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Three-year longitudinal data on the clinical performance of the Abbott RealTime High Risk HPV test in a cervical cancer screening setting

Mario Poljak[a,*], Anja Oštrbenk[a], Katja Seme[a], Anja Šterbenc[a], Nina Jančar[b], Eda Vrtačnik Bokal[b]

[a] Institute of Microbiology and Immunology, Faculty of Medicine, University of Ljubljana, Ljubljana, Slovenia
[b] Department of Obstetrics and Gynecology, University Medical Center, Ljubljana, Slovenia

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ABSTRACT

Background: Testing cervical smears for the presence of high-risk human papillomaviruses (hrHPV) increases the sensitivity for detecting women with underlying high-grade cervical intraepithelial neoplasia (CIN) and provides better and longer protection against invasive cervical cancer compared to cytology testing alone. The Abbott RealTime High Risk HPV test (RealTime) is a hrHPV DNA test with concurrent partial genotyping for HPV16 and HPV18 and aggregate detection of 12 other hrHPV types that have been extensively analytically and clinically evaluated over the last 6 years.
Objectives and study design: To provide the first 3-year longitudinal data regarding the clinical performance of RealTime, the risk of CIN2+ according to various negative baseline characteristics, and baseline and future risk for CIN2+ at 3 years for women with baseline infection with various hrHPV types were assessed in a cohort of 3,920 Slovenian women that had hrHPV DNA and/or cytology in 36- to 48-month follow-up results after a baseline screening round in 2009/2010.
Results: A total of 36 CIN2+ cases were identified in the second screening round. Of these, 17 CIN2+ cases were identified passively through questionnaires/data registries and 19 cases actively as the result of actions triggered by second-round cytology and/or HPV test results. Accumulation of CIN2+ cases during follow-up occurred predominantly in woman with normal cytology at baseline. Among women >30 years old, significantly better protection against CIN2+ at 3 years was associated with a negative hrHPV DNA result at baseline (risk for CIN2+ 0.04% [95 CI, 0.00–0.22%]) than by normal cytology at baseline (risk for CIN2+ 0.68% [95 CI, 0.40–1.08%]). Women with baseline HPV16 infection had a significantly higher risk of CIN2+ at baseline (21.9% [95 CI, 15.2–30.4%]) and baseline plus future risk at 3 years for CIN2+ (33.3% [95 CI, 24.7–44.0%]) in comparison to women with baseline non-HPV16/18 hrHPV infection (7.0% [95 CI, 4.6–10.2%]) or those that were hrHPV-positive (11.7% [95 CI, 9.1–14.9%]).
Conclusions: 3-year longitudinal data reinforce evidence from previous studies that RealTime can be safely used in primary HPV-based cervical cancer screening. Concurrent partial genotyping for HPV16/18 should be strongly considered as a triage method for HPV screen-positive women.

1. Background

The development of cervical cancer and its immediate precursors is inevitably associated with persistent infection with high-risk human papillomavirus (hrHPV) types, mainly HPV16 and HPV18 [1,2]. These findings led to a recent paradigm change in cervical cancer screening so that several countries are now in the process of switching from cytology-based to hrHPV-based cervical cancer screening [3–10]. Primary hrHPV-based cervical cancer screening is an important scientific and clinical advance because it offers better reassurance of low cancer risk compared to cytology-only cervical cancer screening conducted at the same interval [11]. The effectiveness and safety of HPV-based primary cervical cancer screening has been assessed in large-scale randomized clinical trials performed in several European countries, Canada, and India [12–25] and in screening cohorts with longitudinal follow-up data from Europe and the United States [26–39]. According to their results, hrHPV testing as a stand-alone test or in combination with cytology (co-testing) increases the sensitivity for detecting women with underlying cervical intraepithelial neoplasia (CIN)

* Corresponding author. Tel.: +386 1 543 7453; fax: +386 1 543 7401.
E-mail address: mario.poljak@mf.uni-lj.si (M. Poljak).

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grade 2 or worse (CIN2+) and grade 3 or worse (CIN3+), provides better and longer protection against invasive cervical cancer, and reduces cervical cancer mortality compared to cytology testing alone [12,13,15,18,19,26,32,33,40,41].

A recent inventory of commercial HPV tests currently available on the market identified 193 distinct commercial HPV tests and at least 127 test variants [42]; however, only a handful of them can be recommended at present as reliable tools in HPV-based primary cervical cancer screening using clinician-collected cervical samples [43], based on criteria defined in 2009 by an international expert team [44]. The Abbott RealTime High Risk HPV test (RealTime; Abbott, Wiesbaden, Germany) [45,46] is now considered one of the eight HPV assays that fully matches all cross-sectional criteria for primary cervical cancer screening defined by this expert team [43] due to its non-inferior clinical sensitivity and clinical specificity compared to the two clinically validated HPV tests: Hybrid Capture-2 (hc2; Qiagen, Gaithersburg, MD, USA) [47,48] and GP5+/6+ PCR-EIA [49], as well as its high intra-laboratory reproducibility and inter-laboratory agreement for detecting targeted hrHPV assessed in three independent studies [47–49]. However, for hrHPV DNA assays such as RealTime, for which equivalent cross-sectional accuracy as hc2 or GP5+/6+ PCR-EIA are accepted as sufficient evidence to allow their use in primary cervical cancer screening, the generation of longitudinal data remains useful, provides further reassurance, and corroborates the evidence level [43]. Thus, here we provide for the first time 3-year longitudinal data on the clinical performance of RealTime assessed in a cohort of 3,920 Slovenian women 20–64 years old attending the routine organized national cervical cancer screening program, with more than 70% coverage. The three-year longitudinal data of RealTime showed better protection against CIN2+ and CIN3+ associated with a negative hrHPV DNA baseline result than by normal baseline cytology and additionally strengthened the evidence that RealTime is a test that can be safely used in primary HPV-based cervical cancer screening.

2. Objectives

To provide the first 3-year longitudinal data regarding the clinical performance of RealTime, the risk of CIN2+ according to various negative baseline characteristics and current (baseline) and future risk for CIN2+ and CIN3+ at 3 years for women with baseline infection with various hrHPV types were assessed in a cohort of 3,920 Slovenian women that had hrHPV DNA and/or cytology at 36–48 months in follow-up results after baseline testing with cytology, hc2, and RealTime in 2009/2010.

3. Study design

3.1. Study population and protocol

A total of 4,432 women 20–64 years old, who attended the routine organized national cervical cancer screening program in Slovenia between December 2009 and August 2010, were enrolled in the baseline screening round in 16 outpatient gynecology services [48]. The baseline study was provisionally closed in August 2010 to allow publication of data [48]. All of the women that participated in the baseline screening round were invited after 36 months to participate in the second screening round, which started in December 2012 and finished in late October 2014. According to the study protocol, we excluded from the second screening round all women with CIN2+ detected in the baseline screening round before provisional closure of the study in August 2010, all pregnant women, and all those with diseases or other clinical conditions in which a cervical smear was impossible to obtain (Fig. 1). The same gynecologists that participated in the baseline study were responsible for patient recruitment and management in the second screening round. Written standardized informed consent was obtained from all of the women by the participating gynecologists, and patient identities were kept secret from all study participants except the participating gynecologists. The second screening round study was approved by the Medical Ethics Committee of the Republic of Slovenia (consent number: 109/08/12).

A similar approach as in the baseline screening round [48] was also used in the second screening round. Briefly, after visual inspection of the cervix, two cervical specimens from as many eligible women (Fig. 1) as possible were collected for routine traditional cervical cytology and HPV DNA testing. A sample for HPV DNA testing was obtained with either a Cervex-Brush (Rovers Medical Devices, Oss, Netherlands) or a Pap Perfect Plastic Spatula and Cytobrash Plus GT Gentle Touch (Medscand sample collection kit; Medscand Medical, Berlin, Germany) and placed into ThinPrep PreservCyt solution (Hologic, Marlborough, MA, USA). In contrast to the baseline screening round, the presence of 14 hrHPV types was determined using RealTime only, which allows concurrent partial genotyping for HPV16 and HPV18 and aggregate detection of 12 HPV types: HPV31, HPV33, HPV35, HPV39, HPV45, HPV51, HPV52, HPV56, HPV58, HPV59, HPV66, and HPV68 [45]. To determine the HPV type(s) present in the samples, all RealTime-positive specimens were tested using the Linear Array HPV Genotyping Test (Linear Array; Roche Molecular Diagnostics, Branchburg, NJ, USA) and if necessary with additional genotyping tests. The detailed algorithm of HPV testing, three-step genotyping strategy, discordant analysis, cytological examination, and colposcopic referral were described previously [48]. Briefly, all cervical smears were examined under routine conditions by certified cytologists normally used by each participating gynecology practice and blinded to HPV results. The estimated cross-sectional sensitivity of traditional cytology for CIN2+ and CIN3+ in Slovenia is 66.2% and 80.6%, respectively [48]. Women were referred for colposcopy at the cytology threshold of “atypical squamous cells, cannot exclude high-grade lesion (ASC-H)” or worse in accordance with the national screening standards [50]. In addition, according to the study protocol, immediate colposcopy for all HPV16- and/or HPV18-positive women regardless of their cytology result was strongly recommended and, for those positive for hrHPV other than HPV16 and HPV18, colposcopy was performed at the physician’s discretion. Colposcopically directed punch biopsies obtained from the suspicious areas were histopathologically assessed by certified pathologists, who were blinded to the HPV status.

To document any clinically relevant events (e.g., cytology, colposcopy, histology, treatment, or HPV testing) that occurred between two screening rounds, detailed patient- and physician-based questionnaires were designed and data were collected from all participating women. In addition, for non-responders, data regarding all recorded cytology smear and HPV DNA testing results were obtained passively from the national centralized cervical cancer screening registry through three search rounds, the last one being performed on October 3rd, 2014.

The final disease status after 3-year follow-up was determined for all enrolled women. To be included in the final second screening round analysis, a woman must have had either (i) a consensus CIN2+ result, or (ii) at least one valid follow-up hrHPV DNA and/or cytology result on cervical smear collected 36–48 months after the baseline testing with cytology, hc2, and RealTime, including all required colposcopies for those with ASC-H or more severe cytology and/or those HPV16- and/or HPV18-positive regardless of their cytology result. Women vaccinated against HPV between two screening rounds and those with a high probability of therapeutic procedure or known therapeutic procedures between two screening rounds were excluded from the final analysis (Fig. 1).

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