

A tool for the extraction of new disease biomarkers from ex vivo and in vivo data

Francesca Gallivanone^{1✉}, Carla Canevari², Isabella Sassi², Alberto Marassi², Maria Picchio², Maria C Gilardi¹, Isabella Castiglioni¹

¹Institute of Molecular Bioimaging and Physiology (IBFM), CNR, Milan, Italy

²Ospedale San Raffaele, Milan, Italy

Motivation and Objectives

Recent studies have demonstrated the correlation among features of diseases obtained from ex vivo and in vivo Molecular Imaging (MI) studies (e.g. Genomics and Positron Emission Tomography, PET, Strauss et al., 2008; Genomics and Computerized Tomography, CT, Segal et al., 2007), opening a new role to non invasive clinical MI technologies in the current approach of personalized medicine.

At present, proper databases and statistical methodologies are on hand to deal with the different modalities of ex vivo and in vivo MI data but tools for the extraction of new disease biomarkers are still not available for the purpose of clinicians. The main interest of clinical specialists is in finding biomarkers of disease with diagnostic and prognostic values, and this can be performed with an interdisciplinary approach offered by ex vivo and in vivo MI and then translated into the clinical environment.

Aim of this work was the development of a software tool ("cOuch" Correlative and Collaborative Touch System) (Castiglioni et al., 2011) designed to be used by clinicians to find new diagnostic/prognostic biomarkers of disease from the comparison of ex vivo and in vivo data of patients. In this work, as representative example, "cOuch" has been applied to assess the prognostic and diagnostic value of the Standardized Uptake Value (SUV, Graham et al., 2000), a parameter of regional metabolic uptake measured by PET and 18F-labeled fluorodeoxyglucose for breast cancer lesions.

Methods

A. Couch functions and requirements

cOuch has been developed in Matlab R2008b. The Matlab standard toolbox was used, including Processing Toolbox, Statistic Toolbox and Curve Fitting Toolbox. Ad-hoc utilities were implemented with Java languages. A user friendly graphi-

cal interface (GUI) has been designed for clinical users. The GUI consists of different sections: the population of a Database of patient data (different modality data from different in vivo and ex vivo diagnostic tests), a section for Data Pre-processing (to select the category of the Variable Type for a specific correlation test) and a section for Data Processing (to select the specific correlation test). cOuch can be implemented with a standard or touchscreen hardware, on condition that a minimum Ram of 1GB (2GB is recommended) is installed for a 32-Bit system, due to the large software memory footprint required. A 64-Bit operating systems allows to achieve the best performances. Touchscreen system compliance offers the possibility to use cOuch by touchscreen tablets and mobile phones.

B. The Database

The cOuch database is a Relational MySQL Database that is populated through the GUI by registered and authorized clinical specialist users. In vivo and ex vivo MI data, including PET metabolic parameters of the oncological lesions, CT anatomical lesion volume, histopathological indexes and up/down regulated proteins extracted from surgical report samples, can be stored in the database. Every data is standardized and archived in standard formats. Patient data are anonymized and identified by an ID. Couch software is connected to the database and allows to perform statistical analysis and to save analysis reports.

C. Data Pre-processing

The variable type can be considered using its intrinsic type or can be transformed on the basis of proper options, in order to perform different statistical tests. Histological type, as assessed using the pTNM pathological classification, is treated as nominal data in all statistical analysis. Histological grade is treated as nominal data in some statistical analysis but reduced to nominal dichotomous data (only two categories) in

other statistical test. pT stage, as assessed using the pTNM pathological classification, is treated as categorical (four categories) in the statistical analysis. Lymph node status, as assessed using the pTNM pathological classification, is considered as nominal dichotomous variable, distinguishing the cases with no involvement of lymph node from the cases with involvement of lymph nodes. The expression of receptors (e.g. ER, PgR in the case of breast cancer), whose values range from 0% to 100, are considered as ordinal numerical continuous variables, except when not found expressed they are considered as nominal dichotomous data. Tumours are considered to over-express oncoproteins (e.g. c-erbB-2 positive in the case of breast cancer) if more than 30% of invasive tumour cells show definite membrane staining (Score 3 +) or if a definite membrane staining is found smaller than 30% (Score 2+), resulting in the so-called fishnet appearance. SUV is considered as an ordinal continuous variable.

D. Data Processing

Mann-Whitney tests and Kruskal-Wallis tests were implemented to assess the relationship between in vivo MI features with histopathological, immuno-histochemical parameters and up/down regulated proteins. Multivariate linear regression and clustering algorithms were implemented to assess multiple dependences of groups of parameters, also following some methods reported in literature considering matrix of protein expression as input for correlation analysis (Kim et al., 2012). In order to assess the diagnostic and prognostic role of the potential considered biomarkers, a ROC analysis was implemented with the purpose to find cut-off values for the biomarker. Specificity and Sensitivity were also calculated. This analysis allows to differentiate groups of patients on the basis of the values of the biomarker, higher or lower to the cut-off, being the two groups correlated to different biological characteristics of tumor (e.g. as assessed by histopathology).

E. Application of cOuch to real clinical studies

A protocol for the collection of in vivo and ex vivo MI data with the purpose of integration has been designed for one representative population of breast cancer patients. The study protocol, approved by the Institutional Review Board of the Scientific Institute H San Raffaele, involved 40 patients with biopsy-proven breast cancer designed for surgical intervention without per-

forming any treatment before surgery. Eligible patients underwent a total-body 18F-FDG PET/CT exam before surgery, and, during surgical intervention, biological samples were collected and analyzed at the Pathological Anatomy Unit and sent to the proteomics laboratory. For each patient, the senology, the anatomo-pathologist, and the biologist submitted their own reports to the nuclear medicine physician who calculated the SUV of the primary breast lesion from the PET/CT images and imported it in the database together with the other ex vivo data. The nuclear medicine physician performed the correlation analysis with cOuch between the considered parameters.

Results and Discussion

SUV was found correlated with histological type. Mann-Whitney test on SUV and the histological type (Invasive Lobular Carcinoma, ILC vs Invasive Ductal Carcinoma, IDC) showed that SUV was significantly ($p < 0.02$) lower in ILC (2.92 ± 0.94 g/cc) with respect to IDC (7.45 ± 6.16 g/cc). ROC curve analysis showed that a threshold of 3.87 g/cc for SUV allowed to distinguish ILC from IDC histological types with a Specificity of 67% and a Sensitivity of 100%. SUV was found correlated with histological grade. Kruskal-Wallis test on SUV and the histological grades (G1 vs G2 vs G3) showed that SUV was significantly ($p < 0.01$) different in G1 (3.90 ± 3.72 g/cc) with respect to G2 (5.61 ± 5.52 g/cc) and to G3 or G3 (11.30 ± 5.85 g/cc). Mann-Whitney test on SUV and the histological grade (G1 vs G3) showed that SUV was significantly ($p < 0.03$) lower in G1 (3.90 ± 3.72 g/cc) with respect to G3 (11.30 ± 5.85 g/cc). ROC curve analysis showed that a threshold of 3.99 g/cc for SUV allowed to distinguish G1 from G3 histological grades with a Specificity of 83.9% and a Sensitivity of 88.9%. No significant correlation were found between SUV of primary BC lesion and lymph node status either as detected by histopathology either as detected by PET. SUV was found correlated with ER and PgR hormone receptors. ER positive tumors showed a lower 18F-FDG (5.60 ± 5.14 g/cc) with respect to ER negative tumors (13.90 ± 5.65 g/cc) ($p < 0.005$) and PgR positive tumors showed a lower 18F-FDG (5.55 ± 5.13 g/cc) with respect to PgR negative tumors (14.04 ± 5.66 g/cc) ($p < 0.005$). As expected, the same relationship was found considering the total expression of hormone receptors

but with a lower statistical significance ($p < 0.02$). An analysis of ROC curves showed that a threshold of 7.75 g/cc for SUV allows to distinguish both ER positive from ER negative and PgR positive from PgR negative with a Specificity of 100.0% and a Sensitivity of 78.1%. No correlations were found between SUV and c-erbB2 but this could be due to the poor sample. In fact, excluding patient with incomplete information on c-erbB2 expression, only four patient presented an over-expression of c-erbB2 index. Using a threshold of 18% for MiB-1 proliferation index, we found that SUV was significantly ($p < 0.05$), correlated with MiB-1 proliferation index in particular $SUV = 4.52 \pm 2.92$ g/cc for tumor with an expression of MiB-1=18% and $SUV = 9.30 \pm 7.40$ g/cc for tumor with an expression of MiB-1 > 18%. An analysis of ROC curves was performed on the two clusters of data obtained using a 18% threshold. A value of 4.06 g/cc for SUV allows to distinguish positive or negative values of MiB-1 proliferation index, with a Specificity of 70.6% and a Sensitivity of 65.0%. Univariate linear regression analysis was performed on MiB-1 and SUV. Even if the significance of the estimated parameter for MiB-1 proliferation index was strong ($p < 0.001$), indicating the correlation already evaluated using Mann-Whitney test, the significance of the regression was low (R-square < 0.3) showing that linear relationship is not effective. Hierarchical clustering was applied involving SUV and the variables which were found one-to-one correlated with SUV by univariate tests: histological type and grade, hormone receptor status and MiB-1 proliferation index. K-means pre-processing on SUV was performed following the results obtained by univariate analysis. K-means algorithm allowed to define three intervals of SUV

values: a) SUV from 0.78 g/cc to 3.07g/cc; b) SUV from 3.40 g/cc to 4.38 g/cc; c) SUV from 7.03 g/cc to 27.53g/cc. A hierarchical cluster analysis allowed to define two different clusters of multiple-correlated indexes: a) a first cluster including ILC and IDC G1 tumors with a negative expression of MiB-1 and SUV in the first interval. Index of positive expression of hormonal receptors and SUV in the second interval are linked to this cluster but with a lower significance; b) a second cluster including IDC tumors G2 and G3 tumors with a positive expression of MiB-1, a negative expression of hormonal receptors and SUV in the third interval. In conclusion, cOuch allowed to prove that SUV is correlated with many features obtained from ex vivo histopathological tests, suggesting SUV is a good diagnostic/prognostic biomarker to be obtained in vivo by 18F-FDG PET. Further studies will be devoted to apply cOuch methodology to the analysis of proteins differentially expressed in breast cancer tissues.

References

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