Zeinab Elsawaf
Dr. med.

Molecular Pathology, Phenotype, Classification, and Prognosis of Triple-Negative Breast Cancers

Promotionsfach: Pathologie.
Doktorvater: Herr Prof. Dr. med. Hans-Peter Sinn.

Triple-negative breast cancers are a special group of breast cancers with an adverse prognosis that are characterized by their lack of estrogen and progesterone receptor expression (ER-, PR-), as well as their lack of HER2 overexpression. In this study we have examined a series of 142 triple-negative breast cancers (TNBCs) with regard to their morphology, immunophenotype (especially regarding intermediate filament proteins), molecular characteristics (such as cell cycle markers), proliferation, clinical characteristics, and prognosis. This was compared to conventional prognostic markers (type, grade, and tumor stage), with the aim of detecting a biologically oriented, and clinically significant subclassification of TNBCs. In persuing four different tracks (immunophenotype, survival, cluster analysis, and quantitative analysis) the following main results were found:

TNBCs display a wide variation for patterns of expression of intermediate filaments, with the majority of tumors being positive for luminal cytokeratins, followed by basal cytokeratins, and vimentin being least commonly found. Co-expression of different intermediate filaments was the rule, single expression patterns (such as a pure basal phenotype), the exception. Basal cytokeratin expression patterns were correlated with the expression of cell-cycle and differentiation antigens (such as p16, Ki-67, CD117, and others). Luminal cytokeratin expression was associated with better tumor differentiation.

Prognosis in this series of TNBCs was dependent on conventional prognostic factors, such as node positive disease (pN1-3), large tumor size (pT2-3), and advanced tumor stage (II-III), but also luminal cytokeratins (CK7 and CK19) were shown to adversely influence survival, whereas basal cytokeratins had no effect on survival as single markers.

In order to find natural subtypes of TNBCs, a hierarchical unsupervised cluster analysis was performed. This has revealed the presence of four, significantly different

clusters of differentiation based on the patterns of expression of 13 immunohistochemical markers examined. The main distinguishing feature of these four clusters was their intermediate filament expression, therefore these clusters were characerized accordingly as luminal, basoluminal, immature and basal clusters. This classification provided a strong correlation with prognosis in both univariate and multivariate analysis. Tumors in clusters 1 and 2 (luminal and basoluminal clusters) affected older patients and were associated with a worse overall survival (5-year OS $p<0.05$) compared to patients with tumors in the immature and basal cluster groups (5-year OS $p>0.05$). In the multivariate cox model, these cluster groups had a stronger influence on survival than conventional prognostic factors.

The validity of the immunohistochemical analysis of the expression of differentiation markers in TNBCs was confirmed by immunofluorescence and double immunoflourescence studies on the expression of intermediate filaments and related antigens. Additionally, these studies have revealed details of the differentiation patterns in TNBCs and their relationship to myoepithelial markers.

In order to confirm the expression patterns found with semiquantitative analysis, and to get deeper insights into the quantitative distribution of intermediate filaments with regard to the differentiation clusters found, an automated quantitative analysis of nuclear and cytoplasmatic antigens under study was performed. This has revealed a strong correlation of intermediate filament proteins with the cluster analysis, confirming the importance of cytokeratins for the different types of TNBCs.

In conclusion, we were able to delineate biologically, clinically, and prognostically distinct groups of TNBCs, based on their immunohistological characteristics.