

# Evaluation of HPV mRNA in histologically negative sentinel lymph nodes of cervical cancer patients as a predictive marker for recurrence



Häfner N, Altgassen C, Müller B, Greinke C, Schneider A, Dürst M and the German Association of Gynecologic Oncology (AGO)

Friedrich-Schiller-Universität Jena, Klinik für Frauenheilkunde und Geburtshilfe, Abteilung Frauenheilkunde, Bachstraße 18, 07743 Jena, Germany



## Background

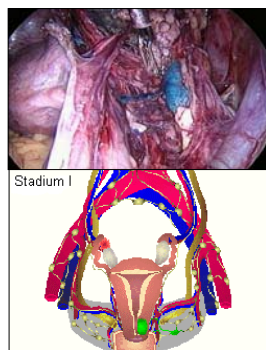
Human Papillomavirus (HPV) are the main cause for cervical cancer (CxCa). HPV DNA is found in 99.7% of CxCa worldwide (1). The oncogenic risk of HPV is mediated by the oncoproteins E6 and E7 that are expressed during all steps of carcinogenesis (2). Histologically positive lymph nodes (LN) represent the most important prognostic factor for recurrent disease in CxCa patients. Overall survival of patients with affected lymph nodes is in the range of 20 - 60%. However, 15% of patients suffer from recurrent disease although their lymph nodes were free from histologically evident cancer metastasis (pN0).

## Aim

Evaluation of the prognostic relevance of HPV mRNA in histologically negative sentinel lymph nodes (SLN) of patients with cervical cancer (RT-nested PCR), quantitative discrimination of E6 expression levels in sentinel lymph nodes and correlation with SLN status (qRT-PCR).

## Study Design and Methods

- Prospective multicenter study
- 400 patients. Follow up 3 years
- Identification of the first draining lymph node(s) SLN after application of Patentblau® and Technetium labelled Albu-Res®.
- Of each SLN and one representative distal LN a thin section was removed for HPV-mRNA analysis.
- RT PCR with oligo-dT primer using 1µg total RNA
- primarily nested PCR (sensitivity: 1 in 10<sup>6</sup>-10<sup>7</sup> cells)
- HPV16 positive samples (70% of patients) were also analysed by real time PCR.



## Results - nested RT PCR

- All histological positive SLN contained HPV-E6-E7 mRNA (H+ /M+)
- Viral mRNA positive but histologically negative SLN (H-/M+) were found in 11 of 57 patients (19.3%) (Fig. 2)
- 4 of 6 patients with histological false negative SLN (pN1) contained viral mRNA (H- /M+) (Fig. 2)
- HPV-type-specific mRNA detection is sensitive and highly specific and provides strong evidence for the presence of isolated tumor cells and tumor cell clusters.
- Although the data are still incomplete it is interesting to note that a higher rate of recurrence is seen in pN0/M+ patients (2/11) than in pN0/M- patients (2/46). The recurrence rate is highest in H+/M+ patients (8/23). (Fig. 2)

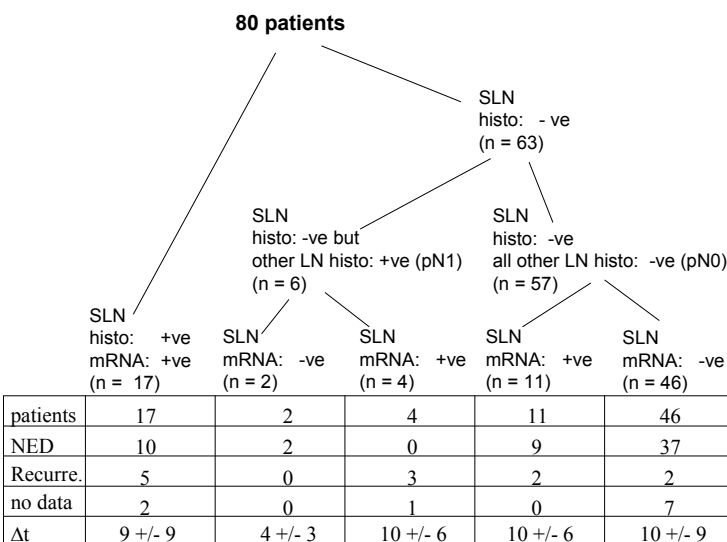
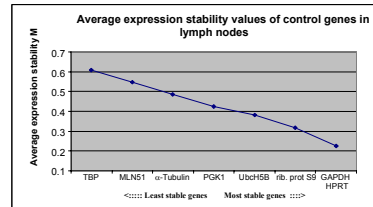


Figure 2: SLN status and preliminary follow up data of 80 patients

## Results - real time PCR

- Determination of housekeeping gene expression in lymph nodes and calculation of normalisation factors based on the geometric mean of most stable housekeeping genes showing the lowest pairwise variation in LN samples (3). (Fig. 3)



Gen	R	efficiency [%]
GAPDH	0.999	89.5
HPR1	0.997	92.1
MLN51	0.997	101.2
PGK1	0.999	90.2
S9	0.998	84.9
TBP	0.987	100
Tubulin	0.998	86.4
Ubch5B	0.999	94.7

Figure 3: Determination of stable housekeeping genes in lymph nodes

- Real time PCR for HPV16 E6 is independent of used template highly efficient (>90%), reproducible, has a high sensitivity (1 in 10<sup>5</sup> cells) and a broad dynamic range (Fig. 4)

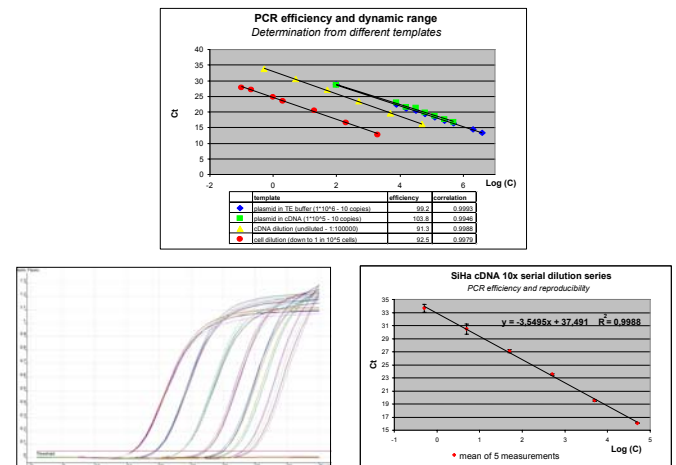


Figure 4: Characterisation of HPV16 E6 real time PCR

- There is a clear correlation between E6 expression level and sentinel lymph node status of patients. (Fig. 5)

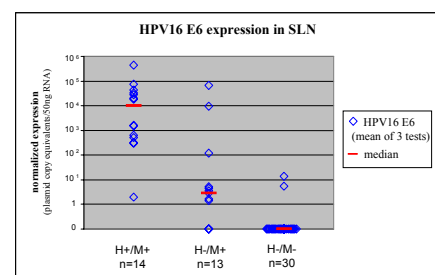


Figure 5: HPV16 E6 expression levels in representative SLN of patients  
H+ histologically positive, H- histologically negative, M+ RT nested PCR positive, M- negative in RT nested PCR

## Conclusions and Open questions

- Preliminary data suggest that patients with histologically negative lymph node status (pN0) but with HPV-mRNA positive SLN have increased risk of recurrence.
- Is there a correlation between recurrence and the amount of HPV-mRNA in lymph nodes?
- Does HPV positivity in SLN correlate with detection of isolated p16 positive cells in these nodes?
- Is there a correlation between HPV-mRNA positive SLN and CK19 mRNA positivity?

## References

- (1) Walboomers *et al.* J. Pathol. 1999;189(1)
- (2) Dürst *et al.* Virology 1992;189(1)
- (3) Vandansompele *et al.* Genome Biology 2002;3(7)