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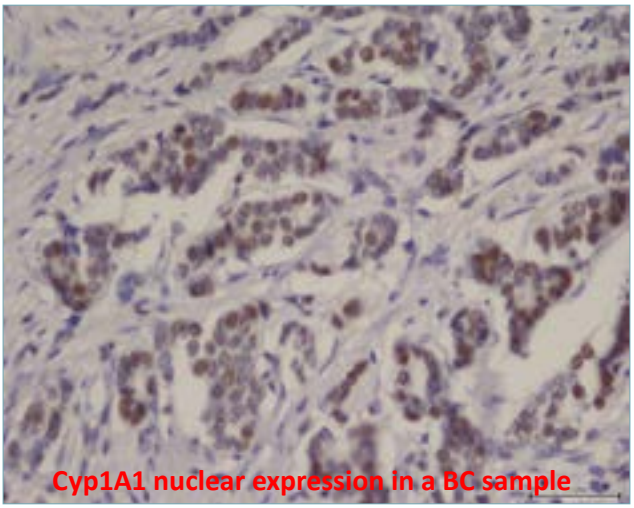

Fig. 1.

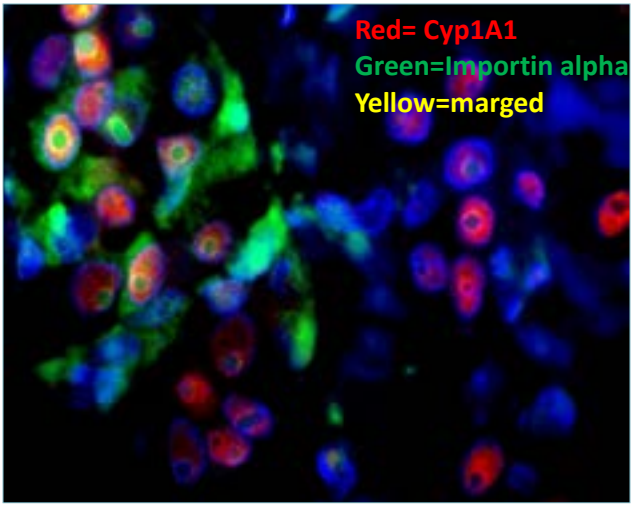

Fig. 2.

functional in vitro studies to better understand associated molecular mechanisms.

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COMPARISON BETWEEN ROUTINE METHODS AND RT-QPCR ASSESSMENT (MAMMATYPER®) OF BREAST CANCER BIOMARKERS: NEW APPROACH FOR IMPROVING MOLECULAR CLASSIFICATION

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Aims. The standards of immunohistochemistry (IHC), the main method used for biomarkers testing in breast cancer[1-3], have been efficiently ameliorate by decades of quality control efforts. However, computational pathology and reverse transcription quantitative PCR (RT-qPCR) may also hold promise for additional substantial improvements. In this regard, the present study compared routine methods and RT-qPCR evaluation of breast cancer biomarkers. The original Results (ORI) of the routinely used IHC for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), proliferation marker Ki67 and fluorescence in situ hybridization (FISH) with the findings of manual (REV) and semi-automated digital image analysis (DIA) re-evaluation of the original IHC slides; moreover, both the IHC (ORI, REV and DIA) and the FISH Results were related to the RNA expression data of *ERBB2*, *ESR1*, *PGR* and *MKI67* from MammaTyper®(MT) gene expression assay.

Materials and methods. The available material of 96 women who underwent surgery for invasive breast carcinoma in the period from 2010 to 2012 (formalin-fixed and paraffin-embedded - FFPE - blocks, Hematoxylin and Eosin - H&E - slides and IHC slides) was retrieved from the archive. The previously reported Results for ER, PR, Ki67, HER2 and FISH were registered as ORI. An experienced pathologist (EC) performed both REV and DIA blind re-evaluation, which involved both tumor classifications and grading on the original H&E slides, according to international recommendations[1-5]. Furthermore, a re-evaluation of ER, PR, Ki67 and HER2 on the existing IHC slides were performed[1-3]. The HER2 equivocal cases, after re-evaluation, were subjected to FISH analysis (dual-probe, Leica HER2 FISH system), the Results of which were recorded according to the ASCO/CAP 2013 updated guidelines.

The MT test was performed in all 96 cases, using 10 µm sections from FFPE blocks, in order to extract total RNA, following the step described by the manufacturers' instructions[6]. The determination of the expression levels of *ERBB2*, *ESR1*, *PGR* and *MKI67* by RT-qPCR was obtained using the CE-marked MammaTyper® IVD kit (BioNTech Diagnostics) on the CFX96™ (BIO-RAD®) platform.

Statistical analysis both to compare ORI, REV, DIA data and to correlate Results from ORI, REV, DIA, FISH with RNA expression data was performed.

Results. Correlation for ER and PR was excellent between ORI IHC and Results from REV, DIA and RT-qPCR. As regards HER2, 10 out of 96 discrepant cases were detected in ORI versus REV comparison. Among these, only 1 case was finally classified as equivocal after comparison of ORI, REV, DIA and RT-qPCR. For Ki67, 22 (23%) cases were categorized differently by

either REV alone, DIA alone or both. Most of the discrepant Ki67 cases changed from low to high between ORI and REV/DIA. Thirty-two (33%) cases resulted discrepant between RT-qPCR and any IHC assessment of Ki67, 29 cases of these showed high *MKI67* expression.

Conclusions. Assessment of the breast cancer biomarkers ER, PR, HER2 and Ki67 at the RNA level shows high degree of correlation with IHC and compares well with correlations between original with subsequent independent manual or semi-automated IHC re-evaluations. Intrinsic marker properties, such as the type of protein and RNA frequency distributions or spatial heterogeneity in whole sections may interact with interpretation bias to shape the extent of inter-observer or inter-method variability. The use of methods with wider dynamic range and higher reproducibility such as RT-qPCR may offer more precise information about endocrine responsiveness, improve Ki67 standardization and help resolve HER2 cases that remain equivocal or ambiguous by IHC/FISH. Moreover, the use of RT-qPCR for the breast cancer biomarkers determination could both improve the molecular classification and allow a more appropriate patients'treatment.

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RANKL, BMP2 AND PTX3 AS BONE METASTASIS MARKERS IN INFILTRATING LOBULAR BREAST CARCINOMA: PRELIMINARY RESULTS

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Introduction and objectives. About 10% of breast cancer patients without evidence of bone metastases at the time of diagnosis will have a first disease relapse in bone within 5 years from primary diagnosis [1]. Bone metastasis (BM) are more frequent in Infiltrating Lobular Carcinoma (ILC), bone representing 92% of metastatic site, if compared with Infiltrating Ductal Carcinoma (IDC) [2]. Recently, cancer cells with osteoblastic differentiation - Breast Osteoblastic-like Cells (BOLCs) - have been described as a potential reliable biomarker predictive of BM [3]. Indeed high expression of Receptor Activator of Nuclear Factor kB Ligand (RANKL), Bone Morphogenetic Protein-2 (BMP-2) and PTX3, antigens involved in the mineralization and osteoblastic differentiation, has been reported in breast cancer [3]. The Aim. of this study was to investigate the potential role of RANKL, BMP-2 and PTX3 as biomarkers of BM risk in ILC.

Materials and methods. Sixteen ILC breast samples from the Breast Unit of San Giovanni-Addolorata Hospital (Rome) database de-identified were included in the study. All experimental procedures were carried out according to the Declaration of Helsinki.

Patients were stratified according to the presence of bone metastasis. Group 1 included 6 cases with bone metastasis (40 months median follow-up). Group 2 included 10 cases of ILC free of disease at the same follow-up time. The clinic-pathologic record included: age at diagnosis, pT and pN at surgery, histological grade (G), multifocality, Lymphovascular Invasion (LVI), Hormone Receptors (ER and PR), Ki67, c-erbB2(neu)

Tab. I. List of antibodies.

Antibody	Clone	Source
Estrogen Receptor	SP1	Ventana-Roche
Progesteron Receptor	1E2	Ventana-Roche
Ki67	Mib-1	Ventana-Roche
c-erbB2(neu)	4B5	Ventana-Roche
BMP-2	Rabbit clone N/A	Novus Biologicals
PTX3	MNB1	AbCam
RANKL	12A668	AbCam

Tab. II. Clinical and molecular markers results.

	Group 1	Group 2	P
Age	61	66	n.s.
pT1	2/6 (33%)	5/10 (50%)	n.s.
pN+	4/6 (66%)	4/10 (40%)	n.s.
multifocality	2/6 (33%)	6/10 (60%)	n.s.
LVI	1/6 (16%)	1/10 (10%)	n.s.
G3	1/6 (16%)	4/10 (40%)	n.s.
ER+	6/6 (100%)	10/10 (100%)	n.s.
PR+	5/6 (84%)	6/10 (60%)	n.s.
Ki67	18%	14%	n.s.
c-erbB2(neu)	Negative (0)	Negative (0)	n.s.
BMP-2	627/mm2	171/mm2	0.03
RANKL	270/mm2	0	0.05
PTX3	214/mm2	105/mm2	n.s.