Il Triage delle lesioni borderline

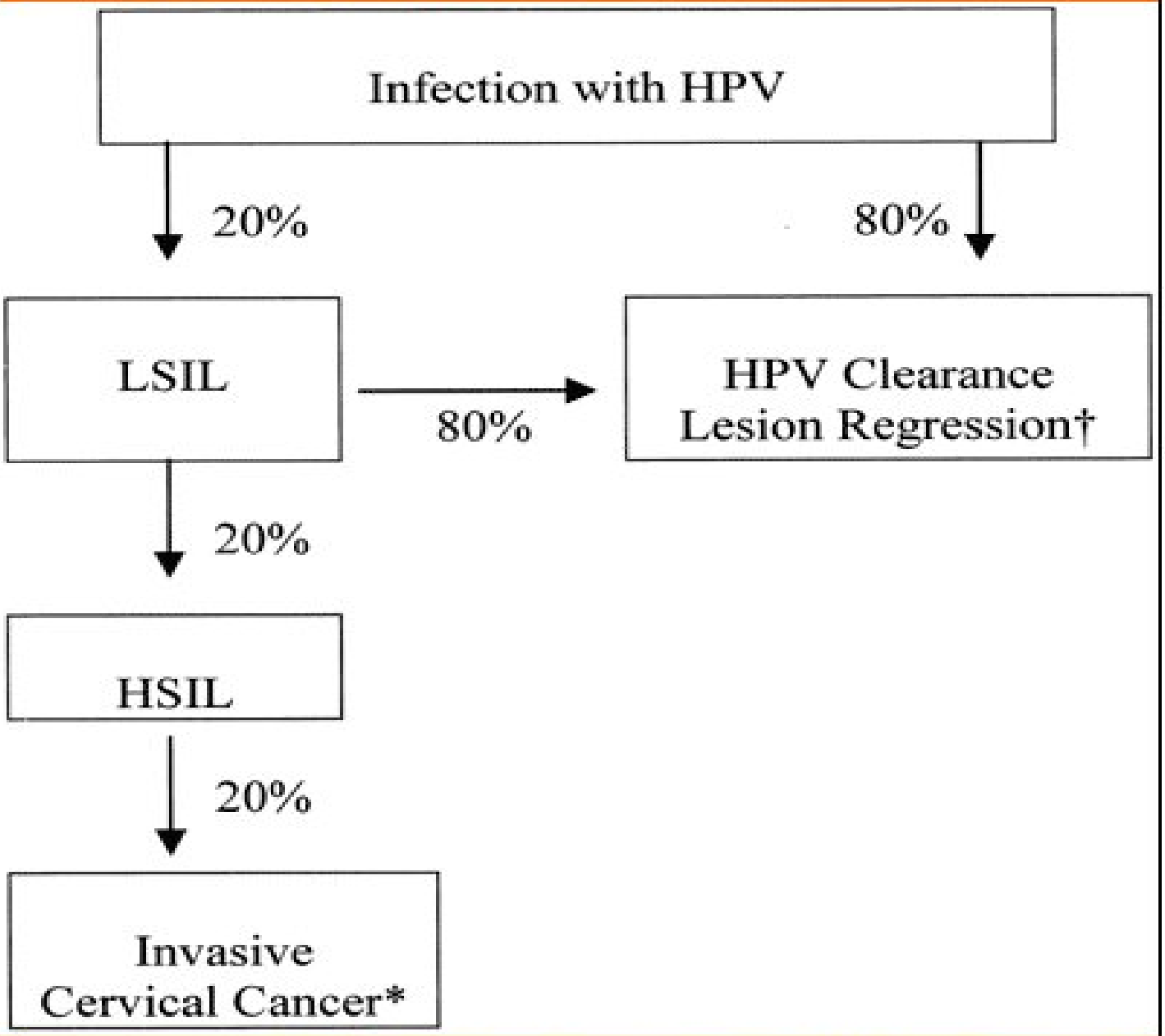
Paolo Dalla Palma

Screening:
dai Programmi alla Ricerca

BOLOGNA, 13-15 DICEMBRE 2004

Sala Auditorium
Regione Emilia-Romagna

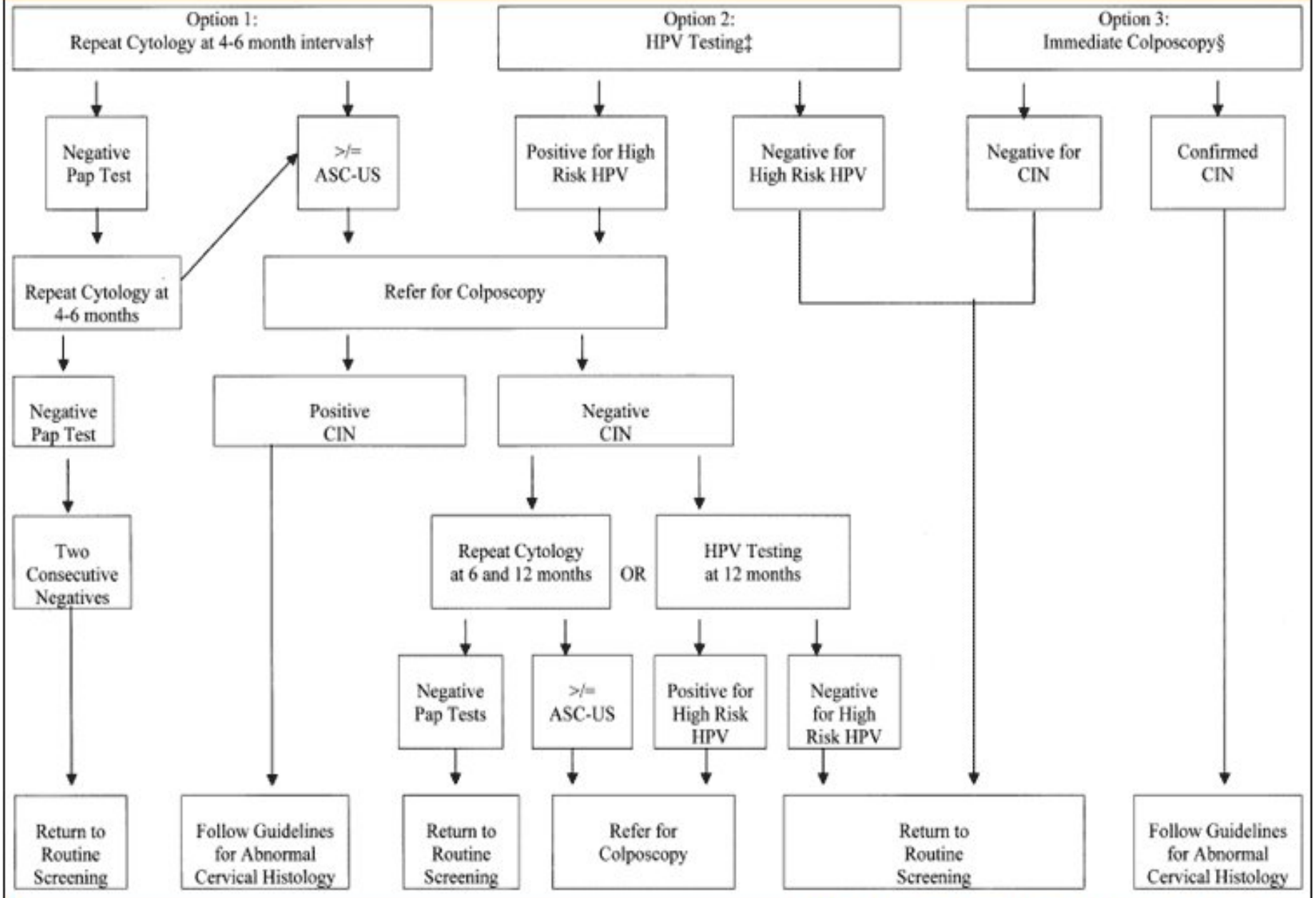
CONVEGNO CONGIUNTO
GISCi
GISMa
OSSERVATORIO NAZIONALE



ASC-US e HPV

Le questioni più importanti sono:

- **Quali sono le linee guida per il test HPV?**
- **Chi deve farlo?**
- **Qual'è il turnaround time accettabile?**
- **Chi deve sostenere i costi?**
- **Il test deve essere fatto in modo automatico o va richiesto con qualche criterio?**



Scopo del Triage

Il trattamento delle donne con alterazioni citologiche “minori” (ASC-US) rimane controverso:

1. **Atteggiamento conservativo con follow-up citologico ravvicinato (possibile sottodiagnosi di alcuni CIN2+!).**
2. **Colposcopia immediata con biopsia (possibile overtreatment di lesioni che regrediscono!)**

Cosa si può fare in questi casi???

Triage HPV

- **E’ la soluzione per le ASCUS ?**
- **E’ la soluzione per i LSIL ?**
- **Qual è l’accuratezza (sensibilità, specificità, valori predittivi, probabilità) di selezionare i casi CIN2+ istologici?**
- **E’ superiore al solo controllo citologico?**

Non serve per i LSIL

- **Troppo alta la percentuale di positività (>80%!) quindi non seleziona.**
- **Lo studio ALTS andrebbe modificato in ATS per esclusione LSIL.**
- **Il Bethesda sostiene che la lesione virale equivale almeno ad un LSIL (CIN1).**
- **La diagnosi LGEA (Australiano) comprende i LSIL e quindi inficia il valore del test HPV.**

E per gli ASCUS ??

The Role of Human Papilloma Virus Testing in Cervical Cancer Prevention

Molly C. Fey, FNP, MSN; Margaret W. Beal, CNM, PhD

J Midwifery Womens Health 49(1):4-13, 2004.
Posted 02/24/2004

Abstract and Introduction

Abstract

A clear causal relationship has been established between human papilloma virus (HPV) infection and the development of cervical cancer. Genital HPV infection is currently the most common sexually transmitted disease worldwide. The recent 2001 American Society for Colposcopy and Cervical Pathology Consensus Guidelines have included HPV testing for management of women with cervical cytological abnormalities. Clinicians now face the challenge of deciding when to use HPV testing in follow-up of abnormal Pap tests. This article includes updates on HPV, cervical cancer screening, and HPV testing technology. Recommendations for integration of HPV testing into clinical practice are provided.

Archives of Pathology and Laboratory Medicine:
Vol. 127, No. 8, pp. 991–994,2003

Successfully Integrating Human Papillomavirus Testing Into Your Practice

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Have We Resolved How To Triage Equivocal Cervical Cytology?

Diane Solomon, Mark Schiffman

Editoriale
Pag. 250-1

Journal of the National Cancer Institute, Vol. 96, No. 4, February 18, 2004

The role of triage is to identify which women with ASCUS are at risk and require colposcopy and which women can be spared the anxiety and costs associated with intensified follow-up.

Virologic Versus Cytologic Triage of Women With Equivocal Pap Smears: A Meta-analysis of the Accuracy To Detect High-Grade Intraepithelial Neoplasia

Marc Arbyn, Frank Buntinx, Marc Van Ranst, Evangelos Paraskevaidis, Pierre Martin-Hirsch, Joakim Dillner

15 studi con 5454 donne con diagnosi di ASCUS e controllo istologico: Negativo versus CIN2+.

Conclusion: The published literature indicates that the Hybrid Capture II assay has improved accuracy (higher sensitivity, similar specificity) than the repeat Pap smear using the threshold of ASCUS for an outcome of CIN2+ among women with equivocal cytologic results. The sensitivity of triage at higher cytologic cutoffs is poor. JJ Natl Cancer Inst 2004;96:280–931

Dati di Trento
2000-2003

Bethesda 1991 fino a giugno 2001

Bethesda 2001 da luglio 2001

Table 1. Cases (909) of atypical squamous borderline lesions

	ASCUS (Bethesda 1991)	ASC-US (Bethesda 2001)	ASC-H (Bethesda 2001)
HPV +	78	147	16
HPV −	231	371	32
HPV n.v.	6	27	1
Total	315	545	49

Bethesda 1991 until June 2001 and Bethesda 2001 subsequently. HPV n.v. = test not valuable because the quantity of residual PreservCyt® (Cytyc) <4 ml.

Table 2. Age of women

	HPV + (%)	HPV −	HPV n.v.	Total
<35 years	135 (20.9)	484	26	645
>35 years	106 (40.2)	150	8	264
Total	241 (26.5)	634	34	909

Table 3. Histological control at colposcopy according to HPV status (156 cases)

	Negative	CIN 1 +	CIN 2 +	Total
HPV +	46	31	32	109
HPV −	40	5	2	47
Total	86	36	34	156

Table 4. Histological control at colposcopy according to HPV status and Bethesda 2001 classification (107 cases)

	Negative	CIN 1 +	CIN 2 +	Total
ASC-US				
HPV +	30	15	23	68
HPV −	20	2	1	23
Subtotal	50	17	24	91
ASC-H				
HPV +	4	3	3	10
HPV −	6	–	–	6
Subtotal	10	3	3	16

Table 5. Specificity, sensitivity and predictive value for CIN2+ cases at biopsy control

	Specificity	Sensitivity	PPV	PNV
156 cases*	0.941	0.369	0.3	0.957
91 ASC-US	0.958	0.328	0.338	0.956
16 ASC-H	1	0.462	0.3	1
107 cases**	0.963	0.35	0.333	0.966

PPV, predictive positive value; PNV, predictive negative value.

*All 156 with histological control.

**Only cases classified according to Bethesda 2001.

Table 6. Likelihood ratio and prevalence of disease defined as presence of CIN2 +

	Positive likelihood ratio	Negative likelihood ratio	Test pos rate CIN2 +	Prevalence of CIN 2 +
156 cases*	1.49	0.16	0.675	0.217
91 ASC-US	1.55	0.06	0.747	0.264
16 ASC-H	2.63	–	0.381	0.188
107 cases**	1.48	0.11	0.729	0.252

The positive likelihood ratio [PLR sensitivity/(1−specificity)] and the negative likelihood ratio (NLR_ [1 − sensitivity]/ specificity) express the likelihood of the presence of CIN2 versus the absence of CIN2 when tests are positive or negative respectively. The positive likelihood ratio should be greater than unity and as large as possible, whereas the negative likelihood ratio should be less than unity and tend towards zero.

*All 156 with histological control.

**Only cases classified according to Bethesda 2001.

HPV triage of women with atypical squamous cells of undetermined significance: a 3-year experience in an Italian organized programme

P. D. Palma, A. Pojer and S. Girlando
Anatomia ed Istologia Patologica, Ospedale S. Chiara, Trento, Italy

Accepted for publication xx xxx 200x

Cytopathology

Hybrid capture II assay on liquid-based cytology improves the accuracy (higher sensitivity, similar specificity) than the repeat conventional Pap smear in smears with atypical squamous cells (ASC) of undetermined significance diagnosis. Human papillomavirus (HPV) testing could be used to manage women, especially the older ones, with cervical abnormalities detected through a triennial organized screening avoiding unnecessary colposcopy and excessive follow-up if the woman is HPV-negative. In our region the HPV DNA triage is offered without any charge to women with ASC since October 2000 and our accuracy, with a specificity of 94%, a sensitivity of 37% and a positive predictive value for CIN2+ lesions of 30%, is comparable with a recent meta-analysis, confirming the promising approach to the treatment of these patients even in terms of health technology assessment.

ASC-US e HPV

Le questioni più importanti sono:

- **Quali sono le linee guida per il test HPV?**
- **Chi deve farlo?**
- **Qual'è il turnaround time accettabile?**
- **Chi deve sostenere i costi?**
- **Il test deve essere fatto in modo automatico o va richiesto con qualche criterio?**

Archives of Pathology and Laboratory Medicine: **Vol. 128, No. 11, pp. 1224–1229.**

Bethesda 2001 Implementation and Reporting Rates: 2003 Practices of Participants in the College of American Pathologists Interlaboratory Comparison Program in Cervicovaginal Cytology

Diane D. Davey, MD,[a] Margaret H. Neal, MD,[a] David C. Wilbur, MD,[a] Terence J. Colgan, MD,[a] Patricia E. Styer, PhD,[a] and Dina R. Mody, MD[a]

[a]*From the Department of Pathology and Laboratory Medicine, University of Kentucky, Lexington (Dr Davey); KWB Pathology Associates, Tallahassee, Fla (Dr Neal); Department of Pathology, Massachusetts General Hospital, Boston (Dr Wilbur); Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, Toronto, Ontario (Dr Colgan); College of American Pathologists, Northfield, Ill (Dr Styer); and Department of Pathology, Baylor College of Medicine, Houston, Tex (Dr Mody)*

Accepted July 19, 2004

Human Papillomavirus (HPV) Testing Practices

	No. (%) of Laboratories
Is HPV testing performed?*	
Testing not offered	150 (20.1)
Testing done in cytology laboratory	35 (4.7)
Testing done in another section	97 (13.0)
Testing referred to reference laboratory	463 (62.2)
HPV testing method†	
Digene Hybrid Capture on liquid vial	493 (85.9)
Digene Hybrid Capture on separate specimen	123 (21.4)
Polymerase chain reaction	31 (5.4)
In situ hybridization	33 (5.7)
Other	10 (1.7)

* Seven hundred forty-five laboratories answered the first part of the question.

† Multiple answers were possible, and percentage was calculated based on number of laboratories that provided an answer (n = 574).

The Bethesda System 2001 recommends an integrated report, rather than separate reports.

Probabilistic Model.—This model is less labor intensive and consists of the result plus a probabilistic statement of an underlying carcinoma intraepithelial neoplasia (CIN) 2 or 3.

•Interpretation: ASCUS with detection of high-risk oncogenic HPV DNA.

Note.—These findings are associated with a 10% to 20% risk of underlying CIN 2 or higher lesion.[1]

Interpretive Model.—This model requires multiple steps and reevaluations before a final interpretation is issued that reflects both cytomorphology and the HPV status.

•Interpretation: LSIL.

Note.—The preliminary cytologic interpretation is ASCUS. High-risk oncogenic DNA has been detected. In combination, these results are most consistent with a diagnosis of LSIL.

The final report will include a revised interpretation with the HPV results. This method is more labor intensive but provides a polished report, which some clinicians prefer.

Percent of Atypical Squamous Cells of Undetermined Significance Cases Testing Positive for High-Risk Human Papillomavirus (HPV) DNA

HPV Positive, %	No. (%) of Laboratories	Median ASC/SIL+ (n*)
<10	64 (9.7)	1.92 (29)
10–24	45 (6.8)	1.36 (33)
25–40	99 (15.0)	1.53 (71)
41–60	112 (16.9)	1.63 (89)
>60	30 (4.5)	1.16 (21)
Unknown	312 (47.1)	Not applicable
Total No. of Laboratories	**662**	**243**

* n indicates number of laboratories with known data for both percent HPV positive and atypical squamous cells/squamous intraepithelial lesions and carcinomas (ASC/SIL+) ratios.

- **Of those laboratories that know their HPV-positive ASC-US rates, the most common response was 41% to 60%, similar to results obtained in the ALTS study. Monitoring this rate is considered a useful quality improvement parameter. Very high HPV-positive ASC-US rates may indicate a tendency to underdiagnose ASC-US cases, which could decrease the sensitivity of cytology screening, or a tendency to classify SIL cases as ASC-US. Low rates of HPV-positive ASC-US may indicate overuse of the ASC-US category. Comparison of HPV-positive ASC-US rates with ASC/SIL ratios suggested some trends, in that laboratories with higher HPV-positive rates tended to have lower ASC/SIL ratios.**

- **There is no single correct ASC/SIL+ ratio, and this quality improvement monitor may vary somewhat according patient population and among individual cytologists. A laboratory with well-screened or older women may have fewer SIL and more borderline cases, and therefore higher ASC/SIL+ ratios. In contrast, laboratories with referred or high-risk patients may have more definitive SIL cases and show lower ASC/SIL+ ratios.**

ASC-US e HPV

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Costi Americani

The reimbursement issues that impeded conversion to liquid cytology will not affect HPV testing, because the *Current Procedural Terminology* (*CPT*) code for HPV testing has existed for years (*CPT* code 88621, Medicare **$48.50**). HPV testing has some regional variations in reimbursement coverage, but individual payer rates should be established (average reimbursement **$41.74**).

It will cost between **$40** and **$50** per test for established in-house testing, and **$50** to **$60** per test to send out to a reference laboratory.

Costi Italiani

?

Trento, 6 aprile 2000

All'ASSESSORATO ALLE POLITICHE SOCIALI E ALLA SALUTE

Oggetto: modifica programma di screening Pap test ed inserimento ulteriori prestazioni di assistenza specialistica ambulatoriale

1) Utilizzo dei preparati in Strato sottile e tipizzazione virale: L'introduzione del sistema di valutazione dei pap test in Strato Sottile automatico (cod. 91.38.9) migliora la qualità della diagnosi e riduce la percentuale di inadeguati.
Tale metodica verrà utilizzata in alternativa all'esame citologico cervico vaginale (cod. 91.38.5) in un "triage" cioè nel gruppo selezionato di donne che abbiano già avuto un pap test inadeguato o nel follow-up di quelle con precedente pap test borderline. In quest'ultimo gruppo di pazienti con lo stesso materiale,può essere effettuata la ricerca e la tipizzazione del virus HPV ad alto e medio rischio oncogeno per personalizzare il trattamento successivo.

PAT Del. 2045 del 29/9/2000

Cod. 91.48.4	Prelievo citologico
Cod. 91.38.5	Esame citologico
Cod. 91.38.9	Esame citologico su preparazione automatica in strato sottile
Cod. 67.19.1	Biopsia mirata della portio a guida colposcopica
Cod. 70.21	Colposcopia
Cod. 89.01	Anamnesi e valutazione definite brevi
Cod. 91.11.5	Virus acidi nucleici in materiali biologici, PCR
Cod. 91.36.1	Conservazione di campioni di DNA o di RNA
Cod. 91.36.4	Digestione di DNA con enzimi di restrizione
Cod. 91.36.5	Estrazione di DNA o di RNA (nucleare o mitocondriale)
Cod. 91.37.1	Ibridazione con sonda molecolare
Cod. 91.44.4	Esame istocitopatologico apparato urogenitale:biopsia cervice utero
Cod. 91.45.5	Esame istocitopatologico apparato urogenitale:biopsia vaginale
Cod. 91.46.3	Esame istocitopatologico apparato urogenitale:biopsie cervicali multiple.

Tariffe e Tempo

- **PCR**
  - **Se negativo: (91.36.5 + 91.36.1 +91.11.5) =149,50**
  - **Se positivo: (+ 91.36.4 x 2) = 84,70**
  - **Se neg. Due giorni;**
  - **Se pos. Tre giorni.**

- **HC2 (solo sonda B)**
  - **Positivo o Negativo (91.37.1) = 81,60**
  - **Tempo 6/8 ore**

ASC-US e HPV

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Costo/Beneficio

- **Ogni Kit per HC2 HPV ad alto rischio sonda B contiene 96 pozzetti ma ogni volta debbono essere messi 6 calibratori tre positivi e negativi.**
- **Quindi l'ottimizzazione si ha per 90 tests.**
- **Antiecomico eseguire meno di 26 test.**
- **Quanto si può far attendere una donna per una risposta?**

Representation of a Digene Hybrid Capture 2 Human Papillomavirus Kit Test Plate

Kit Rows	96 Wells								Total No. of Patient Tests
1	c	c	c	c	c	c	p	p	2
2	p	p	p	p	p	p	p	p	10
3	p	p	p	p	p	p	p	p	18
4	p	p	p	p	p	p	p	p	26
5	p	p	p	p	p	p	p	p	34
6	p	p	p	p	p	p	p	p	42
7	p	p	p	p	p	p	p	p	50
8	p	p	p	p	p	p	p	p	58
9	p	p	p	p	p	p	p	p	66
10	p	p	p	p	p	p	p	p	74
11	p	p	p	p	p	p	p	p	82
12	p	p	p	p	p	p	p	p	90

* Prepared by Richard Lozano to illustrate the minimum number of controls and the number of patient samples required to complete a row. c indicates control; p, patient.

Conclusioni 1

- **Il triage HPV nei casi con prima diagnosi di ASCUS è utile perché seleziona maggiormente i casi CIN2 + alla colposcopia.**
- **Il test non è utile per i LSIL.**
- **Lo studio dimostra come i casi ASC-H sono in realtà una categoria a parte (mai CIN dopo HPV-) e pertanto non beneficiano del test.**
- **Il test ha bassa specificità: troppi casi ASCUS e HC2 + sono < di un CIN 2 + (FP).**
- **Esistono (pochissimi!) FN (2 su 157 casi) → altri tipi di HPV? Insuffciente sensibilità del test? Guarigione dell'infezione? Lesioni non dovute al virus HPV?**

Conclusione 2

Have We Resolved How To Triage Equivocal Cervical Cytology?

Diane Solomon, Mark Schiffman

Additional diagnostics research should evaluate combinations of cytology and HPV testing for screening the general population. HPV infection is highly prevalent, but only persistent infections with oncogenic HPV types pose a risk of neoplastic progression. Strategies that focus on identifying HPV persistence rather than prevalent infection may provide greater specificity without compromising sensitivity. Eventually, we hope to identify and validate markers of cancer risk that are even more accurate than either cytology or HPV DNA testing.