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High prevalence of abnormal cervical smears in a hospital cohort of French women beyond the upper age limit screening program

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ABSTRACT

Objective. To determine the prevalence of cytological abnormalities and high risk Human PapillomaVirus (hrHPV) in cervical smears from French women aged over 65 years who attended the referent Gynecology Clinic of the Besançon University Hospital.

Methods. Between 2002 and 2012, 796 French women aged 66–99 years were cotested for cytology and hrHPV by Hybrid Capture 2 (hc2). hc2-positive cases were subjected to real time PCR for specific HPV 16/18/45 genotyping. Women with normal Pap smears and positive for hrHPV were followed-up every 12 months.

Results. Cytological abnormalities were detected in more than 30% of women and cervical cancers (CC) in 2.9% of women. Benign lesions were more frequent in women aged 66–75 years while (pre)-malignant lesions were preferentially found in women over 76. The prevalence of hrHPV was 22.7%. HPV 16 was the most frequent (23.8%), followed by HPV 45 (7.7%) and HPV 18 (3.9%). The rate of hrHPV increased with the lesion severity and HPV 16 was identified in 50% of CC. Among the followed-up women, those who developed CIN3 were HPV16 positive at study entry.

Conclusion. The study provides important estimates of the prevalence of cervical abnormalities and hrHPV positivity in a French hospital based-population over 65. Findings suggest to consider this high risk population in regards to cervical cancer.

Introduction

Cervical cancer (CC) is a public health problem in women worldwide, affecting 530,000 women and responsible for 275,000 deaths each year. Wide variations exist between high and low-burden countries and incidence rates range from <3 to >50 per 100,000 (Arbyn et al., 2011; Ferlay et al., 2010). In USA, most cases of CC occur in women younger than 50 years of age, but more than 15% are found in women over 65 (American Cancer Society, 2014). Knowledge about pathogenesis has shown that nearly 100% of CC are caused by a persistent high risk Human PapillomaVirus (hrHPV) infection (Schiffman et al., 1993; Walboomers et al., 1999; Wallin et al., 1999). HPV 16 is the most frequently detected genotype worldwide followed, in general, by HPV 18 and HPV 45 (Guardado-Estrada et al., 2014; Monsonego et al., 2015; Munoz et al., 2003; Pretet et al., 2008).

At present, the best way to reduce the number of CC is to implement screening programs to detect precancerous lesions. The Papanicolaou (Pap) test, which was introduced in the 1940s, led to a reduction of up to 60% in the incidence of invasive squamous cervical cancer and mortality due to the disease (La Vecchia et al., 1984; Quinn et al., 1999). In France, CC incidence decreased from an estimated 5100 in 1980 to 3000 cases in 2005 and mortality declined from 2200 to 1000 cases over the same period (Belot et al., 2008). Because of limitations relating to the sensitivity of cervical cytology (Spence et al., 2007), it has recently been recommended to introduce HPV test in clinical practice, including in primary screening (Arbyn et al., 2012; Saslow et al., 2012). Studies in the USA, Canada and Europe indicate that the high risk HPV test may be more

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effective than cytology alone in detecting CIN3 or progressively worse histological findings (Katki et al., 2011; Leinonen et al., 2012; Mayrand et al., 2007; Ogilvie et al., 2012; Ronco et al., 2014). In addition, HPV16, 18 and 45 genotyping would be advantageous to manage hrHPV positive women (Rijkaart et al., 2012; Saraiya et al., 2014; Wright et al., 2015).

The French guidelines for CC screening recommend a Pap smear every three years after two annual consecutive normal smears for women aged 25 to 65 years (Haute Autorité de Santé, 2013). American guidelines also recommend cessation of CC screening at age 65 for adequately screened women with no history of CIN2+ within the last 20 years (Saslow et al., 2012).

In Europe, the number of elderly patients being diagnosed with CC is increasing and the age of screening exit may be raised up to 70 years in some countries (Elfstrom et al., 2015). In 2014, a review of the literature concluded that cervical cancer screening is beneficial for women aged over 60 in order to prevent the occurrence of, and mortality from CC (Elit, 2014).

In this hospital-based retrospective cohort investigation we estimated the rate of pathological smears and the prevalence of hrHPV including HPV 16, 18 and 45 by cotesting in a population of French women aged over 65 who attended the referent Gynecology Clinic of the Besançon University Hospital. This study contributes to the current debate about the value of CC screening after 65 years, taking into account personal characteristics of patients and public health issues.

Patients and methods

Study population and sample collection

Data on cytology and HPV testing were obtained from 796 women aged over 65 (age range 66–99 years, median age 70 years) who attended the Gynecology Clinic of the Besançon University Hospital between January 2002 and December 2012. Patients had been referred to a referent gynecologist for symptoms suggestive of gynecological disorders (i.e. menopausal vaginal symptoms, vaginal itching, abdominal and pelvic pain) or for follow-up of chronic diseases (i.e. grafted patients, autoimmune disorders).

The cohort consisted of 693 women (87%) with no history of cervical cancer screening in our gynecology/pathology medical records and 103 women (13%) having had one Pap smear. As a whole we considered that women were not adequately screened.

Women underwent a pelvic examination and two cervical samples were obtained and used for conventional cytology and HPV testing as we previously described (Dalstein et al., 2004). When necessary, a short course (3–4 weeks) of intravaginal estrogen cream was given. Women with abnormal cytology were clinically managed according to the French guidelines (Anaes, 2002). Women with normal cytology were followed-up by co-testing in line with usual practice in our reference Clinic (Dalstein et al., 2004; Riethmuller et al., 2013).

Cytology data

Cytology results were reported based on the Bethesda System as Negative for Intraepithelial Lesion or Malignancy (NILM) or epithelial cell abnormalities, while abnormalities were classified as Atypical Squamous Cells of Undetermined Significance (ASC-US), Atypical Squamous Cells – cannot exclude High Grade Squamous Intraepithelial Lesions (ASC-H), Atypical Glandular Cells (AGC), Low grade Squamous Intraepithelial Lesions (LSIL), High grade SIL (HSIL), and Squamous Cell Carcinoma (SCC) and ADenoCarcinoma (ADC), without knowledge of HPV status.

HPV DNA testing

HPV DNA testing was performed with the *digene*® hc2 High-Risk HPV DNA Test® (hc2) (Qiagen, Gaithersburg, MD, USA) using the specific HPV RNA probe cocktail for hrHPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68. Presence or absence of HPV DNA in the specimen was defined according to the strength in relative light units (RLU) compared to 1 pg/mL HPV16 DNA-positive control. The sample was considered positive when the ratio of RLU/CO was ≥1.

Samples were stored into a biobank approved by the local ethics committee (Comité de Protection des Personnes), and for which a declaration of collection and storage of human samples for research use has been sent to the French Ministry of Higher Education and Research (Ministère de l'Enseignement Supérieur et de la Recherche) (declaration number DC-2014-2086).

DNA extraction from cervical exfoliated cells

DNA was extracted from hc2 positive cases with the QIAamp DNA Mini Kit (Qiagen, Courtaboeuf, France). Three hundred and twenty microliters of denatured solution were neutralized with 80 μL of 5 M acetic acid and 3 M potassium acetate. Then, 400 μL of AL buffer (Qiagen) and 40 μL proteinase K (Qiagen) were added and the mix was digested overnight at 56 °C. All lysates were processed according to the manufacturer's recommendations; DNA was eluted in 80 μL of elution buffer.

Real-time PCR

Detection of HPV16, HPV18 and HPV45 DNA as well as albumin gene (*ALB*) DNA copies were performed by real-time PCR with an AB7500 thermocycler (Applied Biosystems, Saint-Aubin, France) using TaqMan technology. Primers and hydrolysis probes were designed to target a polymorphic region of the E6 gene and *ALB* (Supplementary Table).

A real-time duplex PCR assay was carried out for simultaneous detection of HPV16 and HPV18 DNA as detailed previously (Jacquin et al., 2013).

Detection of HPV45 DNA was conducted in a final volume of 25 μL containing the 1X Taqman® Gene Expression Master Mix (Applied Biosystems), 300 nM of each primer (Eurogentec, Seraing, Belgium), 250 nM of probe (Eurogentec) and 4 μL of DNA sample. The thermal cycling program was 2 min at 50 °C followed by 10 min at 95 °C and then 40 cycles of amplification (95 °C–15 s, 60 °C–1 min).

To discriminate between failed amplification due to technical error and an absence of HPV16 and/or HPV18 and/or HPV45, each cervical sample was also subjected to real-time PCR for the detection of human *ALB* DNA copies as previously described (Laurendeau et al., 1999).

Statistical analysis

Prevalence of NILM and abnormal cytologies, as well as hrHPV and HPV 16/18/45, was calculated with the corresponding 95% confidence intervals (CI), stratified by age category. Prevalence of hrHPV and specific genotypes according to cytology with the associated 95% CI was also calculated. The chi-square test or Fisher's exact test were used to compare trends in HPV frequency by 5-year intervals, and trends in cytology frequency according to age groups. A p-value < 0.05 was considered statistically significant. All analyses were performed using the R statistical software version 3.2.0 on the website BiostaTGV (The R Foundation, Vienna, Austria).

Results

Cytology results according to age groups

Age of the 796 women was categorized into 5-year age groups (66–70, 71–75, 76–80 and >81). Among the Pap smears, 546 (68.6%, 95% CI 65.4–71.8) were NILM and 250 (31.4%, 95% CI 28.2–34.6) were abnormal. One hundred and twenty-six samples (15.8%, 95% CI 13.3–