Background

Cardiovascular diseases (CVD) cause nearly one-third of all deaths worldwide. Cardiovascular risk factors such as hypertension, smoking, diabetes mellitus and obesity are major causes of these diseases. Population-based global longitudinal studies have established the role of conventional and novel risk factors and measures of subclinical disease in the prediction of CVD. Risk assessment with short-term or long-term risk prediction algorithms may help to identify individuals who would benefit most from risk-factor interventions. Evaluation of novel risk factors can also help to identify individuals at higher cardiovascular risk. Prevention of CVD focuses on identifying and managing risk factors both at the population and individual levels through primordial, primary, and secondary prevention.

Elevated blood plasma levels of fibrinogen have been associated with a wide variety of thrombotic diseases, from stroke, peripheral vascular disease and abdominal aortic aneurysm, to pulmonary embolism. Despite the clear links between fibrinogen levels and CVD, whether this association represents cause or effect remains a subject of debate. Increased levels of plasma fibrinogen result in changes in blood rheological properties (e.g. increase in plasma viscosity and erythrocyte aggregation). These alterations exacerbate the complications in peripheral blood circulation during cardiovascular pathologies. Erythrocyte aggregation has become an issue of increasing interest, especially to assess the risk of primary or secondary cardiovascular events, since it is influenced mostly by fibrinogen concentration and plasma lipids. It is in such clinical situations, and in a non-acute phase of the diseases that it is more useful to determine erythrocyte aggregation as an indicator of the associated cardiovascular risk. Presently, a detailed study of the interactions between fibrinogen and erythrocytes to understand the consequences of the observed increase of erythrocyte aggregation on patients with some cardiovascular risk factors is extremely relevant.

Objectives

We intend to study how fibrinogen-induced erythrocyte-erythrocyte interaction constitutes a risk factor for cardiovascular disorders. More specific goals are:

1) To conduct a 6-months follow-up study of cardiovascular disorders patients, such as acute myocardial infarction and stroke, focusing on the changes on erythrocytes aggregation and on fibrinogen-erythrocyte interactions.

2) To correlate clinical, hemorheological, inflammatory, hemostatic and atomic force microscopy (AFM) data profiles on each patient.

3) To evaluate the possible association between changes in erythrocyte-erythrocyte adhesion and fibrinogen-erythrocyte interactions with the reduction and/or prevention of cardiovascular risk events.
The project draws together the collaboration of Instituto de Medicina Molecular (IMM) and clinicians from Hospital de Santa Maria, Centro Hospitalar Lisboa Norte (HSM/CHLN). The development of the project will be divided into five distinct tasks:

**Task 1** – Fibrinogen-erythrocyte interactions evaluation by AFM on patients after acute myocardial infarction (AMI) (duration: 8 months). We intend to do a six-month follow-up of AMI patients, taking blood on the first medical consultation (upon first diagnostic, before starting anti-hypertensive medication) and after 6 months of medication. An extensive clinical, hemorheological, hemostatic and inflammatory evaluation will be done and the results will be compared with those achieved by the force spectroscopy studies (fibrinogen-erythrocyte and erythrocyte-erythrocyte interactions).

**Task 2** – Fibrinogen-erythrocyte interactions evaluation by AFM on patients after stroke (duration: 8 months). In order to evaluate differences on hemorheological factors after the hospitalization event, we intend to analyze the intensity of the inflammatory response, fibrinogen concentration and erythrocyte aggregation as a function of time.

**Task 3** (time of the task included on tasks 1 and 2) – Evaluation of cell-cell interactions (duration: 8 months). We will compare the interaction forces between two erythrocytes (one of them attached to the AFM cantilever and the other to the solid substrate), in the absence or presence of different fibrinogen concentrations. Differences on elasticity and deformability of the erythrocytes isolated from each patient will also be assessed. The time dispended to conduct this task is the time indicated on tasks 1 and 2, since cell-cell experiments will be done with the same samples from the cardiovascular patients.

**Task 4** – Interrelation of the clinical, hemorheologic, inflammatory and hemostatic profiles with the experimental AFM data (duration: 2 months).

In general, the time necessary to develop each task of the project will be distributed in accordance with the availability of the patients’ blood samples and of the number of samples collected from patients on each week. At the end of the project, we expect to have all the clinical, hemorheologic, inflammatory and hemostatic data profiles and the fibrinogen-cell and cell-cell adhesion AFM studies completed for both cardiovascular disorders. After analyzing the data profile for each studied patient and correlating with the AFM results, we may eventually be able to suggest future therapeutic approaches to reduce the risks of fibrinogen-driven erythrocyte hyperaggregation.