

## 00200 Multiplex Immunohistochemistry for Evaluating Mitoses in Asian Triple Negative Breast Cancers

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**Aims:** Mitotic rate is routinely employed and widely used in grading breast cancers. Phospho-Histone 3 (PHH3) has been identified as an effective and sensitive marker to detect mitotic figures. Through the usage of multiplex-immunohistochemistry (IHC), we investigate the role of mitotic rate as an independent prognostic marker in triple negative breast cancers (TNBC).

**Methodology:** A retrospective cohort of 56 TNBC patients diagnosed between 2003 and 2015 in Singapore General Hospital (SGH) was studied. FFPE samples of breast cancers were analysed on tissue microarray using multiplex-IHC, to combine pHH3 with cytokeratin (CK) and leucocyte common antigen (CD45). The prognostic value of PHH3 scoring as a surrogate marker of mitotic rate will be evaluated as a standalone parameter, as well as adjusted to patient age, tumour size, grade and lymph node stage.

**Result:** Multivariate analysis showed that every incremental 1% in pHH3 expression on CK+ tumour cells of TNBC patients was associated with worse DFS (HR=1.92; 95% CI 1.24-2.96, p=0.004). Meanwhile, every incremental 1% in total pHH3 expression and every incremental single PHH3+ cell of TNBC patients were associated with unfavourable DFS respectively (every 1%; HR=2.79, 95% CI 1.15-6.77; p=0.023); (every single cell; HR=1.02, 95% CI 1.00-1.03; p=0.009), regardless of the colocalization with CK or CD45.

**Conclusion:** Mitotic rates determined by pHH3 expression in both epithelial cells and immune cells were significantly associated with poorer survival in Asian TNBC. Multiple-IHC of PHH3 in combination with CK and CD45 could assist pathologists to accurately and objectively quantify mitotic rates and might potentially be translated to the routine diagnostic work in the near future, pending validation on larger tumour cohorts.