



HPV E6/E7 mRNA detection and concurrent Pap smear cytology in a population of 19,152 Norwegian women aged 13 to 87 years

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ABSTRACT

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OBJECTIVES

The aim of the study was to compare detection of HPV E6/E7 mRNA with cytology diagnosis.

METHODS

Cervical specimens from 19,152 women 13-87 years of age were analyzed for the presence of E6/E7 mRNA from high-risk (hr) HPV 16, 18, 33, 35 and 45 by multiplex NASBA (PreTect HPV-Proofer, NorChip AS, Klokkestua, Norway) during May 2003 to December 2004. Data was matched with the Norwegian Cervical Cancer Screening Program for concurrent cytology diagnosis.

RESULTS

Altogether, 1,590 (8.3 %) women tested positive for E6/E7 mRNA. The proportion of mRNA positives was highest in the age group of 20-24 (20 %) and it decreased with increasing age up to 59 years. The proportion of E6/E7 mRNA positives increased with increasing cytological severity. Among women with Normal, ASC-US, L-SIL, ASC-H, and HSIL cytology, 5.1 %, 25.9 %, 29.3 %, 47.6 %, and 63.2 % were positive for hr HPV mRNA, respectively. Women with Normal, ASC-US, and L-SIL cytology were decreasingly E6/E7 mRNA positive with increasing age after 20-24 years. This was not the case for women with ASC-H and HSIL cytology, where the proportion of E6/E7 mRNA positives, was constant and high across the age groups.

DISCUSSION

The observed age dependent reduction of hr HPV E6/E7 mRNA was in agreement with previous hr HPV DNA and mRNA findings in Norway. Our study indicates difference in HPV E6/E7 mRNA proportions between low and high risk Pap diagnostic groups. Expression of E6/E7 mRNA was high in every age group when Pap diagnose was HSIL or ASC-H. We also observed elevated OR for being diagnosed with histology CIN2+ in the HSIL group when HPV E6/E7 mRNA is positive compared to negative. This indicates E6/E7 mRNA as an adequate tool for diagnosing women with high risk lesions.

INTRODUCTION

HPV is a common virus infection, particularly in younger females, where most infections are transient and asymptomatic^{1,2}. Infection with high-risk HPV is the main cause of cervical intraepithelial and invasive neoplasias^{3,4} and HPV DNA has been detected in >90 % of cervical carcinomas, with the most common HPV types identified as genotypes 16, 18, 31, 33, and 45^{5,6}. Patients infected persistently with these high-risk (hr) HPV types have a clearly enhanced risk of developing invasive cervical carcinoma (ICC)⁷. Further, hr HPV infection is necessary for the development of ICC^{8,9,10} and the expression of the hr HPV E6/E7 oncogenes is necessary for conversion to and maintenance of malignancy in cervical tissue^{11,12,13}. Therefore, detection of the E6/E7 mRNA from hr-HPV is proposed to serve as a better risk evaluation factor than cytology for the development of high-grade squamous intraepithelial lesion (HSIL) and ICC¹⁴. Our objective was to characterize the detection of hr HPV E6/E7 mRNA and corresponding cytology diagnosis in a broad age group.

MATERIAL AND METHODS

Study Population

The study subjects comprise 19,152 women 13-87 years of age visiting their gynaecologist or general practitioner between 1st of May 2003 and 31st of December 2004. Women were included after an individual assessment by the patient's general practitioner and/or gynaecologist. Included women were subject to drawing of HPV cervical samples. In retrospect, results from viral laboratory diagnostics were matched with the Norwegian Cervical Cancer Screening Program for concurrent cytology and histology. To reflect normal practice more closely, we used the original cytological diagnosis and histology diagnosis from the laboratories rather than the review diagnosis by an expert pathology quality control group

HPV Sampling and Analysis

The cervical samples were collected with a Cervex Brush (Rovers Medical Devices, Oss, the Netherlands) that was immersed in 20 ml of PreserveCyt solution (Cytoc, USA). Samples were transported and stored according to manufactures instructions. The isolation of DNA/RNA was done according to Boom's isolation method¹⁵ using the Nuclisens Extractor (BioMérieux, Lyon, France) or by magnetic silica beads using The GenoM-48 robot (Qiagen GmbH, Hilden, Germany), with the respective protocols for automated isolation. DNA/RNA was isolated from 5 ml sample and eluted in 50 µl elution buffer (Buffer ME) and analyzed with PreTect HPV-Proofer (NorChip AS, Klokkestua, Norway). Human U1A served as mRNA internal control. Artificial and standardized oligonucleotides were used as positive control for HPV 16, 18, 31, 33, and 45. Water was used as negative control.

Cytology and Histology

Cytology and histology diagnosis done approximately simultaneously with the HPV test were obtained from the Cytology Register and Histology Register kept at the Cancer Registry of Norway¹⁶. Cytology diagnoses are presented according to the Bethesda terminology¹⁷, while histology diagnosis according to the CIN classification.

RESULTS

In our study population the overall HPV mRNA positives was 8.3 % (Table 1). Expression of HPV E6/E7 mRNA was seen in patients aged 13 to 80 years, and was highest in the age groups between 10-29 years, peaking in age group 20-24 with 20 % positive. With increasing age, the proportion of mRNA positives decreased until it again increased in the age group 75-84.

Altogether 86.9 % of the women had a concurrent normal Pap smear (Table 2). Among them, 5.1 % were positive for HPV mRNA. Among 258 HSIL women, 63 % were positive to HPV mRNA. 7.6 % (1,453) of the women had no concurrent Pap smear.

Among women with concurrent normal Pap smear mRNA prevalence was decreasing with increasing age (Figure 1). The same was observed for women with a concurrent LSIL Pap smear (Figure 2). For women with a concurrent HSIL Pap smear, HPV mRNA prevalence was high, regardless of age (Figure 3).

For women with a concurrent HSIL Pap smear, histology diagnosis was obtained (Figure 4). 72.8 % of women with a histological CIN 2+ were HPV mRNA positive compared to 40.3 % of the women who where histological normal or CIN 1, yielding an OR of 4.0 (95 % CI: 2.2-7.3).

Table 1: mRNA hr HPV ⁺ positives by age groups			Table 2: Results and distribution of Pap smear diagnosis and concurrent HPV mRNA positives			
Age	N	% positive	Pap diagnosis*	N	Distribution	% HPV mRNA positive
10-14	6	16.7	Normal	15 372	86.9	5.1
15-19	439	13.4	Unsatisfactory	727	4.1	4.0
20-24	1 828	20.0	ASC-US	491	2.8	25.9
25-29	2 742	13.9	LSIL	735	4.2	29.3
30-34	3 160	9.7	ASC-H	82	0.5	47.6
35-39	2 870	7.2	HSIL	258	1.5	63.2
40-44	2 437	4.7	AGUS	34	0.2	58.8
45-49	1 985	3.5	Total	17 699	100.0	8.3
50-54	1 462	2.3	*1,453 women had no concurrent Pap smear diagnosis			
55-59	1 142	1.9				
60-64	565	3.0				
65-69	313	2.9				
70-74	127	2.4				
75-79	54	7.4				
80-84	18	5.6				
85-89	4	0.0				
Total	19 152	8.3				

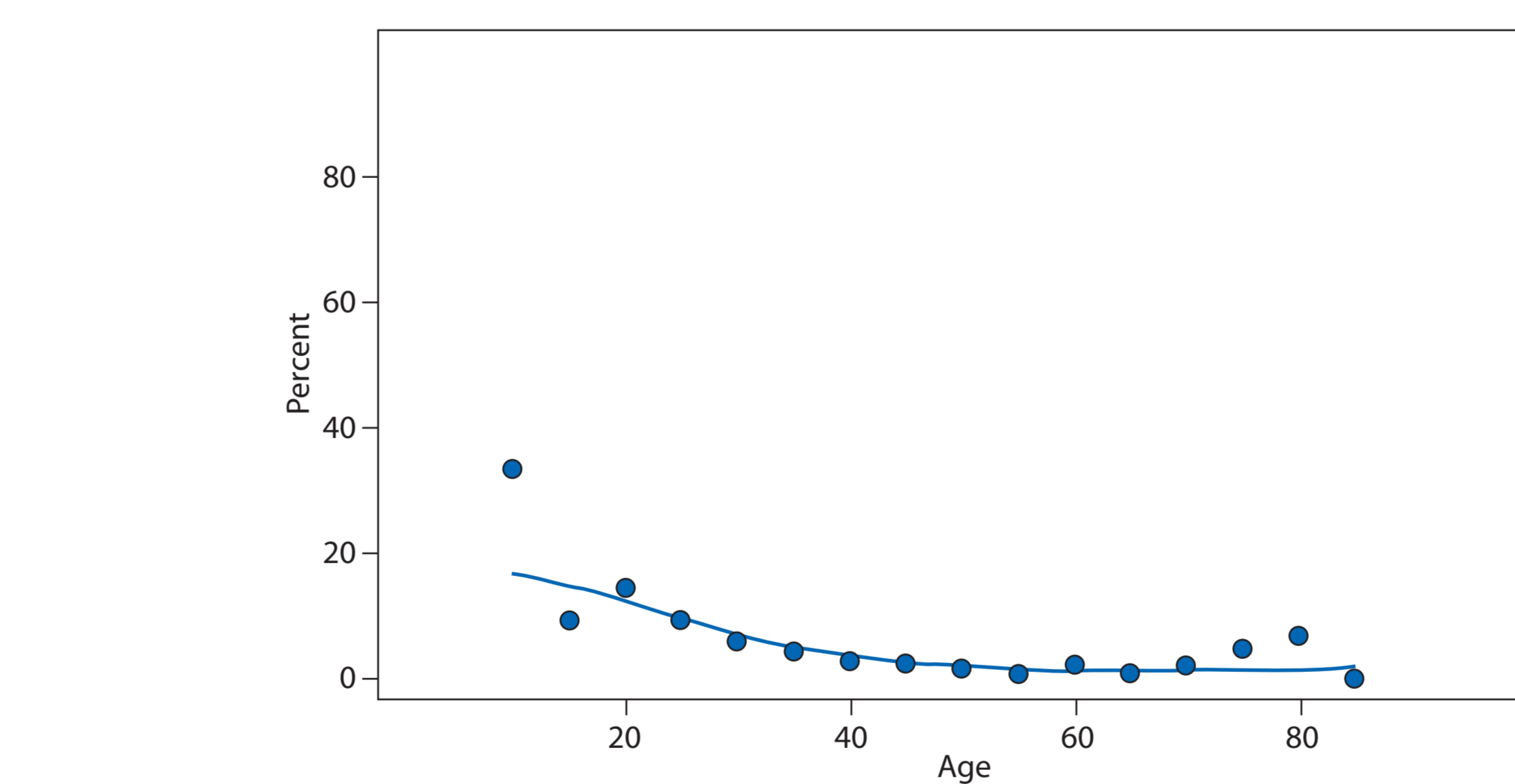


Figure 1 Percent mRNA positive among women with normal Pap smears (n=15,372). Statistics were carried out by locally weighted (LOWESS) regression. Both the regression line and point estimates are presented.

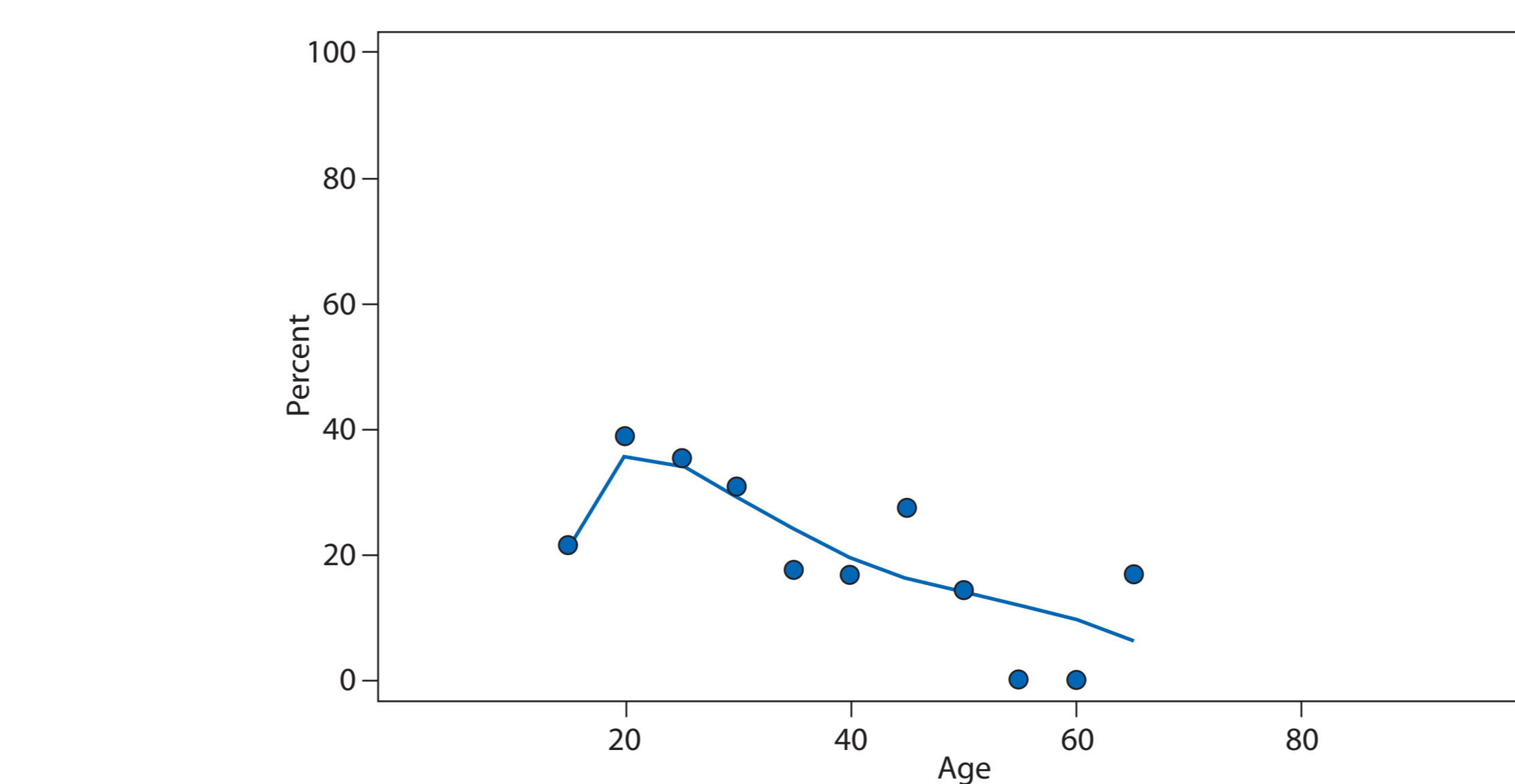


Figure 2 Percent mRNA positives among women with LSIL Pap smears (n=735). Statistics were carried out by locally weighted (LOWESS) regression. Both the regression line and point estimates are presented.

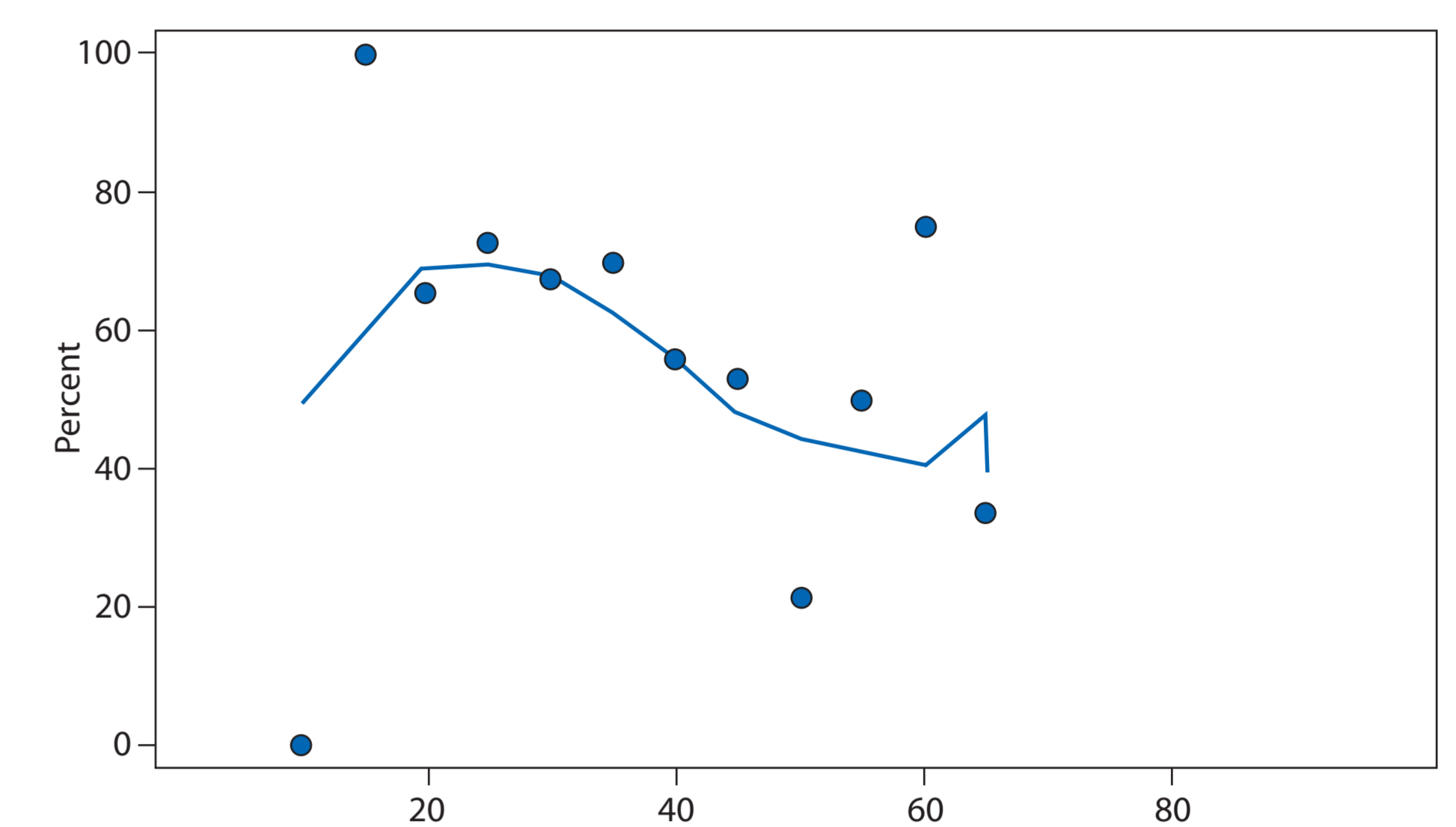


Figure 3 Percent mRNA positives among women with HSIL Pap smears (n=258). Statistics were carried out by locally weighted (LOWESS) regression. Both the regression line and point estimates are presented.

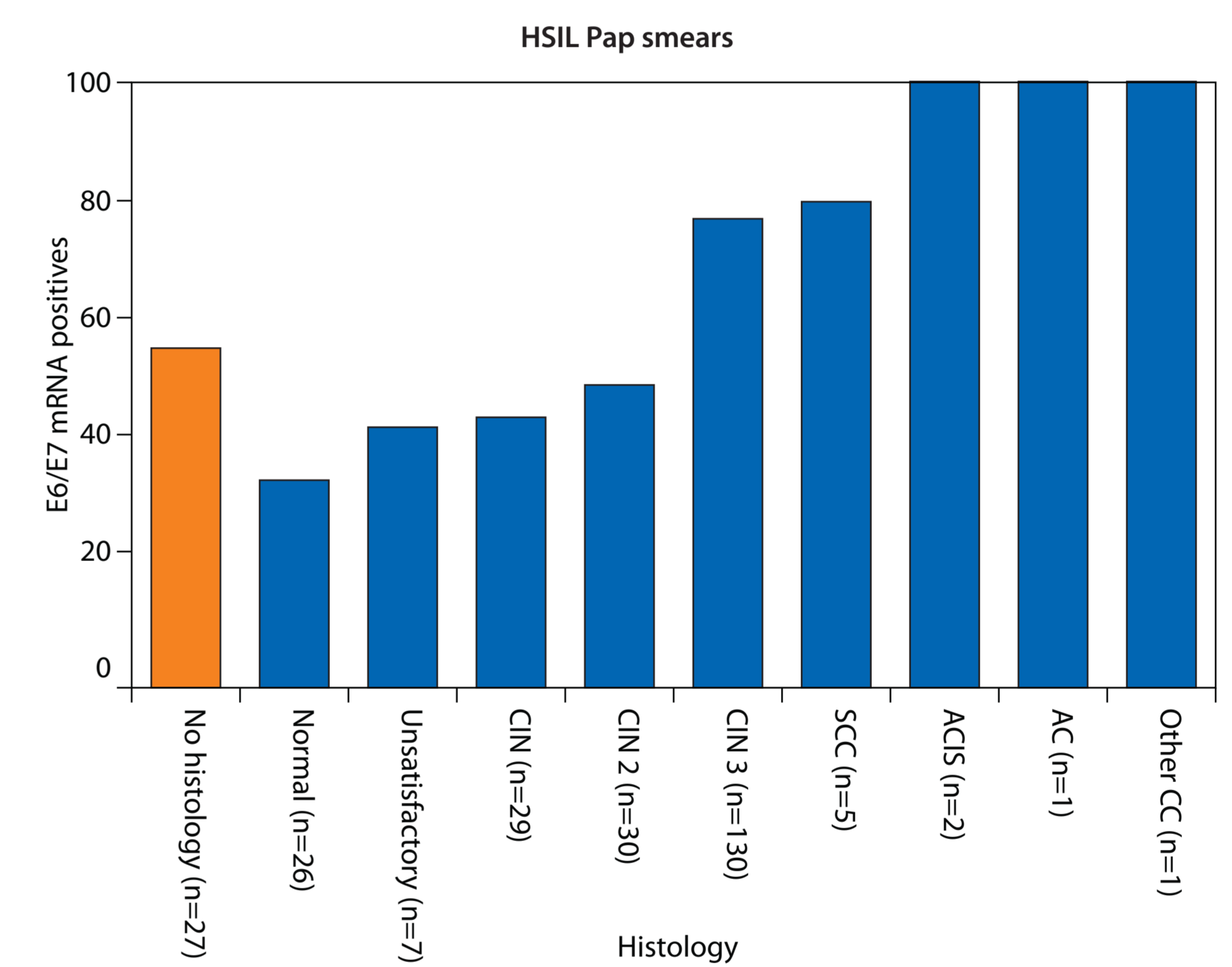


Figure 4 Percent mRNA positives and Histology among women with HSIL Pap smears (N=258).

DISCUSSION

Subjects included in this study originated from routine clinical practice of GPs and gynecologists in Norway. HPV test was performed on 15,372 patients (86.9 %) with concurrent normal cytology. In addition to cytology indications, an individual risk evaluation was done by the doctor prior to HPV testing. This is reflected by higher proportion of women with cytological abnormalities compared to the general screening population.

The observed age dependent reduction of hr HPV E6/E7 mRNA was in agreement with previous hr HPV DNA and mRNA findings in Norway. The increased positive women past 60 years of age could be explained by higher degree of patient selection than in lower age groups. The age independent distribution of HPV mRNA of women with HSIL, and the age dependent distribution within ASCUS and LSIL, should be further investigated.

We observed a significantly higher risk of having histological CIN 2+ when the HPV mRNA test was positive in the HSIL Pap group. However, the impact of this finding may be uncertain as 17 % of the women with HSIL and mRNA positive had normal or CIN 1 histology. A study prospectively monitoring Pap diagnosis and histology is planned to investigate the prognostic value of HPV mRNA testing in a routine clinical setting.

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