OP32. A COMBINED STRATEGY FOR THE DETECTION OF BRAF FUSIONS IN PILOCYTIC ASTROCYTOMA USING RT-PCR AND FISH
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INTRODUCTION: Pilocytic astrocytomas can show a wide morphological spectrum making definitive histological diagnosis challenging. The FISH test for KIAA1549-BRAF fusions is most commonly used, but this is difficult to interpret. We aimed to develop a real-time PCR (RT-PCR) test as a first-line screen for the three most common KIAA1549-BRAF fusion variants.

METHOD: A RT-PCR method for detecting KIAA1549-BRAF fusions from formalin-fixed paraffin-embedded (FFPE) brain tumour tissues (pilocytic astrocytoma). The three most common fusion variants are detected using this assay: exon 16 of KIAA1549 fused to exon 9 of BRAF, exon 15 of KIAA1549 fused to exon 9 of BRAF and exon 16 of KIAA1549 fused to exon 11 of BRAF fusion. GAPDH expression was used as a control.

RESULTS: The RT-PCR assay was initially validated on 12 samples previously tested by FISH or RT-PCR in a different laboratory. The RT-PCR assay had a sensitivity of 89% (8/9 - one sample tested positive by FISH but negative on RT-PCR) and a specificity of 100% (2/2). The failure rate was 8.3% (1/12). Sensitivity experiments showed that the fusion can be detected when present at a least 5% of the total cDNA content. 51 Neuropathology diagnostic FFPE samples from 42 pilocytic astrocytoma patients were then tested using the BRAF fusion RT-PCR assay. The overall pick-up rate was 54% (20/37 patients). Of the positive patients (20), 55% (11/20) had the 16-9 fusion and 45% (9/20) had the 15-9 fusion. Two patients had multiple fusions (2/20 positive patients, 10%) showing the 16-9 fusion and a low-level 16-11 fusion. No patients exclusively had the 16-11 fusion.

CONCLUSION: We propose RT-PCR first line for fusion analysis followed by FISH, for pilocytic astrocytoma.