GISCI ; Finale Borgo (SV) 21-22 Maggio 2015

Uno screening, due percorsi: Test HPV e Pap Test a confronto nella pratica. Valutazione e analisi della co-esistenza dei due percorsi nella pratica corrente

Can biomarkers play a real role for triage of HPV positive women?

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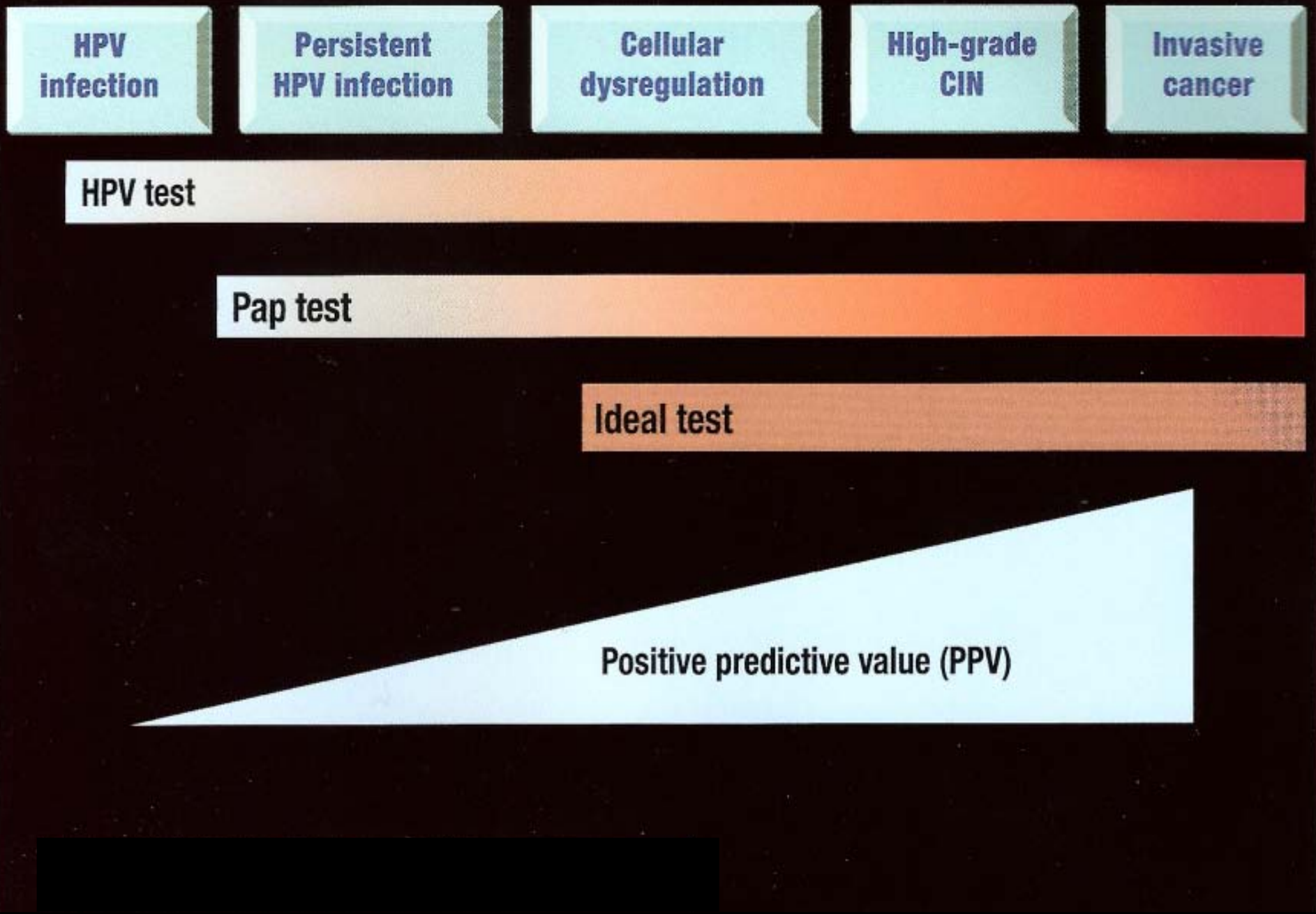

HPV infection
Persistent HPV infection
Cellular dysregulation
High-grade CIN
Invasive cancer
HPV test
Pap test
Ideal test
Positive predictive value (PPV)

HPV testing: recommendations

To improve specificity of HPV test as "stand-alone" primary test for cervical cancer screening a triage of HPV positive women is recommended

Aims:

1. to identify subjects with disease at high risk of progression and to avoid over-treatments to subjects
2. to limit colposcopy referral rate

At present:

- HTA Italy (2012) and european guidelines: Citology as triage in women 30-64 years
- FDA (2014) Genot 16 e 18 e Citology as triage of other HR HPV in women >25 years

Citology: accuracy

Study nested in the NTCC trial (*Bergeron JNCI 2015*)

Methods

- Informed Cytology: Knowledge of HPV positivity
- Revision of 1276 thinprep slides from PreservCyt samples (NTCC phase 2)
- External revision by a cytotechnician (FC).
- Revision abnormal cytology (ASCUS+) by an expert pathologist (CB).

Results

- Accuracy and relative sentitivity vs Pap screening

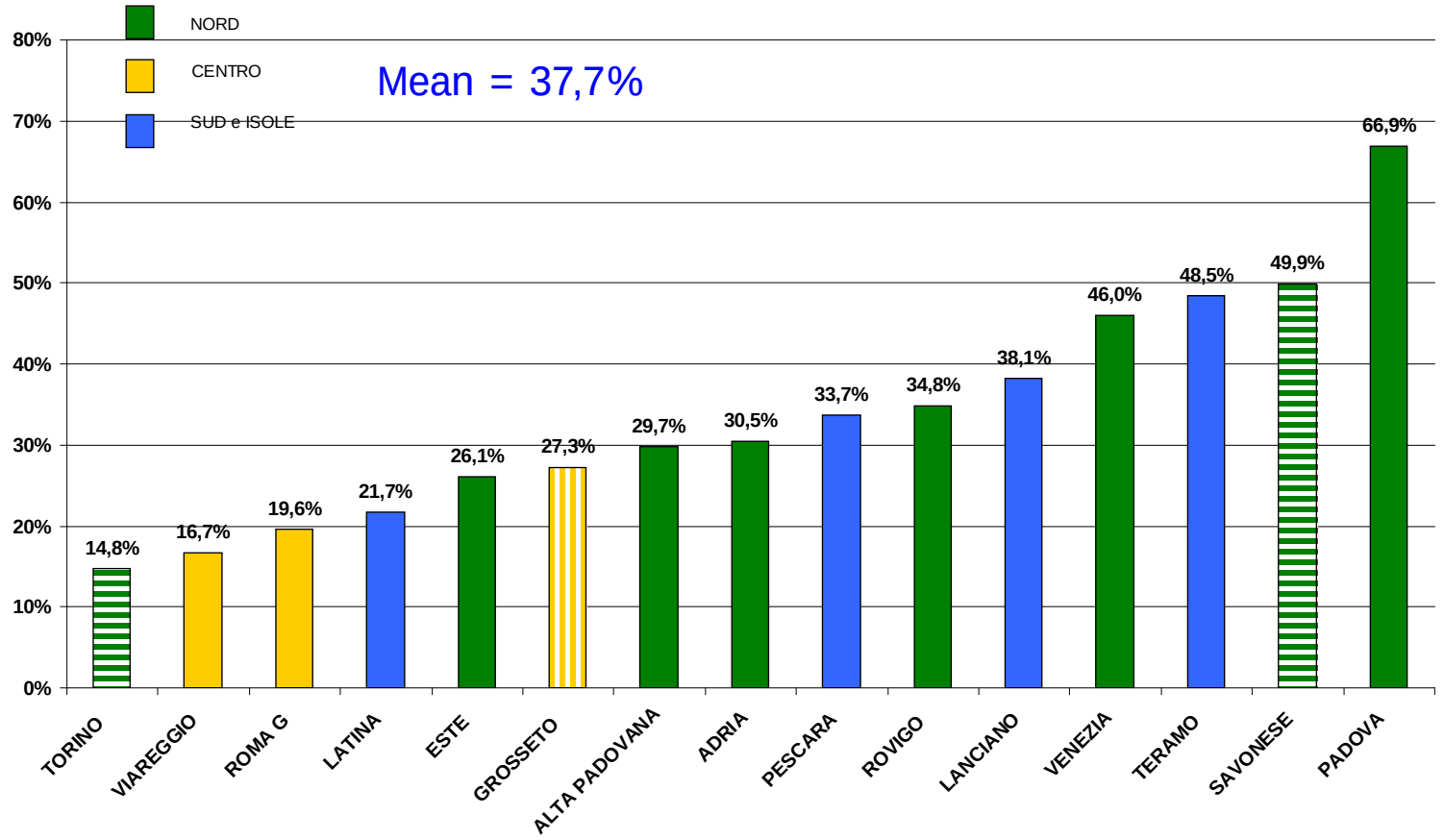
Histological Endpoint	Sensitivity	Specificity	PPV	Relative Risk	Relatve sensitivity vs pap screening	Relative Referral rate
CIN3+ Cross Sectional	88.1% (74.4 - 96.0)	64.0% (61.2 - 66.7)	7.8% (5.5 - 10.6)	12.20 (4.83 - 30.84)	**1.41** (0.99 - 1.96)	**0.95** (0.86 - 1.04)
CIN3+ Longitudinal	61.5% (40.6 -79.8)	67.1% (64.1– 69.9)	4.5% (2.6– 7.1)	3.16 (1.45– 6.89)	**2.36** (1.58-3.51)	**--** (*)

(*) Only women referred at baseline can be referred atl follow up

Informed cytology is more sensitive than blind cytology

Triage Cytology in Italy: Referral rate for colposcopy

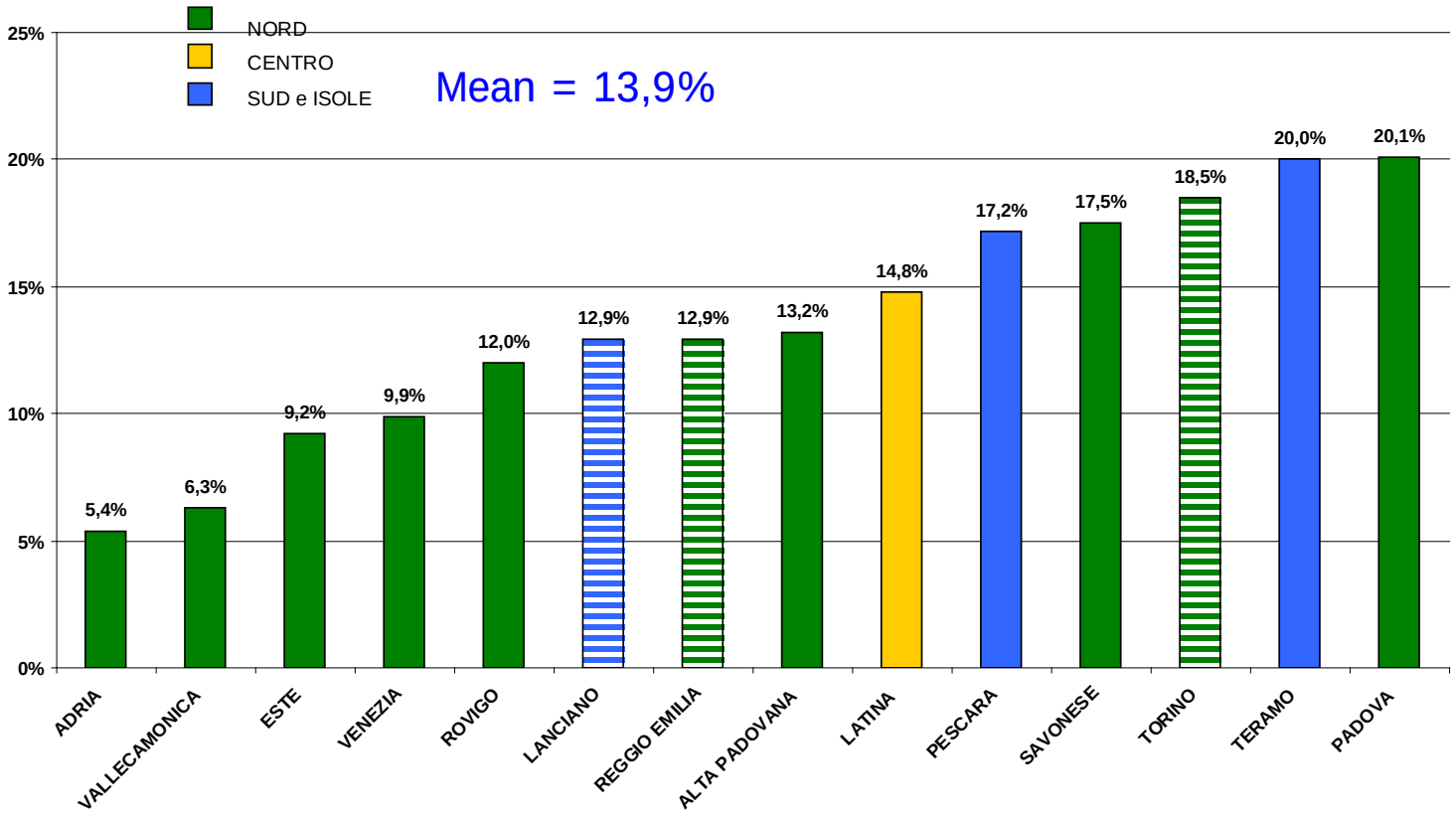
Year 2013, *Ronco ONS - Meeting Perugia 2015*



In Italy high variability in referral rate

Triage Cytology in Italy: PPV

Year 2012, *Ronco ONS - Meeting Perugia 2015*



In Italy high variability in Positive Predictive Value

Screening performance indicators with hrHPV-DNA testing: baseline results .

Regione Umbria; USL Umbria 1 (HTA)	first round (2010-2012)	
	baseline (2010-2011)	
	n	%
Invited women	9979	
total tested women	6272	62,8
hrHPV-Dna positive	396	6,4
Triage cytology positive	**141**	**35,6**
colposcopy referral		**2,2**
compliance to colposcopy referral	136	96,4
CIN2+ detection rate	52/6272	8,3‰
Positive predictive value for CIN2+	**52/136**	**38,2**
Women referred to 1-year follow up	251/6272	4

Are biomarkers
reliable as
alternative or adjunctive test
to Pap cytology

for triage of HPV positive women

? ? ?

Biomarkers investigated for HPV triage application

Biomarker of HPV infection (DNA)

- HPV typing
- Viral Load

Biomarkers of chromosomal instability

- Virus integration

Biomarkers of active/persistent HPV infection

- mRNA → viral oncogene expression
- p16 *INK4a*
- DNA Methylation: epigenetic modifications
- microRNA : expression modifiations
- oncogenic proteins

} Surrogates of viral oncogene expression

Comparison of triage meth: Biomarker of HPV infection (DNA) [*a*]

Endpoint CIN3+: Cross Sectional Accuracy and Relative Sensitivity vs Pap screening

Genotyping

Triage Method	Age	Sensitivity	Specificity	PPV	Rel Sensitivity vs pap screening	Relative Referral for colposcopy	Data Origin
Cito ASCUS+ Informed of HPV+	>25	88.1% (74.4 - 96.0)	64.0% (61.2- 66.7)	7.8% (5.5- 10.6)	**1.41** (0.99 - 1.96)	**0.95** (0.86 - 1.04)	NTCC
	>35	96.3 % (81.0 - 99.9)	68.5 % (63.5-73.2)	18.4% (12.4-25.8)	**2.20 ***	**2.20**	USL Umbria1
HPV16	>35	66.7% (55.1-76.9)	73.4% (71.5-75.2)	8.2% (6.2-10.7)	1.01 (0.66-1.46)	0.63 (0.57-0.71)	NTCC
	>35	55.6% (35.3-74.5)	82.7% (78.4-86.4)	20% (11.9-30.4)	0.64	1.27	USL Umbria1
HPV16 or 18	>35	70.5% (59.1-80.3)	66.0% (64.0-68.0)	6.9% (5.3-8.9)	1.06 (0.69-1.53)	0.80 (0.72-0.89)	NTCC
	>35	63.0% (42.4-80.6)	76.7% (72.0-80.9)	16.3% (9.8-24.9)	0.73	1.65	USL Umbria1
HPV16 or 18 or 31 or 33 or 45	>35	84.6% (76.6-92.6)	49.2% (47.1-51.4)	5.6% (4.4-7.1)	1.21 (0.81-1.75)	1.17 (1.03-1.24)	NTCC
16 or 18 or 31 or 33 or 35 or 45 or 52 or 58	>35	93.6% (85.7-97.9)	40.9% (38.9-43.0)	5.4% (4.2-6.7)	1.31 (0.89-1.86)	1.36 (1.20-1.43)	NTCC
All HR-HPV (HC2+)	>25	>96.0% (95.0–98.0)		3.6% (3.0-4.5)	**1.52** (1.06-2.19)	**2.38** (2.21-2.57)	NTCC & pooled data (Arbyn 2012)

(*) relative to Endpoint CIN2+

Comparison of triage meth: Biomarker of HPV infection (DNA) [*b*]

Endpoint CIN3+: Cross Sectional Accuracy and Relative Sensitivity vs Pap screening

Viral load

Triage Method	Age	Sensitivity	Specificity	PPV	Rel Sensitivity vs pap screening	Relative Referral for colposcopy	Data Origin
Cito ASCUS+ Informed of HPV+	>25	88.1% (74.4 - 96.0)	64.0% (61.2-66.7)	7.8% (5.5- 10.6)	**1.41** (0.99 - 1.96)	**0.95** (0.86 - 1.04)	NTCC
	>35	96.3 % (81.0 - 99.9)	68.5 % (63.5-73.2)	18.4% (12.4-25.8)	**2.20 ****	**2.20**	USL Umbria1
Viral load (HPV copies) 16		50% cutoff 22000 copies/10^3cells	90%	*N.A.*	*N.A.*	*N.A.*	F (Saunier, JCM2008)
Viral load (HPV copies) 16,18,31,33	>29	91.7% (84.9 – 95.6) cutoff for each type	28.0% (26.0-30.3)	33.7% * (28.1-39.3)	*N.A.*	*N.A.*	NL, POBASCAM (Hesselink, IJC 2009)
Viral load (HPV copies) 16,18,31,45	>25	75% cutoff 0.73 copies/cell	80%	*N.A.*	1.29	*N.A.*	UK (Marongiu ,BMC cancer2014)

(*) relative to HPV positive women for included types

(**) relative to Endpoint CIN2+

Viral load: inconsistent results across studies, previous genotyping needed

Comparison of triage meth: Biomarker of chromosomal instability

Endpoint CIN3+: Cross Sectional Accuracy and Relative Sensitivity vs Pap screening

Virus integration

Triage Method	Age	Sensitivity	Specificity	PPV	Rel Sensitivity vs pap screening	Relative Referral for colposcopy	Data Origin
Cito ASCUS+ Informed of HPV+	>25	88.1% (74.4 - 96.0)	64.0% (61.2-66.7)	7.8% (5.5- 10.6)	**1.41** (0.99 - 1.96)	**0.95** (0.86 - 1.04)	NTCC
	>35	96.3 % (81.0 - 99.9)	68.5 % (63.5-73.2)	18.4% (12.4-25.8)	**2.20 ****	**2.20**	USL Umbria1
Viral Integration 16,18,31,45	>25	72% cutoff E2/E6 0.2 (also 0.5 and 0.8)	50%	*N.A.*	1.16	*N.A.*	UK (Marongiu ,BMC cancer2014
Viral Integration 16		78% cutoff E2/E6 0.5	66%	*N.A.*	1.25	*N.A.*	F (Saunier, JCM2008)
Viral Load/Viral Integration 16, 18	>25	52% cutoff E2/E6 0.5	86%	29% *	0.83%	0.25	NL (Litjens, J Med Viol 2013i)

(*) relative to HPV positive women for included types

(**) relative to Endpoint CIN2+

Integration: inconsistent results across studies, previous genotyping needed

Comparison of triage meth: Biomarkers of active/persistent infection

Endpoint CIN3+: Cross Sectional Accuracy and Relative Sensitivity vs Pap screening

DNA methylation, onco-protein, miRNA

Triage Method	Age	Sensitivity	Specificity	PPV	Rel Sensitivity vs pap screening	Relative Referral for colposcopy	Data Origin
Cito ASCUS+ Informed of HPV+	>25	88.1% (74.4 - 96.0)	64.0% (61.2-66.7)	7.8% (5.5- 10.6)	**1.41** (0.99 - 1.96)	**0.95** (0.86 - 1.04)	NTCC
	>35	96.3 % (81.0 - 99.9)	68.5 % (63.5-73.2)	18.4% (12.4-25.8)	**2.20 *****	**2.20**	USL Umbria1
Genomic DNA Methylation CADM1/MAL	>33	69.4 (57.9–80.8)	71.2 (66.1–76.3)	33.1% *	0.90	1.02	NL Verhoef GynOnc 2015
Viral DNA Methylation HPV 16,18,31,45	>18 >25 >30	64-92.5%	38-82%	38-62% *	*N.A.*	*N.A.*	Mirabello IJC2013,2015, Marongiu ,BMC cancer2014 ,Brentall-Lorincz 2014
Onco- E6 protein HPV 16,18,45	>25	53.5% (43.2–63.6)	98.9% (98.7–99.2)	40.8% * (32.2-49.7)	*N.A.*	*N.A.*	China, Zhao – Castle P CanPrev Res 2013
miRNA (miR424,miR375, miR218)	>30	72.0%	70%	38.9% **	1.03	*N.A.*	China, Tian JNCI 2014

(*) relative to HPV positive women for included types (**) relative to HPV+/ASCUS+ (***) relative to Endpoint CIN2+

Partial or inconsistent results across studies. Previous genotyping needed

Comparison of triage meth: Biomarkers of active/persistent infection

Endpoint CIN2+: Cross Sectional Accuracy and Relative Sensitivity vs Pap screening

p16, mRNA

Triage Method	Age	Sensitivity	Specificity	PPV	Rel Sensitivity vs pap screening	Relative Referral for colposcopy	Data Origin
Cito ASCUS+ Informed of HPV+	>25	85.6% (76.6- 92.1)	65.9.0% (63.1- 68.6)	16.2% (13.0- 19.8)	**1.58** (1.22 - 2.01)	**0.95** (0.86 - 1.04)	NTCC
	>35	77.6 % (65.3 – 86.7)	72.5 % (67.2–77.2)	37.0% (28.9–45.6)	**2.20***	**2.20**	USL Umbria1 AJCP in press
p16 (cutoff 1+)	>35	88%	61%	7.75%	1.53 (1.15-2.02)	1.08 (0.96-1.21)	NTCC
p16/Ki67	>35	87.6% (75.7–93.6)	74.9% (69.0–79.0)	42.5% (33.8–51.0)	2.30	2.10	USL Umbria1 AJCP in press
mRNA (14 HPV types)	>30	91.2% (86.0–94.6)	76.1% (67.6–82.9)	N.A.	1.47	N.A.	D Clad, JCM 2011
mRNA (16,18,31,33,45)	>35	80.8% (67.6–89.8)	60.0% (53.6–66.0)	31.4.6% (23.6–39.2)	1.80	2.30	USL Umbria1 AJCP in press

(*) relative to Endpoint CIN2+

p16 shows similar perfomance as cytology

No biomarker has shown at present better performance (Sensitivity,Specificity,Ref rate) than cytology for triage of HPV-pos

Any biomarker reliable as alternative to cytology for triage of HPV-pos ?

→ p16 = similar performance as cytology in >35 ys

Can biomarkers be proposed as adjunctive test to cytology for triage of HPV-pos ?

Combined approaches

Endpoint CIN2+

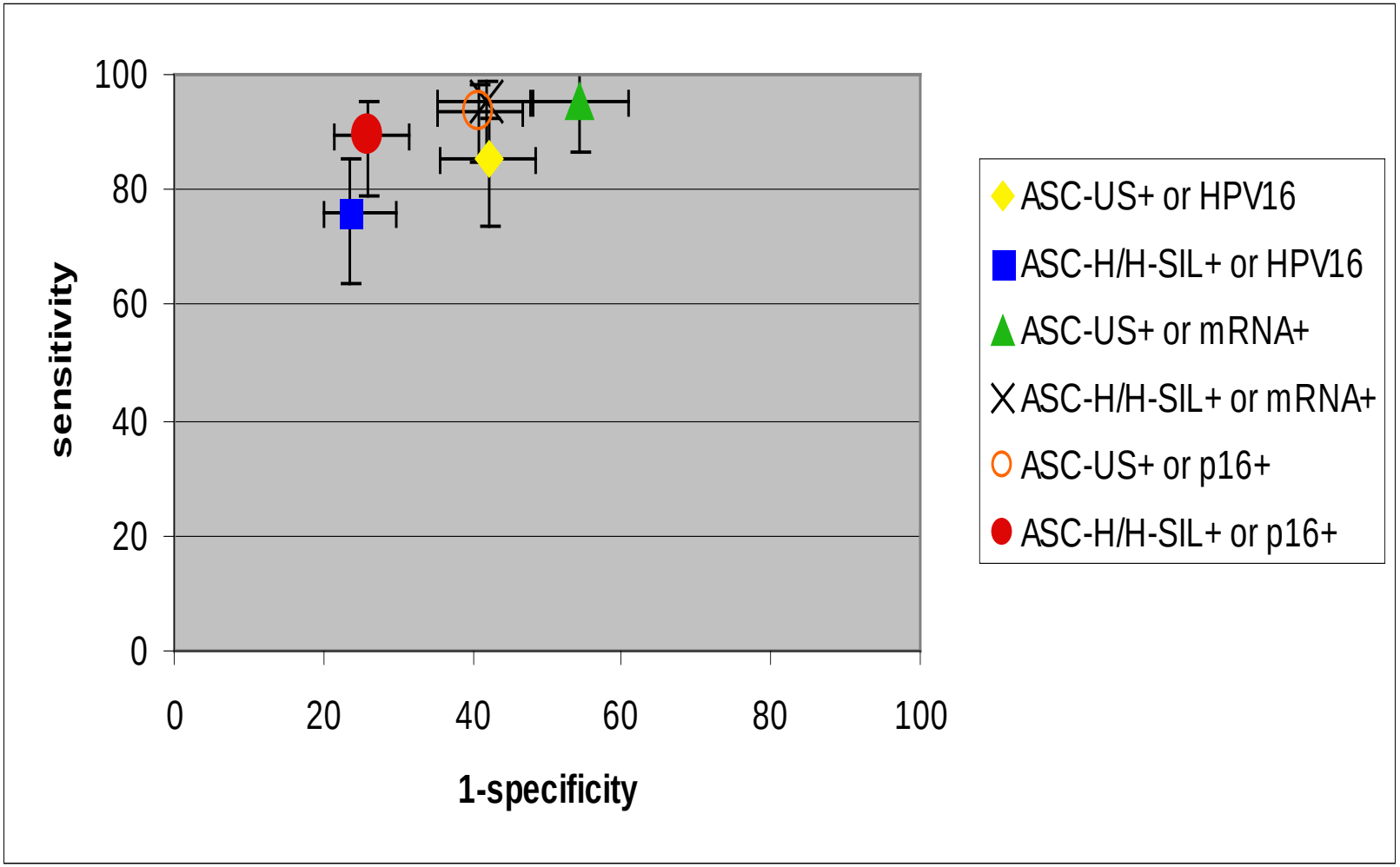
Triage Method		Age	Sensitivity	Specificity	PPV*	Referral for colposcopy	Data Origin
Cytology	ASCUS+	>35	77.6%	72.5%	37.0%	36%	USL Umbria1
	High Grade		53.2%	97.3%	79.5%	11.2%	
Citology/typing	ASCUS+/ 16,18+	>35	85.1% (73.9 - 92.5)	57.8% (51.5-64.5)	29.1% (5.5- 10.6)	49.5 (44.7-54.8)	USL Umbria1
	High grade/ 16,18+	>35	75.9% (63.6 - 85.5)	76.5% (70.4 - 80.0)	39.7% (30.8 -48.4)	32.4% (27.8 – 37.2)	USL Umbria1
Citology/mRNA	ASCUS+/ mRNA+	>35	95.1% (84.9 – 98.9)	45.8% (39.6 – 52.4)	28.4% (22.8 – 36.3)	61.5% (55.7 – 67.0)	USL Umbria1
	High grade/ mRNA+	>35	95.1% (84.9 – 98.9)	58.2% (52.1 – 64.7)	34.6% (27.4 – 42.9)	51.5% (45.7 – 57.3)	USL Umbria1
Citology/p16-Ki67	ASCUS+/ p16-Ki67+	>35	93.8% (85.0 – 98.3)	59.2% (53.4 – 64.6)	33.0% (26.1 – 39.5)	50.1% (44.9 – 55.3)	USL Umbria1
	High grade/ p16-Ki67+	>35	**89.2 %** (79.1 – 95.6)	**74.2 %** (68.4 – 78.5)	**42.5 %** (33.9 – 51.1)	**36.9 %** (32.0 – 42.1)	USL Umbria1

(*) relative to HPV+/ASCUS+ women

Gustinucci,...Passamonti, AJCP in press

Best strategy: High grade/ p16-Ki67

Combined approaches



Combined approaches

Endpoint CIN3+: Estimate of Cross Sectional Sensitivity and Referral

Cuzick, JCV 2014
According to PPV on referral pt

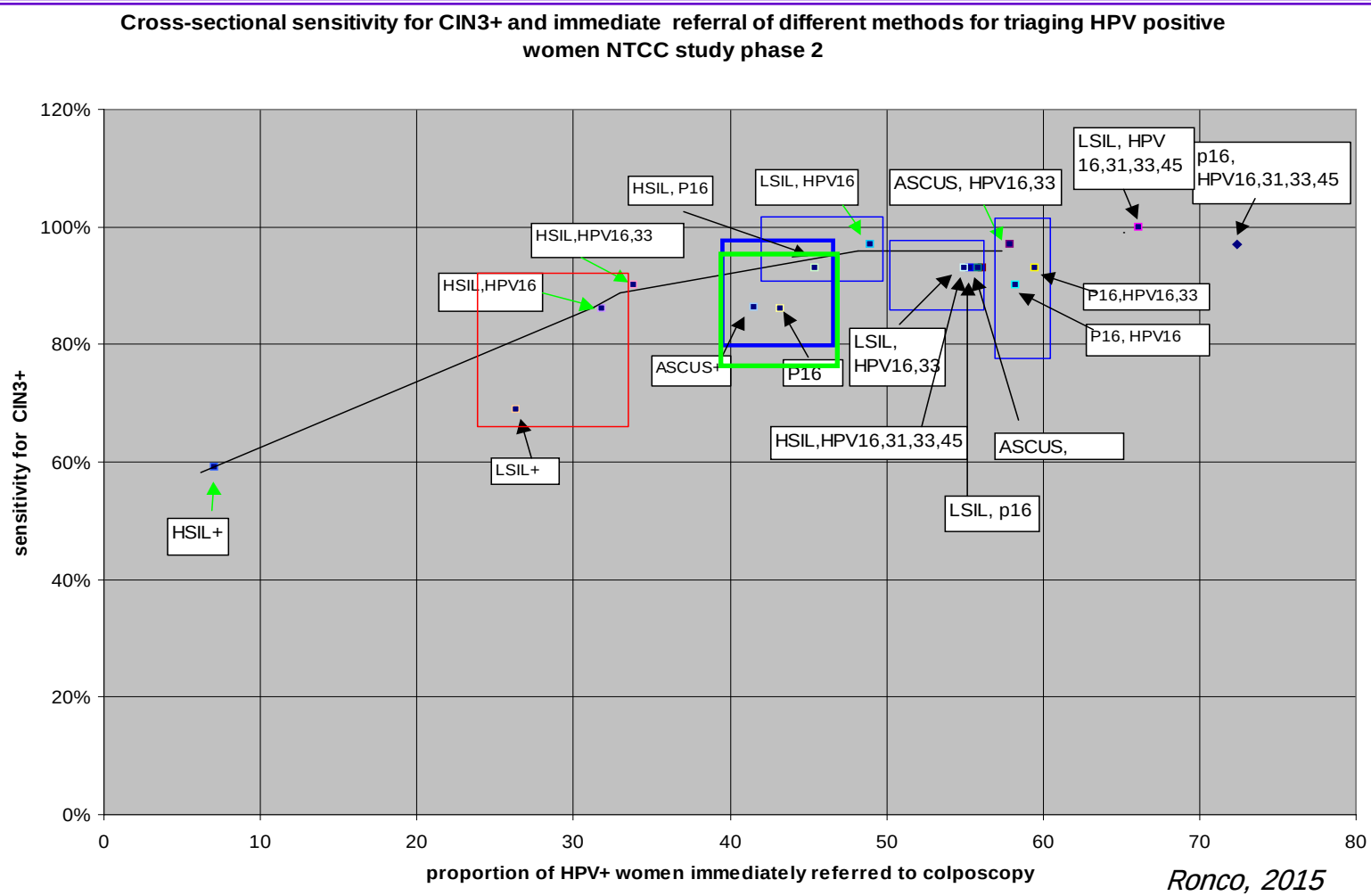
Group A: very high risk HPV16 and 33

Group B: high risk HPV 18,31,35,52,58

Group C: intermediate risk HPV 39,45,51,56,66,68

Triage Methods	Sensitivity CIN3+	Referral Rate for colposcopy	Data Origin
HPV informed cytology	88%	36%	NTCC Phase 2
Hsil+,16,18	86%	32%	NTCC Phase 2
ASCUS+,CADM1/MAL	88%	53%	NL, Verhoef Gyn Onc 2015
Hsil+, 16,33	90%	34%	NTCC Phase 2
p16,16,18	90%	58%	NTCC Phase 2
Hsil+,p16	93%	45%	NTCC Phase 2
LSIL+,16,18	93%	47%	NTCC Phase 2
HSIL+,16,18,31,33,45	93%	55%	NTCC Phase 2
ASCUS +,16	93%	56%	NTCC Phase 2
LSIL+,p16	93%	56%	NTCC Phase 2
p16,16,33	93%	59%	NTCC Phase 2
LSIL+,16,33	97%	49%	NTCC Phase 2
ASCUS+16,33	97%	58%	NTCC Phase 2
p16,16,18,31,33,45	97%	72%	NTCC Phase 2
LSIL+,16,18,31,33,45	100%	66%	NTCC Phase 2

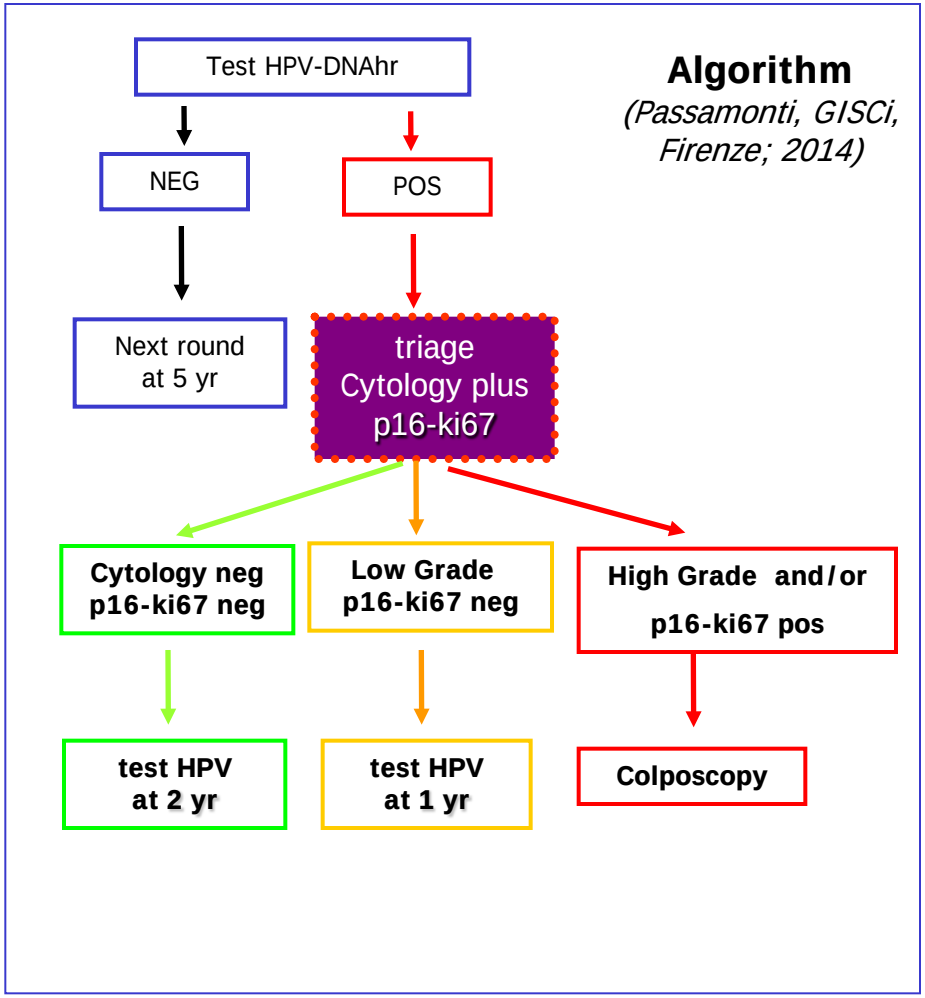
Combined approaches



Ronco, 2015

Combined approaches

Combined Strategies: new algorithms?



HPV 1 yr results regional screening

HPV 1 yr (05/2014-04/2015)	HPV+	HPV -	% positivity
387	238	149	61

HPV 1yr positive	COLPOSCOPY	Neg	CIN1	CIN2	CIN3	PPV
238	157 (66%)	7	19	9	4	8%

Biomarkers Reproducibility

Triage Method	Overall agreement	K agreement	References
Cytology conv. Cytology LBC	78% 84%	0.69 0.82	2 laboratories Heard, Bergeron et al. Cytopathology 2014
Genot HPV16 Genot HPV18	97% 87%	*N.A.*	WHO - 2011 HPV LabNet Internat Proficiency Study Eklund, J Clin Microbiol 2014
Genotyping 16 HPV types	42% (same test)	*N.A.*	WHO - 2011 HPV LabNet Internat Proficiency Study Eklund, J Clin Microbiol 2014
Viral load HPV16, 18	N.A	>0.9	2 laboratories. Gravitt J Virol Meth 2003
P16/ki67 dual stain	83-91% N.A.	0.71 0.70	10 evaluators . Wentzensen, Cancer Cytopathol. 2014 7 evaluators. Allia, Ronco, Ghiringhello... Cancer Cytopath 2014
mRNA	>96% (company)	*N.A.*	Kit Aptima - Hologic-Gene Probe No inter-laboratories studies
DNA Methylation	*N.A.*	*N.A.*	No inter-laboratories studies
Onco-E6 protein	*N.A.*	*N.A.*	No inter-laboratories studies
Viral Integration	*N.A.*	*N.A.*	No inter-laboratories studies
microRNA	*N.A.*	*N.A.*	No inter-laboratories studies

Conclusions

For triage of HPV pos women at present no biomarker showed a better performance than cytology

→ no biomarker can be proposed as alternative to cytology

Some biomarkers can be considered as adjunctive to cytology in combined strategies

→ improvement of triage accuracy (sensitivity, specificity)

→ delay of follow up controls → lower referral rate for colposcopy (cost efficiency)

Good candidates : p16
Geno HPV16,18, 33
DNA methylation
E6 protein

Conclusions

Limits of combined approach

- Clinical validation is still lacking for many biomarkers, as well as inter-laboratory reproducibility assessment
- Longitudinal studies to ensure safety for negatives available only for some biomarkers
- More test = more complexity and + delay in diagnostic results
- Simple analysis = more compliance to screening

Aknowledgements

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