Operative Protocol

Title: **NON-IONIZING DIAGNOSIS IN RHEUMATOLOGY**

Short Title: **ECHO-BONE**

Version 1 1/4/2018

1 AIM OF THE STUDY

This is a protocol for an observational study employing a CE marked medical device (MD). The aim of the study is to perform a clinical validation of a recently introduced method for non-ionizing diagnosis of osteoporosis against the current gold standard, dual-energy X-ray absorptiometry (DXA) investigations performed on lumbar spine and/or femoral neck. We plan to recruit 1000 Southampton patients as part of an international multicenter study. The expected study duration is approximately 12 months.

2 STUDY DESIGN

2.1 Involved centers

Centers involved in the study are listed below:

- Metabolic Bone Diseases Unit, Department of Surgery and Translational Medicine, University of Florence, Italy (Contact Person: Prof. Maria Luisa Brandi) – Scientific Coordinator;
- National Council of Research-Institute of clinical Physiology (CNR–IFC), Lecce, Italy (Contact Person: Dr. Marco Di Paola);
- O.U. of Rheumatology, “Galateo” Hospital, San Cesario of Lecce, Italy (Contact Person: Dr. Maurizio Muratore);
- Department of Internal Medicine, Hospital del Mar, Barcelona, Spain (Contact Person: Prof. Adolfo Diez-Perez);
- University of Liège and Bone and Cartilage Metabolism Unit, Liège, Belgium (Contact Person: Prof. Jean-Yves Reginster);
- MRC Lifecourse Epidemiology Centre, University of Southampton, Southampton General Hospital, Southampton, United Kingdom (Contact Person: Prof. Elaine Dennison).

2.2 Patients’ informed consent

Patients will receive an information sheet about the study with their DXA appointment details. At their DXA appointment they will be asked if they have any questions about the study; they will then be invited to sign a consent form prior to having the ultrasound scan.

2.3 Safety

Ultrasound imaging techniques are non-ionizing diagnostic methods which are used widely, including in pregnancy. The present study involves the simple execution of external echographic scans without the use of intracavitary probes and without the need of contrast agents. The study does not involve changes in the standard diagnostic protocol: a simple echographic exam will be carried out by a clinical member of the Osteoporosis Centre Staff.

2.4 Enrollment Criteria

The following inclusion/exclusion criteria will be applied.

Patients' inclusion criteria

- ✓ both women and men,
- ✓ all ethnicities,
- ✓ age range from 30 to 80 years,
- ✓ body mass index (BMI) < 40 kg/m^2,
- ✓ absence of significant walking impairment,
- ✓ medical prescription for a spinal and/or femoral DXA,
- ✓ signed informed consent.

2.4.1 Patients' exclusion criteria

- ✓ Significant walking impairment
- ✓ BMI > 40 kg/m^2

2.5 Materials and equipment to be used

Echographic scans on anatomical reference sites (lumbar vertebrae and/or femoral neck) will be carried out using the EchoStation system, an echographic device that has been developed in Italy within the ECHOLIGHT Project through a collaboration between CNR-IFC and Echolight S.p.a. The device is equipped with a 3.5-MHz broadband convex ultrasound transducer and configured to provide both echographic images and "raw" unfiltered radiofrequency (RF) signals, simultaneously processed through a series of automatic algorithms for reaching the final diagnosis and other clinical indicators.

The proper coupling between probe and patient's skin will be assured by using a common hydrosoluble and hypoallergenic coupling gel.

2.6 Data Acquisition

All the recruited patients will undergo an echographic scan of lumbar vertebrae (transabdominal) and/or proximal femur. Each scan will last between 40 to 80 secs for femoral acquisition and spine acquisition respectively. Each acquisition will generate a variable number of data frames (more than 100) of B-mode and RF data depending on scan depth and other analytic specific experimental conditions. All the acquired data will be stored in a PC hard-disk which is password protected for subsequent off-line analysis.

2.7 Automatic Diagnostic Measurements

Acquired EchoSound data will be analyzed through novel automatic algorithms that performs a series of image, signal spectral and statistical analyses, involving both the echographic images and the corresponding RF signals, in order to calculate a new ultrasound parameter, called the "osteoporosis score" (O.S.), which represents the percentage of analyzed bone regions classified as "osteoporotic" (EchoSound technology [2,3]).

The adopted algorithm performs diagnostic calculations on RF signal segments corresponding to specific regions of interest (ROIs) internal to the target bone structure (i.e., a lumbar vertebra or the femoral neck), which are automatically identified by the algorithm itself. The aim of such calculations is to measure the percentage of bone segments whose signal spectral features correlate better with those of an osteoporotic bone model rather than with those of a healthy one. The algorithm actually compares RF spectra calculated from the considered patient dataset with corresponding reference models of healthy and osteoporotic bone structures obtained from previous echographic acquisitions on DXA-classified patients.

The main data analysis steps that will be performed on each patient dataset are the following:

1. Automatic identification of target bone structures within the acquired echographic images;
2. For each bone structure image, automatic identification of a specific RF signal portion for each scan line crossing the bone surface (the set of identified RF signal portions represents the ROI for the considered image frame);
3. Classification of each RF signal portion as "osteoporotic" or "healthy" on the basis of the correlation between its frequency spectrum and each of the two age-matched models stored in a previously obtained reference database;
4. For each bone structure, calculation of the O.S. value;
5. Calculation of the O.S. value for the considered patient as the average of the single bone structure values;

6. Calculation of the conventional diagnostic parameters Bone Mineral Density (BMD), T-score and Z-score, as a function of the O.S. value, through specific equations depending on patient age and BMI.

The next two paragraphs will describe the fundamentals of the automatic segmentation of the target bone interface and the calculation of O.S. values.

2.7.1 Automatic segmentation of target bone interface

For a generic patient dataset, once the age-, BMI- and gender-matched spectral models for the considered anatomical site have been identified in the reference database, the first operation performed by the algorithm is the automatic segmentation of the target bone interfaces within the sequence of acquired images. The automatic segmentation procedure is based on the following main steps, carried out on each considered frame and specifically tailored to search lumbar vertebra or femoral neck interfaces:

- Noise removal and signal enhancement through operations such as brightness masking, image smoothing, histogram equalization, etc.;
- Thresholding, in order to transform the image into a binary map;
- Morphologic evaluations, to verify whether among the white pixel clusters present in the thresholded image there is one that has the typical features of the sought bone interface in terms of length, thickness, position, etc.;
- Spectral validation, consisting in a check of the RF data corresponding to the ROI selected below a "possible bone interface" identified in the previous step, in order to verify if the associated spectral content resembled the typical features of the target bone structure.

2.7.2 O.S. calculation

Once all the frames belonging to the analysed patient dataset have been segmented, the algorithm will proceed to the following diagnostic calculations on the RF signals corresponding to the ROIs selected under the identified bone interfaces. The frequency spectrum of each RF signal portion belonging to the considered ROI will be classified as "osteoporotic" if the value of its Pearson correlation coefficient with the appropriate osteoporotic model (r_{ost}) is higher than the corresponding correlation value with the related healthy model (r_{heal}), otherwise it will be classified as "healthy". Then, the O.S. value for the *i*th identified ROI will be calculated through the following formula:

$$O.S._{ROI_i} = \frac{E_{i_{ost}}}{E_i} \cdot 100$$

where:

$E_{i_{ost}}$ = number of spectra classified as "osteoporotic" for the *i*th identified ROI (ROI_i);

E_i = total number of spectra belonging to the *i*th identified ROI (ROI_i).

Then, the O.S. value for the *k*th considered patient is:

$$O.S._k = \frac{\sum_{i=1}^{n_k} O.S._{ROI_i}}{n_k}$$

where n_k represents the number of bone interfaces identified in the dataset corresponding to the patient *k*. Finally, the obtained O.S. value is used to calculate the ultrasound-estimated values of BMD, T-score and Z-score through mathematical equations incorporated in the reference model database (these equations had been obtained through linear regression approaches employed on database patients).

2.8 Reference database

The adopted algorithm will be integrated with a previously built reference database, which, for each combination of ethnicity, age, BMI, gender and investigated anatomical site, includes the "osteoporotic" and "healthy" spectral models to be used for O.S. calculation and the mathematical equations to obtain BMD, T-score and Z-score.

Database patients have been grouped on the basis of their age into 5-y intervals. For each age interval 100 patients will be included in the reference database. The size of the sample to be included in the reference database (100 patients per age group) was calculated according to the indications provided by Hou et al. (2008) [2], who show that a reference database can be obtained from a population of 458 women of the same ethnicity aged in the range 6–85 y, in which the most populated 5-y age interval includes 44 subjects. We rounded off this value to 50 and multiplied it by a safety factor of 2. In this way, we also respected the indications provided by Engelke and Gluer (2006) [3], stating that sample sizes including at least 100 patients per age group can be considered fairly robust.

2.9 Reproducibility Studies

The present study will also include a dedicate assessment of the clinical precision of the device, according to the updated Official Positions of the International Society for Clinical Densitometry [4]. The first 30 patients enrolled, and classified as "healthy" by DXA, will undergo two consecutive echographic investigations

performed by the same operator with patient repositioning between the scans, in order to quantify the intra-operator repeatability (i.e., technique precision).

In order to quantify also the inter-operator repeatability, a further subsequent group of 30 patients classified as “healthy” by DXA will undergo two consecutive echographic scans performed by two different operators (with patient repositioning between the scans).

Finally, the whole reproducibility test will be repeated on further 60 patients classified as “osteopenic” or “osteoporotic” by DXA (30 for the intra-operator repeatability and 30 for the inter-operator variability), in order to verify that the obtained reproducibility results are independent on BMD level.

Final variability results will be presented as a fraction of the standard deviation of the young normal reference group. Clinical testing of reproducibility will be assessed at different conditions of temperature and humidity.

DISSEMINATION OF THE RESULTS OBTAINED IN THE STUDY

Acquired data will be presented at national and international conferences and the obtained results will be submitted for publication in international scientific journals.

3 REFERENCES

[1] Clinical Trial Sample Size Calculation Program. Consultabile dal sito web: http://www.biostat.wisc.edu/landemets/. University of Wisconsin, Department of Biostatistics; 2004.

[2] Hou YL, Liao EY, Wu XP, Peng YQ, Zhang H, Dai RC, Luo XH, Cao XZ. Effects of the sample size of reference population on determining BMD reference curve and peak BMD and diagnosing osteoporosis. Osteoporos Int 2008;19:71–78.

[3] Engelke K, Gluer CC. Quality and performance measures in bone densitometry: Part 1. Errors and diagnosis. Osteoporos Int 2006;17: 1283–1292.

[4] Official Positions of the ISCD (International Society for Clinical Densitometry) as updated in 2013. Available at: http://www.iscd.org/official-positions/2013-iscd-official-positions-adult/. Accessed February 29, 2016.