ALK-FISH borderline cases in non-small cell lung cancer: Implications for diagnostics and clinical decision making.

Abstract:
Fluorescence in-situ hybridization (FISH) for the detection of ALK-rearrangements in non-small cell lung cancer (NSCLC) is based on at first sight clear cut-off criteria (\(\geq 15\\%\) of tumor cells) for split signals (SS) and single red signals (SRS). However, NSCLC with SS-counts around the cut-off may cause interpretation problems. Tissue microarrays containing 753 surgically resected NSCLCs were independently tested for ALK-alterations by FISH and immunohistochemistry (IHC). Our analysis focused on samples with SS/SRS in the range between 10\% and 20\% (ALK-FISH borderline group). To better understand the role of these samples in routine diagnostics, we performed statistical analyses to systematically estimate the probability of ALK-FISH-misclassification (false negative or positive) for different numbers of evaluated tumor cell nuclei (30, 50, 100, and 200). 94.3\% (710/753) of the cases were classified as unequivocally (\(=20\%\)) ALK-FISH-negative (93\%; 700/753) or positive (1.3\%; 10/753) and showed concordant IHC results. 5.7\% (43/753) of the samples showed SS/SRS between 10\% and 20\% of the tumor cells. Out of these, 7\% (3/43; ALK-FISH: 14\%, 18\% and 20\%) were
positive by ALK-IHC, while 93% (40/43) had no detectable expression of the ALK-protein. Statistical analysis showed that ALK-FISH misclassifications occur frequently for samples with rearrangements between 10% and 20% if ALK-characterization is based on a sharp cut-off point (15%). If results in this interval are defined as equivocal (borderline), statistical sampling-related ALK-FISH misclassifications will occur in less than 1% of the cases if 100 tumor cells are evaluated. While ALK status can be determined robustly for the majority of NSCLC by FISH our analysis showed that ~6% of the cases belong to a borderline group for which ALK-FISH evaluation has only limited reliability due to statistical sampling effects. These cases should be considered equivocal and therapy decisions should include additional tests and clinical considerations.