

# hr-HPV testing in the management of women with ASC-US+ and in the follow-up of women with cytological abnormalities and negative colposcopy. Recommendations of the Italian group for cervical cancer screening (GISCi)

Test hr-HPV nella gestione delle donne con citologia ASC-US+ e nel follow-up delle donne con citologia anormale e colposcopia negativa. Raccomandazioni del Gruppo italiano per lo screening del carcinoma della cervice uterina (GISCi)

Francesca Maria Carozzi,<sup>1</sup> Anna Iossa,<sup>1</sup> Aurora Scalisi,<sup>2</sup> Mario Sideri,<sup>†</sup> Karin Louise Andersson,<sup>1</sup> Massimo Confortini,<sup>1</sup> Annarosa Del Mistro,<sup>3</sup> Giovanni Maina,<sup>4</sup> Guglielmo Ronco,<sup>5</sup> Patrizio Raggi,<sup>6</sup> Maria Luisa Schiboni,<sup>7</sup> Marco Zappa,<sup>1</sup> Paolo Giorgi Rossi<sup>8,9</sup>

<sup>1</sup>Istituto per lo studio e la prevenzione oncologica, Firenze

<sup>2</sup>Azienda sanitaria provinciale, Catania

<sup>3</sup>Istituto oncologico veneto IOV - IRCCS, Padova

<sup>4</sup>Azienda ospedaliera Città della scienza e della salute di Torino - Presidio S. Anna

<sup>5</sup>Centro di riferimento per l'epidemiologia e la prevenzione oncologica in Piemonte, Torino

<sup>6</sup>Azienda sanitaria locale, Viterbo

<sup>7</sup>Azienda ospedaliera San Giovanni - Addolorata, Roma

<sup>8</sup>Servizio interaziendale di epidemiologia, AUSL Reggio Emilia, IRCCS

<sup>9</sup>Arcispedale S. Maria Nuova, Reggio Emilia

## Abstract

Compared to spontaneous screening, an organized screening programme is characterized by the presence of protocols and recommendations for all stages including follow-up. Despite the availability of well-functioning screening programmes throughout the country, the follow-up protocol after an abnormal Pap test and negative colposcopy is not clearly defined in Italy, and there is no uniformity of indications.

HPV testing for oncogenic human papillomavirus (hr-HPV) has a high negative predictive value (NPV) and high positive predictive value (PPV) for CIN2+ and its employment can reduce follow-up assessments.

In order to provide indications about the management of women with ASC-US+ and the follow-up of women with cytological abnormalities and negative colposcopy, a literature analysis was carried out, taking into consideration European and American guidelines and good practice recommendations from the most important scientific associations and regulatory agencies. GISCi (Italian Group for Cervical Screening) drafted recommendations for the management of women with ASC-US, L-SIL, ASC-H, AGC, and H-SIL until their return to the routine screening interval. This protocol can be applied not only in the management of abnormal Pap smears in cytology-based programmes, but also in the management of abnormal Pap test triage after HPV positive test when HPV is the primary screening test. The protocols approved within the screening programmes must have an extensive consensus among all involved professionals, including any that women might meet outside the programme.

(*Epidemiol Prev* 2015; 39(3) Suppl 1: 84-90)

**Keywords:** cervical cancer screening, colposcopy, human papillomavirus, follow-up, Italy

## Riassunto

Rispetto allo screening spontaneo, un programma di screening organizzato è caratterizzato dalla presenza di protocolli e raccomandazioni per tutte le fasi, incluso il follow-up. Nonostante l'ampia diffusione dei programmi di screening su tutto il territorio, il protocollo di follow-up dopo Pap test anormale e colposcopia negativa in Italia non è chiaramente definito e non c'è uniformità nelle indicazioni date dai programmi. Il test HPV per la ricerca di papillomavirus oncogeni ha un elevato valore predittivo negativo (NPV) e un elevato valore predittivo positivo (PPV) per CIN2+ e il suo utilizzo può ridurre i controlli di follow-up. Al fine di fornire indicazioni sulla gestione delle donne con ASC-US+ e nel follow-up delle donne con citologia anormale e colposcopia negativa è stata effettuata una analisi della letteratura, delle linee guida europee e americane e delle raccomandazioni di buona pratica delle principali associazioni scientifiche. Il Gruppo italiano per lo screening del carcinoma della cervice uterina (GISCi) ha prodotto le raccomandazioni per la gestione delle donne con ASC-US, L-SIL, ASC-H, AGC e H-SIL fino al loro ritorno al normale intervallo di screening. Questo protocollo può essere applicato non solo nella gestione del Pap test anormale nello screening con Pap test primario, ma anche nella gestione del Pap test di triage anormale dopo hr-HPV test positivo, quando HPV è il test di screening primario. I protocolli approvati nell'ambito dei programmi di screening devono avere un ampio consenso tra tutti i professionisti coinvolti, compresi coloro che potrebbero entrare in contatto con le donne al di fuori del programma.

(*Epidemiol Prev* 2015; 39(3) Suppl 1: 84-90)

**Parole chiave:** screening carcinoma cervice uterina, colposcopia, papillomavirus umano, follow-up, Italia

## INTRODUCTION

No clear guidelines concerning the follow-up protocol after an abnormal Pap test and negative colposcopy exist in Italy, and actual management of these cases is highly variable.

Purpose of this article is providing recommendations on the use of hr-HPV testing in the management of colposcopies after an abnormal cytology (either applied as primary screening test or as triage of HPV-positive women) and in follow-up after colposcopy. The rationale of recommendations is the high negative predictive value for cervical intraepithelial neoplasia grade 2 or more severe (CIN2+) of HPV testing for oncogenic human papillomavirus (hr-HPV).<sup>1,2</sup> This makes it possible to reduce and standardize follow-up controls.

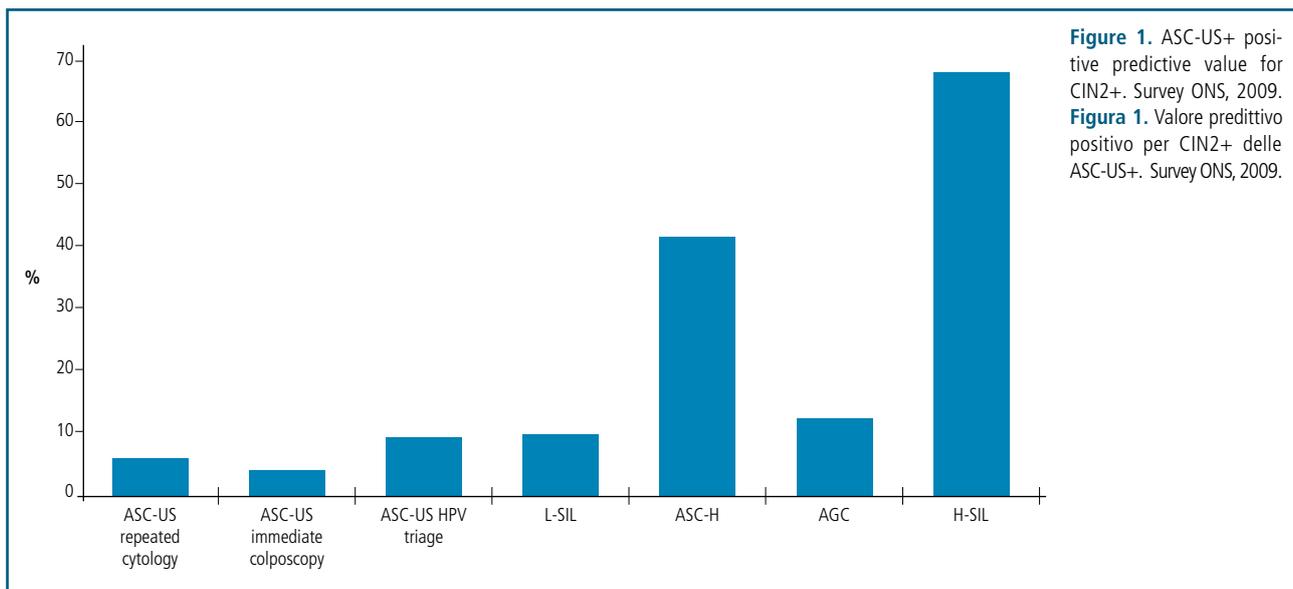
Persistent infection with one of 12 high-risk HPV types is a necessary cause of invasive cervical cancer.<sup>3</sup> Thus, hr-HPV testing can be used as a negative triage test to determine whether a woman can be safely returned to routine screening<sup>4</sup> in the follow-up of abnormal cytology and after a negative colposcopy.

## MATERIALS AND METHODS

A literature review was carried out: European and American guidelines were considered, along with good practice recommendations from the most important scientific associations and regulatory agencies.

Recommendations are based on the risk of harbouring a CIN2+ (i.e., on PPV) by primary cytology result. The PPV of cytology is highly variable between Italian screening programmes (2.8% to 52.7% for ASC-US or higher). Nevertheless, the difference between cytological categories is very large: PPV for CIN2+ is <10% in women with ASC-US and L-SIL cytology and >40% in women with ASC-H, and H-SIL cytology (figure 1).

For cytology classification we refer to the 2001 Bethesda system.<sup>4</sup> «Second-level negative for CIN2+» means that no CIN2+ was detected, either because histology was negative or because no biopsy was taken as no colposcopic abnormality was observed.



**Figure 1.** ASC-US+ positive predictive value for CIN2+. Survey ONS, 2009.  
**Figura 1.** Valore predittivo positivo per CIN2+ delle ASC-US+. Survey ONS, 2009.

## MANAGEMENT OF WOMEN WITH AN ABNORMAL SCREENING TEST

### Atypical squamous cells of undetermined significance (ASC-US)

ASC-US is the most common cytologic abnormality and entails a low risk of CIN2+. In 2006, national guidelines and GISCi recommended three possible management strategies: immediate referral, repeat cytology at 1 year or hr-HPV triage.<sup>5</sup>

In 2011, a report of the English NHSCSP pilot study on hr-HPV triage of women with ASC-US and L-SIL,<sup>6</sup> pointed out that hr-HPV triage makes it possible to return about one third of women with ASC-US to routine screening, with a considerable reduction in colposcopies. The study also showed a good acceptability of triaging to women.

The 2012 American Cancer Society Guidelines<sup>7</sup> recommend to return women with ASC-US and negative hr-HPV to the normal screening interval, i.e., 3 years. For the management of ASC-US/hr-HPV positive women who have a negative second-level assessment for CIN2+, American<sup>7</sup> and European guidelines<sup>8,9</sup> provide two options: repeat an hr-HPV test after 12 months or repeat cytology after 6 and 12 months. American guidelines<sup>7</sup> also recommend not to repeat hr-HPV testing earlier than 12 months. Since 2005/2007, GISCi<sup>5</sup> has recommended hr-HPV testing as one of the three possible options for the management of ASC-US, and endorsed the use of hr-HPV tests validated for screening. Data from the 2010 GISCi survey showed that triage with hr-HPV has a PPV for CIN2+ greater than the other two options (figure 1), and reduces variability between centres.

### Recommended management

If cytology is the primary test, triage by hr-HPV testing (HPV triage) is recommended (figure 2). Women with ASC-US cytology and negative hr-HPV should return to routine screening,<sup>10</sup> while women with ASC-US and positive hr-HPV should have colposcopy.

For women that are ASC-US/hr-HPV positive and second-level negative for CIN2+, re-testing for hr-HPV test after 1 year is strongly recommended. If the hr-HPV test is negative, return to normal screening is recommended. If it is positive, colposcopy should be repeated. In the latter case, if the new second-level analysis comes out negative for CIN2+, women are invited to repeat hr-HPV testing after 12 months. If the repeat hr-HPV test is negative, women return to routine screening. If the repeat hr-HPV test is positive, women are invited to repeat colposcopy and cytology.

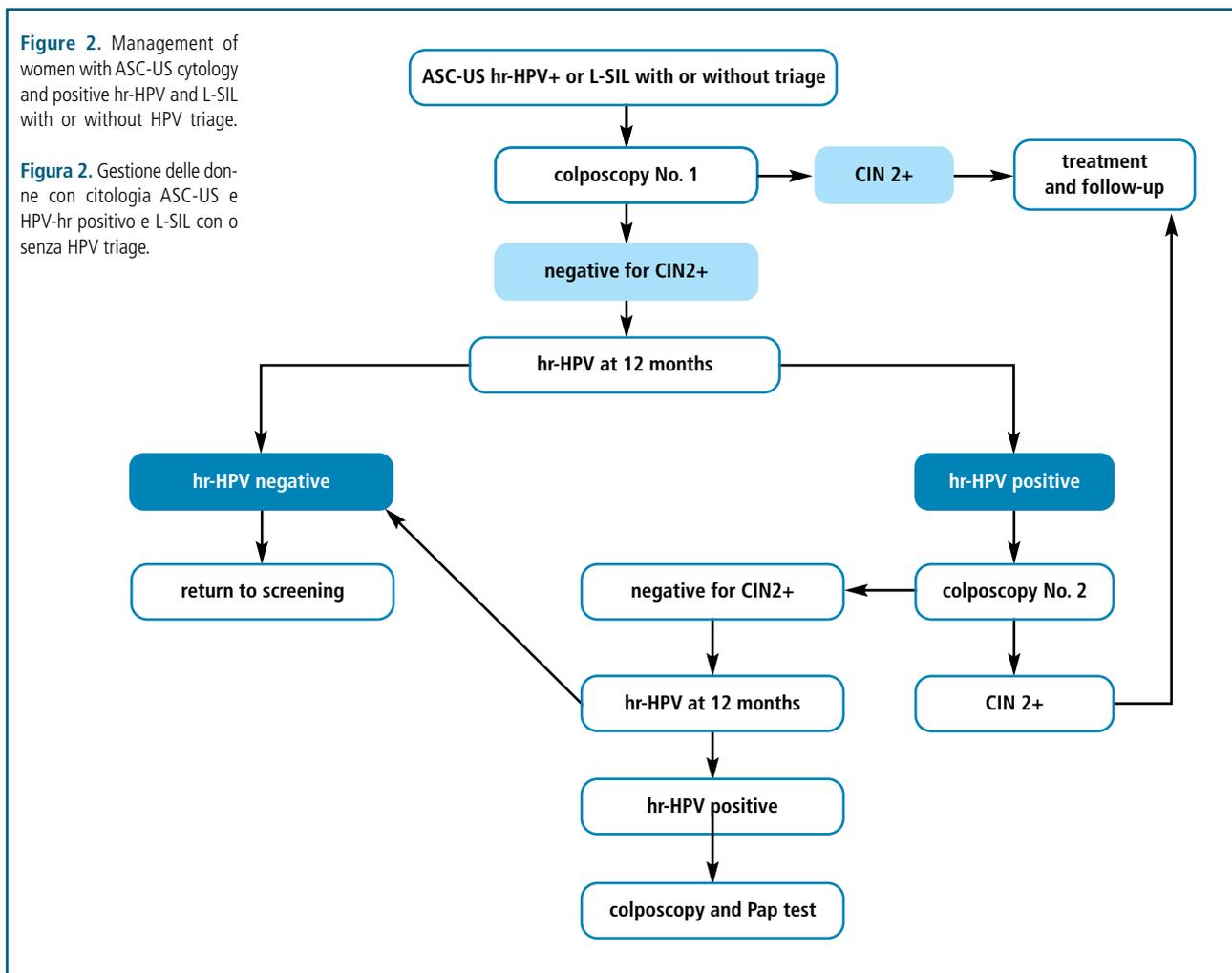
### Low-grade squamous intraepithelial lesions

hr-HPV triage is recommended for women with L-SIL cytology if age is  $\geq 35$  years, according to the GISCi 2005-2007 document.<sup>5</sup> Triage is not recommended in younger women. In the Italian programmes that have adopted this approach, the proportion of hr-HPV positive women is variable, and in many cases high.<sup>11</sup> This likely reflects different criteria for reporting cytology. Depending on the local situation, hr-HPV triage could be proposed for older (i.e., above the age of 45 years) women only.<sup>1</sup>

For women with L-SIL cytology and negative colposcopy, European and American guidelines<sup>7-9</sup> recommend repeating an hr-HPV test after 1 year. If the test is negative, the woman returns to routine screening; if it is positive the woman will be referred to colposcopy.<sup>7</sup> English guidelines<sup>10</sup> did not initially provide protocols, pending the results of a pilot study. The protocol proposed at study<sup>6</sup> publication (2011) recommended, in the case of negative 2nd level result for CIN, to return the woman to routine screening while, in the case of CIN 1 without treatment, repeating cytology in 12 months was recommended.

### Recommended management

hr-HPV triage for L-SIL is recommended for programmes where L-SIL cytology has a low PPV (<5-10 %) and after an evaluation of the local proportion of hr-HPV-positive L-SIL



through a pilot study. For women  $\geq 35$  years of age and L-SIL-hr-HPV negative, return to routine screening is recommended (figure 2). For women  $\geq 35$  years and L-SIL-hr-HPV positive and for women with L-SIL cytology and no HPV result, colposcopy is recommended.

If colposcopy is negative for CIN2+, the woman is invited for an hr-HPV test after a year. If the test is negative, the woman returns to routine screening. If it is positive, the woman is referred for colposcopy. If the second colposcopy is also negative for CIN2+, the woman is invited to repeat an hr-HPV test at 12 months. In case this further hr-HPV test is positive, the woman is referred to a new colposcopy and Pap test. This follow-up protocol, which uses the hr-HPV test after an in-depth analysis of 2nd negative level for CIN2+, can be applied even where there is no initial triage with hr-HPV.

### Atypical glandular cells (AGC)

AGC is an uncommon cytology<sup>12</sup> and is often associated with benign conditions, such as reactive cellular changes or polyps. In the literature, however, 9% to 38% of women with AGC are reported to have CIN2+, and 3% to 17% to have an invasive carcinoma.<sup>12</sup>

Atypia on glandular cells may affect endometrial as well as en-

docervical cells. European guidelines<sup>8,9</sup> make a distinction between «AGC, favour neoplasia or AIS» and «AGC not otherwise specified (NOS)». For women older than 35 years, in case of AGC favour neoplasia, a colposcopy with endocervical sampling is indicated. Even if this colposcopy is negative for CIN2+, a diagnostic conisation is recommended in this age group. In case of AGC NOS with negative colposcopic findings, European guidelines recommend a Pap test every 6 months for 2 years. American guidelines suggest for both categories of AGC a colposcopy with endocervical sampling. An endometrial sampling in all women over the age of 35 years or those with clinical elements suggestive for neoplastic pathology of the endometrium is also encouraged. A negative hr-HPV test can be useful in identifying women who have a greater risk of endometrial cancer rather than cervical disease.<sup>7</sup>

### Recommended management: initial workup

For women with AGC cytology, colposcopy is recommended; at the time of colposcopy hr-HPV testing is also recommended: the hr-HPV test will assist in excluding an origin from cervical glandular lesions in case of initial negative colposcopy workup.

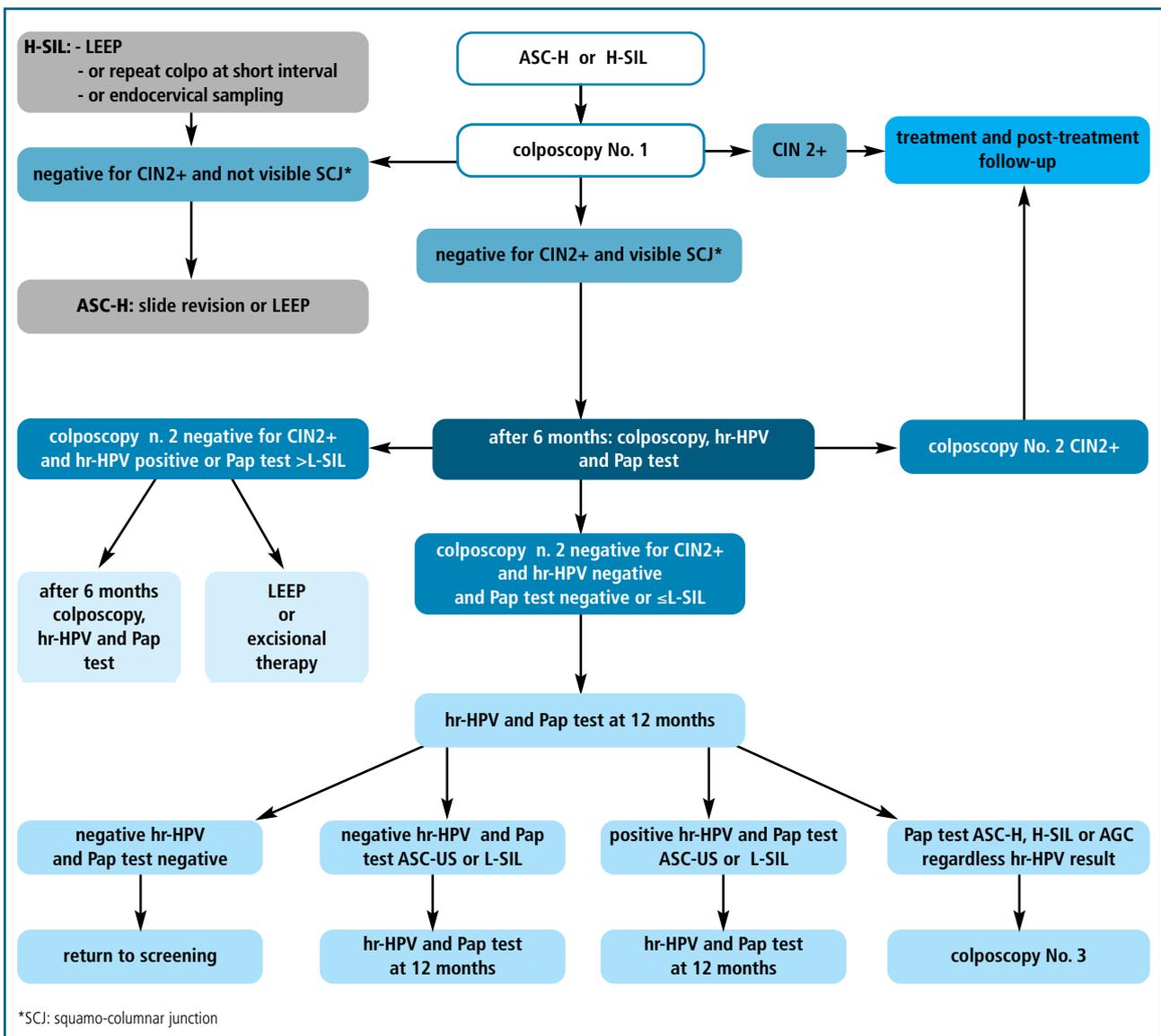


Figure 3. Management of women with cytology ASC-H and H-SIL. / Figura 3. Gestione delle donne con citologia ASC-H e H-SIL.

**Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion (ASC-H) and high-grade squamous intraepithelial lesions (H-SIL)**

Given their high positive predictive value (PPV), women with ASC-H and H-SIL cytology should be referred directly to colposcopy. While there is consensus for the management in case of CIN2+ (excisional therapy), there is no uniformity of indications in case of negative 2nd level workup for CIN2+. American guidelines<sup>12</sup> in case of H-SIL always recommend, in addition to colposcopy, an examination of the cervical canal. As for ASC-H with negative 2nd level for CIN2+, American and European guidelines<sup>7-9</sup> recommend a Pap test after 6 and 12 months or, alternatively, hr-HPV testing after 12 months. If both Pap tests or the hr-HPV test are negative, guidelines suggest a return to routine screening (1 or 2 years at the time of the 2006 guidelines expanded to 2/3 in 2010). In case of Pap

test ≥ASC or positive hr-HPV a new colposcopy is recommended. For H-SIL with negative 2nd level for CIN2+, guidelines offer three options:

- combined cytology and colposcopy at 6 and 12 months with return to screening in case of negativity of both tests;
- excisional therapy;
- review of cytology and histology (recommended by European guidelines).

**Recommended management for ASC-H and H-SIL**

Women with ASC-H or H-SIL should be referred to colposcopy. If colposcopy is positive for CIN2+, excisional treatment must be provided. The management of women with ASC-H and H-SIL and negative 2nd level for CIN2+ differs according to squamo-columnar junction visibility. To exit follow-up and return to routine screening, in any case, two negative colposcopies, two negative hr-HPV tests, and a negative cytology are needed.

If, during the first colposcopy, the squamo-columnar junction is visible and no CIN2+ is identified histologically, the woman is invited after 6 months for a new colposcopy (figure 3, p. 88), an hr-HPV test and a Pap test, recommended especially in case of initial ASC-H cytology:

- if after 6 months the 2nd level in-depth analysis turns out to be positive for CIN2+, the woman should be referred to treatment;

- if after 6 months the 2nd level in-depth analysis remains negative, i.e., histology does not identify any CIN2+, colposcopy does not locate suspicious areas on which to perform a biopsy, and hr-HPV test and Pap test are negative or  $\leq$ L-SIL, the woman should be asked to repeat an hr-HPV test and a Pap test after 12 months. If after 12 months there is H-SIL, ASC-H or AGC cytology, the woman should be referred to colposcopy, regardless of the hr-HPV test result. If after 12 months the hr-HPV test remains negative and the Pap test is negative, the woman can return to routine screening. If after 12 months the hr-HPV test is confirmed negative but the Pap test shows ASC-US or L-SIL, hr-HPV test and Pap test repeat at 12 months are recommended. If after 12 months the hr-HPV test turns out positive and the Pap test is negative or ASC-US or L-SIL cytology, the woman is recommended to repeat an hr-HPV test and a Pap test after 12 months;

- if after six months the 2nd level in-depth analysis is negative for CIN2+ but the hr-HPV test is positive, the gynaecologist can either (figure 3):

- a) schedule a diagnostic LEEP (loop electrosurgical excision procedure) or excisional therapy

or:

- b) repeat colposcopy, hr-HPV, and PAP test at 6 months.

If, during the first colposcopy, the squamo-columnar junction is not visible and no CIN2+ is detected, different options can be considered (figure 3) on the basis of initial cytology. For H-SIL there are 3 options:

- repeat colposcopy after a short interval;
- perform endocervical sampling;
- perform a diagnostic LEEP.

For initial ASC-H it is suggested to perform diagnostic LEEP or to review the slide. If the review is negative, or ASC-US, or L-SIL, an hr-HPV test should be repeated after one year. If the review confirms ASC-H cytology, then endocervical sampling is carried out.

## DISCUSSION AND FUTURE PERSPECTIVES

Organized screening programmes are more effective than opportunistic activity. The availability and quality of field and laboratory facilities for screening and diagnostic follow-up, as

well as the available treatment facilities, are key elements of any screening programme. Monitoring the management of each patient with an abnormal screening result is of crucial importance.<sup>13</sup>

Pap smear testing is widely available and has shown high efficacy in reducing cervical cancer incidence. Nevertheless, every year in Italy many new cervical cancers are diagnosed (2,200 in 2012), and 5-year relative survival rates have only slightly increased, from 64% in 1990-1994 to 67% in 2000-2004.<sup>14</sup> Reasons for Pap-test-based screening failure include lack of Pap testing, failure of the Pap smear to detect an abnormality, and lack of adequate follow-up after an abnormal Pap test.

Compared to spontaneous activity, organized screening is characterized by protocols and guidelines for all its stages, including follow-up. Protocols to be applied within screening programmes must have an extensive consensus among all involved professionals, including those that women might meet outside the programme. It is of utmost importance to verify compliance to follow-up protocols. In 2014, GISCi conducted a specific survey to evaluate the workload induced by follow-up after a negative colposcopy. Evidence to set forth the optimal management of women with negative colposcopy after abnormal cytology or with CIN1 is poor. A recent paper<sup>2</sup> confirmed that hr-HPV testing is able to identify, among women with cytology  $\geq$ ASC-US and no evidence of high-grade disease, those at risk of developing CIN2+. Performing hr-HPV testing within 1 year could avoid 30% of follow-up colposcopies in women with ASC-US and 33% in selected women with the remaining cytological abnormalities (ASC-H, L-SIL, H-SIL, AGC).<sup>15</sup> We stress that only tests for the DNA of oncogenic HPV types, validated according to European guidelines as for sensitivity and specificity for high-grade lesions, should be applied, even when the test is used for follow-up.

Determining which hr-HPV-positive women are at future clinical risk and identifying robust markers of disease progression is the challenge for the future. Follow-up studies of women managed by HPV genotyping, p16 immunostaining, and methylation markers are needed to establish their role in the management of cervical abnormalities. New HPV DNA tests, including direct partial genotyping for types 16 and 18,<sup>15</sup> or p16<sup>INK4a</sup>,<sup>16</sup> have also been shown to be promising triage test methods. Hence, with the introduction of new biomarkers for cervical cancer, more screening options will become available. As the number and sophistication of tools applied to cervical cancer prevention continue to increase, the complexity of management promises to grow.

Conflicts of interests: none declared

## References/Bibliografia

1. Ronco G, Cuzick J, Segnan N et al. HPV triage for Low grade (L-SIL) cytology is appropriate for women over 35 in mass cervical cancer screening using liquid based cytology. *Eur J Cancer* 2007; 43:476-80.
2. Carozzi F, Visioli CB, Confortini M et al. hr-HPV testing in the follow-up of women with cytological abnormalities and negative colposcopy. *Br J Cancer* 2013;109(7):1766-74.
3. Bouvard V, Baan R, Straif K et al. WHO International Agency for Research on Cancer Monograph Working Group. A review of human carcinogens-Part B: biological agents. *Lancet Oncol* 2009; 10:321-22.
4. GISCi Gruppo italiano screening del cervicocarcinoma. *Documento operativo GISCi per l'applicazione nei programmi di screening del sistema Bethesda 2001*. April 2006, updated June 2009. [www.gisci.it/documenti-gisci]
5. GISCi Gruppo italiano screening del cervicocarcinoma. *Utilizzo del test HPV HR nel triage delle diagnosi citologiche di ASC-US e delle diagnosi di LSIL in donne con più di 35 anni nonché nel follow-up delle lesioni CIN2-3 come indicatore di rischio di recidiva*. April 2005, updated 2012. [www.gisci.it/documenti-gisci]
6. Kelly RS, Patnick J, Kitchener HC et al. HPV testing as a triage for borderline or mild dyskaryosis on cervical cytology: results from the Sentinel Sites study. *Br J Cancer* 2011;105:983-88.
7. Saslow D, Solomon D, Lawson HW et al. American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology. Screening guidelines for the prevention and early detection of cervical cancer. *Am J Clin Pathol* 2012;137:516-42.
8. Arbyn M, Anttila A, Jordan J et al. European Commission. *European guidelines for quality assurance in cervical cancer screening*. Second edition. European Communities, Luxembourg 2008.
9. Arbyn M, Anttila A, Jordan J et al. European Guidelines for Quality Assurance in Cervical Cancer Screening. Second edition-summary document. *Ann Oncol* 2010;21:448-58.
10. NHS Cancer Screening Programmes. *Colposcopy and programme management. Guidelines for the NHS Cervical screening program* (second edition). NHSCSP Publication No. 20, May 2010.
11. Giorgi Rossi P, Chini F, Bisanzì S et al. Distribution of high and low risk HPV types by cytological status: a population based study from Italy. *Infect Agent Cancer* 2011;6(1):2.
12. Wright TC Jr, Massad LS, Dunton CJ et al. 2006 consensus guidelines for the management of women with cervical cytological abnormalities. *JAMA* 2002;287:2120-29.
13. Nygård M. Screening for cervical cancer: when theory meets reality. *BMC Cancer* 2011;11:240.
14. Altavilla G, Bernardo G, Bracarda S et al. *I numeri del cancro in Italia*. Brescia, Intermedia editore 2012.
15. Castle PE, Stoler MH, Wright Jr et al. Performance of carcinogenic human papillomavirus (HPV) testing and HPV16 or HPV18 genotyping for cervical cancer screening of women aged 25 years and older: a subanalysis of the ATHENA study. *Lancet Oncol* 2011; 12:880-890.
16. Carozzi F, Gillio-Tos A, Confortini M et al. Risk of high-grade cervical intraepithelial neoplasia during follow-up in HPV-positive women according to baseline p16-INK4A results: a prospective analysis of a nested substudy of the NTCC randomised controlled trial. *Lancet Oncol* 2013;14(2):168-76.