

Prognostic value of HPV-mRNA in sentinel lymph nodes of cervical cancer patients with pN0-status

Supplementary Material

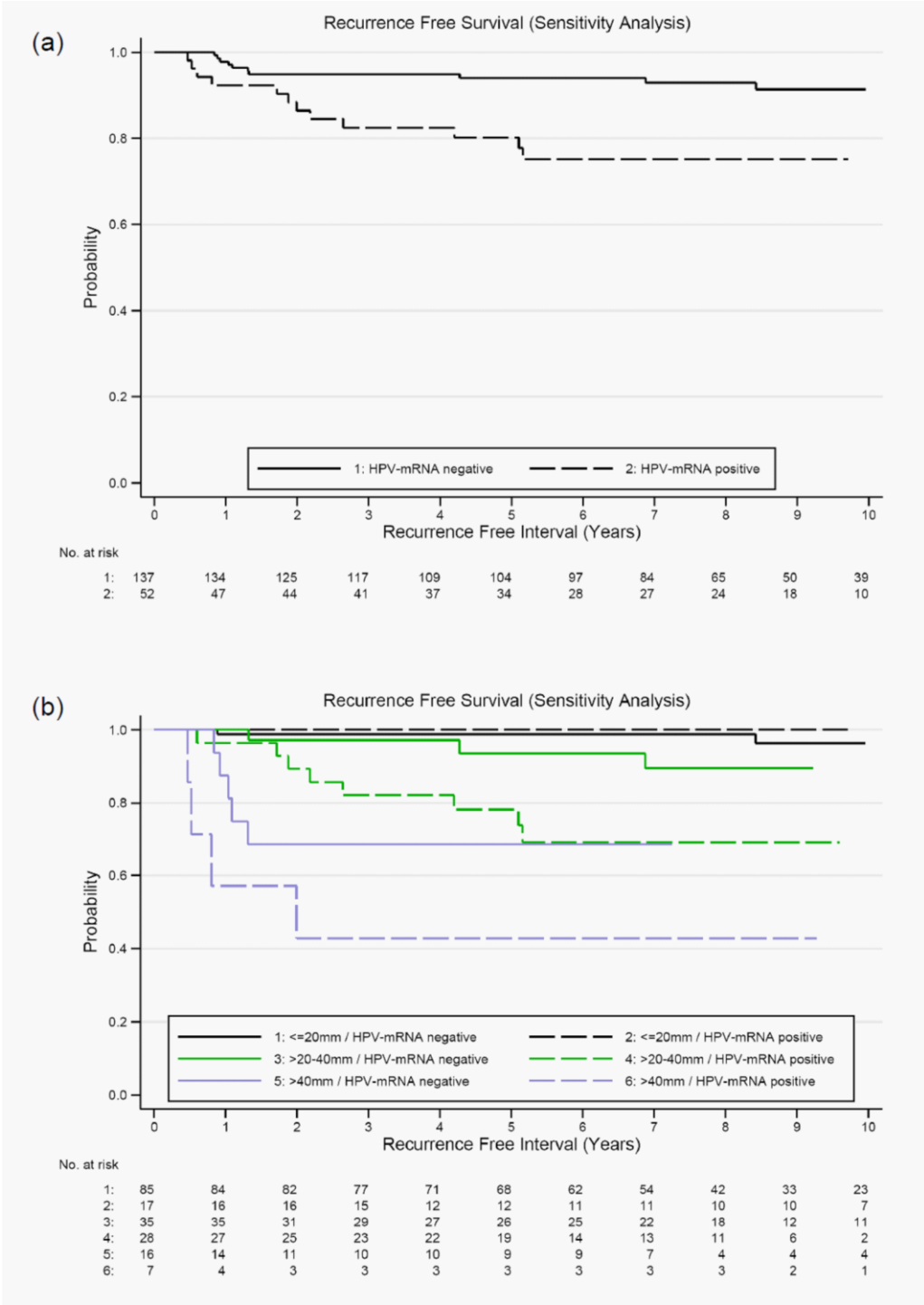
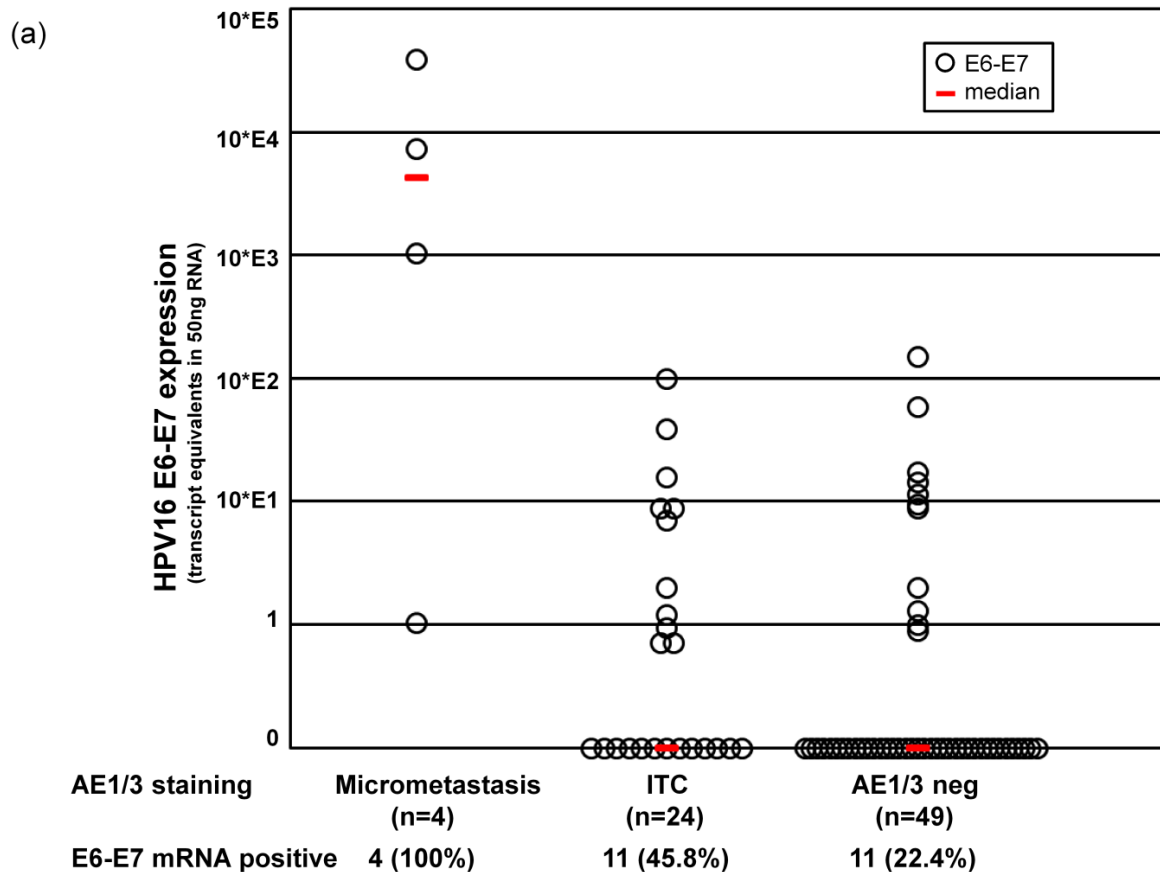


Figure S1: (a) Sensitivity analysis - Kaplan Meier curves of recurrence-free survival according to HPV-mRNA status of sentinel lymph nodes; log rank test $p=0.002$ (study population $n=189$) and (b) Sensitivity analysis - Kaplan Meier curves of recurrence-free survival according to HPV-mRNA status of sentinel lymph nodes stratified by tumor size; log rank test for HPV-mRNA adjusted for tumor size $p=0.028$ (study population $n=189$).



(b)

		nested PCR		
		+	-	
qPCR	+	24	2	26
	-	5	46	51
		29	48	77

kappa = 0.802

Figure S2: HPV16 E6-E7-mRNA expression as determined by qRT-PCR and RT-nested-PCR in relation to immunohistochemical staining for cytokeratins (AE1/3) in 77 SLN scored negative by conventional histopathology. (a) Expression levels (qRT-PCR) corresponding to HPV16-E6-E7 transcripts in 50ng of total RNA (see Häfner et al. [16]) are shown on the y-axis. 50ng of total RNA corresponds to 5×10^3 cells. On the x-axis the results of AE1/3 staining and quantitative RT-nested-PCR are shown. ITC refers to single dispersed tumor cells. (b) Overall agreement between qRT-PCR and RT-nested PCR.

Supplementary Table S1: Primers for the detection of hrHPV-E6-mRNA by RT-nested-PCR and oligonucleotide probes for Southern blots

HPV type	1st PCR			2nd PCR			Southern Blot hybridisation probe
	forward	reverse	length FLT (E6*) [bp]	forward	reverse	length FLT (E6*) [bp]	
16	ACAGGAGCCACCAAGAGTTACC	ATGGTTCTGAGAACAGATGGGGC	739 (556)	TGGGGCACACAATCTAGTGTGC	AGTTACCAGATTATGCACAGAGC	704 (521)	TCATGCAATGAGGTGTCTCC
18	ACAAGCTACCTGATCTGTGC	CTGAGTCGCTTAATTGCTCG	554 (373)	GGAACTGAACACTCACTGTC	TGACATAGAAGGTCAACCGG	512 (331)	GCAGCATGCGGTATCTGCTC
31	AATCTGCGAAGAACCTCGG	GGCACACGATCCAAATGAGC	717 (514)	CGGAAATGCATGAACTAAGC	CCAAATGAGCCATTAAACAGC	688 (485)	ACTCCGTGTGGTGTCTGTC
33	TGCACTGATTTGTCCAAAG	TTTGTACCTTCAGGATCG	759 (481)	CCAAGCATTGAGACAAC	GATCGGCCATTGTAGATG	733 (455)	CACTAAITTTAGATAAGAACCGGAAA
35	TCAGGACCCAGCTGAACGACC	TATGCTCTCTGGAACAGCGG	746 (563)	GAACGACCTTACAACTGCATG	CACACTATTCCAAATGTGCC	704 (521)	GGTCAGTTGCCTGGGTTC
39	TCACAATCTGCAGAACGGC	ACAATTTCTGCAAGGTGGG	509 (326)	TGCCAGACTGTGCAACAGC	AGGTGGGCTTTGGTCCACGC	467 (284)	GGTCGTGCAATAGACACAGG
45	ATGGCGCGCTTTCAGATCC	CTGACTCGCTTAATTGCTCG	591 (406)	GCAACGACCTTACAAGTAC	CATTCTGAGGTTCCAAATGC	520 (335)	TACCTCTGCGCTTCCAATGTT
51	GGGAAAGCACCAACAGCTGC	CTAGTTGTCGCAACACAGG	753 (528)	GCTGCATGAATTAATGGAAGC	CAAACGAGGTTAGTTCGCC	716 (491)	GCGAAGGGTGTCTCACTGC
52	CCAGCAACACGCCCGGACC	AATGTGCCCAACAGCATTGTC	705 (450)	CCCTGCACGAATTGTGTGAGG	TAGAGTACGAAGTCCGTCGC	660 (405)	TGTCTCCAGCATGACGTTACA
53	CATCCATGGATCGCAATTGTTG	TTGCAGTTATCGCTTGTGTC	790 (607)	AAGAGCCACCGATACATTGC	CACCTGTGCCATAAGCATTG	715 (532)	GAGGACTGCCGATCACTGAAC
56	CAACAATCCACAGGAACCTCC	GCTTGTGTGGCCGCTCTGC	605 (350)	GCCTGACCACTGAGTGTGAGG	TCCTCATCTCTGAGTGTCC	541 (286)	GTTTGTCTCCAGCACCCAAAC
58	ATTTGTGTGAGGCTTTGG	CCGTTGTACCTTCAGGG	759 (481)	CGTTGGAGACATCTGTGC	CAGGGTCACTCATGTCAG	734 (456)	ACTCACTATTTTATAGATGCAATCGTAAG
59	TGAGGATCCACACAACGACC	AAACAGCTGCTGAAGGCTCG	752 (353)	ACAACGACCTACAACTGCC	CTCGCAATCCGCTTGCAGC	727 (328)	TCTTTCTTGAGTCCCTT
66	CATATTCAGCAATCAACAGG	TCAATTCCTGTTGGCGTGC	527 (272)	ACGTCCAGCAAGCTGCACC	TGCAACGTGGTACTTTACC	467 (212)	TCCAACTGCAAAACGACCC
68	AGTTGTCTATACCAATGGCG	GGTTCCGTTGCACACCAAGG	825 (642)	CGCTATTCACAACCTGAGT	AGCAGTTTACGTTCCGACGG	764 (581)	GACGCTGTTGTGCTCCGCTC
73	GACTGTGTGTTTGGCAAGC	AGGTCAATTCGGTGTGTC	429 (246)	TGATTTTGATTTAGTATTGTT	CACCACAGTTGCAGATGTC	313 (130)	GCCTATACTGATTTGCTATATGCTC
82	CATAAGAGAAAGCACGAAACG	GGTGTAACTCCAACTATGTC	502 (277)	TGTGAAGCCTGCAATATGTC	TACCACGATGGCGTTATTA	428 (203)	TTCTTAGGCCCAAGTGGTCTC

The RT-nested-PCR assay for hrHPV-E6-mRNA allows the detection of solely matured spliced (E6*) and un-spliced (E6-full length) viral oncogene transcripts which can only be found in intact tumor cells

Supplementary Table S2: Frequency of adjuvant therapy, para-aortic lymph node dissection and death in relation to HPV mRNA status

	HPV mRNA status of sentinel lymph nodes			
	Positive	Negative	Probably negative	Total
Adjuvant therapy post OP	19 / 52 (36.5%)	26 / 119 (21.8%)	7 / 17 (41.2%)	52 / 188 (27.7%)
Para-aortic lymph node dissection	16 / 52 (30.8%)	28 / 119 (23.5%)	7 / 18 (38.9)	51 / 189 (27.0%)
Death* (due to cervical cancer)	7 (6) / 52	6 (2) / 119	2 (1) / 18	15 (9) / 189

* without percentage due to different duration of observation

Supplementary Table S3: Five-years rate of recurrence free survival according to HPV mRNA status of sentinel lymph nodes stratified by tumor size (sensitivity analysis population, n=189)

Tumor size [mm]	HPV mRNA of SLN	Disease recurrence (n)	5-years rate of recurrence free survival (95% CI)
	all	22 (189)	90.1 (84.8 – 93.7) %
**	all		
	negative*	10 (137)	94.0 (88.4 – 97.0) %
	positive	12 (52)	80.2 (66.2 – 88.8) %
***	≤ 20		
	negative*	2 (85)	98.8 (91.9 – 99.8) %
	positive	0 (17)	100.0 %
	>20 to 40		
	negative*	3 (35)	93.5 (76.3 – 98.4) %
	positive	8 (28)	78.2 (57.8 – 89.6) %
	>40		
	negative*	5 (16)	68.8 (40.5 – 85.6) %
	positive	4 (7)	42.9 (9.8 – 73.4) %

Abbreviations: SLN – sentinel lymph node, CI – confidence interval

* Probably or definitely negative

** Log rank test for HPV mRNA: p=0.002

*** Log rank test for HPV mRNA adjusted for tumor size: p=0.028

Supplementary Table S4: Strength of association between prognostic factors and recurrence free survival determined by Cox regression analysis (sensitivity analysis population, n=189)

		n	Hazard Ratio (95% CI)	p-value***
<i>Single factor models</i>				
HPV mRNA of SLN	probably or definitely negative	137	1	0.004
	positive	52	3.45 (1.48 – 7.98)	
Tumor size [mm]**	≤ 20	102	1	<0.001
	>20 to 40	63	9.43 (2.08 – 42.54)	
	>40	23	27.01 (5.82 – 125.19)	
Histology	Squamous cell ca.	152	1	0.487
	Adenocarcinoma	29	1.28 (0.42 – 3.82)	
	Others	8	2.41 (0.55 – 10.47)	
Grading**	G1	16	1	0.194
	G2	85	0.38 (0.09 – 1.52)	
	G3	82	0.88 (0.25 – 3.08)	
Age [years]		189	1.03 (0.99 – 1.06)	0.074
<i>Two factor models</i>				
HPV-mRNA of SLN**	negative*	136	1	0.034
	positive	52	2.54 (1.07 – 6.03)	
Tumor size**	≤ 20 mm	102	1	<0.001
	>20 – 40 mm	63	7.09 (1.53 – 32.87)	
	>40 mm	23	24.42 (5.24 – 113.60)	
HPV-mRNA of SLN	negative*	137	1	0.006
	positive	52	3.32 (1.41 – 7.75)	
Histology	Squamous cell ca.	152	1	0.747
	Adenocarcinoma	29	1.24 (0.41 – 3.71)	
	Others	8	1.72 (0.38 – 7.59)	
HPV-mRNA of SLN**	negative*	131	1	0.010
	positive	52	3.05 (1.31 – 7.10)	
Grading**	G1	16	1	0.291
	G2	85	0.43 (0.10 – 1.74)	
	G3	82	0.90 (0.25 – 3.16)	
HPV mRNA of SLN	negative*	137	1	0.005
	positive	52	3.30 (1.42 – 7.64)	
Age [years]		189	1.03 (0.99 – 1.06)	0.106

Abbreviations: SLN – sentinel lymph node, CI – confidence interval, ca. – cancer

* Probably or definitely negative

** Valid datasets: Tumor size and related two factor model n=188, grading and related two factor model

n=183

*** Wald test