MARIA DONATELLA BECCATI, MD

DIAGNOSTICA CITOPATOLOGICA
Dipartimento Patologia e Oncologia

AZIENDA OSPEDALIERO-UNIVERSITARIA
ARCISPEDALE S.ANNA
FERRARA

donatella.beccati@unife.it

Sviluppo della citologia cervicale a Ferrara

TECNOLOGIA	CONTESTO	ANNO
Strato sottile ThinPrep® Cytyc	Clinico	1997
HPV ISH INFORM® I-II Ventana	Clinico	1998
Marcatori biopatologici di cancerogenesi	Translazionale	2002
Strato sottile SurePath® TriPath	Screening	2003
Prescreening FocalPoint™ GS TriPath	Screening	2004
PreTect HPV-Proofer® NorChip	Translazionale	2005
Test hrHPV HCII® Digene	Screening e clinico	2006
ProExC® TriPath Oncology	Translazionale	2006

Azienda Ospedaliera Reggio Emilia
Azienda USL Reggio Emilia

in collaborazione con
Regione Emilia-Romagna - Assessorato Politiche per la salute

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA

CORSO DI FORMAZIONE
PER IL PERSONALE SANITARIO
ADDETTO AL PROGRAMMA
DI SCREENING DEI TUMORI DEL
COLLO DELL'UTERO

Reggio Emilia, 16 – 17 ottobre 2006
Palazzo Rocca Saporiti

LE NOVITA' TECNOLOGICHE NELLA REGIONE EMILIA-ROMAGNA

La lettura automatica

MARIA DONATELLA BECCATI, MD

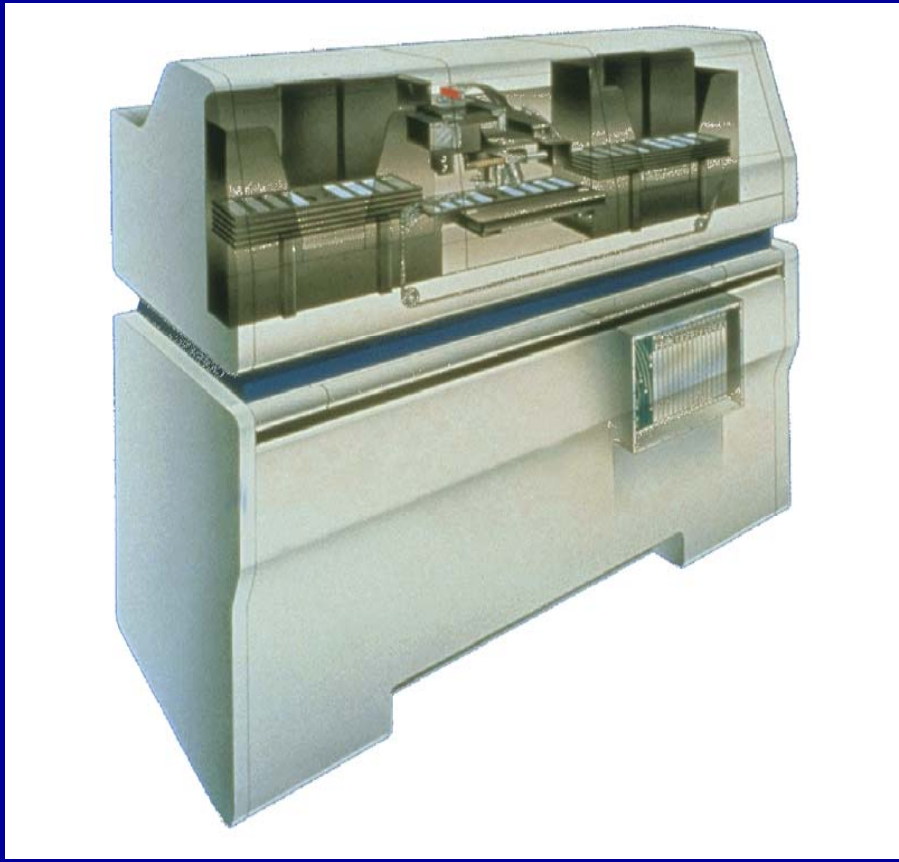
DIAGNOSTICA CITOPATOLOGICA

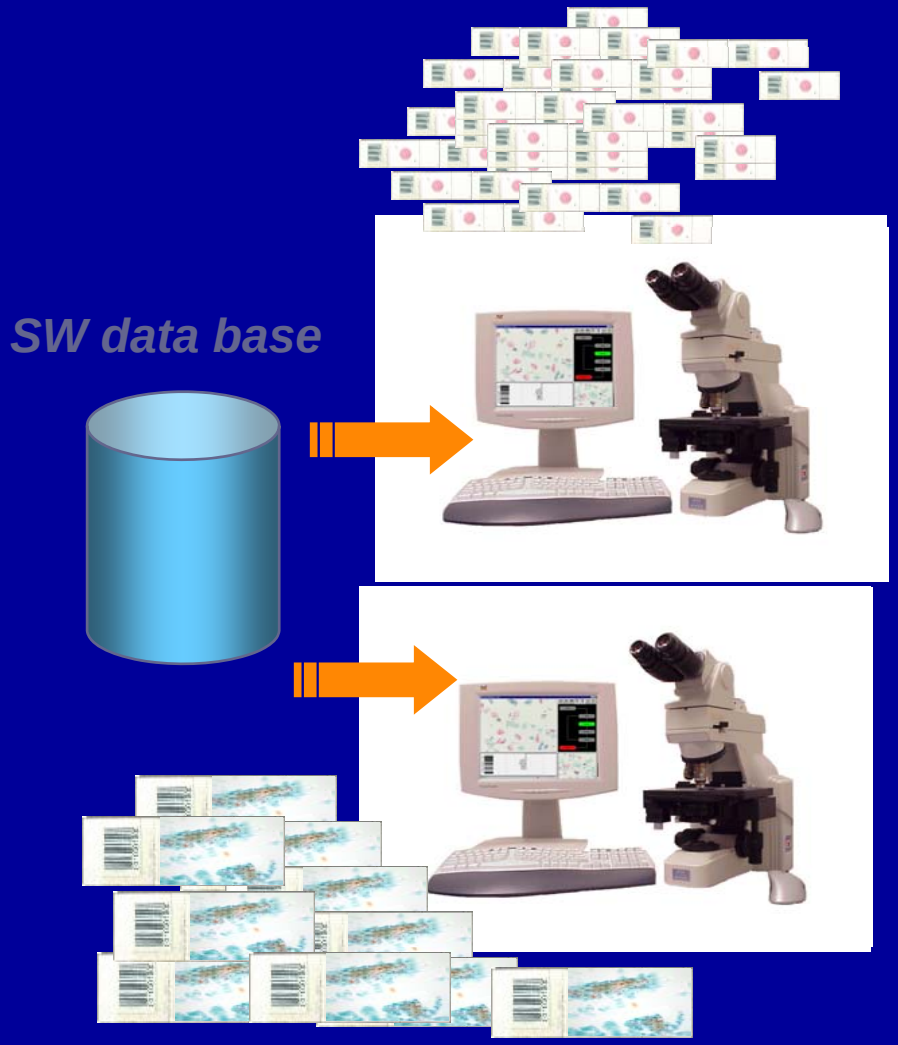
AZIENDA OSPEDALIERO-UNIVERSITARIA

ARCISPEDALE S.ANNA

FERRARA

FocalPoint GS





PARAMETRI

Target: anomalie delle cellule epiteliali squamose
Soglia ASC+

- Distribuzione delle categorie diagnostiche
- Tasso di invio ad accertamento
- Tasso di biopsia nei casi inviati ad accertamento
- **Valori predittivi positivi della citologia**
- **Valori predittivi positivi del FocalPoint**
- **Tempi di refertazione**
- **Giudizio dei Professionisti impegnati nella diagnostica**
- **Tasso di lesioni individuate**

VALORI PREDITTIVI POSITIVI CIN1+
Pap test 44.609
Correlazione cito-istologica casi 815

	Strato Sottile		*Convenzionale*	
VPP	**Computer assistita**	**Tradizionale**	**Computer assistita**	**Tradizionale**
Validazione	**80,8**	**73,2**	**81,8**	**73,5**
Monitoraggio	**87,5**	**85,0**	**-**	**-**

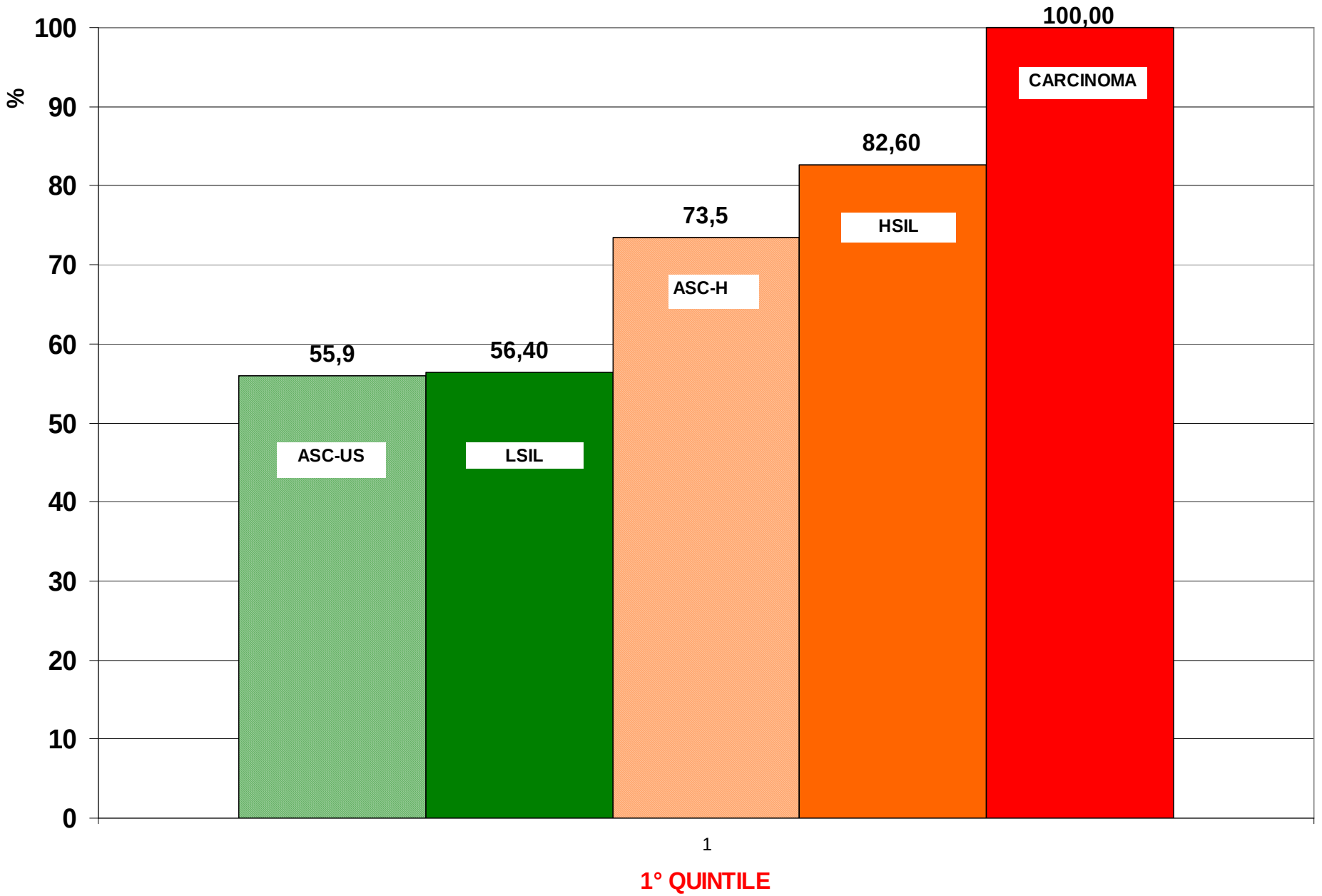
Strato Sottile SurePath
Valori Predittivi Posititvi del
Prescreening assistito da FocalPoint GS

	CIN1/LSIL+	CIN2+	CIN3
Tutte le diagnosi	87.47	26.22	14.39
ASC-US	79.75	8.72	4.65
ASC-H	93.54	38.71	19.35
LSIL	89.87	17.72	4.63
HSIL	98.57	82.85	58.57

FocalPoint-GS

ANALISI DEI QUINTILI

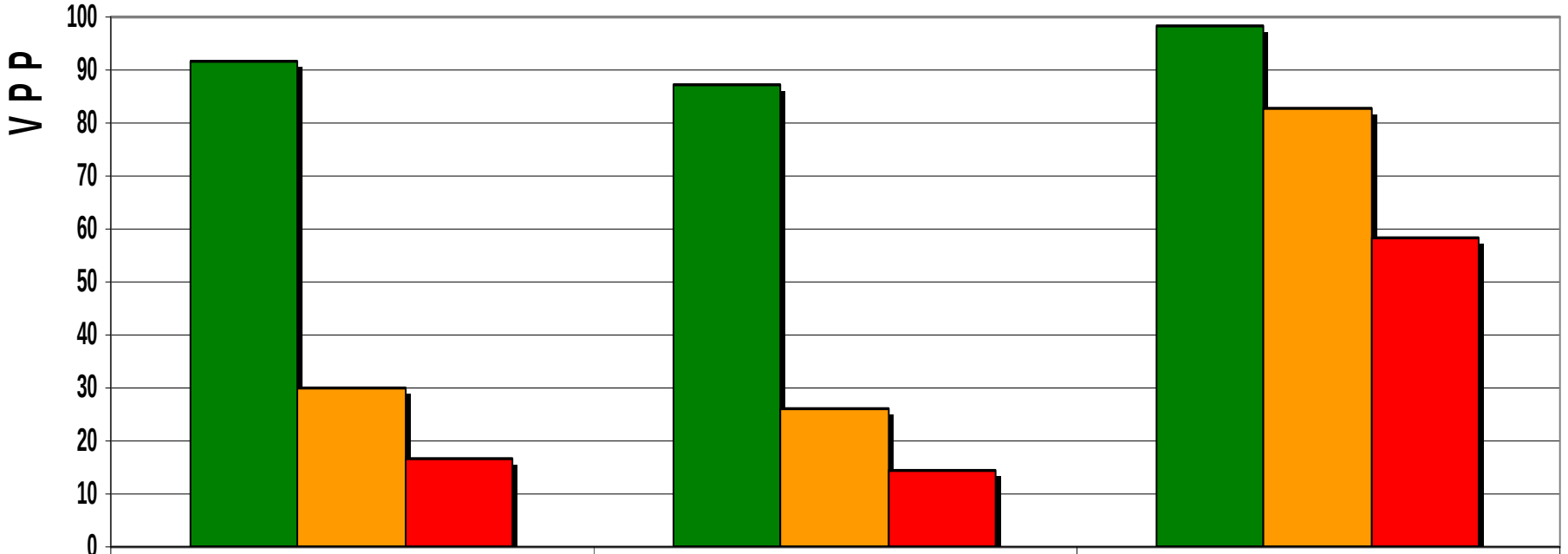
Casistica	**489 casi con istologia**
Metodo	**correlazione FP/istologia**


%
100
90
80
70
60
50
40
30
20
10
0
55,9
56,40
73,5
82,60
100,00
ASC-US
LSIL
ASC-H
HSIL
CARCINOMA
1
1° QUINTILE

PREDITTIVITA' DEI QUINTILI

Quintili	CIN1/LSIL+	CIN2+	CIN3
1°	91.53	30.00	17.00
2°	79.16	27.8	11.00
3°	84.21	10.5	5.30
4°	82.35	23.5	18.00
5°	80.00	4.00	0

CONFRONTO TRA VALORE PREDITTIVO POSITIVO DEL 1° QUINTILE E DELLA CITOLOGIA



	1° QUINTILE	ASC+	HSIL
CIN1+	91,53	87,47	98,57
CIN2+	30,00	26,22	82,85
CIN3+	16,53	14,39	58,57

TEMPI DI REFERTAZIONE

Osservato Prescreening FocalPointGS	**5.82 gg**
Atteso lettura tradizionale	**7.77 gg**
O/E	**0,75**

Tasso di lesioni individuate
Detection Rate

N. Pap-test refertati	*Detection Rate*
Lettura Focal Point	4,3 ‰
Lettura Tradizionale	3,1 ‰
O/E	1.38

GIUDIZIO DEI PROFESSIONISTI

- Da *locator* a *interpreter*
- Breve addestramento pertinente
- Approccio attivo/interattivo
- Diminuzione dei carichi di lavoro
- Aumento dell’attenzione in tempo ridotto

Azienda Ospedaliera Reggio Emilia
Azienda USL Reggio Emilia

in collaborazione con
Regione Emilia-Romagna - Assessorato Politiche per la salute

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA

CORSO DI FORMAZIONE
PER IL PERSONALE SANITARIO
ADDETTO AL PROGRAMMA
DI SCREENING DEI TUMORI DEL
COLLO DELL'UTERO

Reggio Emilia, 16 – 17 ottobre 2006
Palazzo Rocca Saporiti

LE NOVITA' TECNOLOGICHE NELLA REGIONE EMILIA-ROMAGNA

Nuove metodiche diagnostiche molecolari

MARIA DONATELLA BECCATI, MD

DIAGNOSTICA CITOPATOLOGICA

AZIENDA OSPEDALIERO-UNIVERSITARIA

ARCISPEDALE S.ANNA

FERRARA

DIGENE® Italia

Hybrid Capture II

Tipi di HPV a rischio alto/intermedio
16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68

Significato biologico della positività

INFEZIONE

IL TEST HPV IN EMILIA-ROMAGNA

Città	Struttura	Denominazione	Indirizzo	Telefono
Bologna	Ospedale	Ospedale Maggiore	L.go Nigrisoli, 2	051 41 64 85 3
Bologna	Ospedale	Ospedale S.Orsola Malpighi	Via G. Massarenti, 13	051 63 64 36 4 Lun-Ven 08.30-12.00
Cesena	Ospedale	Osp. M. Bufalini U.O. Anat.Pat. e Citologia/Centro Screening Oncologici	V.le Ghirotti, 286	0547 352 737
Ferrara	Ospedale	Arcispedale S. Anna	C.so Giovecca 203	Lab.Citopatologia 0532 237 092 Segr.Anat.Patologica 0532 291 502
Piacenza	Ospedale	Ospedale Guglielmo da Saliceto Segreteria Laboratorio Centrale	Via Taverna, 49	0523 303816 (lun-ven ore 10-13)
Reggio Emilia	Ospedale	Arcispedale Santa Maria Nuova Centro Citologia Vaginale	V.le Risorgimento, 80	0522 29 62 42 (lun-ven ore 9-13)

IL TEST HPV A FERRARA

- **INIZIO 01 GENNAIO 2006**
- **TRIAGE ASC-US**
- **PROGRAMMA DI SCREENING E CLINICA**
- **INDICAZIONE DEL PATOLOGO**
- ***REFLEX TEST* SU MATERIALE RESIDUO STRATO SOTTILE SUREPATH**
- **REFERTO CONTESTUALE AL PAP TEST**
- **COMUNICAZIONE FILTRATA ALLA PAZIENTE**

RISULTATI PRELIMINARI

VALORE PREDITTIVO POSITIVO CIN2+ DI ASC-US

periodo 1 gennaio - 31 maggio 2006

	N.	%	Biopsia	%	VPP CIN2+
ASC-US	150				
hr-HPV Pos	*59*	*39,33*	*31*	*52,54*	*31,03*
hr-HPV Neg	*91*	*60,67*	*18*	*19,78*	*0*

Vol. 98, No. 11 > Ronco et al., pp. 765-774. Journal of the National Cancer Institute, Vol. 98, No. 11, 765-774, June 7, 2006

Human papillomavirus testing and liquid-based cytology: results at recruitment from the new technologies for cervical cancer randomized controlled trial.

Ronco G, Segnan N, Giorgi-Rossi P, Zappa M, Casadei GP, Carozzi F, Dalla Palma P, Del Mistro A, Folicaldi S, Gillio-Tos A, Nardo G, Naldoni C, Schincaglia P, Zorzi M, Confortini M, Cuzick J; New Technologies for Cervical Cancer Working Group.

Unit of Cancer Epidemiology, Centro per la Prevenzione Oncologica Piemonte, 10123 Turin, Italy.
guglielmo.ronco@cpo.it

BACKGROUND: Although testing for human papillomavirus (HPV) has higher sensitivity and lower specificity than cytology alone for detecting cervical intraepithelial neoplasia (CIN), studies comparing conventional and liquid-based cytology have had conflicting results. METHODS: In the first phase of a two-phase multicenter randomized controlled trial, women aged 35-60 years in the conventional arm (n = 16,658) were screened using conventional cytology, and women in the experimental arm (n = 16,706) had liquid-based cytology and were tested for high-risk HPV types using the Hybrid Capture 2 assay. Women in the conventional arm were referred to colposcopy with atypical cells of undetermined significance (ASCUS) or higher and those in the experimental arm were referred with ASCUS or higher cytology or with a positive (> or = 1 pg/mL) HPV test. Sensitivity and positive predictive value (PPV) for detection of cervical intraepithelial neoplasia grade 2 or higher (CIN2+) were calculated. RESULTS: The screening methods and referral criterion applied in the experimental arm had higher sensitivity than that in the conventional arm (relative sensitivity = 1.47; 95% confidence interval [CI] = 1.03 to 2.09) but a lower PPV (relative PPV = 0.40; 95% CI = 0.23 to 0.66). With HPV testing alone at > or = 1 pg/mL and at > or = 2 pg/mL, the gain in sensitivity compared with the conventional arm remained similar (relative sensitivity = 1.43, 95% CI = 1.00 to 2.04 and relative sensitivity = 1.41, 95% CI = 0.98 to 2.01, respectively) but PPV progressively improved (relative PPV = 0.58, 95% CI = 0.33 to 0.98 and relative PPV = 0.75, 95% CI = 0.45 and 1.27, respectively). Referral based on liquid-based cytology alone did not increase sensitivity compared with conventional cytology (relative sensitivity = 1.06; 95% CI = 0.72 to 1.55) but reduced PPV (relative PPV = 0.57; 95% CI = 0.39 to 0.82). CONCLUSIONS: HPV testing alone was more sensitive than conventional cytology among women 35-60 years old. Adding liquid-based cytology improved sensitivity only marginally but increased false-positives. HPV testing using Hybrid Capture 2 with a 2 pg/mL cutoff may be more appropriate than a 1 pg/mL cutoff for primary cervical cancer screening.

“Pap Test Bio-molecolare”

- **L’infezione persistente da HPVhr hrHPV è la condizione patogenetica *“sine qua non”* per l’instaurarsi della lesione cervicale CIN3+. La positività del test hrHPV-DNA certifica questo step necessario ma non sufficiente nel percorso da infezione a sviluppo di CIN, senza fornire informazioni sulla progressione indotta dal virus, che può essere dimostrata dall’aumentata espressione delle proteine oncogene virale E6/E7.**

- **Il test hrHPV E6/E7 mRNA, il *“Pap test Bio-molecolare”*, potrebbe essere l’anello di congiunzione tra la semplice infezione e la vera trasformazione cellulare indotta dal virus.**

PreTect HPV-Proofer® assay (NorChip, Norway)

- **Identification of E6/E7 mRNA transcript from HPV 16, 18, 31, 33, and 45 was performed as *reflex test***

Results

ut of 67 samples were hrHPV E6/E7 mRNA positive, 8 (50%) HPV45, .6%) HPV16, 1 (6.2%) HPV18, 1 (6.2%) HPV 31 and 0 HPV33.

Tests	67
Positive	16 (23.9%)
Hpv 45	*8*
Hpv 16	*6*
Hpv 18	*1*
Hpv 31	*1*
Hpv 33	*0*
Negative	51 (76,1%)

***erus bicollis* Patient showed left cervix L-SIL (histology CIN1) which**

Results

The correlation between hrHPVmRNA and the first tologic diagnosis shows that 42.8% of the Patients ated for H-SIL were positive for the test, 6-42 months er the first diagnosis and treatment.

Note that the 40% of H-SIL is well known to progress wards infiltrating carcinoma

50 patients/51 samples were hrHPV mRNA negative.

Comparison between hr-HPV mRNA Positive and Negative Cases

ytology cannot demonstrate if the lesions is caused by ogenic or non-oncogenic HPVs

o difference was evident in the clinical and pathological racteristics between positive and negative Patients.

The outcome of the lesions, modified from therapy, cannot nguish the lesions with activated HPV oncogenic virus from bare HPV infection.

uch distinction could be provided by the HPVmRNA PreTect ofer positivity. In fact, only those Patient with HPV-induced

CLINICAL IMPACT

rospectively, all Patients were followed up from many years olposcopy, cytologic and/or histologic procedures (mean ber of events 4.12, range 1-17)

hrHPVmRNA test carried out at the time of the first ment could have modified the follow up schedule, i.e. to the Positive Patients at risk of progression, and to promptly sure the negative ones.

ur study the hrHPVmRNA test could have spared Patients xcess of follow up, (185 clinico-pathological events, 55% in tive Patients) .

MCM2 and TOPO2A ProExC™ in cervical pre-neoplasia

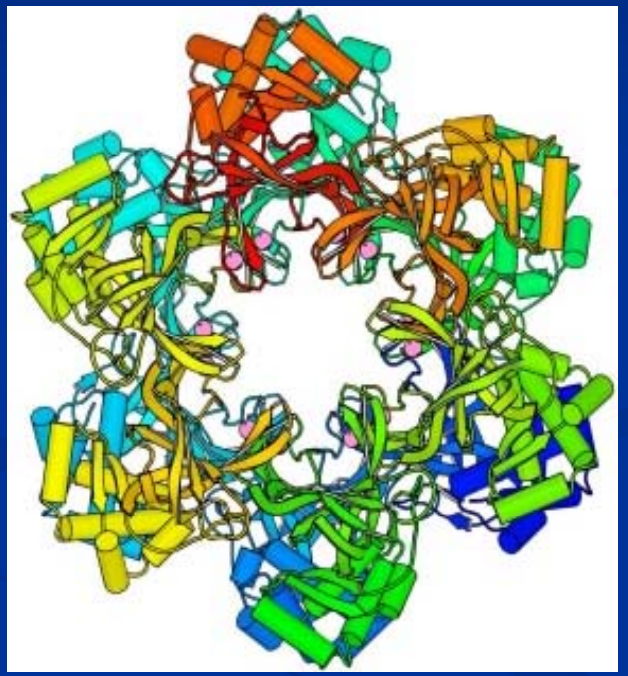
Maria Donatella Beccati, MD

Carolina Buriani, BSc

Diagnostic Cytopathology
Department of Pathology and Oncology,
S.Anna University Hospital, Ferrara,

Minichromosome Maintenance Proteins (MCM)

- MCMs 2-7 function in initiation of DNA replication during the cell cycle
- Members of the DNA licensing factor family
- Markers of cell proliferation in high grade cervical dysplasia and carcinoma



http://www-rcf.usc.edu/~forsburg/main3.html

Topoisomerase II (TOP2A)

- Modulates DNA topology
- Unknots and decatenates DNA for DNA replication, transcription, chromosome segregation, and cell cycle progression
- Regulated by Rb
- Over-expression may reflect HPV E7-mediated Rb degradation in cervical dysplasia and carcinoma

Objectives

dentify a bio-molecular profile
nformative for diagnosis of HSIL+

Translate this profile into a morphologic
Pap test adjunct
(“bio-molecular Pap test”)

Set a marker threshold for the diagnosis
nd the assessment of progression risk

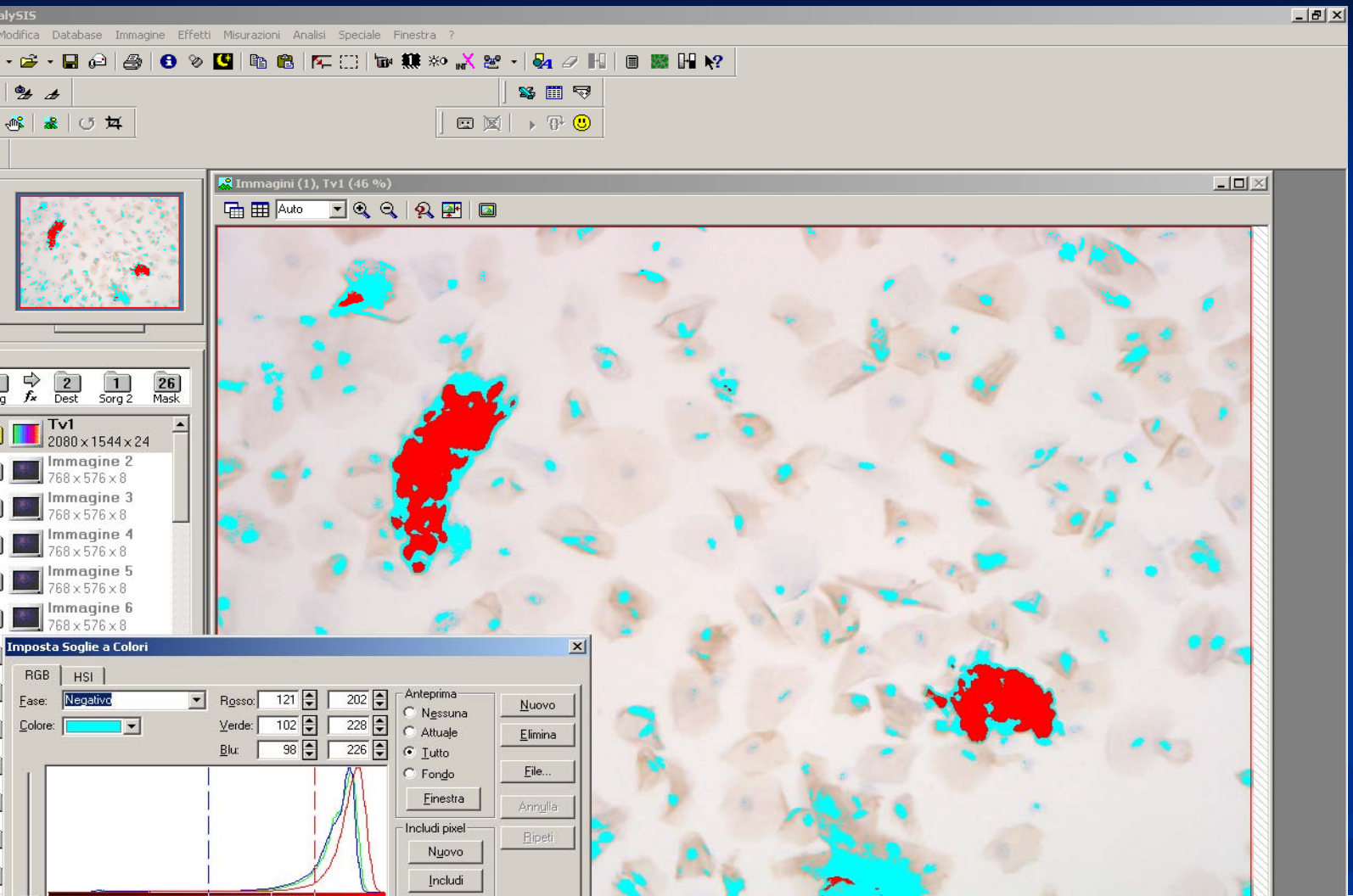
Materials and methods

76 liquid based SurePath Pap tests
- 20 NILM taken from routine samples
- 56 ASC+ selected with matching histology

Appropriate measures for antigen preservation

ProExC immunocytochemical assay performed in unstained residual specimens with Ventana Benchmark XT

Olympus AnalySIS (Soft Imaging System)



tologic Diagnosis
CUS
L
L
L
L
L
L
M
M
M
M
M
M
M
M
M
M
M
M
M
M
M
CUS
CUS
CUS
CUS
CUS
M
CUS
CUS
CUS
CH
L
CUS
CUS
L
L
CUS
L
CUS
CH
CH
CUS
CUS
CUS
M
IL
IL
CH
M
IL
CUS
IL
CH
IL
CH
CH
CH
CH
IL
CUS
IL
IL

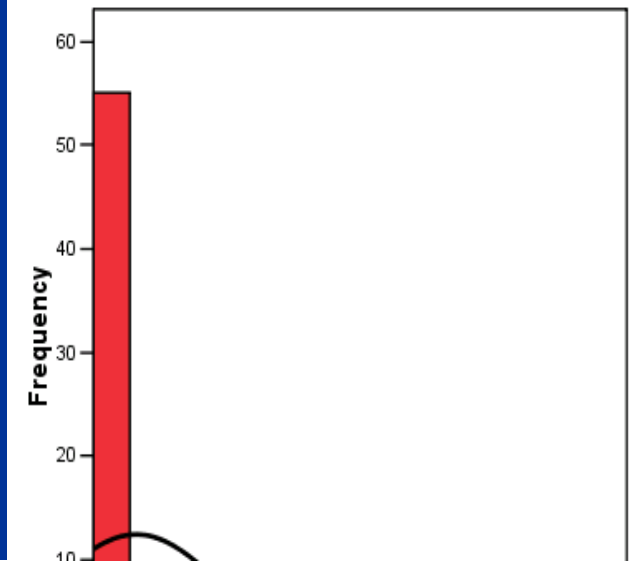
CASE STUDY: ProExC distribution

- NILM
- ASC-US
- LSIL
- ASC-H
- HSIL

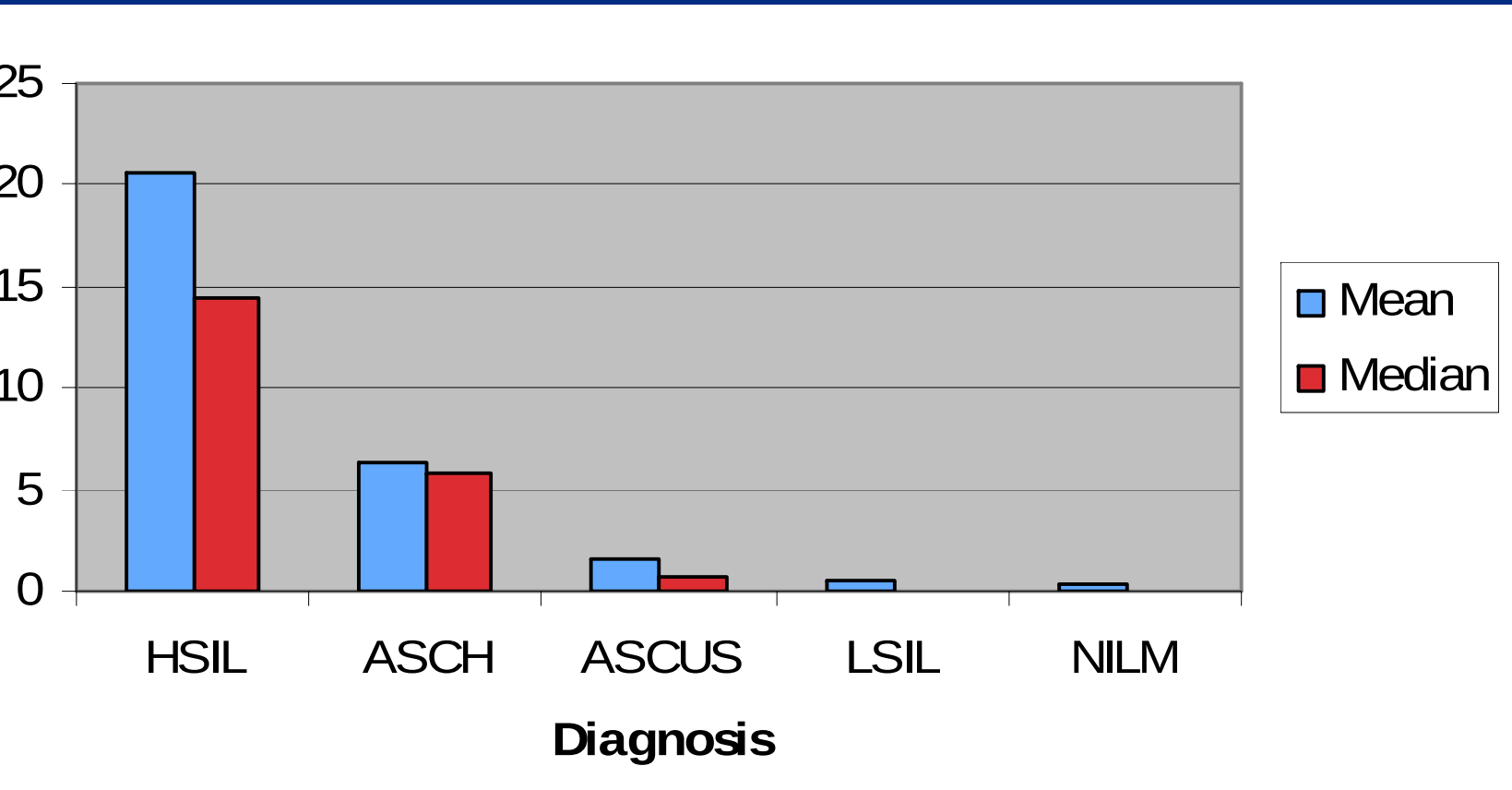
Statistics

ProExC

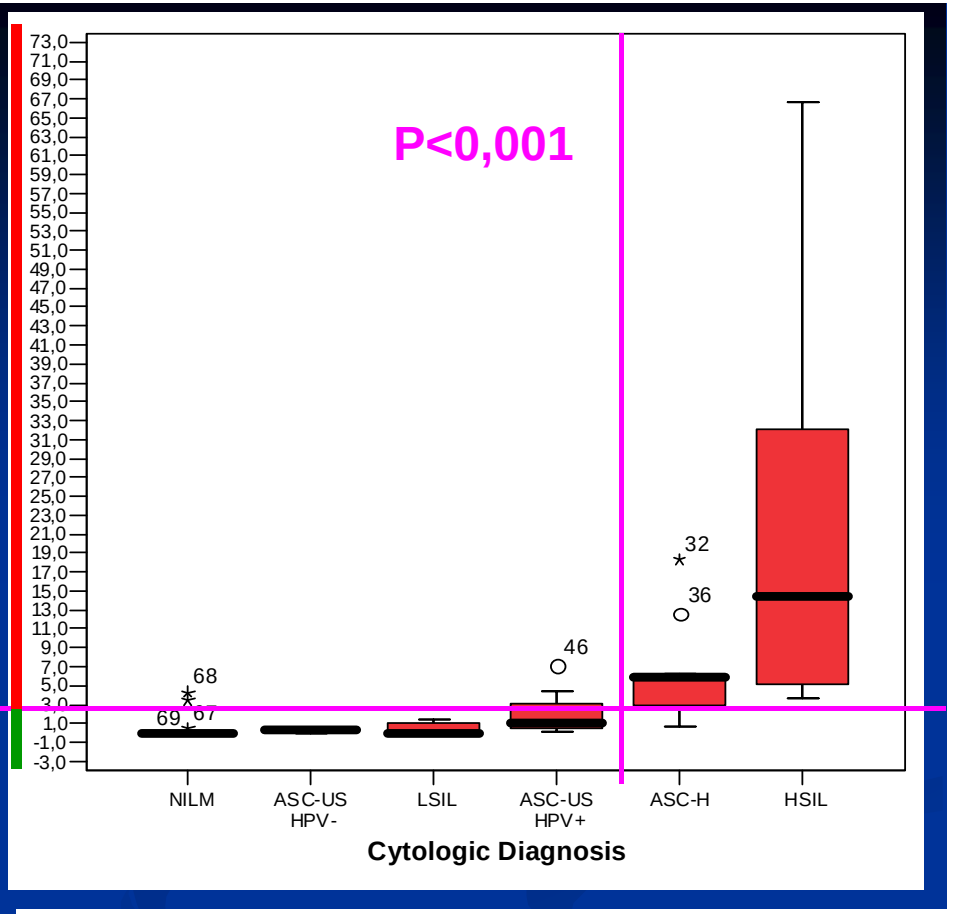
N	Valid	75
	Missing	1
Mean		5,8726
Median		1,1261
Mode		,00
Std. Deviation		12,03710
Minimum		,00
Maximum		66,60



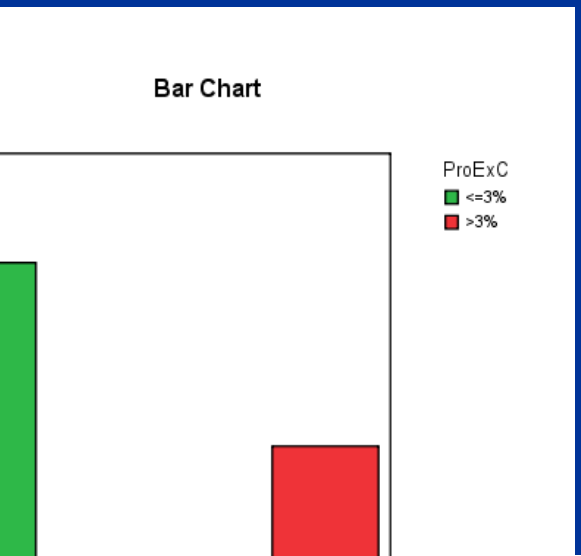
Mean and Median ProExC in cytology categories



Boxplot of ProExC distribution in cytology categories



ggested analytical threshold 3.0%

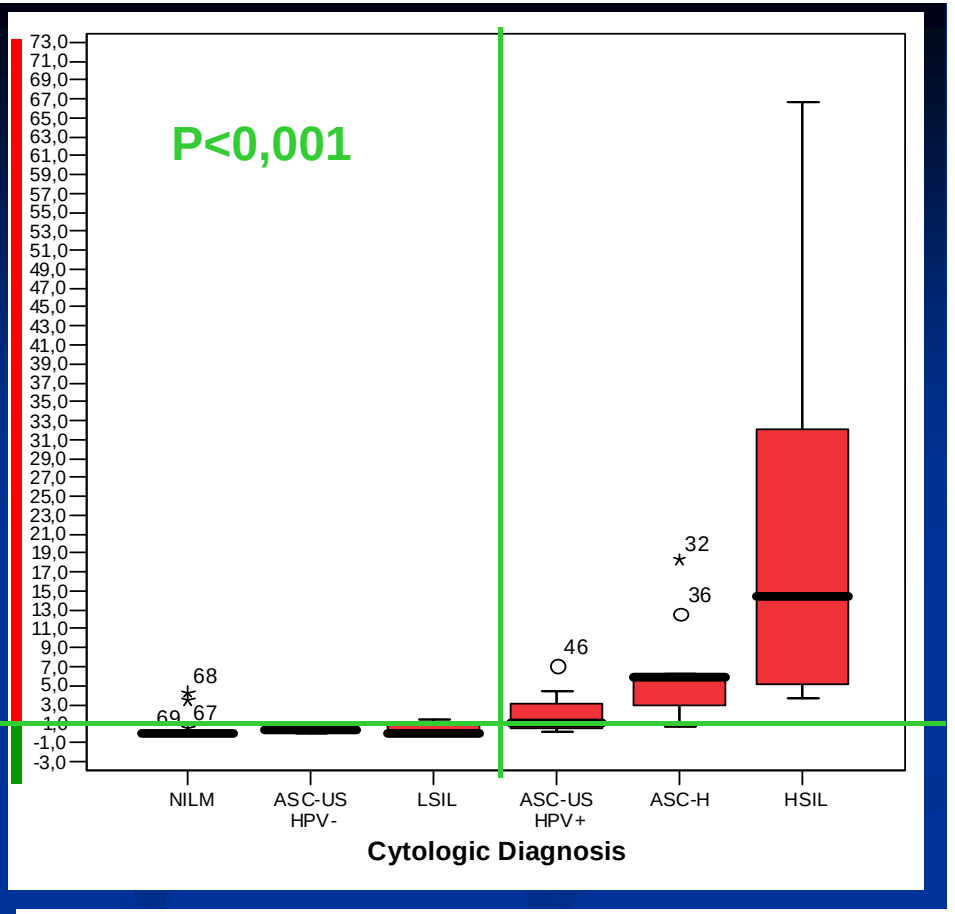


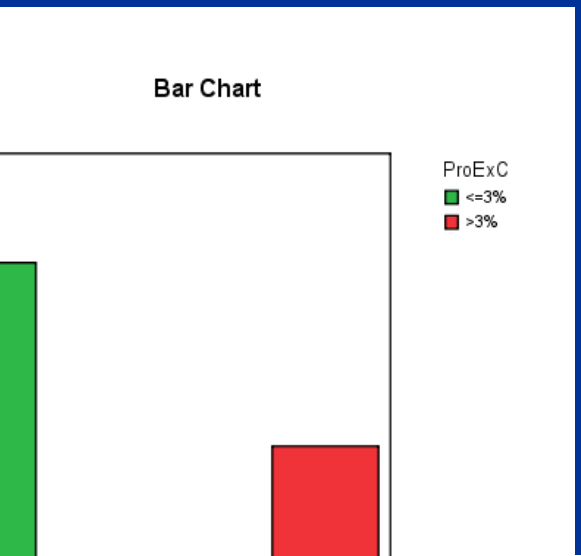
Cytologic Diagnosis * ProExC Crosstabulation

			ProExC		Total
			<=3%	>3%	
Cytologic Diagnosis	NILM/ASCUS/LSIL	Count	42	6	48
		% within Cytologic Diagnosis	87,5%	12,5%	100,0%
		% within ProExC	93,3%	20,0%	64,0%
		% of Total	56,0%	8,0%	64,0%
	ASC-H/HSIL	Count	3	24	27
		% within Cytologic Diagnosis	11,1%	88,9%	100,0%

Boxplot of ProExC distribution in cytology categories

Suggested clinical threshold 1.0%





Cytologic Diagnosis * ProExC Crosstabulation

			ProExC		Total
			<=3%	>3%	
Cytologic Diagnosis	NILM/ASCUS/LSIL	Count	42	6	48
		% within Cytologic Diagnosis	87,5%	12,5%	100,0%
		% within ProExC	93,3%	20,0%	64,0%
		% of Total	56,0%	8,0%	64,0%
	ASC-H/HSIL	Count	3	24	27
		% within Cytologic Diagnosis	11,1%	88,9%	100,0%

Conclusions

roExC detects aberrant S-phase in LBC

roExC ICC is a valid adjunt to
norphologic Pap for progression risk
dentification

modified FocalPointGS may integrate
ne bio-molecular Pap test interpretation.

Conclusions

- threshold set at 3.0% ProExC of positive uclei clearly splits NILM/ASC-US/LSIL rom ASC-H/HSIL
- threshold set at 1.0% ProExC of positive uclei clearly splits NILM/LSIL/ASC-US IPV hr Negative from ASC-US HPV hr ositive/ASC-H/HSIL

Riassumendo….

escreening automatico con FocalPointGS

Efficace, efficiente

PV alto rischio

pecifico, promettente come futuro test di screening

RNA E6/E7

nfezione attiva con HPV oncogeni

oExC

Informativo di permanente attivazione di fase S


FINE

Introduction and Objectives

V persistent infection is the *"sine qua non"* pathogenetic condition for - cervical lesions. The hrHPV-DNA test positivity testifies for this ssary but not sufficient step along the pathway from infection to CIN opment, without informing about the viral-induced progression, which be shown by increased expression of E6/E7 viral oncogenic proteins.

V E6/E7 mRNA test, a new *"Biomolecular Pap test"*, could fill the gap een the bare HPV infection and the true viral-induced cell formation.

f the study is to investigate the significance and clinical implications of viral oncogenic mRNA expression, for specific risk assessment and nts monitoring.

ACKNOWLEDGMENTS

Prof. Italo Nenci, MD, Director of Department of Pathology and Oncology, for stimulating discussion.

Dr. Massimo Pedriali, MD, for statistical analysis, informatic expertise and enthusiasm

Mrs. Rossella Parolini, TSLB, for technical assistance and untired help

Dr Cristina Zampini, TSLB, for technical assistance

Mr. Larry Costache, for friendly help and assistance

LIDE WIZARD REVIEW STATION

-conventional PC with Windows based operating system

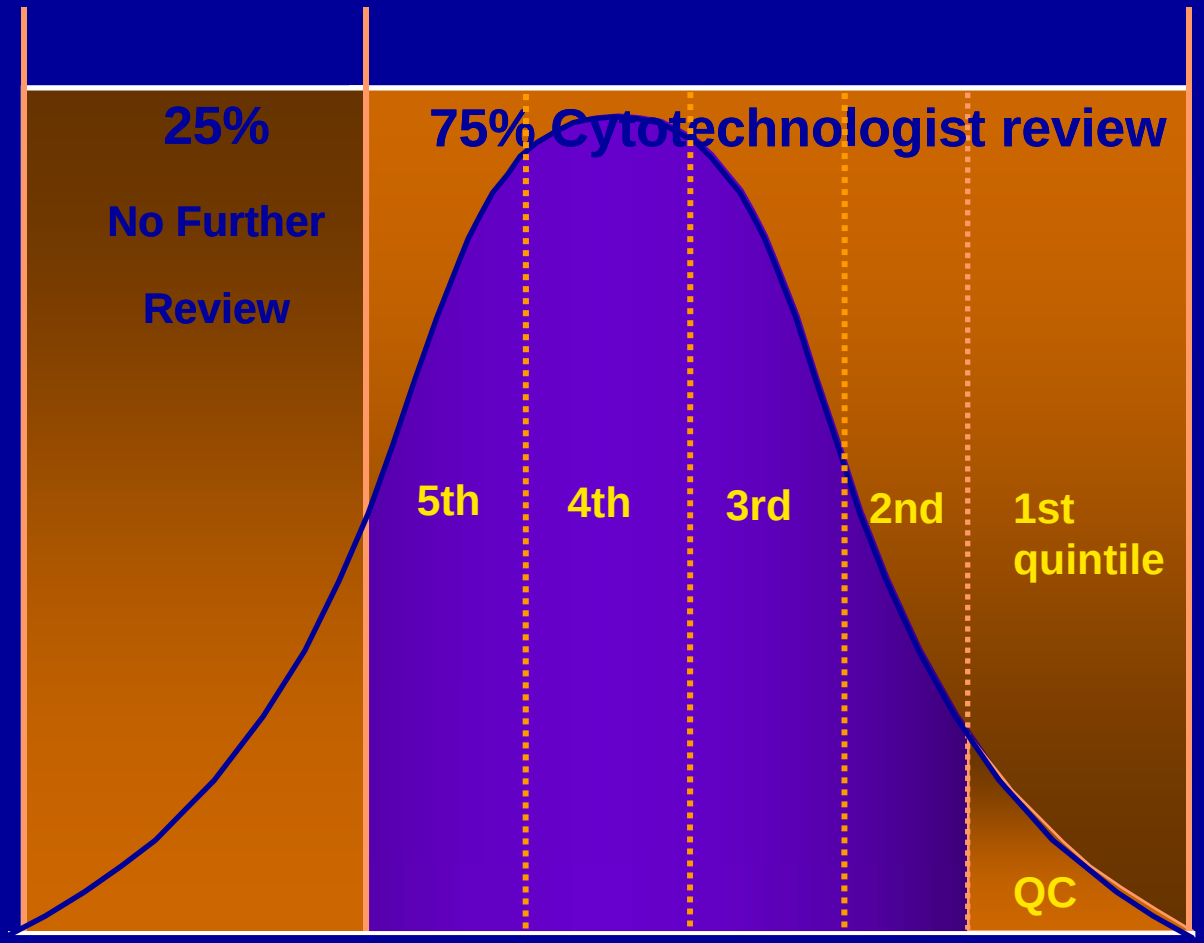
-barcode reader

-foot switch

-standard off the shelf microscope

-specific stage

FocalPoint™ Slide Scoring



TEMPI DI REFERTAZIONE

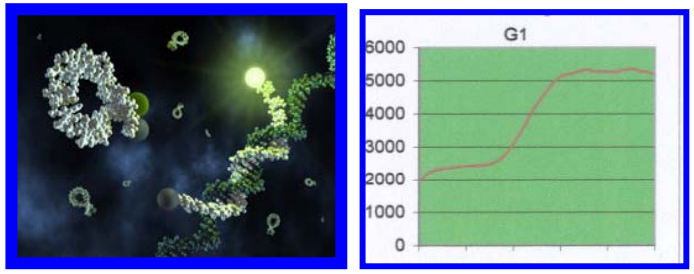
Prescreening con FocalPoint GS: 5.82 gg

Tempo Atteso: 7.77 gg

O/E 0.75

Methods

ntification of E6/E7 mRNA transcripts from HPV 16, 18, 31, 33, and
was performed as *reflex test* by means of the PreTect HPV-
oofer® assay (NorChip, Norway), a real time NASBA technique



Patients

ients/67 ThinPrep® samples ASC+ Pap Test (1 *uterus bicollis* Patient,
parated samples, and 1 Patient with two split endocervical and
rvical samples)

VALIDAZIONE E MONITORAGGIO

PROGRAMMA FERRARA SCREENING

Confronto tra analisi “tradizionale” ed analisi con il supporto del Lettore automatico FocalPointGS

Materiale

- Striscio convenzionale

- Strato sottile SurePath™

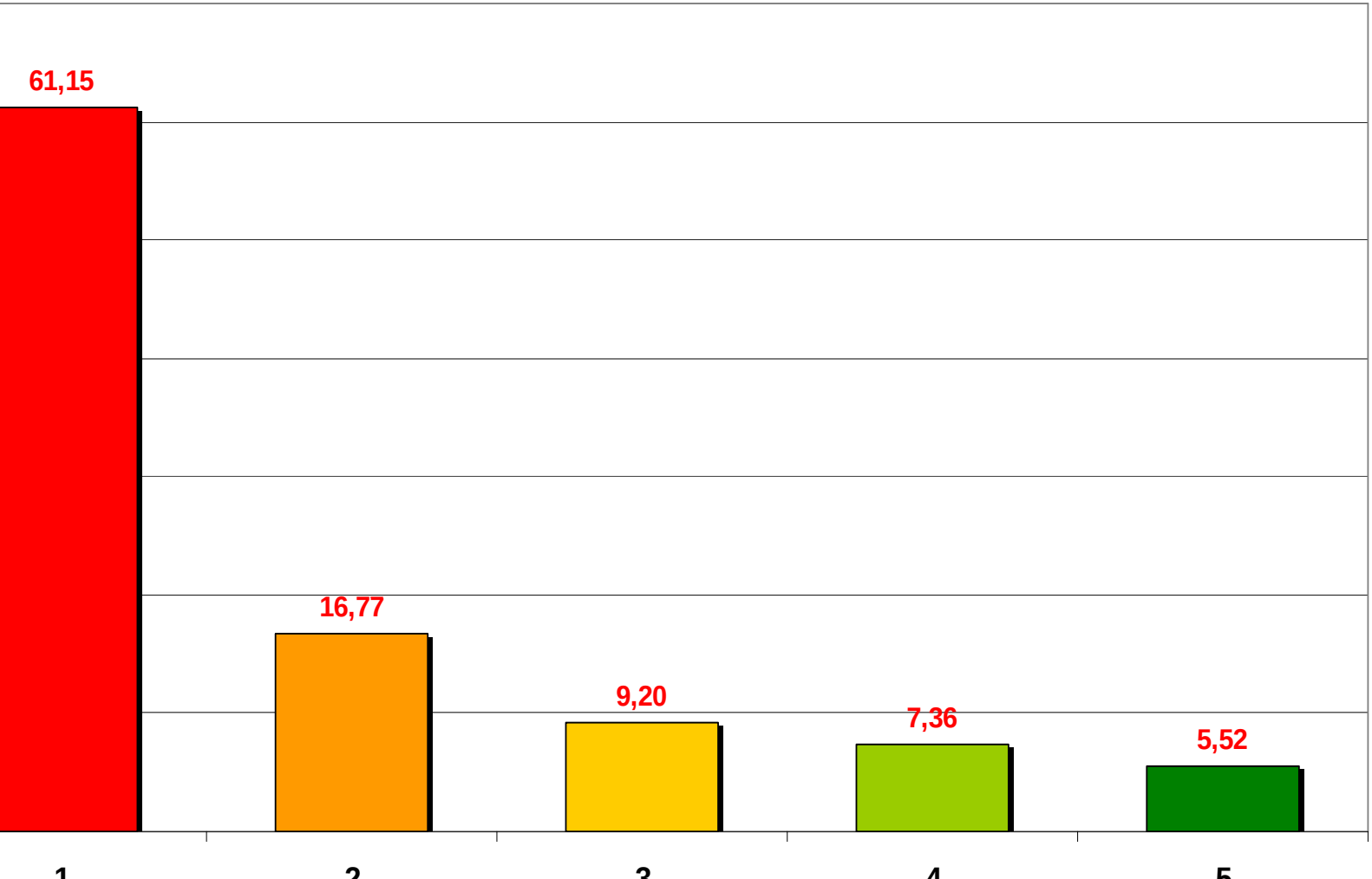
Metodo

correlazione cito-istologica

Periodo

Gennaio 2004-Giugno 2005

FREQUENZA DEI QUINTILI NEI CASI CON ISTOPATOLOGIA



nmary: MCM and TOP2A as Biomarkers of Cervical Dysplasia and Carcinoma

MCM2, MCM6, MCM7 and TOP2A **mRNAs are increased in cervical squamous cell carcinoma and cervical adenocarcinoma vs benign cervical mucosa.**

The encoded proteins for MCM2, MCM6, MCM7 and TOP2A are also over-expressed in cervical dysplasia and carcinoma.

Biomarkers show a quantitative difference in protein expression in CIN2+ lesions.

MCMs and TOP2A could be useful immuno-cytochemical targets, as cytologic diagnostic adjuncts.