The impact of the determination of human papillomavirus infection in cervical cancer screening strategy and follow-up strategy of precancer lesion procedures

PhD-Thesis

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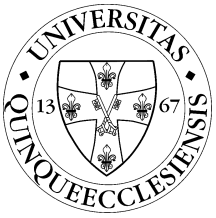

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Introduction

The cancers have been detected in early stage due to the development and widespreading of cancer screening on the world , despite it is the second largest cause of mortality in the world.

1.Objective

The aim of my research is to show that the HPV DNA determination as a diagnostic method to improve significantly the effectiveness of cervical screening and could reduce the number of surgical procedure done on cervix. Could determination of HPV genotyping and mapping of the prevalence of HPV types of cervical cancer and precancerous cases influence the screening strategy? During my research, I would like to clarify which and what kind of frequency of HPV DNA can be detected in abnormal cytology and histological samples. How can the persistent HPV infection detected after conisation influence the prognosis of the disease?

2. Materials and Methods

Materials and Methods 1.

These are the first, multicentric, retrospective parallel studies to estimate HPV type prevalence in more than 6000 women diagnosed with either HG-CIN or ICC across 17 European countries, using standardised and validated methods for specimen preparation, with centralised pathology review and HPV DNA testing (HERACLES-SCALE study). Prevalence ratios of specific HPV types in ICC versus HG-CIN and the median age of HG-CIN and ICC diagnoses for different HPV types were estimated. Centralised expert histopathological review and standardised HPV DNA typing for 14 HR-HPV types were applied to more than 6000 parallel samples of HG-CIN and ICC. The pooled prevalence of individual HPV types was estimated using meta-analytic method to control for heterogeneity observed among the countries. For each participating country, samples were collected from consecutive archived formalin-fixed paraffin-embedded cervical specimens of HG-CIN or ICC from women (aged ≥18 years) who had been diagnosed between 2001 and 2008. Information about age at diagnosis, year of diagnosis, and original histological diagnosis was obtained. The majority of these sites were part of a cervical screening programme that had stored specimens representative of the total population.In each participating country, only study sites which maintained an archive of cervical excision specimens were selected. The majority of these sites were part of a cervical screening programme that had stored specimens representative of the total population. From each participating country, consecutive archived formalin-fixed paraffin-embedded cervical specimens of HG-CIN and/or ICC diagnosed between 2001 and 2008 were selected. The selection procedures were standardised. If several specimens were available for a subject, the most recent paraffin block containing the area with the highest grade of CIN or the primary ICC obtained prior to chemo/radiotherapy, was selected. Information about age at specimen collection, year of specimen collection, and original histological diagnosis were obtained. A convenient sample of 290 HG-CIN and 290 ICC were collected for each country, starting with the most recent specimen and dating back until reaching their respective case numbers. Inclusion criteria into the study (*Screened Cohort*) were a pathological confirmation of HG-CIN or ICC with the availability of a cervical/excision specimen at the country level. If several specimens were available for a subject, the most recent paraffin block containing the area with the highest grade of CIN or the primary invasive cancer, obtained prior to chemo/radiotherapy, was selected.Reasons for exclusion from the *Total cohort* were that the specimen blocks were too large (>2cm diameter), too thin (<2mm), held highly irregular tissue, had been inadequately preserved (in terms of paraffin conditions) or were too worn down to cut into sections. Specimens for which histological examination of sections failed to confirm the same grade or type of abnormality as the section cut for PCR analysis and/or inability to perform HPV PCR testing were rejected from the *Histologically-eligible cohort* .

Laboratory procedures

Following anonymisation, specimens were shipped to a central laboratory (DDL Diagnostic Laboratory, Voorburg, The Netherlands) for histopathology review and HPV-DNA detection and typing.

At DDL the tissue blocks were sectioned and analysed according to the sandwich cutting procedure which ensured that PCR for HPV was performed within a sandwich of histology diagnosis.H&E sections were examined by an experienced gynaecological histopathologist, blinded to the initial diagnosis and HPV status, to confirm presence of the lesion in the section used for HPV testing. When multiple areas of abnormality were present in one slide, the worst grade of lesion diagnosed represented the study clinical diagnosis.

Cervical samples with a confirmed histopathological diagnosis were tested for HPV using PCR methodology, SPF_{10}-DEIA/$LiPA_{25}$-PCR system (SPF_{10}-$LiPA_{25}$)(version 1, Laboratory Biomedical Products, Rijswijk, Netherlands) The SPF_{10}-PCR primer set amplifies a small fragment of 65 bp from the L1 region of at least 54 HPV genotypes, as described earlier .DEIA positive SPF_{10}-amplifiers were used to identify the HPV genotype by reverse hybridisation probe assay (SPF_{10}-$LiPA_{25}$) which detected 25 HR and low risk (LR) HPV types (6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68/73, 70, and 74).

Statistical analysis

The primary population for analysis was the *Histologically-eligible HPV+ cohort* comprising women with a validated diagnosis of HG-CIN or ICC and positive HPV PCR test . From each country, 290 women with an initial diagnosis of HG-CIN and 290 women with an initial diagnosis ICC (with 260 of these being SCC) were enrolled to reach the target of 210 HPV-positive women in each study allowing for histological misclassification and a proportion of HPV-negative cases. Sample size calculations demonstrated that with 210 HPV-positive women the precision of the 95% confidence interval (CI) of percentages for each HPV type would not exceed 6% when percentages are lower than 20% and 5% when percentages are lower than 10%. Data were summarised by means, medians, standard deviations and range for continuous variables, and by frequency and percentages for categorical variables. Two-sided 95% confidence intervals (95%CIs) for percentages were computed using either Chi-square or an exact method based on the binomial distribution (Clopper-Pearson method [Newcombe 1998]). Cochran Q statistics were computed to test potential differences among countries with respect to the proportion of each HPV type, at 5% significance level. All statistical analyses were performed using SAS software (version 9.1).

Material and Methods 2.

The aim of our study was to evaluate the HPV DNA detection could decrease the number of the reconisation. 438 cervical conisation was done, aged 22-65 year, from March 2008 to Augustus 2010 The indication of the procedure was citological abnormality mostly. In every cases loop electrosurgical procedure (LLETZ) was used. Before the cone biopsy a HPV test was taken from the cervical canal and the surface of cervix. HPV samples were analysed by Genoid ELISA-PCR method.

In every cases loop electrosurgical procedure (LEEP) was used.

LEEP conisation was performed under local anesthaesia using wire loop electrodes, with diathermy apparatus set to 50 W for cutting and 50 W for coagulation. Generally only one specimen was removed by a single excision. All specimens were fixed with 10 % buffered formalin and submitted to histopathology examination. Prior to the biopsy an HPV test was taken from the cervical canal and from the surface of the cervix. HPV samples were analysed by Genoid ELISA-PCR method. Spectrum HPV Detection Kit (Genoid) was used according to the instruction manual. The cervical specimen was collected in PreservCyt medium, transferred to the laboratory and after the isolation of the nucleic acids by silica based method a multiple HPV specific PCR was carried out. The amplicon is genotyped using hybridisation based method the biotinilíted amplicons were captured on solid phase and labelled genotype specific oligonucleotides were used as probes. The assay is capable to detect virtually all

mucosal HPV types and high-risk genotypes, too (16,18,31,33,45,51,52,56,58,66,68). Pathological examination verified the histological grade.. The formalin fixed preparations were sliced and embedded in paraffin for histological examination. The sections were stained with hematoxylin and eosin. The aim of our study was to assess the second (pre-reconisation) HPV test an appropriate method to reduce the number of interventions in histologically positive cases.

The indication of the second LLETZ was CIN2/3 cases with positive surgical margins, accepted by the hungarian and international protocols. In all CIN1 cases we have choosen conservative treatment independently the status of surgical margins. The HPV DNA detection was performed from the operation area before the second LLETZ in every cases.

The statistical analysis of the data was performed according to the Chi-square test. A P value of < 0.05 was considered significant.

3.Results

3.1 Results 1.

For 3103 women and 3162 women in the *Histologically-eligible cohorts*, the diagnoses of HG-CIN and ICC were, respectively, confirmed and HPV testing was performed. Among women diagnosed with HG-CIN, CIN3, CIN2, CIN2/3, AIS and 'other' accounted for 73.6%, 15.3%, 9.4%, 0.7% and 0.9% of cases, respectively. Among women diagnosed with ICC, SCC, ADC and 'other' accounted for 77.7%, 13.4% and 8.9% respectively (Table 1).

Table 1: **HERACLES (HG-CIN) and SCALE (ICC) cohort demographics.**

		All countries		**Countries participating in both studies only**	
Cohort		**HERACLES (HG-CIN)**	**SCALE (ICC)**	**HERACLES (HG-CIN)**	**SCALE (ICC)**
Total enrolled cohort	N	3979	3626	1923	2140
	Median age (range) at date of specimen collection, years	35 (18–86)	48 (18–99)	35 (18–86)	50 (20–99)
Histologically-eligible cohort	N	3103	3162	1923	2138
	Median age (range) at date of specimen collection, years	34 (18–86)	49 (19–99)	35 (18–86)	50 (20–99)
	Main diagnosis % (HPV positivity rate %)				
	CIN2	15·3 (95.0)		16·0 (94.1)	
	CIN3	73·6 (99.3)		72·0 (99.1)	
	CIN2/3	9·4 (99.3)		10·0 (99.0)	
	AIS	0·7 (100)		0·9 (100)	
	SCC		77·7 (94.2)		78·3 (94.3)
	ADC		13·4 (81.3)		13·4 (81.9)
	Other	0·9 (89·3)	8·9 (86.9)	1·1 (90.0)	8·3 (86.4)
HPV+ cohort	N	3057	2903	1889	1966
	Median age (range) at date of specimen collection, years	34 (18–86)	48 (19–99)	35 (18–86)	49 (20–99)
	Single HPV infection %	80.0	93.5	80.4	93.5
	Multiple HPV infection %	17.4	4.8	16.9	4.5
	Unknown HPV type %	2.6	1.7	2.7	2.0

The age distribution of women in the two groups was reflected in the well-known fact that the HG-CIN group of mostly 26-30 years of age, and in the eldery age the number of precancerous cases are reduced. In contrast in ICC group with increasing age the number of inavsive cases are increased. Median age (range) at the time of specimen collection was 34 years (18-86) for HG-CIN (33 years (19-84) for CIN2, 34 years (18-86) for CIN3, 36 years (19-77) for CIN23 and 35 years (22-55) for AIS), and 49 years (19-99) for ICC (45 years (22-90) for ADC and 50 years (19-99) for SCC).

1.5% of women diagnosed with HG-CIN and 8.1% of women diagnosed with ICC were HPV negative. The number of women diagnosed with HPV negative (HPV−) ICC increased with increasing age, reaching the highest number in women aged >61 years (45.6%). Multiple HPV infections occurred in 17.1% of HG-CIN and 4.4% in ICC diagnoses. They were more frequently observed in younger women with HG-CIN, decreasing in frequency with increasing age.

The *HPV+ cohorts* included 3057 (98.5%) women with HG-CIN and 2903 (91.8%) women with ICC. 2445 (80%) HPV+ women with HG-CIN and 2715 (93.5%) HPV+ women with ICC were infected with a single HPV type. In women infected with a single HPV type and diagnosed with HG-CIN, the most common types were HPV16 (59.9%), HPV33 (10.5%), HPV31 (9.0%), HPV52 (3.9%) and HPV18 (3.6%).

In women infected with a single HPV type and diagnosed with ICC lesions, the most common types were HPV16 (63.3%), HPV18 (15.2%), HPV45 (5.3%), HPV33 (4.6%) and HPV31 (3.7%).

In women infected with a single HPV type, the pooled prevalence of LR-HPV types was 0.65% (95% CI: 0.00-3.63) in HG-CIN and 0.73% (0.00-4.30) in ICC.

In each country studied, HPV16 was the most common type to be associated with both HG-CIN and ICC in women infected with a single HPV type. The prevalence varied from 47.1% in Norway to 71.9% in Estonia for HG-CIN, and from 54.4% in Norway to 72.8% in Poland for ICC. Similar variations across the countries were observed for HPV18/31/33/45 and 'other' types.

Patterns of age-specific HPV prevalence differed between HG-CIN and ICC. This pattern was also observed for women with single HPV16/31/33-related HG-CIN and ICC diagnoses.

In contrast, the highest number of women with single HPV18-related HG-CIN and ICC diagnoses was observed in the 31-35y age group (24.4%) and in the 51-60y groups (19.3%), respectively, showing that there is less time between diagnoses of HPV18-related HG-CIN and ICC than for those related to HPV16/31/33. A similar pattern, as for HPV18-related HG-CIN and ICC, was observed for women diagnosed with HPV45-related HG-CIN and ICC (26-30y (22.2%) for HG-CIN; 51-60y (20.4%) for ICC).

In the 534 women infected with multiple HPV types and diagnosed with HG-CIN, the most frequent HPV types were HPV16 (59.9% [55.6-64.1]), HPV31 (26.0% [22.4-30.0]), HPV52 (23.4% [19.9-27.2]), HPV33 (18.0% [14.8-21.5]), HPV51 (15.0% [12.1-18.3]) and HPV18 (14.2% [11.4-17.5]).

In the 138 women infected with multiple HPV types and diagnosed with ICC, the most frequent HPV types were HPV16 (52.9% [44.4-61.4]), HPV18 (26.8% [19.6-35.0]), HPV52 (21.0% [14.5-28.8]), HPV31 (19.6% [13.3-27.2]), HPV45 (18.8% [12.7-26.4]) and HPV33 (17.4% [11.5-24.8]).

In the eight countries participating in both HERACLES and SCALE studies, the total number of women diagnosed with HG-CIN and ICC and included in the *Histologically-eligible cohorts* was 1923 and 2138, respectively. The demographic and clinical characteristics of these two populations were similar to those in the overall population in the respective studies. The frequencies of HPV types in HG-CIN and ICC were also similar to those seen in the overall population.

In women infected with a single HPV type, the ICC/HG-CIN prevalence ratio varied for individual HPV types. HPV39/18/45 were 4.8/3.5/2.5 times more prevalent in ICC than HG-CIN, respectively. HPV16 was prevalent in both ICC and HG-CIN with a prevalence ratio of 1.1, while HPV31/33/35/52 were 0.4/0.4/0.5/0.5 were less prevalent in ICC than HG-CIN respectively

The median age at diagnosis varied according to HPV type, with CIN3 being diagnosed earlier in women infected with HPV16 (34y), HPV31 (33y) and HPV33 (35y), and at a later median age in women with HPV18 (38y) and HPV45 (42y). In contrast, SCC tended to be diagnosed earlier in women infected with HPV16 (49y), HPV18 (47y) or HPV45 (43y) and at later age in women infected with HPV31, HPV33 and 'other' HPV types.

The difference in age at specimen collection between CIN3 and SCC for HPV18 (9y) was statistically significantly narrower when compared to the difference in age at specimen collection for HPV31 (23y), HPV33 (20y) and 'other' (17y) (p-values of <0.001, 0.001 and 0.011, respectively) and the difference in age at specimen collection between CIN3 and SCC for HPV45 (1y) was statistically significantly narrower when compared to the difference in age at specimen collection for HPV16 (15y), HPV31 (23y), HPV33 (20y) and 'other' (17y) (p-values of 0.005, <0.001, <0.001 and 0.001, respectively). The difference, though not statistically significant, in age at specimen collection between AIS and ADC for HPV18 (6y) was lower than for HPV16 (13y) (p = 0.162).

3.2 Results 2.

The results in the second clinical trial was the next.

119 (**27,2 %)** out of **438** cases were reconisations. The indication of the second conisation was positive surgical margins at the first conisation in every case. The second loop conisation was performed 8 weeks later after first conisation. Median age was 34.7 y(22 y-65 y). Respect to the median age the patients were divided to two subgroups, younger or older than 35 years.

In 90 cases of the 119 reconisated patients (75,6 % of the total amount of reconisated patients) residual dysplasia was not detected at reconisation in spite of the surgical margins positivity of the first biopsy.

In 77 out of this 90 patient cohort the repeated HPV test did not confirm any HPV infection. In 13 out of this 90 patients HPV infection was detected repeatedly but only in 3 cases could we confirm the same HPV type. In this 3 cases the first histology proved severe cervical dysplasia.

In most of these 13 a new HPV type was detected at the pre-reconisation HPV test, showing the break of continuity of the persistent infection relating to previosuly detected HPV type.

In cases of histologically proven residual dysplasia (**29** of **119**) high-risk HPV infection was detected by HPV test, too.

In 29 patients (25,4 % of the total amount of reconisated patients), where residual dysplasia was confirmed high risk HPV infection was detected by 100 %.

Where the histology revealed persistent high grade CIN the repeated HPV test detected the ***same HPV*** type as occured at first time.(64%)

Futhermore in those cases where reconisation detected lower grade of dysplasia as seen previously, a ***new HR-HPV*** type was observed (38%)

Analyzing the HPV distribution we realized that HPV 16, 31 and 33 types were very common (92 %) in precancerous lesions. . There was no significant difference in occurrence of residual dysplasia between the two subgroups (below and above 35 years) by chi-square test (P<0,01).

4. Summary

HPV was identified in 98·5% of HG-CIN and 91·8% of ICC specimens. The most common single-type HPV in HG-CIN and ICC following meta-analysis were HPV16/33/31 (59·9%/10·5%/9·0%) and HPV16/18/45 (63·3%/15·2%/5·3%). We have found strongly difference of prevalence of HPV between SCC and ADC cases. In SCC cases HPV16/18/33 (66.2%/10.8%/5.3%), in ADC cases HPV16/18/45 (54.2%/40.4%/8.3%) were indentified. HPV16/18/45 were 1.1/3.5/2.5 times more strongly associated with ICC than HG-CIN.

Consistent with other studies, we found that HPV16 was the most frequent type detected across Europe in both HG-CIN and ICC. Women with HPV16/18/45 infection in ICC were younger than women with other HPV types in ICC, and those with HPV18/45 infection in CIN3 were older than those with other HPV types in CIN3.

The higher prevalences of HPV18 and HPV45 in ADC compared to SCC are consistent with other recent data, and suggest that these HPV types may differ from HPV16 in their target cell specificity. Hence, HPV16 infection results in predominantly squamous cervical neoplasia, while HPV18 and HPV45 have a greater tendency to induce glandular cervical neoplasia.

Margin positivity was the obvious indication for reconisation in CIN3 cases.

In our study we have performed, that second- pre-reconisation- HPV DNA test can influence our therapeutic decision. In those cases, where the second HPV test did not confirm any HR-HPV infection, the reconisation is unnecessary procedure because the low risk of the residual severe dysplasia. In those cases, where the second HPV test confirmed the same HPV infection, than at the first one, the reconisation is confirmed because of the high risk of the severe residual dysplasia. The type specific HPV detection has strongly impact in this clinical trial. The same HPV type, detected before the first conisation and positive surgical margins are together the indicators of reconisation.

According to our experience of these two clinical trial the type specific HPV detection has strongly impact and performing in cervical screening program is valuable for therapeutic and follow up procedure due to having an excellent prognostic ability.

5. Conclusion

These are the first parallel studies to estimate HPV type prevalence in more than 6000 women diagnosed with either HG-CIN or ICC across 17 European countries, using standardised and validated methods for specimen preparation, with centralised pathology review and HPV DNA testing. The pooled prevalence of individual HPV types was estimated using meta-analytic method to control for heterogeneity observed among the countries. A comparison of age at diagnosis for different HPV types was undertaken to investigate type-specific age distributions of HPV-associated HG-CIN and ICC. Consistent with other studies, we found that HPV16 was the most frequent type detected across Europe in both HG-CIN and ICC. HPV16/18/45/39/59/68 were more frequently detected in women diagnosed with ICC than HG-CIN; whereas HPV31/33/35/51/52/58/66 were more frequently detected in women diagnosed with HG-CIN than ICC.

This is in keeping with the results of other studies of type-specific HPV prevalence across the spectrum of HPV-related cervical diagnoses

Our studies also aimed to determine the prevalence ratios of specific HPV types in ICC compared to HG-CIN in Europe. In women infected with a single HPV type, the prevalence ratios of HPV39/18/45 in ICC versus HG-CIN were >1. This observation has paid attention to intensive carcinogenetic ability of above mentioned HPV types. This is especially the case for ADC, which is more likely to be missed by screening, and for which the precursor of AIS is infrequent. In contrast the prevalence ratios of HPV31/33/35/52 in ICC versus HG-CIN were ≤1, reflecting their importance in HG-CIN and a lesser role in ICC.

In addition to their overall prevalence of specific HPV types in ICC versus HG-CIN, their age-specific prevalence in ICC and HG-CIN reflects another distinguishing aspect of HPV16/18/45-related cervical lesions compared to lesions infected with other HPV types. This analysis performed new datas in European population. ICC associated with HPV16/18/45 tended to be diagnosed at an earlier age than ICC related to 'other' HPV types. This, together with the above findings, reinforces the hypothesis that HPV16/18/45 may have a greater potential for rapid neoplastic transformation than other types as found in other studies. The results confirm the later presentation of HPV18- and HPV45-related CIN3 and the narrow age range between the detection of HPV18- and HPV45-related CIN3 and HPV18- and HPV45-related ICC. It was also notable that the study identified no cases of HPV45-related AIS despite identifying several cases of HPV45-related ADC. These observations are consistent with data on HPV type specific integration suggesting that HPV18 and HPV45 may promote a higher degree of chromosomal instability than other types.

Our observations on the prevalence ratios and differences in median age at diagnosis for HG-CIN and ICC associated with HPV18/45/39 – which are all of the same alpha-7 clade – also suggest that this group of HPV types may have a distinct natural history different from that of HPV16 or other HPV types. HPV16 predominates in both HG-CIN and ICC, especially in SCC. HPV types of the alpha-7 clade (18/39/45) are much more prevalent in ICC than in HG-CIN, being relatively infrequent in the latter. In comparison, HPV31 and HPV33 have a large median age difference between HG-CIN and ICC, and are more prevalent in HG-CIN than in ICC.

The strengths of the present studies include the large number of subjects and the diversity of selected European countries with and without screening programmes, to which a standardised study protocol was applied. Pooled data analysis allowed the inclusion of a large sample size and a wide geographical coverage across Europe whilst controlling for possible discrepancies and biases between the countries and study populations (e.g. differing geographical HPV type distribution, variations in cervical cancer screening policies and methods, etc.). The study design did not include a normal control population and was cross-sectional so that any consideration of actual progression from HG-CIN to ICC for individual HPV types remains hypothetical. However, our findings provide important information regarding differences in the prevalence of the individual HPV types in ICC versus HG-CIN, and differences in median age of diagnosis of lesions associated with individual HR-HPV types. Estimates of the prevalence of infrequent HPV types and results on rarer histological diagnoses (e.g., AIS) are limited by number of cases and resulting wide confidence intervals. This resulted from consistent, expert central pathology review being able to exclude cases of CIN1 that had been erroneously submitted.

As in other clinical trial the histological diagnosis of CIN1 cases has also chosen the follow-up regardless of the status of the surgical margins. In recent years, several studies have dealt with the development of precancerous risk factors. Positive correlation was found between the surgical margin, HPV positive after treatment and the remaining histological differences. In our clinical study the patients referred to second conisation because of positive surgical margins were taken HPV DNA status before second conisation proved to be a good predictive factor of potentially precancerous residual image.

In our clinical study, we in addition to HPV DNA positivity is confirmed in the remaining histological differences, it has also been shown that the type specific HPV DNA detection is good in the definition of the degree of severity.

This observation correlates well with the our European, large epidemiological analysis, wherein the type specific HPV caused by the progression of pre-malignant condition could be monitored.

It is also in the second study, we were able to demonstrate that in case of repeated HPV test negativity we did not find any residual severe histologic abnormalities. This observation is a significant step forward in the positive surgical margins cases on the choice of a treatment strategy. The reconisation has had significant risk for miscarriage and premature births respectively.

In our study we demonstrated that the sensitivity of the second (pre-reconisation) HPV test is 94%to predict severe residual dysplasia. The negative predictive value of the second HPV test for prediction of severe residual dysplasia has been 100%.

According to the age distribution, in contrast to other authors, we were unable to distinguish between the under or over 35 year. We could not find the older age as a pathognomonic factor for prediction of the residual dysplasia.

In conclusion, we can say that in all cases where the positive surgical margins was occured at the conisation, the first and the second (pre-reconisation) HPV DNA test result comparison crucially affects another indication of conisation. Only those cases suggested the re-conisation where the high-risk HPV infection is identified at the second HPV test. It has been obviously demonstrated if HR-HPV DNA has not been detected at the second HPV DNA test, it is not recommended to perform re-conisation because of no chance of the severe residual cervical dysplasia. The experience gained from our observation, helping every practicing gynecologist, it is important to note that all patients treated for HG CIN must be carefully followed up for at least 10 years because a British study revealed that the risk of developing an invasive cevical cancer among these women during the next 8 years is about five times higher than that of the general population.

In our clinical investigations have shown that the pre-cancerous conditions (CIN2 / 3) was detected in child-bearing age so we feel it essential that the indication of the conisation and aleatory re-conisation should be determined by strict criteria.

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1. Marek E., Gőcze P., Bózsa Sz., Molnár G., Stefanovits Á., Benczik M., **Koiss R.,**Gőcze K., Survey of knowledge about the HPV infection and cervical cancer among students and parents in Hungary,Poster in Bridges in Life Sciences US –CEE Regional Networking Meeting IV April 4, 2009 Debrecen, Hungary

2. Marek E., Gőcze P, Bózsa Sz, Molnár G.,Stefanovits Á, Benczik M, **Koiss R,** Gőcze K: Survey of knowledge about HPV infection in Hungary. Poster The 25th International Papillomavirus Conference,May 8-14 2009, Malmö, Sweden.

3. C.Jeney;C.Józsa;N.Varga;A.Kovács;J.Mózes;**R.Koiss:** „Evalutaion of a new screening biomarker panel"- Poster The 25th International Papillomavirus Conference May 8-14, 2009, Malmö, Sweden

4. C.Jeney;C.Józsa;N.Varga; M.Benczik J.Mózes; **R.Koiss:** „Detection and evaluation of a new screening biomarker panel" Poster,The 26 th IPV Conference, July 05-08, 2010 Montreal,Canada

5. **R.Koiss** T.Wiebren; A.Fiander; O. Reich; B. Kirschner;„ Human papillomavirus type distribution in women with high-grade cervical intraepithelial neoplasia and invasive cervical cancer in Europa" Poster, The13th biennal meeting of the IGCS, okt. 23-27, 2010 Prague, Czech Republic

6. **Koiss R,** Babarczi E, Jeney C,Gőcze P.Horányi L,Siklós P: Reconisation or repeated HPV test? P6-21 EUROGIN 2011 Lisbon Portugal, 8-11., May, 2011

Chapter in book:

Robert Koiss (2012). Screening Methods in Prevention of Cervical Cancer, Human Papillomavirus and Related Diseases - From Bench to Bedside - A Clinical Perspective, Davy Vanden Broeck (Ed.), ISBN:978-953-307-860-1,InTech,Availablefrom: http://www.intechopen.com/articles/show/title/screening-methods-in-prevention-of-cervical-cancer

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R Koiss:. " Occurance of " high-risk" HPV types in the specimens of the conisation of patient suffering CIN". XI. Congress of EAGO, 09.2001. Hungary

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