VALIDATION OF A MOLECULAR SIGNATURE (EHT Dx14) ON FINE-NEEDLE ASPIRATE SAMPLES FROM BREAST TUMORS

Fabrice André 1, Olivier Sol 2, Pascale Beurdeley 2, Raphaël Haddad 2, Jennyfer Carrière 2, Véronique Scott 1, Suzette Delaoge 1, Philippe Vieil 1.

1 Instituts Gustave Roussy, Villejuif, France; 2 ExonHit SA, Paris, France.

Abstract

One 1228-probes molecular classifier (EHT Dx14) able to discriminate benign breast lesions from breast cancer with a 96% performance was previously identified in a panel of fine-needle aspirate (FNA) samples collected at Institut Gustave Roussy (IGR). This signature, which enabled the signature to be generated, was based on the genomewide RNA analysis using the ExonHit’s SpliceArray™ technology.

The objective of the current study was to validate the performance of the EHT Dx14 molecular classifier on an independent cohort of FNA samples stored at the Centre de Ressources Biologiques at IGR.

The samples were collected from women undergoing investigations for suspicion of breast cancer at mammography and ultrasonography. The cytopathological analysis of FNA samples was found to be benign, malignant or indeterminate. All samples were collected with the best clinical diagnosis of the breast tumor.

On the first part of the study, the EHT Dx14 transcriptomic signature performance was evaluated on a set of suspicious breast tumors (n=47) or cancerous (n=71) on cytology. The clinical status of the tumors was confirmed by subsequent investigations or follow-up. Results were respectively 97.87% (95% CI : 90.77-99.93%) for sensitivity and 94.68% (95% CI : 88.02-98.25%) for specificity. Results on indeterminate FNA samples are being investigated in the second part of the study.

Overall, the study did confirm the high performance of EHT Dx14 signature in identifying malignant from benign breast tumors in an independent cohort of FNA samples.

Introduction

The current first-line investigation of breast tumors is based on the performance of different complementary assessments: clinical examination, breast imaging (mammography, ultrasonography) and guided biopsy. FNA biopsy using FNAC and/or core biopsy (1-3). When this 3-way approach gives concordant results, the level of diagnostic accuracy may exceed 97 % (4). A single core biopsy provides a very low level of information and is therefore not always diagnostic. Nevertheless it is associated with some limitations, including significant false-positive and false-negative results, invasive procedure necessitating local anesthesia and one period of follow-up after biopsy, it also generates adverse events which may be severe in haemorrhagic pain (5). FNA appears as a safer and quicker procedure for breast cancer diagnosis and it is also preferred by patients which have already largely experienced cancers.

The most relevant issue is due to its higher rate of indeterminate or false negative results which also depends on the experience of the investigator (6). Performance of FNA is improved by the use of subsequent core-needle biopsy (CNB) in patients having indeterminate FNA results or in CNB samples insufficient for analysis with clinical and/or radiological findings. Although CNB is preferred in some institutions because it allows the study of a piece of tissue by pathologists, the availability of a trained cytopathologist performing FNA with an on-site, immediate assessment, may be helpful for the clinician. Moreover an additional and expensive advantage of FNA compared with CNB is its ability to obtain high percentages of tumor cells with much lower contamination of stromal cells for molecular analysis (7).

One 1228-probes molecular classifier (EHT Dx14) based on ExonHit’s SpliceArray™ technology and able to distinguish breast cancer from benign lesions was developed at Institut Gustave Roussy (IGR) (8). This genomewide expression signature was identified from a large population of women referred at IGR for suspicion of breast cancer prior to any specific breast cancer treatment (chemotherapy or surgery). FNA was indicated because of suspicious aspect of breast node at mammography/ultrasonography. Confirmation of breast cancer diagnosis was based on the identification of cancer cells on subsequent biopsy or surgical specimen. The diagnosis of benign lesion was based on the identification of benign breast cancer cells at biopsy or Pratexia® which lead to no pathological change of the lesion after a 3-month follow-up.

Dx14 signature was generated using a training set of 184 samples (24 benign lesions and 160 breast cancers) and the performance of the signature was assessed on a validation set of 71 additional samples (21 benign tumors and 50 breast cancers). All samples were stored frozen in the IGR Biobank and were associated with a written consent from women who accepted that the remaining cells from FNA are to be considered for research use. 68 of 71 samples were accurately classified (95%, 85–99%) with a sensitivity of 96% (86–100%) and a specificity of 91% (75–100%). Of three mismatched samples, one was a benign tumor (inflammatory granulomatosis) classified as malignant, and two were malignant tumors classified as benign. The two mismatched cancers were a grade 1, serous/cystic type breast cancer in a premenopausal woman and a grade II tumor. This latter malignant lesion was also misclassified by cytopathological investigation.

Cytological investigation was compared with the molecular classifier. Cytological investigation failed to provide a definitive diagnosis in five of 71 patients; four of these five were accurately classified with the molecular classifier. Moreover, differences in expression results obtained with EHT Dx14 signature in 181 women patients were observed: two were benign lesions and two malignant lesions for which cytopathological evaluation was inconclusive (9). The last patient was a misclassified result of cytological investigation. This last case was also misdiagnosed by the molecular classifier.

Main Objective

The objective of this first part of the project was to confirm the highly significant results obtained with EHT Dx14 signature on a set of 71 FNA negative samples (benign) and positive samples (malignant).

The other main objective (not presented here) will be to assess the performance of the EHT Dx14 signature on a set of indeterminate FNA samples.

The results of this study will be published in a peer-reviewed scientific journal.

Results

Classification call built on an independent cohort containing 47 malignant and 47 benign FNA samples.

This was a retrospective research study based on the collection of FNA samples stored at the IGR Biobank (IGR – Centre de Ressources Biologiques) at 80 C in ILT liquid ysis. FNA was extracted from the selected samples and had to fulfil the quality requirements to be eligible for microarray analysis. Amplified cDNA were prepared using the NuGeneTMGeneChip™ Plus WT System. The amplified cDNA were labeled using the Encore™ Bioli Module 2V. Standard methods following recommendations of the manufacturer were used to hybridise the samples to the SpliceArray™. The arrays were scanned and washed using the FS400-0017 FS500 script prior to scanning with the Affymetrix™ GeneChip Scanner 3000 7G. Affymetrix CEL file and the related library files were imported into Partek Genomic Suite™ (Partek Incorporated, St Louis, MI) and preprocessed with background adjustment, RMA background correction, quantile normalization using the reference distribution of the training set, log 2 transformation and probe set summarization (mean).

GenomeWide SpliceArray™ technology (GWAS)

Monitoring the expression of entire transcriptome, including splice variants, requires a specific probe configuration combining exonic body probes with exon junction probes:

- Probes F and T monitor the long and the short isoforms
- Probes B, C and D monitor the long isoform
- Probe E monitors the short isoform

Main Objective

Selection of FNA samples was based on 3 categories that were selected retrospectively at the IGR Biobank including FNA samples from breast tumors issued from women undergoing investigation for suspicion of breast cancer at mammography and ultrasonography. FNA-positive for breast cancer: malignant.

FNA-negative for breast cancer: benign.

FNA-indeterminate: suspicious (Note: the current work only focuses on positive and negative FNA).

All selected samples fulfilled the main following criteria:

- FNA samples were issued from women having given their written informed consent for the samples.

- Samples were stored at the IGR Biobank (Centre de Ressources Biologiques at 80 C).

- No previous breast cancer history was mentioned in the patient’s file.

- Selected FNA samples had to be different from those which were previously evaluated for molecular signature identification.

- FNA was performed based on suspicion of breast cancer at mammography (ARCH view) and ultrasonography.

- FNA samples had to be associated with grading of cytological aspect included in the following 4 type of characterization: benign, suspicious, malignant (unsatisfactory samples – i.e., the fourth category - are not to be considered for the study as being not appropriate).

- Diagnosis had to be confirmed after FNA and results were to be available in patient’s file.

- FNA-positive for breast cancer: subsequent confirmation of breast cancer by either core biopsy or surgical specimen

- FNA-negative for breast cancer (i.e. benign lesion): evidence of absence of malignancy in the breast lesion by imaging at least 3 months after FNA, or pathological evidence (i.e. core biopsy) of benign lesion.

- FNA-unknown for breast cancer: evidence of presence or absence of breast cancer as the major lesion using either subsequent core biopsy or surgical specimen was to be present in patient’s file.


correlation of the clinical and the classification status of the clinical samples of patient.

<table>
<thead>
<tr>
<th>PERFORMANCE OF DX</th>
<th>Index Results</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>97.87%</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>91.49%</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
<td>94.68%</td>
</tr>
</tbody>
</table>

The results indicate an excellent correlation between the classification of the patient sample and the related clinical status. Only 4 false positive and 1 false negative samples were identified.

Overall, the high sensitivity and specificity of EHT Dx14 signature shown previously were able to be reproduced in an additional and independent cohort of cancerous and benign FNA samples.

Conclusion and perspectives

This work confirmed the ability of the gene-expression EHT Dx14 signature to distinguish benign tumors from breast cancer in negative and positive FNA samples in an independent cohort. The high performance obtained (94.74%) confirms the robustness of this molecular signature.

The next step of the project will be to assess the capability of EHT Dx14 to discriminate the benign versus the malignant nature of breast tumors in indeterminate FNA samples and thus will enable to precise the clinical utility of the signature in the diagnostic process of breast tumors.

References