HYPMED

Topic: PHC-11-2015:
Development of new diagnostic tools and technologies: in vivo medical imaging technologies

HYPMED

Digital Hybrid Breast PET/MRI for Enhanced Diagnosis of Breast Cancer

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D 4.2 Correlation of histopathological parameters, tumour micro-environment and PET–MR imaging

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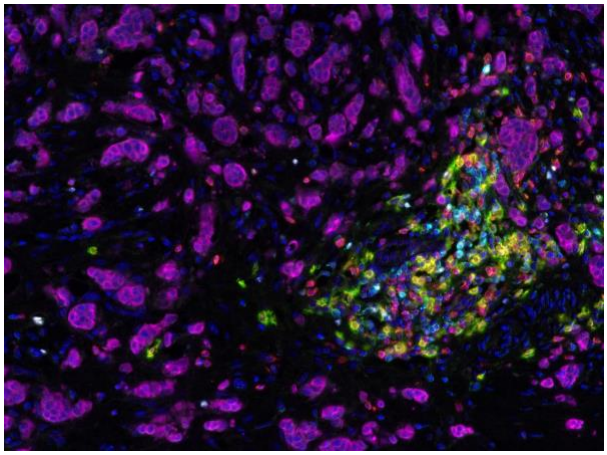
1 Introduction

Different components of breast lesions account for different imaging behaviour in the different imaging procedures, known as the "imaging phenotype" of lesions or tumors. Especially tumor microenvironment including inflammatory cells such as lymphocytes and macrophages but also fibroblasts and vessels in preinvasive and invasive tumours might contribute to distinctive features in MR as well as PET imaging.

Inflammatory cells are known to play a role in microenvironment and have well established prognostic implications. To characterise patterns of the inflammatory infiltrate that may lead to a specific imaging signature, UKM investigated this infiltrate by means of histology, immunhistochemistry and immunfluorescence in different benign and malignant breast lesions.

As a first step, a retrospective control group has been generated to establish a panel of antibodies to characterize the inflammatory cells. Besides the analysis of the inflammasome in serial sections, the OPAL-system for multispectral immunfluorescence was used to analyse the interconnection of the different cellular components in the same slide. For this, the staining procedures have been established.



Legend:
Opal520 CD4 green
Opal570 CD8 red
Opal540 CD20 yellow
Opal 650 CD45 cyan blue
Opal 690 PanCK purple
DAPI nucleus blue

In a second step, the results from the above mentioned retrospective series were used to develop an immunoscore.

According to the initial project plan, we plan to apply this scoring system in a later step to breast tissue samples obtained from patients being examined by the novel HYPMED device. In a next step, we will then correlate the results of the histologic and immunohistochemical samples from the different lesions with the respective imaging phenotype patterns as provided by the MR and PET imaging component , and finally with factors indicative clinical/ prognostic relevance. These latter steps will unfortunately not be possible within the timeframe of the ongoing HYPMED project due to the unsettled medicolegal requirements of using medical device prototypes under the new MDR.

2 Correlations

2.1. Introduction

Due to the pandemics and other challenges encountered during the engineering phase of the new HYPMED device, the project suffered from multiple delays – with the result that the finalization of the device was delayed, and thus, the new MDR came into effect before the clinical trial could be started. As a result, with the new regulatory framework, the actual clinical trial could not be performed, and thus no tissue samples of patients undergoing imaging with the HYPMED device could be collected for correlation with immunoscores and biomarkers. For this reason, we changed the approach to still achieve results with relevance for the project. We were able to establish a cooperation with the Department of Pathology in Vienna University. After all requirement were met that regulate the shipping of biosamples across EU countries, the Department of Pathology of the University of Vienna was able to provide us with pathology tissue specimen from the women who had undergone conventional, whole-body contrast enhanced FDG-PET/MR imaging within WP3.

2.2. Münster series

We were able to collect 26 cases of radial scars (a.k.a. “complex sclerosing lesion” – i.e. benign pathologic changes that mimic breast cancer on conventional breast imaging including MRI) – and for malignant lesions, 25 cases with pre-invasive changes (ductal carcinoma in-situ, DCIS), 13 cases of women with invasive breast cancer who had undergone biopsy before and after completion of neoadjuvant treatment – yielding 26 tissue samples, and then samples from 31 invasive breast cancers, NST (previously designated as ductal carcinomas). All these cases were stained by an extended set of markers to identify those of potential relevance for an immunoscore (CD 4, CD 8, CD 20, CD 31, CD 34, CD 68, CK 7, CK 14, SMA, p 63, ERG, FOXP3, PD-L1, Nestin).

2.3. Vienna series

We were able to process up to now 42 tissue samples shipped from Vienna, of chemo-naïve patients who had undergone whole-body PET/MR imaging including contrast enhanced breast PET/MR imaging before biopsy, and who had undergone image guided biopsy, and with information on tumor subtype, tumor stage, and patient outcome. In these samples, we performed immunohistochemistry as identified in our Münster series to be suitable for an immunoscore. The markers we applied were the following: CD4, CD8, CD20, CD68, CD34, CD31, ERG. In addition, tumor-infiltrating lymphocytes (TILs) and stromal components for a tumor-stroma ratio were quantified in H&E-stained slides. By using different endothelial markers (CD34, CD31, ERG), we were able to evaluate the microvessel density.

Interestingly, the vast majority of breast cancer patients nowadays receives neoadjuvant treatment which allowed us to compare these cases with those without systemic treatment before surgery. We recognized that in this subgroup, TILs is go down after treatment whereas macrophages increase in number possibly due to resorptive processes.

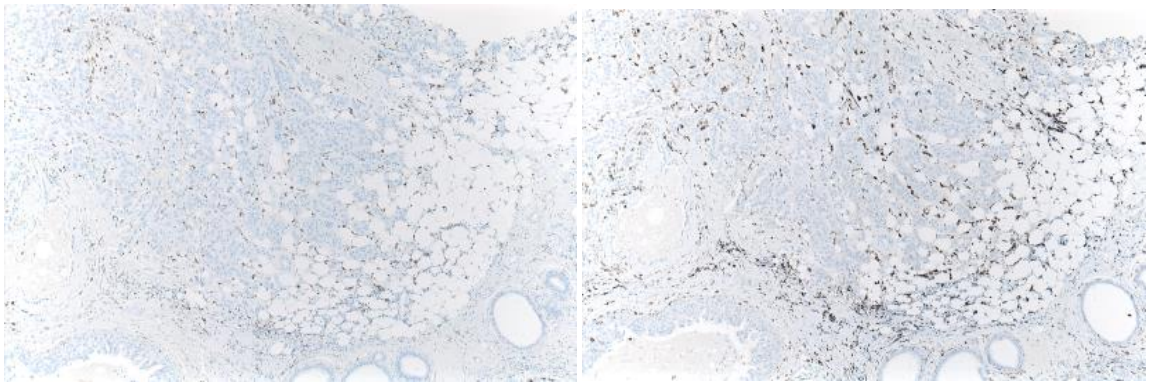


Figure 1. Different types of macrophages (M1 left, CD68; M2 right, CD 163) in an invasive breast cancer, NST.

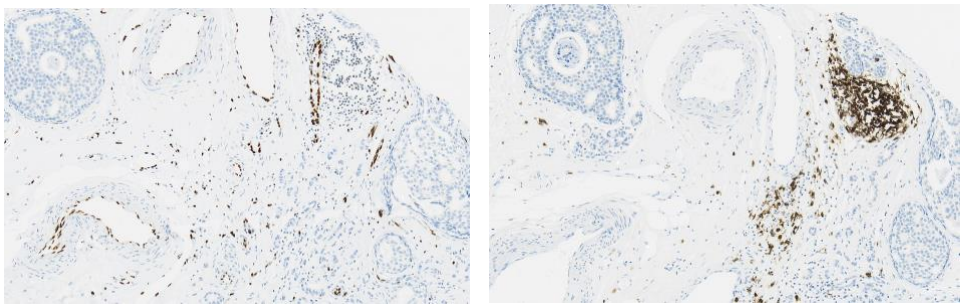


Figure 2. Invasive BC and DCIS: ERG for microvessel density and CD20 for B-lymphocytes.

Furthermore, the number of vessels per stroma is decreasing after treatment which will likely influence imaging parameters such as contrast enhancement kinetics as well as diffusion coefficients; the correlation with respective patients' imaging findings in MR and PET is currently ongoing.

Accordingly, this exploratory analysis was useful to define specific hypotheses that will now be validated by further cases that we will process in close cooperation with WP3; our goal is to increase the number of cases to be evaluated by this standardized approach to 100.

2.4. Aachen series

Another goal of our WP was to correlate our immunoscore data with biomarkers identified by Edgar Dahl's group in Aachen (see also D4.3). They had generated an own series of benign and malignant breast lesions to include them into TMAs. We received 7 tissue micro arrays (TMA) including 118 benign lesions that were selected to reflect benign lesions that are known to be associated with contrast enhancement in breast MRI as well as with hypermetabolic findings in PET. Aim is to find out why – in the absence of malignant angiogenesis in these benign changes – there is at all contrast enhancement, or even contrast enhancement that can mimic that of breast cancers, as a notorious cause of false-positive findings on MRI imaging – associated with these lesions. These benign histologic changes are radial scars (complex sclerosing lesions), adenosis, fibroadenomas, apocrine metaplasias, and flat epithelial atypias (FEA). In addition, 32 DCIS cases could be provided. All these TMAs were stained in Münster and then sent back to Aachen to be scanned. This procedure allows both sites to analyse the digitalized slides in parallel, and to apply artificial intelligence for pathologic

data analysis within a future conjoint project. Once the whole series is evaluated these data are ready to be correlated with the biomarker results from the same samples.

3 Conclusions

During the project we were able to collect numerous annotated samples from three different university hospitals, from patients who did undergo conventional breast imaging including contrast enhanced MRI, and/or conventional whole-body PET/MR, carefully selected to reflect current challenges of breast cancer imaging: Benign lesions that can mimic breast cancer based on their specific morphology (e.g. radial scars), and benign lesions that can mimic breast cancer or DCIS due to their enhancement on breast MRI or PET (radial scars, adenosis, fibroadenoma, FEA), and pre-invasive as well as invasive breast cancer, the latter before and after neoadjuvant treatment. We have been able to perform quantitative evaluation of parameters defining the tumor microenvironment, and were able to develop interesting hypotheses that may help explain the respective radiologic phenotypes of different lesion types and stages under systemic treatment, and thus completed the first part of MS15 (Classification system based on biomarker panel and immunoscore as correlate of differential PET/MRI imaging). The remaining step (correlation of this histopathological and immunological classification system with the respective PET/MR imaging phenotypes) is still ongoing.

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