International Journal of Public Health and Epidemiology ISSN 2326-7291 Vol. 9 (4), pp. 001-007, April, 2020.
Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Diagnosis of invasive squamous cell carcinoma: Impact of opportunistic screening in >70 years old women in Trentino (northern region of Italy)

Teresa Pusiol[1], Doriana Morichetti[2] and Maria Grazia Zorzi[2]

[1]Section of Cytopathology, Institute of Anatomic Pathology, Rovereto Hospital, Italy.
[2]Institute of Anatomic Pathology, Rovereto Hospital, Italy

Accepted 07 February, 2020

To assess the value of opportunistic screening in diagnosis of invasive squamous carcinoma found in >70 years old women in Trentino (northern region of Italy) in the period 2007 to 2010, the cytopathology Section of Rovereto Hospital examined 28589 opportunistic Pap smears. papillomavirus (HPV) genotyping by polymerase chain reaction (PCR) was performed in all histological specimens with diagnosis of low grade and high grade intraepithelial lesions and invasive cervical carcinoma. 111 cases (0.38%) of cervical intraepithelial neoplasia-3-squamous cell carcinoma were identified in 28589 opportunistic Pap smears. The cytological diagnosis of cervical intraepithelial neoplasia-3 was performed in three cases, confirmed by cone biopsy in two patients with the presence of HPV-16 and HPV-58 with PCR. The diagnosis of keratinizing squamous cell carcinoma was performed with Pap smear in three patients, histologically confirmed by the biopsy with the presence of HPV-58 in one case. Non-keratinizing squamous cell carcinoma was diagnosed with Pap smear in two cases, histologically confirmed by hysterectomy with bilateral salpingo-oophorectomy in absence of HPV. It is necessary to note that early diagnosis decrease to mortality, morbidity and management costs of new cases of cervical cancer diagnosed in > 65 years old women. The present study supports the screening policy to perform Pap test every 3 years until aged 69 years, independently to sexual activity.

Key words: Invasive cervical carcinoma, opportunistic screening, cancer in elderly women.

INTRODUCTION

Since 1996, Italian national guidelines have recommended to different regions, the implementation of organised screening programmes for cervical cancer. These recommendations, largely based on European guidelines, include personal invitations to women aged 25 to 64 years for a Pap smear every three years, a monitoring system and quality assurance for each phase of the programme. Surveys designed to assess the level of implementation of organised programmes in Italy and to collect process indicators have been conducted by Italian Group for Cervical Screening since 1997 (Ronco et al., 2007). Their results have been published by the Osservatorio Nazionale Screening (ONS; National Centre for Screening Monitoring) since 2002. Since 1993 in Trentino region (North Italy), an Organized Screening (OrS) exists for women 25 to 65 aged. The target population comprises 146737 women. In the period 1993 to 2006, the Pap-smears of OrS were examined in the Institutes of Anatomic Pathology and Cytopathology of S. Chiara Hospital Trento and Rovereto Hospital. Since 2007, the Cytopathology Section of Institute of Anatomic Pathology of Rovereto Hospital has examined only Pap-tests of Opportunistic Screening (OpS); left to the woman's initiative. OpS may be considered as all Pap-test performed outside an OrS program. For example, some women have Pap-test at their doctor's office during their physical examination independently of personal

*Corresponding author. E-mail: teresa.pusiol@apss.tn.it. Tel: 001-0464-403501.

letter invitation of OrS. An estimated 2,927 new cases of cervical cancer occurred in Italy in 2005 (crude incidence 9.7/100,000; world age-standardized incidence 6.0/100,000). 1014 (34.64%) has been diagnosed in >65years old women (AIRTum, 2006; ISTAT, 2005). Early diagnosis is necessary to decrease the direct management costs of disease. In the present study we have examined the screening histories, treatment, human papillomavirus (HPV) detection of cervical intraepithelial neoplasia (CIN)3- invasive squamous cell carcinoma (SCC) in >65 years-old women, diagnosed in the period 2007 to 2010 with opportunistic Pap-tests in the Cytopathology Section of Institute of Anatomic Pathology in Rovereto Hospital. The aim of the paper is to study the incidence of invasive SCC diagnosed with opportunistic screening in order to decrease the direct management cost of disease in Trentino region in >65 years-old women.

MATERIALS AND METHODS

The Pap smear was performed by gynaecologist to the woman's initiative. An experienced cytopathologist (TP) whose diagnostic experience exceeds 20 years examined all abnormal smears and 10% of the normal smears previously observed by a senior cytotechnologist. Colposcopic and cervical biopsies were taken by an experienced colposcopist (in practice for more than 10 years) and review by a senior colposcopist as part of the routine. Cervical biopsy was performed in two cases, cone biopsy in two patients and hysterectomy with bilateral salpingo-oophorectomy in three cases. Consensus polymerase chain reaction (PCR) and direct sequencing of PCR products (DNA HPV typing) were used to determinate the type or types of HPV in histological specimens. PCR consensus primer sets which hybridize to highly conserved regions of the HPV genome has been designed to detect many known HPV types in a single amplification procedure (Gravitt et al., 1991; Lungu et al., 1992; Rady et al., 1995; Zheng et al., 1995). In the present study, we used the consensus primer sets to detect HPV in histological specimens. These primers promote the amplification of a 450 bp fragment spanning the LI open reading frame (ORF) from at least 25 distinct genital HPV types (Manos et al., 1989). Additionally, samples positive by L1-PCR were digested with three restriction enzymes (Hae III, BstN I and Dde I) for accurate HPV typing. Cervical tissue samples were obtained from 8 patients (Table 2). Specimens were stored in liquid nitrogen until use. DNA extraction was carried out according to standard procedures (Strauss, 1987). DNAs of several cloned HPV types were tested by the PCR using general HPV primers. The PCR amplifications, which target a portion of the HPV L1 region (approximately 450 bp), were performed on 5 to 10 µl of each sample preparation as previously described (Manos et al., 1989). Reactions contained aliquots of clilnical samples or of control DNAs in 100 µl of solutions containing 50 mM KCl, 1.5 mM MgCl2, 10 mM Tris (pH 8.3), 200 µM of each dNTP (dATP, dCTP, dGTP, dTTP), 100 mg/ml gelatin, 2.5 units Taq polymerase (Perkin Elmer Cetus Instruments, Norwalk, CT) and 100 pmoles of each consensus primer (MY 11 and MY 09). In addition to HPV-negative and HPV-positive control samples, no-DNA controls (that is, reaction mixtures to which no DNA were added) were included during each amplification series for the detection of contamination during reaction set-up. Furthermore, to minimize contamination, we used only positive displacement pipettes and disposable pipettes in the assembly of amplification reactions. Pre- and post- amplification reagents were kept physically separated throughout the experiments to avoid contamination (Kwok and Higuchi, 1989) Each L1 amplification reaction contained L1 degenerate primers MY11 (GCMCAGGGWCATAAYAATGG) and MYO9 (CGTCCMARRGGAWACTGATC) (Manos et al., 1989), with the following modification: 5 pmol each of the β-globin primers GH2O (5' GAAGAGCCAAGGACAGGTAC 3') and PCO4 (5' CAACTTCATCCACGTTCACC 3') were included for the simultaneous amplification of a β-globin product of 268 bp that served as an internal control. Each reaction was subjected to 30 amplification cycles in a DNA Thermal Cycler, using thermocycle-step parameters of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min. An additional 5 min was included at the final 72°C elongation cycle. If the initial result was HPV-negative, a portion (usually, $1/10^{th}$) of the first amplified reaction mixture was subjected to another 30 cycles of amplification under the same conditions with freshly supplemented primers, deoxyribonucleotide triphosphates, and Taq polymerase. 10 ml of PCR product was visualized with UV-light after agarose gel electrophoresis (2%) containing ethidium bromide (5 µg/25 ml). Three restriction enzymes (Hae III, BstN I, Dde 1) were chosen based on their restriction patterns of the amplified product produced from L1-PCR. Samples that were L1-PCR-positive for any HPV type assayed and produced a visible band on agarose gel, it was digested with the 3 chosen restriction enzymes for confirmation of the results. In L1-PCR, amplification products were almost free from nonspecific bands and therefore aliquots of the amplification reaction were subjected to restriction digestion without further purification. Volumes of 12 µI of each sample were added to a restriction enzyme cocktail under conditions specified by the manufacturer. Reactions were stopped with 12 µl of gel loading buffer and separated on a 12% polyacrylamide gel. DNA was visualized with ethidium bromide staining.

RESULTS

The distribution of women for decades is reported in Table 1. Between the women (> 64 years old) with CIN3-SCC cytological diagnosis all were > 70 years old and were not invited to OrS because of age > 64 years. We have reported in Table 2 the age, histological diagnosis, treatment and HPV detection of 8 patients over 70 years with CIN 3 SCC cytological diagnosis. Each specimen was tested for amplification of the L1-ORF of HPV 6, 11, 16, 18, 31, 33, 35, 42, 51 and 58 using consensus primers. PCR product of each sample was revealed by 2% agarose gel electrophoresis. About 100 pg of HPV types 6b, 11, 16, 18 and 33 in viral plasmids as positive controls were routinely detected by PCR. Negative controls were satisfactory. Tissues tested for the presence of HPV DNA by PCR were also tested for the presence of the β-globin DNA to determine if the cellular DNA in the specimens was accessible for PCR. The β-globin sequences were successfully amplified in all 8 cases. The amplified β-globin fragment (268 bp) and HPV amplification products (450 bp) of the expected size was visible in the ethidium bromide-stained gels. In cervical biopsies obtained from 8 women, an HPV rate of 37.5% (3 of 8) was found. One of these HPVs was HPV 16 and two was HPV 58. Detection rates for HPV infection were not increased after second sampling. HPV detection by PCR correlated with that by restriction enzyme analysis.

Table 1. Opportunistic screening: Decades of age of 28.589 women in the period 2007 to 2010.

Total number opportunistic Pap-tests	≤ 20 Years (%)	21 – 40 Years (%)	41 – 70 Years (%)	> 70 Years (%)
28.589	892 (**3.2%**)	11240 (**39.3%**)	14848 (**51.9%**)	1620 (**5.6%**)

Table 2. Age, treatment, histological diagnosis, HPV detection in women over 70 years-old with CIN3-squamous cell carcinoma.

No. of patients	Age	Treatment	Histological diagnosis (pT)	HPV finding
1	81	Hysterectomy with bilateral salpingo-oophorectomy	CIN3	HPV16
2	82	Cone biopsy	CIN3	Negative
3	71	Cone biopsy	CIN3	HPV58
4	79	Cone biopsy + radiotherapy	Keratinizing squamous cell carcinoma NOS	Negative
5	75	Hysterectomy with bilateral salpingo-oophorectomy	Non keratinizing squamous cell carcinoma (pT1b1)	Negative
6	82	Biopsy	Keratinizing squamous cell carcinoma NOS	Negative
7	72	Biopsy + radiotherapy	Keratinizing squamous cell carcinoma NOS	HPV58
8	75	Hysterectomy with bilateral salpingo-oophorectomy + radiotherapy	Non keratinizing squamous cell carcinoma (pT1b1)	Negative

Samples that were L1-PCR positive for 2 HPVs were digested with 3 restriction enzymes, Hae III, BstN I and Dde I. The restriction fragment lengths are useful for typing the HPV DNA fragments amplified by L1-PCR.

DISCUSSION

The annual incidence of invasive cervical cancer in women between 30 and 50 years of age in high-risk areas is 1/1,000. From an epidemiological point of view, an HPV infection meets the criteria as a causal agent for cervical cancer (Schiffman et al., 1993; Bosch et al., 2002). Having sexual contact is the main source of HPV infection. HPVs are a group of host specific DNA virus with remarkable epithelial cell specificity. More than 120 different HPV genotypes have been identified and almost 45 subtypes, isolated from the low genital tract have been grouped into high- and low- risk HPV types, considering their risk potential to induce an invasive cervical cancer. In a recent study Muñoz et al. (2003) classify HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 as high risk viruses, detectable in high grade squamous intraepithelial lesions or in invasive cancer; HPV 26, 53 and 66 as potential high risk with a not well known oncogenic potential; while types 6, 11, 40, 42, 43, 44, 54, 61, 70, 72, 81 and 89 can be considered as viruses with low oncogenic risk and they can be isolated from low grade epithelial lesions. Functionally high risk HPV types infection contributes to carcinogenesis and tumour progression predominantly through the action of two viral oncogenes, E6 and E7. The E6 protein exerts rapid degradation of p53, in corporation with E6-associated protein (E6-AP), via ubiquitin-mediated proteolysis pathway (Scheffner et al., 1993; Huibregtse et al., 1993). The E7 protein mediates the release of the E2F transcription factor from pRb-E2F complex (Nevins, 1992). Mutational analysis of HPV 16 E6 protein revealed that a certain level of the activity to degrade p53 is required for E6 to manifest its transforming function (Nakagawa et al., 1992). The p53 mutations are the most frequent genetic abnormalities found in a wide variety of human malignant tumours (Harris, 1993). Once DNA damage occurs, p53 protein is induced and arrests cells in the G1 phase to enhance DNA repair (Kuerbitz et al., 1992), or triggers apoptosis following DNA damage (Lowe et al., 1993). These functions of p53 protein are important to maintain the genomic integrity. Mutant p53 proteins are devoid of these functions, because they lose the ability of DNA contact or destabilize the structure of the core domain (Cho et al., 1994). In this way, once p53 is mutated, DNA damage is fixed and subsequent genetic rearrangement progress which may be putative mechanisms to initiate cancer. Thus far, exceptionally low prevalence (0 to 6%) of the p53 mutations had been documented in cervical carcinomas (Fujita et al., 1992; Choo et al., 1993; Helland et al., 1993; Paquette et al., 1993; Miwa et al., 1995). The p53 protein in cervical carcinoma is thought to be inactivated presumably due to complex formation with HPV E6 oncoprotein. The most common member of the high-risk group is HPV 16, which accounts for more than 60% of all cervical cancers. The high-risk types account for more than 95% of all cases of cervical cancer. One of the main differences between

high- and low-risk types is the possibility of integration in the genome. Approximately 1% of the high-risk HPV types and only 0.1% of the low-risk HPV types will lead to the development of cervical cancer (Ferlay et al., 2000). In our case HPV 16 and HPV 58 were detected in two cases of CIN3 and HPV 58 in one case of SCC. In one case of CIN3 and in 4 cases of squamous cell carcinoma the HPV type has been not detected. Two hypotheses may be made. There is a subset of SCCs that is unrelated to HPV. Other hypothesis takes into account that HPV 16 is only integrated in 72 percent of all invasive cervical cancers (Walboomers et al., 1999). The finding of the absence of HPV 16 DNA integration in some carcinomas implies that integration is not always required for malignant progression, but does not exclude the importance of HPV integration in the initiation of cervical cancer. Hypothetically, after the development of a carcinoma, the abnormal clone could lose the viral DNA. HPV 18, on the other hand, shows 100% integration. In light of recent studies demonstrating that mutation of p53 gene was found in over 20% of the patients with vulvar carcinoma (Lee et al., 1994; Milde-Langosch et al., 1995), a disease of elderly women and a known HPV- related malignancy, Nakagawa et al. (1999) analysed mutation of the p53 gene in 46 women with cervical carcinomas at the age of 60 or more (mean; 71 years, range; 60 to 96 years). Of the 46 patients, 41 had squamous cell carcinoma and 5 had adenocarcinoma. Point mutation of the p53 gene was detected in 5 out of 46 (11%) cervical carcinomas: 1 of 17 (6%) samples associated with high-risk HPVs (HPV 16 and HPV 18) and 4 of 27 samples (15%) with intermediate-risk HPVs, whereas no mutation was found in 2 HPV negative cases. Although falling short of statistical significance reduces the strength of the conclusion. Data presented by Nakagawa et al. (1999) imply that p53 gene mutation may constitute one pathogenetic factor in cervical carcinoma affecting elderly women. To clarify the age-related genetic events in cervical cancer in elderly (>65 years) women, a large variety of nucleic acid hybridization assays has been employed for the detection and specific identification of HPV DNAs. These include Southern, dot blot, filter *in situ* (FISH) and tissue *in situ* hybridizations. Recently, much attention has focused on the potential utility of PCR methods to amplify HPV-specific DNA sequences (Manos et al., 1989). PCR promises to be much more sensitive than previously used methods of HPV DNA detection. Morever, PCR consensus primer sets, which hybridize to highly conserved regions of the HPV genome, have been designed to detect many known HPV types in a single amplification procedure. Saito et al. (2000) have analyzed for HPV typing via PCR, the expression of p53, 66 tissue specimens obtained from patients with stage Ib-IIb cervical carcinoma. Of this group, 50 women aged 64 years and younger were designated as the younger group (mean age 46.7) and 16 women aged 65 years and older were designated as the older group (mean age 67.6). The prevalence of HPV DNA was higher in the younger group than in the older group (84.0 vs. 50.0%) as was the detection rate of HPV 16 (44.0 vs. 6.3%). In contrast, HPV 18, 33, 52, 58, were frequently detected in older patients. The positive rate of p53 overexpression in the older group was similar to that in the younger group (46.7 vs. 48.8%). There was no significant difference in the incidence of lymph node metastasis, histology, and the distribution of clinical stage between the two groups. EGFR and Cox-2 overexpression has been reported in many neoplasms (Tsujii and DuBois, 1995; Tsujii et al., 1997). To find information on invasive SCC in the elderly, Giordano et al. (2011) have analyzed 110 invasive SCCs obtained from 2 groups of patients for HPV status by PCR study, for immuno-histochemical EGFR, Cox-2 expression and clinicopathologic features. In this study, 64 women aged 60 years or younger were designated as the younger group and 46 who were 61 years or older were designated as the older group. The HPV status and the expression of Cox-2 and EGFR in the younger and older women were compared and correlated with the grading, staging neoplasm, lymph nodal status and overall survival. The number of neoplasms with higher staging was significantly greater than those in the younger women. The mortality was higher in the older group than in the younger patients. In the elderly, the presence of HPV DNA in 65% of cases and in the absence of sexual activity could be due to reactivation of latent HPV infection. In accordance with data provided by the literature, this finding demonstrated that HPV DNA can be detected in elderly women and can be associated with cervical carcinoma (Baay et al., 2001; García-Piñeres et al., 2006; Subbaramaiah and Dannenberg, 2007). Thus, it is possible that, in elderly women, HPV presence, in the absence of sexual activity, could be due to reactivation of latent HPV infection because of impairment of host immunologic response (Mubiayi et al., 2002). Inadequate immunologic control of HPV infection resulting in viral persistence is likely an important determinant of risk of progression to cervical neoplastic disease. Immunologic competence has been reported to decrease with aging. Garcia-Piñeres et al. (2006) examined the association between lymphoproliferative responses to antigens/mitogens and persistent HPV infection in women older than 45 years. Women included in this study were participants in a 10,000 woman population-based cohort study of cervical neoplasia in Costa Rica. Women older than 45 years and HPV DNA positive at a screening visit were selected as cases (n = 283). Garcia Piñeres et al. (2006) selected a comparably sized control group of HPV DNA–negative women, matched to cases on age and time since enrollment (n = 261). At an additional clinical visit, women were cytologically and virologically re-screened and cervical and blood specimens were collected. Proliferative responses to phytohemagglutinin (PHA), influenza virus

Table 3. Organised cervical cancer screening programmes in Italy: Value of some process indicators in Trentino region between 2005-2008 (National Centre for Screening Monitoring).

	TRENTINO			
	2005	**2006**	**2007**	**2008**
Nominal extension (%)	37	30	30,1	29,8
Number of invitated woman	52.305	43.455	45.104	44.852
Compliance with recommendation to repeat cytology (%)	35,7	36,2	37	53,2
Inadequate citology (%)	4,8	5,7	5,5	5
Recommendation to repeat cytology(%)	1,5	1,3	1,3	1,2
Compliance colposcopy with referral for ASCUS+	75,5	78,5	79	76
DR° for cytologyc lesions CIN2+ unadjusted	3,4	2,4	3,2	2,4
PPV for CIN2+ of ASCUS+ referred to colposcopy	29,6	23,9	31,5	28,3

(Flu), and HPV16 virus-like particle (VLP) were lower among women with persistent HPV infection than for the control. The decreases were most profound in women with long-term persistence and were only observed for the oldest age group (≥ 65 years). The results of this study indicate that impairment in host immunologic responses is associated to persistent HPV infection. Since 1993, at least 7 studies have described the screening histories of women with invasive cervical cancer (Ciatto et al., 1993; Kenter et al., 1996; Stuart et al., 1997). In 2007 the almost 30% of the Italian population not included in organised programmes is partly the result of an implementation process still in progress in some Regions in Southern Italy, but mainly of a very limited or completely absent implementation in a few Regions in Northern Italy. In 2007, 121 active programmes had a target population 11,872,810 women, corresponding to 71.8% of Italian women aged 25 to 64 years vs. 69% in 2006. During 2007, 39.8% of invites women were screened vs. 38.5% in the previous year. The last report of National Centre for Screening Monitoring as been published in 2008 and various process indicators of all regions have been described with exclusion of Liguria. Only 39.7% of invited women were screened vs. 39.8% in the previous year. The data of Trentino Region has been reported in Table 3. The nominal extension varied from 9,9% (Puglia) to 54,4% (Basilicata), the compliance with recommendation to repeat cytology from 17.2% (Puglia) to 73.3% (Valle D'Aosta), the inadequate cytology from 0.8% (Valle D'Aosta) to 12.1% (Molise), the recommendation to repeat cytology varied from 1.2% (Trentino and Puglia) to 4.5% (Abruzzo). The main examined process indicator has not been reported in all the regions.

In conclusion, the data of National Centre for Screening Monitoring provides information regarding the deludent performance of the organized screening programmes for cervical cancer. The distinction between OpS and OS screening has not been done. Ricciardi et al. (2009) have examined the direct cost of managing invasive cervical cancer in Italy. An estimated 2,927 new cases of cervical cancer occurred in Italy in 2005. The estimated numbers of new cases by FIGO stage were: FIGO I, 1,927; FIGO II, 556; FIGO III, 259; and FIGO IV, 185. Costs for the most frequent procedures were estimated as: € 6,041 for radical hysterectomy or other surgery; € 4,901 for radio-chemotherapy; € 1,588 for brachytherapy; and € 3,795 for palliative chemotherapy. Mean management costs for incident cases (including 10 years follow-up) were estimated at: FIGO I, € 6,024; FIGO II, € 10,572; FIGO III, € 11,367; FIGO IV, € 8707; and € 5,854 for the terminal phase (1 month). The total direct management cost was estimated at € 28.3 million per year. Because the 34.64% of invasive cervical carcinoma has been diagnosed in > 65years old women, it is necessary to consider the extension of screening programs after the 65 years. With regard to screening histories of invasive cervical carcinoma in Italy, no studies have been published except OrS programme of Friuli Venezia Giulia.

In Italy the complete screening history of women diagnosed with invasive cervical cancer has been performed only in Friuli Venezia Giulia – North eastern Italy. In these regions an OrS was initiated in 1999 targeting women aged 25 to 64 years, who are invited to have a Pap-test every 3 years. The screening histories of CIN3 – SCC in > 65 years old women may be made with study of OpS because the OrS offers a free-of-charge Pap-test every 3 years to all women aged 25 to 64 years. Zucchetto et al. (2010) have examined the screening histories of 438 women with invasive cervical cancer diagnosed in Friuli Venezia-Giulia between 1999 and 2005. 82 cases (49.7%) were found in > 65 years old women. 165 (37.7%) women were not screening. 69 (15.8%) women were not invited to OrS because of age >65 years old. Histological type and HPV detection of invasive cervical cancers has been reported. The study of Zucchetto et al. (2010) shows that the lack of screening among older women and of compliance with organized programs among women in the target population are the main limitation in cervical cancer secondary prevention. The results of Zucchetto et al. (2010) are in agreement with research conducted in northen Europe. Bos et al. (2006) have analyzed the screening history of 3.175 women with invasive cervical cancer diagnosed in the

years 1994 to 1997 in the Netherland. 57% of 3175 women with invasive cervical cancer had no previous smears. Given the high proportion of women with invasive cervical cancer older than 64 years at diagnosis, the possibilities of inviting them to have at least one Pap smear test after 64 years, should be taken into consideration. According to American Cancer Society Guidelines for the early detection of cancer and the guidelines of other national regional screening programme, women 70 years of age or older who have had 3 or more normal Pap-test in a known and no abnormal Pap-test results in the last 10 years may choose to stop having Pap-test. According to National Cervical Screening Program, the current policy of screening women of New Zealand is to continue organized regular screening until aged 69 years with Pap test every three years if the women have ever been sexually active. The National Cervical Screening Program of Australian Government believes that at age 70, women should consult with your doctor about whether they need to continue to have a regular Pap smear.

The present study support the screening policy to perform Pap test every 3 years until aged 69 years, independently to sexual activity because 34.64% of invasive cervical carcinoma has been diagnosed in > 65 years old women. Consequently, it is necessarily early diagnosis that decreases mortality, morbidity and direct management costs of disease.

REFERENCES

AIRTum: I Tumori in Italia – Rapporto (2006). I dati di incidenza e mortalità dei Registri Tumori generali, 1998-2002. Epidemiol. Prev. Suppl., 2: 1-148.

Baay MF, Tjalma WA, Weyler J, Pattyn GG, Lambrechts HA, Goovaerts G, Baekelandt M, Buytaert P, Van Marck EA, Lardon F, Vermorken JB (2001). Prevalence of human papillomavirus in elderly women with cervical cancer. Gynecol. Obstet. Invest., 52: 248-251.

Bos AB, Rebolj M, Habbema JD, van Ballegooijen M (2006). Nonattendance is still the main limitation for the effectiveness of screening for cervical cancer in the Netherlands. Int. J. Cancer ,119: 2372-2375.

Bosch FX, Lorincz A, Munoz N, Meijer CJ, Shah KV (2002). The causal relation between human papillomavirus and cervical cancer, J. Clin. Pathol., 55: 244–265.

Cho Y, Gorina S, Jeffrey PD, Pavletich NP (1994). Crystal structure of a p53 tumor suppressor-DNA complex: understanding tumorigenic mutations. Science, 265: 346-355.

Choo KB, Chong KY (1993). Absence of mutation in the p53 and retinoblastoma susceptibility genes in primary cervical carcinomas. Virology, 193: 1042-1046.

Ciatto S, Grazzini G, Cecchini S, Iossa A (1993). Screening history of incident cases of invasive carcinoma of the cervix. Tumori, 79: 311-313.

Ferlay J, Bray F, Pisani P, Parkin DM (2000). Globocan 2000. Cancer Incidence, Mortality and Prevalence Worldwide. IARCPress: Lyon.

Fujita M, Inoue M, Tanizawa O, Iwamoto S, Enomoto T (1992). Alterations of the p53 gene in human primary cervical carcinoma with and without human papillomavirus infection. Cancer Res., 52: 5323-5328.

García-Piñeres AJ, Hildesheim A, Herrero R, Trivett M, Williams M, Atmetlla I, Ramírez M, Villegas M, Schiffman M, Rodríguez AC, Burk RD, Hildesheim M, Freer E, Bonilla J, Bratti C, Berzofsky JA, Pinto LA (2006). Persistent human papillomavirus infection is associated with a generalized decrease in immune responsiveness in older women. Cancer Res., 66: 11070-11076.

Giordano G, D'Adda T, Dal Bello B, Brigati F, Bersiga A, Campanini N, Berretta R, Rocco A, Merisio C (2011). Clinicopathologic implications of the epidermal growth factor receptor, cyclooxygenase 2 expression, and human papillomavirus status in squamous cell carcinoma of the uterine cervix in the elderly. Int. J. Gynecol. Cancer, 21: 337-348.

Gravitt P, Hakenewerth A, Stoerker J (1991). p53: A direct comparison of methods proposed for use in widespread screening of human papillomavirus infections. Mol. Cell Probes, 5: 65-72.

Harris CC (1993). At the crossroads of molecular carcinogenesis and risk assessment. Science, 262: 1980-1981.

Helland A, Holm R, Kristensen G, Kaern J, Karlsen F, Trope C, Nesland JM, Børresen AL (1993). Genetic alterations of the p53 gene, p53 protein expression and HPV infection in primary cervical carcinomas. J. Path., 171: 105-114.

Huibregtse JM, Scheffner M, Howley PM (1993). Cloning and expression of the cDNA for E6-AP, a protein that mediates the interaction of the human papillomavirus E6 oncoprotein with Molecule. Cell Biol., 53(13): 775-784.

National Institute of Statistics, ISTAT (2005): "Multipurpose Household 2005" on the health status and use of health services 2005.

Kenter GG, Schoonderwald EM, Koelma IA, Arentz N, Hermans J, Fleuren GJ (1996). The cytological screening history of 469 patients with squamous cell carcinoma of the cervix uteri; does interval carcinoma exist? Acta Obstet. Gynecol. Scand., 75: 400–403.

Kuerbitz SJ, Plunkett BS, Walsh WV, Kastan MB (1992). Wild-type p53 is a cell cycle checkpoint determinant following irradiation. Proc Natl Acad Sci USA 89: 7491-7495.

Kwok S, Higuchi R (1989). Avoiding false positives with PCR. Nature, 8: 339-490.

Lee YY, Wilczynski P, Chumakov A, Chih D, Loeffler HP (1994). Carcinoma of the vulva: HPV and p53 mutations. Oncogene 9: 1655-1659.

Lowe SW, Schmitt EM, Smith SW, Osborne BA, Jacks T (1993). p53 is required for radiation-induced apoptosis in mouse thymocytes. Nature, 362: 847-849.

Lungu O, Wright TC, Jr, Silverstein S (1992). Typing of human papillomaviruses by polymerase chain reaction amplification with L1 consensus primers and RFLP analysis. Mol, Cell Probes, 6: 145-152.

Manos MM, Ting Y, Wright DK (1989). Use of polymerase chain reaction amplification for the detection of genital human papillomavirus infections. Cancer Cells, 7: 209-214.

Milde-Langosch KM, Albrecht K, Joram S, Schlechte H, Giessing M, Loning T (1995). Presence and persistence of HPV infection and p53 mutation in cancer of the cervix uteri and the vulva. Int. J. Cancer, 63: 639-645.

Miwa K, Miyamoto S, Kato H, Imamura T, Nishida M, Yoshikawa Y, Nagata Y, Wake N (1995). The role of p53 inactivation in human cervical cell carcinomadevelopment. Br. J. Cancer, 71: 219-226.

Mubiayi N, Bogaert E, Boman F, Leblanc E, Vinatier D, Leroy JL, Querleu D (2002). Cytological history of 148 women presenting with invasive cervical cancer. Gynaecol. Obstet. Fertil., 30: 210–217.

Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV (2003). International Agency for Research on Cancer Multicenter Cervical Cancer Study Group, et al: Epidemiologic classification of human papillomavirus types associated with cervical cancer. N. Engl. J. Med., 348(6): 518-527.

Nakagawa S, Watanabe S, Yoshikawa H, Taketani Y, Yoshiike, KKanda T (1992). Mutational analysis of human papillomavirus type 16 E6 protein: transforming function for human cells and degradation of p53 in vitro. Virology. 212: 535-542.

Nakagawa S, Yoshikawa H, Jimbo H, Onda T, Yasugi T, Matsumoto K, Kino N, Kawana K, Kozuka T, Nakagawa K, Aoki M, Taketani Y (1999). Elderly Japanese women with cervical carcinoma show higher proportions of both intermediate-risk human papillomavirus types and p53 mutations. Br. J. Cancer, 79: 1139-1144.

Nevins JR (1992). E2F: A link between the Rb tumor suppressor protein and viral oncoproteins. Science, 258: 424-429.

Paquette RL, Lee YY, Wilczynski SP, Karmakar A, Kizaki M, Miller CW,

Koeffler HP (1993). Mutation of p53 and human papillomavirus infection in cervical carcinoma. Cancer, 72: 1272-1280.

Rady PL, Arany I, Hughes TK, Tyring SK (1995). Type-specific primer-mediated direct sequencing of consensus primer-generated PCR amplicons of human papilloma viruses: a new approach for the simultaneous detection of multiple viral type infections. J. Virol. Methods, 53: 245-254.

Ricciardi A, Largeron N, Giorgi RP, Raffaele M, Cohet C, Federici A, Palazzo F (2009). Incidence of invasive cervical cancer and direct costs associated with its management in Italy. Tumori, 95:146-152.

Ronco G, Giubilato P, Naldoni C, Zorzi M, Anghinoni E, Scalisi A, Dalla Palma P, Zanier L, Federici A, Angeloni C, Prandini S, Maglietta R, Mancini E, Pizzuti R, Iossa A, Segnan N, Zappa M (2007). Extension of organised cervical screening programmes in Italy and their process indicators. Epidemiol. Prev., 31(2): 33-47.

Saito J, Hoshiai H, Noda K (2000). Type of human papillomavirus and expression of p53 in elderly women with cervical cancer. Gynecol. Obstet. Invest., 49: 190-193.

Scheffner M, Werness BA, Huibregtse JM, Levine AJ, Howley PM (1993). The E6 oncoprotein encoded by human papillomavirus types 16 and 18 promotes the degradation of p53. Cell, 63: 1129-1136.

Schiffman MH, Bauer HM, Hoover RN, Glass AG, Cadell DM, Rush BB, Scott DR, Sherman ME, Kurman RJ, Wacholder S (1993). Epidemiologic evidence showing that human papillomavirus infection causes most cervical intraepithelial neoplasia, J. Natl. Cancer Inst., 85: 958–964.

Strauss WM (1987). Preparation of genomic DNA from mammalian tissue. In: Ausubel FM, Brent R, Kingston RE, eds. Current Protocols in molecular biology. New York; Greene Pubblishing and John Wiley & Sons, 2.2: 1-3.

Stuart GC, Mc Gregor SE, Duggan MA, Nation JG (1997). Review of the screening history of Alberta women with invasive cervical cancer. CMAJ., 157: 513–519.

Subbaramaiah K, Dannenberg AJ (2007). Cyclooxygenase-2 transcription is regulated by human papillomavirus 16 E6 and E7 oncoproteins: evidence of a corepressor/coactivator exchange. Cancer Res., 67: 3976-3985.

Tsujii M, DuBois RN (1995). Alterations in cellular adhesion and apoptosis in epithelial cells overexpressing prostaglandin endoperoxide synthase-2. Cell, 83: 493-550.

Tsujii M, Kawano S, DuBois RN (1997). Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc . Natl. Acad. Sci. U.S.A., 94: 3336-3340.

Walboomers M, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJ, Peto J, Meijer CJ, Munoz N (1999). Human papillomavirus is a necessary cause of invasive cervical cancer worldwide. J. Pathol., 189: 12-19.

Zheng PS, Li SR, Iwasaka T, Song J, Cui MH, Sugimori H (1995). Simultaneous detection by consensus multiplex PCR of high- and low-risk and other types of human papilloma virus in clinical samples. Gynecol. Oncol., 58: 179-183.

Zucchetto A, Franceschi S, Clagnan E, Serraino D, Zanier L, Franzo A, Friuli Venezia Giulia Cancer Registry Working Group (2010). Screening history of women with invasive cervical cancer in north-east Italy. Eur. J. Obstet. Gynecol. Reprod. Biol., 152: 200-204.