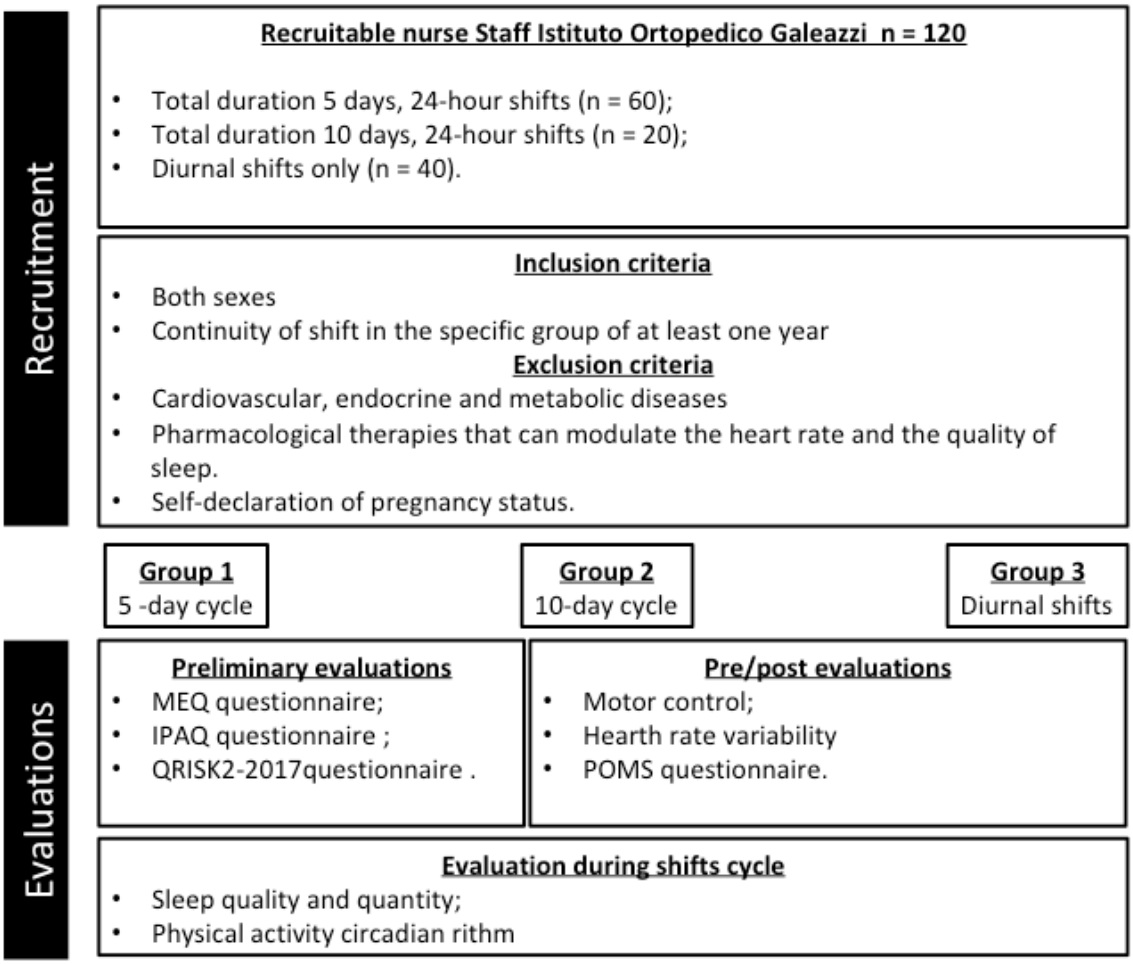
STUDY DESIGN

The project involves the recruitment of nursing staff of the IRCCS Galeazzi Orthopedic Institute of Milan, which is subjected to three types of shift:

- GROUP 1: total duration 5 days, 24-hour shifts;
- GROUP 2: total duration 10 days, 24-hour shifts;
- GROUP 3: diurnal shifts only (control).

The experimental design is schematized as shown in Figure 1.

Figure1: Experimental design.



Upon recruitment, all participants will undergo to the following evaluations (see Figure 2):

- Questionnaire Horne-Ostberg Morningness-Eveningness Questionnaire (MEQ) for the determination of the chronotype (Horne JA, Ostberg O. 1976);
- International Physical Activity Questionnaire (IPAQ) for the assessment of the level of physical activity;
- QRISK2-2017 questionnaire for cardiovascular risk assessment (Hippisley-Cox J, 2010; Hippisley-Cox J, 2008).

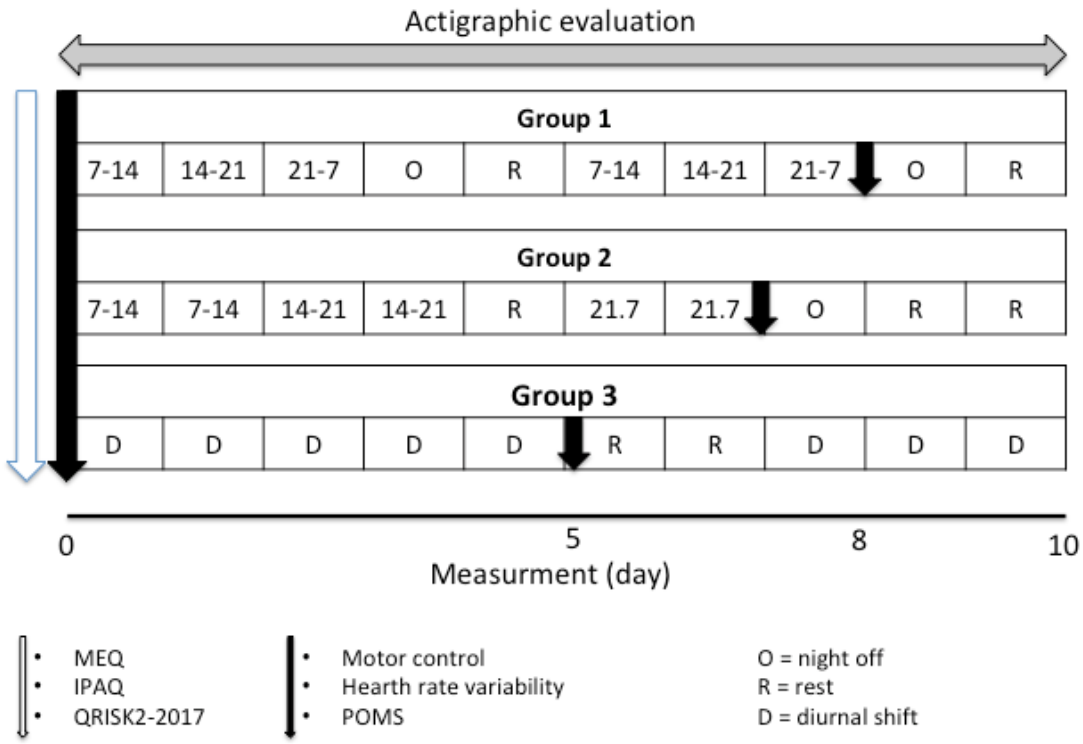
All the questionnaires are validated in Italian except the QRISK2-2017, which will be administered by medical personnel.

During the entire duration of the shift, all participants will be subjected to actigraphic monitoring to assess the quality of sleep and circadian rhythm activity-rest. Monitoring will start from the first day of the shift and will continue for 10 consecutive days for all three groups. During the entire duration of the monitoring the participants will have to fill in a diary on which they will note the hours of alarm, of falling asleep and any intervals of time during which the actigraph will be removed.

At the beginning, after 5, 7 and 8 days of shift for GROUP 3, GROUP 2 and GROUP 1, respectively, participants will undergo to:

- Evaluations of the motor control and level of neuromuscular activation of the finger flexor muscles;
- Hearth rate variability analysis by monitoring heart rate;
- Profile of Mood States (POMS) questionnaire for the determination of the mood profile.

Figure 2



The study will be conducted according to the indications of the Good Clinical Practices.

Duration and timing of the study

- Study start date: January 2018
- Recruitment time: 20 months starting from January 2018.
- End of the study: December 2019
- Final report: At the end of each year a report will be prepared on the progress of the study.

POPULATION

The population consists of about 120 potential participants recruited within the nursing staff belonging to the Galeazzi Orthopedic Institute, of which 60 potentially belong to GROUP 1, 20 to GROUP 2 and 40 to GROUP 3.

Inclusion criteria: Both sexes who have a continuity of shift in the specific group of at least one year will be included in the study.

Exclusion criteria: All participants will be excluded from the project with documented presence of cardiovascular, endocrine and metabolic diseases and / or pharmacological therapies in place that can modulate the heart rate and the quality of sleep. Participants with self-declaration of pregnancy status will also be excluded from the study.

Participation and completion of the study: The participant is considered enrolled in the study when providing written informed consent. No study procedure will be initiated before the Informed Consent is signed. Participation in the study may end before its completion for the reasons described in the dedicated section. The whole study ends when the last subject has completed the study or has been withdrawn from the study.

Interruption of the participation to the study: Participants have the possibility to withdraw from the project at any time. In the latter case, personal data will be completely erased while the scientific data collected until the time of the possible withdrawal, will be used anonymously for purely scientific purposes. Participants must promptly notify the inability to continue the study.

The study does not provide for follow-up but some samples or evaluations could be lost. Any participants lost during the study will not be replaced.

CLINICAL EVALUATIONS

Evaluation of the chronotype: the administration of the MEQ questionnaire will allow, on the basis of the scores obtained, dividing the sample into the three corresponding chronotypes (M-Types, N-Types to E-Types). Morninginess/eveningness can have a major impact on the well being of the individual through sleep-related influences, as it interferes with normal daily functions.

Assessment of the level of physical activity: the IPAQ questionnaire includes 4 sets of questions that investigate four different aspects of the level of physical activity in relation to health.

Assessment of the psychological state: the POMS questionnaire identifies and quantifies affective states such as tension, depression, aggression, vigor, fatigue, and confusion. These six aspects prove to be particularly useful for assessing the subject's state of stress, fatigue and energy.

Cardiovascular risk assessment: The extent of cardiovascular risk will be calculated in all participants via QRISK2-2017 questionnaire. The questionnaire requires the inclusion of parameters such as gender, ethnicity, age, weight and height, smoking and relative frequency, the presence of diabetes, hypertension, episodes of angina, fibrillation, rheumatoid arthritis, chronic diseases in the kidney, cholesterol / HDL ratio and systolic blood pressure. The information will then be inserted into a special algorithm (https://qrisk.org), which will return the percentage of probability of risk involved in a cardiac problem in the next 10 years.

Evaluation of force and motor control: To evaluate the possible variations of force and motor control, the maximal voluntary isometric force (MVC) of the flexor muscles of the dominant hand fingers and their activation will be evaluated in all participants. Once the MVC has been identified, sub-maximal contractions will be required at 20, 40, 60 and 80% MVC, each lasting 20 seconds. During contraction, in addition to the force signal (model SM-2000 N, Interface, Scottsdale, UK) the surface electromyographic signal (sEMG) will be detected by the flexor muscles of the fingers. The analysis of the force signal will be evaluated (i) the ratio between the standard deviation of the force signal during the plateau phase and the mean of the signal itself (coefficient of variation, CV), as an index of stability of the muscle contraction and (ii) the distance of the force signal with respect to the target (DF) as an index of accuracy. The electromyographic signal will be analyzed in the time and frequency domain: during the maintenance period of the force plateau, the quadratic root mean (root mean square, RMS) and the mean frequency (mean frequency, MF). The temporal trend of the two signals will be used as markers of neuromuscular fatigue.

Assessment of hearth rate variability (HRV): The study of HRV consists in the measurement and analysis of the heart rate, in order to deduce some important information such as assessing the risk of cardiac and heart attack arrhythmias or the balancing of the activity between the sympathetic and parasympathetic nervous system (sympathovagal balance). The heart rate will be measured at rest for duration of at least ten minutes through a heart rate monitor (Polar mod 810, Polar, Finland), which allows R-R detection of the heart rate. The measurement will be performed with the participants in a supine position and according to the guidelines of the American Heart Association. The recordings will be analyzed in the time and frequency domain using software (Kubios, http://kubios.uef.fi). In the time domain the mean quadratic root of the standard deviation of all the intervals between the QRS complexes will be calculated (RMSSD). The size of the RMSSD is an indication of the activation of the Parasympathetic Nervous System. In the frequency domain the powers of the frequencies included between 0.01 and 0.4 Hz will be calculated. In this case, the Low Frequency (LF, between 0.04 and 0.15 Hz) will be calculated, considered a marker of activation of the sympathetic nervous system and the activity of regulation of the baroreceptors; the High Frequency, between 0.15 and 0.4 Hz), considered as an expression of the activity of the parasympathetic nervous system and influenced by the frequency and depth of respiration. The LF/HF ratio is generally used as a balancing index between the sympathetic and parasympathetic nervous system.

Assessment of the sleep quality and the circadian rhythm of physical activity levels: for this evaluation we will use the use of actigraphs. Actigraphy is a method used to evaluate the circadian rhythm of activity levels and sleep parameters. The Actiwatch Software will be used to obtain activity data, expressed in activity counts and recorded for each minute throughout the monitoring period. For each subject, the data will be processed to evaluate the circadian rhythm of activity levels. The software for sleep analysis allows to extrapolate some parameters indicative of the quantity and quality of sleep: (i) Sleep Efficiency (SE), percentage of time spent in bed with actual sleep; (ii) Assumed Sleep (AS), difference between beginning

and end of sleep; (iii) Sleep Latency (SL), period of time between bed and sleep; (iv) Movement and Fragmentation Index (MFI), percentage of time spent moving indicative of the fragmentation of sleep and (v) Immobile Time (IT) total time spent without movement, between sleep from start to sleep.

To determine the circadian rhythm in the three chronotypes, the data relating to the activity provided by the actigraph would be analyzed using the single Cosinor method, which identifies and evaluates the cosinusoidal mathematical function that it is better suited to data as a function of time. The function defines three parameters that are characteristic of each rhythm: MESOR (Midline Estimating Statistic of Rhythm), amplitude and acrophase. The rhythmic parameters of the activity levels will be elaborated with the Cosinor Population, in order to evaluate the rhythmic characteristics of the levels of activity for each chronotype.

EVALUATION AND REGISTRATION OF ADVERSE REACTIONS

Safety and evaluation of adverse reactions in relation to the techniques used Participating subjects will be closely monitored for possible side effects.

Criteria for suspension of the study: Suspension from the study is planned based on the doctor's assessment in the cases specified above. Participants will be informed in the consensus session that participation in the study can be interrupted at any time.

Data collection for retired participants: Even if participants can withdraw from the study, undesirable effects will be recorded.

Risk/Benefits Report: As no invasive measures are planned within the study, participants will not be exposed to particular risks. The results will provide useful information to be able to reason on any revaluations of the shift system by making any changes.

STATISTICAL ANALYSIS

The sample size was calculated (Sigmaplot mod software 12.5) on the basis of the results of a previous study (Martin et al., 2015), which is structured with an experimental design similar to that proposed in the present project. In consideration of the main objective of the project, the sleep efficiency was identified as the main outcome. An analysis of variance (ANOVA) model, a minimum detectable change percentage of 5%, a residual standard deviation of 6.9 and a number of groups equal to 3 were considered for sample size quantification. On these basis, a number of 37 participants would guarantee a statistical power> 0.80 with an α value <0.05.

A Shapiro-Wilk test will be applied to check the normal distribution of the sample.

A linear mixed ANOVA with Bonferroni correction, with a Tukey-Kramer post-hoc test will be applied to estimate the effects of the work shift on the different chronotypes (quality of sleep, circadian rhythms of activity levels, motor control, cardiovascular risk and mood profile). In the opposite case, the Box and Cox method will be used as non-parametric approaches.

MONITORING, VERIFICATION, STUDY INSPECTION

Study insurance and monitoring: Given the non-invasive nature of the procedures envisaged by the study, monitoring activities and the stipulation of an ad hoc insurance policy are not considered necessary.

Verification and inspection: The main investigator will allow monitoring, verification and inspections related to the study by the Ethics Committee, the promoter, the bodies required by law, the quality assurance commissions of all documents. The main investigator will ensure the possibility of inspections if applicable to the structures connected to the study. Participation as a principal investigator in this study implies acceptance of a possible inspection by the competent authorities and university offices for quality assurance.

ADHERENCE TO ETHICAL, REGULATORY AND ADMINISTRATIVE FACTORS

Ethics Committee: This study will be conducted according to the ethical principles of the Helsinki Declaration and the indications of good clinical practice, in compliance with regulatory requirements and legal obligations. By subscribing this protocol, the experimenters agree to comply with these requirements and to conduct the study diligently and efficiently and in accordance with this protocol.

This protocol and any amendments will be submitted to the EC in accordance with the legal obligations for formal approval of the study. The EC decision on the conduct of the study will be written to the coordinator and a copy of this decision will be provided to the promoter's representative before the start of the study.

Written informed consent is required for all participants.

Information to the participants and their consent: Complete information on the study and details of the protocol must be discussed with each potential subject, and an informed written consent must be obtained for all participants before carrying out any procedure or registration related to the study.

In order to be registered, the participants give consent to the processing of personal data in anonymous and aggregate form, in accordance with the Guidelines of the Data Protection Authority of 24/07/2008 on the protection of persons and on the processing of personal data.

All the participants of the present study will receive a Consent Form that describes the study and provides the main information to express an informed decision about their participation. The professional will explain in detail, in an individual session, all the procedures. The information must be provided in a language and in terms that are understandable to the subject and at an appropriate time. The researcher must keep the consent

form, also signed by the designated and dated experimenter, as part of the study records. A copy of the consent, signed and dated, must be delivered to the subject. The Consent Form must include all the required elements of informed consent according to regulations. Furthermore the consent form must identify the promoter or his representative. The consent form and the explanation will include: detailed information on the methods of investigation, on the rationale for which the study is carried out, on the potential effects and risks, on the emergency and safety procedures. Detailed information on data collection will be described. The understanding of the consent form will be ascertained with specific questions. The participants will be reassured that their participation is voluntary and that the withdrawal from the study will have no influence on the treatment and on the relationship with the careers. All participants will be informed about the potential risks and benefits of being involved in the study. Participants may request suspension from the study and the assessments will be returned. The Ethics Committee must approve the consent form and the sponsor before the study starts at any location. Any changes subsequent to the approved informed consent form must be reviewed and approved by the Ethics Committee before use.

Privacy - Anonymity and confidentiality of data: The information relating to the participants of the study will be kept confidential and managed according to the relevant provisions (D.L.196 / 30 June 2003, Guidelines of the Data Protection Authority of 07/24/2008). A signed written authorization is required to inform the subject about the personal health information collected by the participants in the study, who will have access to this information and why who will use or disclose this information to whom the data and reasons will be communicated for this communication and, finally, the right of the participants of the study to revoke the authorization to use their personal health information. For the purposes of the research anonymity will be guaranteed, through the use of identification codes.

Data confidentiality: By subscribing this protocol, the investigator declares to the promoter that the information provided to the investigator will remain confidential and that such information will be disclosed to the Ethical Committee or similar commissions only under appropriate protection of confidentiality. The data obtained from this study will be considered confidential by the researcher, except for what is included in the publications, as per the relevant section.

Confidentiality of patient documentation: By signing this protocol the researchers agree that the promoter, the Ethics Committee, can consult and / or copy the study documents in order to verify the data of the CRF. By signing the informed consent forms, the participants consent to this process.

The data of the participants will be kept and the information will not be disclosed to third parties. The information relating to the participants of the study will be kept confidential and managed according to the relevant provisions (D.L.196 / 30 June 2003). To guarantee the anonymity, the subject will be identified only by an identification code; the full name or initials or other personal identifying elements will be masked. The decoding list of the patient code will be present only at the IRCCS Galeazzi in Milan.

Case Report Form (CRF) - Data collection form: A data collection form or CRF (Case Report Form) of the subject will be completed for all participants who have given informed consent and participate in the study. The CRF is the main tool for data collection for the study. All required data must be recorded.

DATA MANAGEMENT

Conservation and security of collected data and research results: The data of the participants will be kept and the information will not be disclosed to third parties. The data will only be accessible to the data manager and to the personnel who for their role within the study have operational needs or data entry (Principal Investigator and designated investigators). It is the responsibility of the principal investigator to keep the essential documents and data resulting from the study for at least 10 years after its conclusion.

Publications and other rights: The possibility of producing manuscripts and abstracts is foreseeable. However, neither the results obtained from this study protocol, complete or partial, nor any information provided by the promoter for the purpose of this study will be published or referred to third parties without his consent. The main investigator undertakes to publish the results according to the agreements contained in this section and will be the main responsible for the publication of the first results of this study. The principal investigator has the right to publicly publish or present the results of the study in accordance with this section of the protocol.

The principal investigator is entitled to use the results of all the work produced by the principal investigator according to this protocol, including, but not limited to, the test results and any raw and statistical data obtained solely for teaching purposes by the principal investigator, of research. The principal researcher consents that, on his part, his own employees, managers, collaborators and representatives, data will not be disclosed except by written authorization.

It is the responsibility of the principal investigator to send the end of study report to the competent authorities.

FINANCING OF THE STUDY

Source of financing: The costs of the study will be supported by the Research Department of the Ministry of Health year 2017.

Compensation to the participants: There is no financial compensation for the participants participating in the study. Participation is voluntary.

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