Study Protocol

Title:

Regional registry-based biobank development and pharmacogenetic analysis: synergistic strategies driving towards personalized medicine in Rheumatoid Arthritis management-PRUA1GR-2013-00000203 (RABiobank)

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Number of patients Involved in the study:

Biobank creation - the total number of patients is not predictable, as it depends on the number of patients that will be eligible for inclusion in the Emilia Romagna Regional Registry of Rheumatoid Arthritis (Reuma Registry).

Pharmacogenetic Analysis - 600 patients

1.8 Sintesi delle premesse teoriche dello studio:

Rheumatoid arthritis (RA) is highly heterogeneous disease with a clinical spectrum ranging from mild and self-limiting diseases to severe and progressive joint destruction leading to disability and great impairment of quality of life, associated to a wide variability in therapeutic response(1).

Treat-to-target paradigm in RA arises by the evidence that early and effective treatment has been recognized a successfully strategy for slowing/halting RA progression. Even if this model is greatly acknowledged to optimize early arthritis management, it remains one of the main challenge in clinical practice(2).

Indeed, despite the availability of highly effective treatment options a sizeable proportion of patients fail to adequately respond to treatment(3).

Over the last years, a growing number of biological agents has become available on the market.

A mounting body of evidence testifies to the effectiveness of these agents in controlling the clinical manifestations and in preventing disease progression both in disease-modifying antirheumatic drug (DMARD)-naive patients and in those that have failed previous DMARD therapy(4).

Nevertheless, data derived by clinical trials and by register-based observational study indicate that only 50-70% of RA patients responds successfully when treated with biologics(5-7). In addition, potential serious toxicity of these drugs has been observed(8). Therefore the identification of response predictors might strongly impact on clinical decision-making process so improving patient quality of life and the cost-benefit performance.

To address this unmet need, pharmacogenetic approach might open promising perspectives, increasing our understanding on genetic-related drug efficacy/toxicity, and providing decision-making tools for therapy optimization(9). Nevertheless, to ensure feasibility and reliability of translational applications of this strategy in clinical practice, large population-based research represents a crucial key step in order to validate the hypothesis of a correlation between genetic variation, environment and drug-related effect(10).
In these context, availability of register-based database and accessibility to a large-scale biobank are essential and primary tools to combine large collections of standardized disease-specific biological samples with comprehensive anthropometric, clinical and life-style information, providing a unique and exclusive opportunity to define disease-related molecular and genetic signature.

In the region Emilia-Romagna, regional RA registry has been established in 2010 to monitor usage, efficacy and safety of biological treatments for RA. Taking advantage of this available resource an ongoing prospective population-based study (granted by Italian Ministry of Health-Ricerca Finalizzata 2009, RF-2009-1549144), has been designed in order to improve the quality and effectiveness of the healthcare for patients with inflammatory arthropathies.
Therefore, the development of a register-based biobank represents a key resource and a paramount added value fostering large-scale and high-quality basic and clinic research studies and enabling a wide variety of technical approaches including molecular genetics, proteomic, serologic and genomics investigations.

Disease-based biobank and registry, as combined strategies, should be considered extraordinary opportunities to target the practice of personalized medicine, that remain one of the main challenge for Healthcare System

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Aim of the study

The main purpose of this project is the building-up of an integrated model of multidisciplinary research tools to support large-scale high quality disease studies.

The main objectives of the project are:

1) to develop a research biobank, integrated with the already existing Regional Registry of Rheumatoid Arthritis of Emilia-Romagna (REUMA Registry http://assr.regione.emilia-romagna.it/it/aree_attivita/governo-clinico/valutaz- qual-assistance / database / db-reuma / intro), starting a collection and storage of multiple biological samples (serum, plasma, whole blood, DNA) according to quality standards

2) perform a preliminary pharmacogenetic study analyzing a panel of gene variants potentially involved in the pharmacological response.

Inclusion Criteria:

As regards the inclusion of biological material in biobanks, the project includes:

a) the enrollment of each patient with rheumatoid arthritis that is inserted "ex-novo" in the REUMA Registry starting from the start date of the operational activities of the biobank;

b) parallel to this, for the part of the project that concerns the study of pharmacogenetics, blood samples will be taken from all patients already present in the REUMA Registry at the time of project start.

All patients will be enrolled according to the inclusion / exclusion criteria specified below.

Aim1-Biobank establishment

Patients will be eligible for inclusion into the registry if they satisfy the criteria of the American College of Rheumatology (ACR) 1987 or the 2010 ACR/European League Against Rheumatism (EULAR) criteria. The following patients will be included:

age over 18
newly diagnosed patients
already diagnosed patients with active fase disease (Disease Activity Score-DAS28 > o = 4,2),
already treated patients when they start therapy with new biological agents

Aim2: pharmacogenetic analysis
patients already enrolled in the Regional Registry

Exclusion criteria

Patients non residing in regione Emilia-Romagna
Patients unable to provide informed consent

Study design

Aim 1: Preparation of the collection of biological material related to the Regional Registry of Rheumatoid Arthritis

Preliminary organization phase

• Planning of the biobank governance plan.

• Definition of standard operating procedures (SOP) for the collection, transport, treatment and storage of different biological samples (whole blood, serum, plasma, peripheral blood mononuclear cells-PBMC, DNA).

• Development of the IT system already in use at Lab Immunorheumatology and Tissue Regeneration-IOR in order to obtain an IT management system fully developed for the workflow of the biobank.

Operating activity

Section A - OBJECTIVE: Development of the research biobank associated with the Regional Registry of Rheumatoid Arthritis (REUMA Registry)

- 1) Collection and delivery of biological material by the Recruitment Units

- 1.1 - Patient selection and enrollment according to the criteria / inclusion / exclusion indicated
- 1.2 - the total number of patients is not predictable, as it depends on the number of patients who
- they will be eligible for inclusion in the Reuma Register from the start of the Project.
- 1.3 - Follow-up and clinical, radiological and laboratory evaluations. All patients will be characterized based on clinical, imaging and laboratory evaluations (following routine clinical course).
- Follow-up time: 3, 6, 12 months after first sight and every 6 months thereafter

(Perspective route defined based on the timing of data entry in the REUMA Registry)

- 1.4 Collection / shipment of blood samples to be kept in the biobank and data collection of the patient.
- Blood samples will be taken from all patients (upon obtaining informed consent) during the first visit and prospectively at the established follow-up time and will be sent to the SC Lab. Immunorheumatology and Tissue Regeneration IOR.
- For each patient, all the data, demographic, anthropometric, radiological, laboratory, clinical and pharmacological, related to the first visit and subsequent follow-up times, will be inserted by the personnel of the individual Units of Rheumatology in the REUMA database.

2) Reception and treatment of samples

Units involved: SC Lab. Immunorheumatology and Tissue Regeneration IOR

- All blood samples received will be immediately treated following Standard Operating Procedures in order to obtain:

• plasma
• serum
• whole blood
• mononuclear cells of peripheral blood

- The different biological samples will be divided and maintained under controlled temperature conditions that guarantee long-term preservation
- Part of the aliquots of the whole blood samples will be subsequently sent to the Department of Medical Genetics of Ferrara where DNA extraction - conservation and all the pharmacogenetic investigations will be carried out.

Section B - OBJECTIVE: pharmacogenetic study

1) Collection and sending of biological material by the Recruitment Units

1.1 - Patient selection and enrollment according to the inclusion criteria indicated

1.2 Number of patients: about 600 patients (estimated number based on the Report on the processing of the Reuma Register indicators provided by the Emilia Romagna Region and updated at30/11/2013)

1.3 - Blood samples collection

Blood samples (2 tubes with EDTA and 2 tubes without anticoagulant) will be taken from all patients (after obtaining informed consent) already entered in the REUMA Registry and belonging to the Rheumatology Units for the follow-up controls foreseen.

The next steps of the study protocol of the study will be the same as described in Section A: points 1.3, 1.4 and paragraph 2.

3) Experimental phase

Units involved: UR2 - Department of Medical Genetics of Ferrara

3.1 - Extraction and conservation of genomic DNA

3.2 - Analysis of the genotype polymorphisms in the candidate gene variants to influence the response to anti-TNFalpha drugs will be evaluated by real-time polymerase chain reaction (PCR):). Other genetic variants potentially involved in influencing the response to anti-TNF-alpha, anti-CD20 or anti-IL-6 drugs will also be tested.

3.3 - Statistical analysis

The association between the response to drug therapy and the genotype will be performed with multivariate and data-mining approaches (eg multifactorial reduction of dimensionality), which will also take into account clinical and demographic data.

All the costs related to the carrying out of the experimental analyzes are totally covered by the funding obtained by the Emilia-Romagna Region in the framework of the Young Researchers Announcement "Alessandro Liberati" 2013 - Project Code PRUA1GR-2013-00000203 (Total funding obtained € 194.320)

Summary of primary and secondary efficacy evaluation parameters:

1) Rate of overlap between the samples inserted in the biobank and the patients entered the register [Indicator: (total number of biobank entries / number of registry entries) x100]
2) Percentage of blood samples not correctly collected or sent by the Operating Units of collection [Indicator: number of blood samples not correctly collected or shipped / total number of biobank records] x100]
3) High quality guarantee of the samples stored in the biobank [Indicators and timing will be defined in the specific operating procedures]
Points 1) and 2) can be strongly influenced by two specific factors: patient compliance and /or physician compliance. In order to deal with these problems, planned strategies will be implemented

All scientific and informational publications (articles in newspapers and websites) presentations at conventions and congresses, etc. related to the research activities carried out within the scope of this Project will report the source of the funding received in the manner and the mandatory wording required from the Emilia-Romagna Region