Evaluation of HPV DNA and mRNA Detection Technologies for Detecting HPV in Cervical Cytology Specimens

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HPV infection is the primary agent in the development of CIN and cervical cancer, with screening programmes moving towards the introduction of HPV testing as part of the screening process. In this study we evaluated two HPV detection technologies for detection of HPV in liquid based cytology specimens. These included HPV DNA by Hybrid Capture II (Digene, HCII), which detects 13 high-risk HPV types and E6/E7 mRNA expression by PreTect HPV Proofer (Norchip), which detects HPV 16, 18, 31, 33, and 45.
In summary, 205/299 cytology specimens representing the broad spectrum of the disease (Normal-CIN3) were positive for HPV DNA and 113/299 specimens were positive for E6/E7 mRNA. We report higher concordance rates between both technologies in CIN3 cases (83%) and Normal cases (88%) than in the BNA or CIN1-2 disease categories. The positive predictive value (PPV) and specificity of the HCII DNA test (41% and 43.7% respectively) were lower than that of the PreTect HPV Proofer mRNA test (53.6% and 75.6%) for detection of high-grade disease (CIN2+), indicating that PreTect HPV Proofer may be more useful than HCII for predicting high-grade disease.
This study forms part of the MicroActive Consortium funded under the EU 6th framework *eHealth* Initiative

Clinical Measurement of Prognostic Immune Signature in Follicular Lymphoma by RT-PCR based Gene Expression Profiling and Immunohistochemistry Demonstrates Favourable T-cell and Unfavourable Macrophage Infiltration

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Gene expression profiling studies have demonstrated immune response gene signatures predictive of outcome in follicular lymphoma (FL) and there is a need for validation of these signatures and for their translation to clinical use. However, measurement of these genes in routine practice remains difficult and to date there have been very few studies validating the hypothesis. In this project we used real-time PCR measurement of gene expression levels in globally amplified polyA cDNA to analyse of immune response signatures in FL. We used real-time PCR to measure expression levels (normalised to the mean of 4 housekeeping genes) of 36 candidate *Indicator* genes, selected from microarray studies, in polyA cDNAs prepared using polyA PCR from 58 archived human frozen lymph nodes, together with immunohistochemistry for CD3, CD4, CD7, CD8, CD10, CD20, CD21 and CD68 in parallel formalin fixed paraffin embedded tissue samples to measure immune response in FL. Immunohistochemical positivity was measured by a semi-automated image analysis method using spectral unmixing to identify areas of immunopositivity. Kaplan-Mier survival analysis was performed against the normalised real-time PCR expression levels of each of the genes and against the percentage immunohistochemical postivity for each of the antibodies except for CD68 survival analysis for which was performed for cases with either 15 or less or more than 15 CD68 positive cells per high power field (hpf). High levels of CCR1, a marker of monocyte actication, were associated with a shorter survival interval ($p<0.02$), whilst immunohistochemistry demonstrated association of high numbers of CD7 positive T-cells with longer survival interval ($p<0.02$) and of high numbers of CD68 positive macrophages with a shorter survival interval ($p<0.032$). The results confirm the role of the host immune response in outcome in FL and identify CCR1 as a prognostic indicator and marker of immune switch between macrophage and T-cell dominant response. The methods used are clinically applicable, whilst the clinical utility of polyA DNA and real-time PCR for measurement of gene signatures and the strength of this approach as a molecular block are confirmed.