HPV Vaccine Protein L1 Predicts Disease Outcome of High-Risk HPV+ Early Squamous Dysplastic Lesions

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Abstract

Prediction of the clinical outcome of nonadvanced, early dysplastic lesions is one of the unresolved problems of cervical cancer screening programs. We examined the influence of human papillomavirus (HPV) L1 capsid protein detection in a randomized, prospective study of 187 high-risk HPV+ early dysplastic lesions during 36 to 46 months.

The difference in the clinical outcome of the HPV L1– cases and the HPV L1+ cases was highly statistically significant (P < .0001) and independent of the classification of low-grade squamous intraepithelial lesion (mild dysplasia) and high-grade squamous intraepithelial lesion of the moderate dysplastic type.

L1+ mild and moderate dysplasias, reflecting productive HPV infection, showed low malignant potential, justifying a wait-and-watch strategy to prevent overtreatment, especially in young women. L1– early dysplastic lesions, as nonproductive infections or precancerous lesions, have a high malignancy potential and close follow-up with colposcopy and histologic evaluation should be advised.

Even with the vaccination era rising, human papillomavirus (HPV) will remain a major global health burden, inducing a range of malignant and benign diseases. Women, in particular, are experiencing the consequences of HPV infection, with about 500,000 new cases of invasive cervical cancers annually.1

Cervical cancer screening programs for the detection of preinvasive cervical neoplasia are highly effective and caused a decline in the incidence of invasive cervical carcinomas.2,3 As a result, cervical precancerous lesions are being diagnosed frequently, and the cost for treatment of low-grade intraepithelial lesions soared in recent years.4 In young women, conizations are being performed frequently for high-grade lesions with a potentially negative impact on reproductive outcomes.

To improve women’s quality of life and health care resources, it would be most useful to have prognostic markers distinguishing patients who will experience progression of an early precursor lesion to cancer from patients who will not.5 Such a risk assessment is equally important for borderline abnormalities such as atypical squamous cell of undetermined significance (ASC-US) and squamous intraepithelial lesion (SIL) of undetermined grade. Data from the ASCUS-LSIL Triage Study (ALTS) confirmed that HPV DNA testing is not a useful triage strategy in low-grade SIL (LSIL) cases6 and that it is still unresolved whether cervical intraepithelial neoplasia grade 2 (CIN 2) represents low- or high-grade disease in individual lesions. Therefore, more specific tools are needed to identify women who are at risk for progressive lesions, ie, to discriminate HPV infection from real precancer.7

Different reporting systems are used for Papanicolaou (Pap) smear diagnoses. Besides the original World Health Organization classification,8 the Bethesda System (TBS)9 is internationally accepted. In Germany, the Munich
Nomenclature II is being recommended,\(^\text{10}\) which puts mild and moderate dysplasia in group IIID, with recommendation for cytologic follow-up and colposcopy. Germany’s “watch-and-wait” strategy offers the opportunity to investigate the clinical outcome of cytologically detected moderate dysplasia because independent of the reporting system, it is known that 10\(^\text{th}\)% of the mild and 20\(^\text{th}\)% of the moderate dysplastic lesions develop CIN 3+ lesions, but most of them regress spontaneously.\(^\text{11,12}\)

Many anogenital cancers, particularly squamous cell carcinoma of the cervix, are induced by epitheliotropic DNA tumor viruses, specifically the HPVs.\(^\text{13}\) A large number of HPV types are known to infect the anogenital tract, but only a subset, the high-risk types, is frequently found in malignant lesions.\(^\text{14}\)

L1, or the major capsid protein, is 1 of 8 known HPV-specific proteins (E1, E2, E4, E5, E6, E7, L1, and L2). During the productive phase of the viral life cycle, the L1 capsid protein (together with L2, the minor capsid protein) is produced within the cytoplasm and translocated into the nucleus, immunochemically visible by a strong nuclear staining reaction in intermediate and superficial squamous epithelial cells. The viral DNA is encapsulated by 360 L1 capsid proteins to build new infectious viral particles that are released in the upper epithelial layer.\(^\text{13}\)

The presence of L1 capsid protein within the dysplastic cells, as evidenced by L1 capsid protein–positive cases, is proof of a completed HPV life cycle. L1 capsid protein–negative cases, however, have lost the ability to produce virions depending on squamous epithelial cell differentiation.

In 2003, Melsheimer et al\(^\text{15}\) reported that most of the high-risk HPV-associated LSILs express HPV L1 capsid protein, but most of the high-risk HPV-associated high-grade squamous intraepithelial lesions (HSILs) failed to synthesize HPV L1 capsid protein. It was suggested that a loss of viral L1 capsid protein, a major target of the immune response in HPV-infected SIL, could function as a prognostic marker for the development of CIN lesions.\(^\text{15,16}\)

We subsequently confirmed this in a retrospective study with a median follow-up of 22.8 months on 84 routinely performed Pap smears. We showed that high-risk HPV-associated mild to moderate dysplastic squamous lesions without immunochemically detectable HPV L1 capsid protein are significantly more likely to progress (76.4\%) than are L1+ cases (23.6\%).\(^\text{17}\) Similar results regarding the prognostic relevance of HPV L1 capsid protein were obtained in different retrospective studies using cytology and biopsy specimens.\(^\text{18-20}\)

**Materials and Methods**

**Patients and Design**

In 2005, we started a randomized, prospective study with cytologic diagnoses according to TBS and the Munich nomenclature because the 2 main criticisms of our initial study\(^\text{17}\) were its retrospective approach and the application of the Munich nomenclature only. On each working day, we recruited the first high-risk HPV+ mild or moderate dysplasia case (as defined by the World Health Organization classification) from the general screening population. A high-risk HPV association was confirmed with the Hybrid Capture II test (Digene/Qiagen, Hilden, Germany).

The 211 conventional Pap smears and ThinPrep slides were independently classified (by H.G. and H.S.) according to the Second Munich Cytological Nomenclature (mild and moderate dysplasia as group IIID) and diagnosed according to TBS as LSIL (mild dysplasia) and HSIL (moderate dysplasia). Established cytologic criteria were applied to define mild and moderate dysplasia.\(^\text{21}\)

Informed consent from the patients was not necessary because the samples were received and analyzed anonymously and patients were treated according to the German Munich nomenclature with cytologic follow-up and colposcopy for mild and moderate dysplastic lesions.

Follow-up smears were obtained at intervals of 3 to 6 months or annually after the first negative smear for intraepithelial lesions. A conization for treatment and/or histologic verification was performed if clinically indicated and with the patient’s consent. Follow-up ended in December 2008, resulting in a follow-up period of 36 to 46 months.

The clinical outcome was understood as possible remission, persistence, or progression of the lesion. Women having at least 2 consecutive smears negative for an intraepithelial lesion were considered to be in remission. Persistence was defined as the state in which mild and moderate dysplasia persisted cytologically during the whole follow-up period or as histologically CIN 1 or CIN 2. Progression was defined as a histologically confirmed CIN 3+ lesion because interrater and intraobserver reproducibility for CIN 3 histology is high and considerably better than that for CIN 2 histomorphology.\(^\text{7}\) All histologic diagnoses were from conization specimens.

The 2-sided Fisher exact test was used for data analysis.

**Immunochemical Studies**

At presentation, routinely processed conventional Pap smears and ThinPrep slides (Hologic, Bedford, MA) were immunochemically stained with the Cytoactiv Screening Set (Cytoimmun Diagnostics, Pirmasens, Germany), which detects the L1 capsid protein of all known HPV types. Staining was performed according to the manufacturer’s protocol.

In brief, slides were subjected to antigen unmasking by microwave treatment after unmounting without prior destaining. Cytoactiv screening antibody was applied to the slides and incubated for 30 minutes at room temperature, followed by incubation with the detection reagents for 10 minutes and AEC chromogen for 5 minutes.
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After counterstaining with hematoxylin, slides were mounted with Aquatex (Merck, Darmstadt, Germany) and coverslipped. Stained slides were studied by light microscopy independently (by H.S. and H.G.). Slides with at least 1 epithelial cell with distinctly positive nuclear staining were scored as positive [Image 1]. This cutoff is recommended for the Cytoactiv assay and was used in all studies published so far, based on our initial work showing that only the presence, not the amount of staining intensity of positive nuclei, correlated with the clinical course.

Results

The mean age of the 211 patients was 33.6 years (range, 16-83 years). The mean was 34.8 years (range, 16-83 years) for the LSIL group (n = 78 [37.0%]) and 32.9 years (range, 18-83 years) for the HSIL group (n = 133 [63.0%]).

Of the 211 high-risk HPV-associated early dysplastic lesions, 119 (56.4%) were L1– and 92 (43.6%) were L1+, with a profoundly different outcome between the 2 groups but independent of the classification as LSIL or HSIL. The mean age in the L1– group was 33.7 years (range, 18-67 years) and 33.4 years (range, 16-83 years) for the L1+ group.

Of the 119 L1– cases, 36 (30.3%) were initially diagnosed as LSIL (mean age, 34.3 years) and 83 (69.7%) as HSIL (mean age, 33.4 years). Initially 42 (46%) of 92 L1+ cases were diagnosed as LSIL (mean age, 35 years) and 50 (54%) as HSIL (mean age, 32 years) [Table 1].

For data analysis, 187 cases with complete clinical records were included [Table 2]. Of 211 cases, 22 (5 HSIL and 17 LSIL) were lost during the follow-up period, and 2 HSIL L1– cases were excluded owing to inconsistencies in clinical records.

Independent of the designation as LSIL or HSIL, 43 (61%) of 71 L1+ but only 6 (5.2%) of 116 L1– cases showed spontaneous remission of the lesion. In contrast, only 18 (25%) of 71 L1+ but 84 (72.4%) of 116 L1– cases progressed to CIN 3+, including 4 invasive carcinomas in the L1– HSIL cases only.

The remission rate was highest among the L1+ LSIL (66% [21/32]) and HSIL cases (56% [22/39]) and lowest in the L1– LSIL (11% [4/36]) and HSIL (3% [2/80]) groups. In contrast, the rate of progression was highest in the L1– HSIL group (79% [63/80]), followed by the L1– LSIL (58% [21/36]), the L1+ HSIL (28% [11/39]), and the L1+ LSIL (22% [7/32]) groups.

[Table 1] Results of 211 Initially Recruited Cases by L1 Staining Result, LSIL/HSIL Grouping, and Age*

<table>
<thead>
<tr>
<th></th>
<th>LSIL</th>
<th>HSIL</th>
<th>Total</th>
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<tr>
<td>L1–</td>
<td></td>
<td></td>
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<tr>
<td>Mean age (y)</td>
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<td>33.4</td>
<td>33.7</td>
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<tr>
<td>L1+</td>
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<td></td>
<td></td>
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<tr>
<td>Mean age (y)</td>
<td>42</td>
<td>50.5</td>
<td>49.8</td>
</tr>
<tr>
<td>Total</td>
<td>78</td>
<td>133</td>
<td>211</td>
</tr>
<tr>
<td>Mean age (y)</td>
<td>34.8</td>
<td>32.9</td>
<td>33.6</td>
</tr>
</tbody>
</table>

HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

* Data are given as number (percentage) unless otherwise indicated.

[Image 1] L1 capsid protein–positive cases with strong nuclear staining. A, High-grade squamous intraepithelial lesion (moderate dysplasia) in a routinely performed Papanicolaou smear, overstained with Cytoactiv (×400). B, Low-grade squamous intraepithelial lesion on a ThinPrep slide (×400).

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The L1– HSIL cases had a 2.8 times higher risk of progression than the L1+ HSIL cases and L1– LSIL cases a 2.6 times increased risk compared with L1+ LSIL.

Within the 71 L1+ cases, only 18 (25%) progressed to CIN 3, and 10 lesions (14%; 2 CIN 1, 6 CIN 2, and 2 with cytologically persistent moderate dysplasia) were classified as stable disease. In detail, 22% of L1+ LSIL cases (7/32) and 28% of the L1+ HSIL cases (11/39) progressed. Of the 32 L1+ LSIL cases, 4 (13%; 2 CIN 1 and 2 CIN 2) were stable disease. Of the 39 L1+ HSIL cases, 6 (15%; 4 CIN 2 and 2 cytologically with persistent dysplasia) were stable disease.

Of the 116 L1– cases, 84 were histologically CIN 3+ (72.4%), qualifying for progression, and 26 (22.4%; 10 CIN 1, 14 CIN 2, and 2 cytologically) were classified as stable disease. Of the 36 L1– LSIL cases, 21 (58%) progressed to CIN 3, as did 63 (79%) of the 80 L1– HSIL cases.

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Of the 36 L1– LSIL cases, 11 (31%; 5 CIN 1, 5 CIN 2, and 1 cytologically LSIL) were confirmed as stable disease. Of the 80 L1– HSIL cases, 15 (19%) were confirmed as stable disease (5 CIN 1, 9 CIN 2, and 1 cytologically as moderate dysplasia).

The difference in clinical outcome for the L1– cases and the L1+ cases was highly statistically significant ($P < .0001$) and independent of the classification of LSIL (mild dysplasia) and HSIL (moderate dysplasia). Analysis of the age-related clinical outcome of the women showed no statistically significant difference related to age younger than 30 years or 30 years and older.

The mean duration from the initial L1 positively stained smear to the recognition of disease progression and to remission was 8.5 months (range, 1-13 months) and 7 months (range, 3-35 months), respectively Table 3. For L1– cases, the interval until progression was 6 months (range, 1-29 months) and was 6.4 months (range, 2-12 months) for remission.

Discussion

Prophylactic HPV vaccines using virus-like particles of the L1 capsid protein as the antigen are highly effective in preventing HPV-associated diseases.

Among nonvaccinated and, possibly, vaccinated women (except for HPV types 16 and 18), high-risk HPVs as facultative but not obligatory pathogens are quite commonly detected. But owing to the low transformation capacity, progressive disease to cervical cancer occurs in only a minor fraction of infected people. This may be the reason that other markers seeking cellular transformation events have thus far failed to predict the outcome of nonadvanced dysplastic lesions.

On the other hand, the presence of infiltrating T lymphocytes and macrophages in spontaneously regressing papillomas22 and the increased incidence and progression of HPV infections in immunosuppressed patients emphasize the critical importance of cell-mediated immune responses in the resolution and control of HPV infections.16,23 Therefore, we focused on one of the major HPV-associated stimuli of the immune system, the L1 capsid protein.
Earlier studies have shown that the immunochemical evaluation of HPV L1 status at presentation is valuable for predicting the outcome of early dysplastic lesions.

HPV has developed several mechanisms to evade the innate immune response and delay the activation of the adaptive immune response. Despite its ability to impede host defense mechanisms, a successful immune response to genital HPV infection is established in almost all cases. But viral clearance can be delayed considerably because the HPV life cycle does not induce keratinocyte death and, especially in high-risk HPV infection, does not result in major proinflammatory signals. Hence, the time required for clearance of high-risk types, particularly HPV-16, averages 8 to 14 months, which is considerably longer than the 5 to 6 months needed for clearance of low-risk types. Consequently, one could envision that in setting an efficient transfer of antigen from HPV-infected keratinocytes to the antigen-presenting cells (APCs) is not triggered.

The only fully accessible antigen sources in the earlier stage of virally induced SIL are free viral particles consisting of 360 L1 capsid proteins released from the L1 capsid protein–positive apical layers of keratinocytes during the productive phase at the end of the natural viral life cycle in HPV infection. To generate an effective virus-specific immune response, the virus particles have to be detected by the APCs of squamous epithelium, the Langerhans cells, or dendritic cells, which can be promoted by microlesions in the epithelial transformation zone. The activated APCs then migrate to the draining lymph node, process HPV antigens en route, and present these to naïve T cells in the lymphatic tissue. The T cells should then differentiate into armed effector cells, migrate back to the site of infection, and destroy the infected keratinocytes, leading to a (spontaneous) remission of the lesion.

When such immunologic activation mechanisms are functional, they may be quite effective, since we found a remission of the lesion in about 61% of the cases within a period of 7 to 9 months, irrespective of the dysplasia being cytologically mild or moderate. That this process is not failsafe is evident in our finding that 25% of the L1+ cases in our study showed early progression to CIN 3 within 8.5 months, compared with 6 months for L1– cases.

A block at any stage of the activation cascade, such as no or diminished antigen release, reduced numbers of APCs, or MHC incompatibility or deficiencies, may result in an ineffective immune response and failure to clear HPV, propagating a progression of the intraepithelial lesion.

The short interval from detection of LSIL to the diagnosis of a CIN 3 lesion could also indicate that multiple dysplastic lesions and dysplasias of different grade coexist in the transformation zone, possibly reflecting a mixture of L1+ and L1– lesions with different progressive potentials. Looking for coexisting CIN 1/CIN 3 lesions, Negri et al reported that about 70% (68.4%) of these CIN 1 lesions were already L1–. It seems that this loss of L1 capsid protein synthesis is an early precancerous event, and CIN 3 lesions may evolve out of such L1– CIN 1 lesions.

Since the capsid protein–negative, L1 messenger RNA–positive murine C3 cells can be destroyed by an L1-specific T-cell response, a beneficial effect of the presence of L1 capsid protein for the immune response could be postulated, even in coexisting L1 capsid protein–negative dysplastic areas. Among the L1– cases in our study, this was an extremely rare event, with 5.2% of cases showing remission of the lesion. Reasons for the remission of these L1– cases are most likely expression levels below the detection limit of the immunochemical assay or a sampling error, with absence of L1-expressing cells in the sample.

Persistence or progression of dysplasia was observed in 94.8% of the L1– cases, further emphasizing the importance of the HPV L1 capsid protein as major stimulus for the activation of the immune system and the clearance of dysplasia. The rapid progression of mild and moderate dysplasias to CIN 3 lesions within 6 months seems to support the notion that disease progression can be linked to a local immune system failure. L1–, nonproductive HPV infection, as a precancerous event, eventually may lead to disturbed viral-cellular interaction, resulting in disorders of cell cycle regulation at transcriptional, translational, and genomic levels.

Taken together, the data from our prospective study confirmed our previously published findings using retrospective approaches and provide further evidence that immunochemical L1 capsid protein detection in situ is a highly useful prognostic tool in predicting the outcome of early dysplastic lesions, particularly LSIL and moderate dysplastic lesions, providing a better risk assessment than the detection of high-risk HPV DNA. We would like to emphasize that no significant prognostic difference between cytologically mild (LSIL) and moderate dysplastic lesions (part of the HSIL category) related to the L1 expression profile was evident in our study. Therefore, prognostic significance of immunochemical L1 detection can be postulated also for borderline lesions such as high-risk HPV+ ASC-US and SIL of undetermined grade or even moderate dysplasia as part of HSIL and the equivalent of CIN 2 biopsied lesions.

L1+ mild and moderate dysplastic lesions, reflecting productive HPV infection, have a low risk of progression. L1– mild to moderate dysplastic lesions, as nonproductive infections or precancerous lesions, have a high progression potential, as high as expected for severe dysplasia and squamous carcinoma in situ. Close follow-up with colposcopy and histologic verification, therefore, should be advised for high-risk HPV+, L1– cases.

Independent of the cytologic classifications used so far, we suggest determining the progressive potential of the
dysplastic lesions with an HPV L1 screening antibody and classifying L1 capsid protein–positive cases as lesions with low malignant potential and L1−, high-risk HPV+ cases as lesions with high malignant potential.

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References


