# Biomedical Studies

*Teresa Pinheiro*

The aims of the Biomedical Studies group are the study of putative biomarkers in order to characterize exposure, diseases and therapy efficacy and to identify potential targets for novel therapies.

Efforts were developed in the translation of basic biomedical research into novel diagnostics and therapies for the benefit of human populations exposed to metals, and of patients with chronic diseases.

Undertaken research is an end product of intense and interactive collaborative work among researchers in Cardiology, Pneumology, Dermatology, Biology, Biochemistry, Chemistry and Environmental Sciences.

Current projects join different groups from three ITN Units, Reactor, UCQR and UFA, which are working in consortium with other research institutes, academia and hospitals.

Major research areas focused:

1) Clinical outcomes research establishing disease progression and clinical response to therapy;

2) environmental health research establishing new biomarkers of exposure.

New technical capabilities recently developed in ITN, such as inductive coupled plasma mass spectrometry (ICP-MS) and flow cytometry, helped consolidating achieved expertise and opened new areas of research.

Continued funding in the areas of environmental health, cardiovascular and skin diseases during the last five years had strengthened existing skills and promoted advanced training of Ph.D. and M.Sc. students.

The main achievements are summarised in the following pages.

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# Biomarkers of Disease, Therapy and Exposure

*T. Pinheiro, L.C. Alves, M.A. Barreiros[1], R. Fleming[2], P. Filipe[3] and R. Silva[3]*

## Clinical outcomes research - Skin iron as a diagnostic tool in hemochromatosis

**Background:** Hemochromatosis is a hereditary disease that causes an inappropriate intestinal absorption of Fe resulting in its accumulation in multiple organs, such as liver, heart and skin. Fe metabolism indicators in the circulation do not provide reliable indication of organ overload as they can be influenced by other clinical conditions. Assessing metabolism organs such as liver often requires invasive procedures which is not adequate to patient's serial observations.

The project finished in June 2009, when final data for the last patients enrolled in the study was collected. Results have been partially published in peer review journals and one comprehensive paper including all results is being prepared to be submitted to a specialty journal.

**Objectives:** Our aim was establishing cross sectional and longitudinal information on the amount of Fe that deposited in skin and liver during a life period, how iron is cleared out by therapy intervention and study the relationship of these changes between the two organs using non-invasive methods.

**Methods:** The study used conventional and innovative laboratory tests to differentiate distortions of iron metabolism. Patients were genetically characterized and studied before initiating therapy (Phase 1) and continued to be surveyed along therapy, at the end of the phlebotomy programme (Phase 2) and 6 months after (Phase 3). Nuclear microprobe (NMP) and nuclear resonance techniques provided iron quantitative imaging and physiological information on skin and liver. Biochemical methods provided hepcidin contents in serum and markers of iron metabolism and organ function.

**Results:** Skin features can be easily observed with NMP techniques, enabling the accurate localization of Fe deposits. At hemochromatosis diagnosis time point patients (25 phlebotomy naïve patients) showed remarkable Fe deposits in skin epidermis (Fig. 1).

At Phase 1, hemochromatosis patients also showed high transferrin saturation values, and remarkably elevated concentrations of ferritin, serum and plasma iron when compared to controls. Hepatic Fe was also high. At this time point before starting the phlebotomy therapy, skin and liver Fe concentrations could be associated to serum indicators of Fe overload, such as ferritin content and transferrin saturation.

As therapy progresses Fe blood indicators, skin and hepatic Fe concentrations sharply decrease. Hepatic Fe content shows a similar trend to skin Fe concentration (Fig. 2).

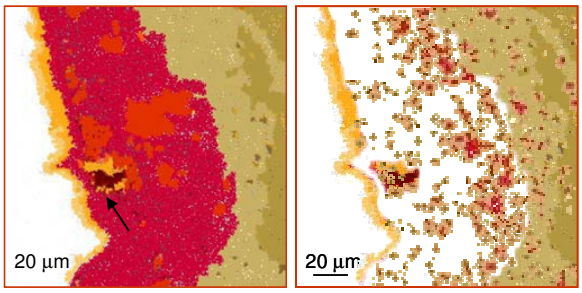


Fig.1 NMP Images of skin morphology and Fe distribution in hemochromatosis. Left image: epidermis in red, dermis in green, stratum corneum and hair shaft in yellow, hair in dark red (arrow). In the right image, iron (red) map overlapped on density image (left), shows that Fe is mainly deposited in epidermis.

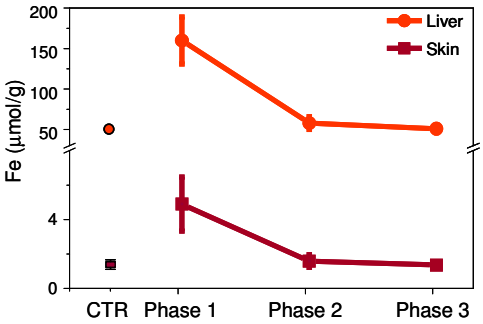


Fig.2 Skin and liver Fe content in patients and controls.

However, the decrease of ferritin and transferrin values did not correlate with the decline of skin and liver Fe content. Opposite, the Fe decrease observed along therapy (from Phase 1 to Phase 3) in skin and liver was correlated at all phases and the Fe decreasing rate from Phase 1 to Phases 2 and 3 was similar in both organs.

**Conclusions:** 1) skin Fe deposits is a non-invasive procedure enabling serial patient's assessment and therapy efficacy evaluation; 2) skin iron concentration alone or in combination with the hepatic Fe content may constitute alternative and reliable biomarkers for iron overload diseases.

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**Clinical outcomes research – New biomarkers for Coronary Artery Disease**

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Continuous efforts are being developed in the translation of basic biomedical research carried out into novel diagnostics and therapies for the benefit of patients with coronary artery disease. Current projects join teams with know-how in intervention cardiology, biochemists, biologists, geneticists and statisticians. Cohort studies were undertaken to study new biomarkers in coronary syndromes. Indicators of inflammation, thrombosis and oxidative stress such as the molecules TNF-a, C-reactive protein, CD40 ligand, P-selectin, oxidized LDL, microparticles released by the endothelial cells and platelets and inflammatory cells surface markers, have been study in acute, instable and stable clinical conditions. Under the scope of a FCT project, specific proteins and molecules that may be indicators of plaque activity (cathepsins) and endothelial dysfunction (nitric oxide and VEGF) will be related to virtual histology intravascular ultrasound (VH IVUS)-derived measurements of the atherosclerotic plaque. The major aim is to find biomarkers that can be associated to plaque composition. Major achievements were 1) the association of the serial changes of inflammatory markers with adverse clinical outcomes in patients with acute myocardial infarction; 2) the differentiation of patients with ST-elevation myocardial infarction based on soluble CD40 ligand variations. During 2009 a Ph.D. thesis in Biology was completed and two M.Sc. thesis were carried out under the current projects.

Project funding: LAHSM/2005-2009 - Liga de Amigos do Hospital de Sta. Marta, FCT/PIC/IC/82734/2007, FCT grant SFRHI/BD/ 18822/2004.

**Environmental health research - new biomarkers of exposure.**

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The project is a joint initiative of ITN units UFA and UR, together with the Instituto de Soldadura e Qualidade and the Hospital de Santa Maria. The primary objective is to develop a new non-invasive human bioindicator - Exhaled Breath Condensate (EBC) - that could be employed for a better risk assessment among workers exposed to lead. Major achievements during 2009, were: 1) the validation of methods of collection and analysis of EBC; 2) decision on reliable biomarkers that can be determined in EBC – metals and cytokines have been examined.
Methodologies to sample, store and analyse the EBC were tested and established. EBC elemental concentrations were determined by ICP-MS in ITN at the ICP-MS laboratory of UCQR, in collaboration with the Environmental and Analytical Chemistry Group. The whole analytical methodology is being controlled by comparing results with TXRF which is accredited at LNEG by the Portuguese Quality System following the regulations of ISO/IEC 17025. Also, cytokine concentrations in EBC were inspected by ELISA. The Pb, Cr, Mn and Cu concentrations in EBC can be used as indicators of occupational exposure. On the other hand cytokine measurements in EBC revealed to be inadequate due to the instability of the analyte or to the low concentration levels.
Detailed information about this project in URSN/ANANE.

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