DOI: 10.1111/j.1471-0528.2010.02521.x
www.bjog.org

Commentary

# Human papillomavirus-based triage of women showing a cervical cytology result of borderline or mild dyskaryosis

**M Arbyn,[a] P Martin-Hirsch,[b] N Wentzensen[c]**

[a] Unit of Cancer Epidemiology, Scientific Institute of Public Health, Brussels, Belgium [b] Department of Obstetrics and Gynaecology, Central Lancashire Teaching Hospitals, Preston, UK [c] Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD, USA

*Correspondence:* Dr M Arbyn, Unit of Cancer Epidemiology, Scientific Institute of Public Health, J. Wytsmanstreet 14, B1050 Brussels, Belgium. Email marc.arbyn@iph.fgov.be

*Accepted 13 January 2010. Published Online 18 March 2010.*

*Please cite this paper as:* Arbyn M, Martin-Hirsch P, Wentzensen N. HPV-based triage of women showing a cervical cytology result of borderline or mild dyskaryosis. BJOG 2010;117:641–644.

## Background

Most women with a Papanicolaou smear showing minor abnormalities, detected in the framework of cervical cancer screening, do not have or will not develop clinically significant disease. Minor cytological abnormalities are classified by UK cytological criteria as borderline or mild dyskaryosis, which can be translated into international terminology as atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesions (LSIL), respectively.

According to a recent meta-analysis, the absolute risk of underlying high-grade cervical intraepithelial neoplasia (grade 2 or 3 or worse [CIN2/3+]), among women with ASCUS, is on average, 9–10% for CIN2+ and 4–5% for CIN3+. For women with LSIL, these risks are about 1.5 to 2 times as high.[1] These risks are 10 to 30 times higher than for women with normal cytology. The probability that high-grade CIN progresses to invasive cancer is not well known because these lesions are usually treated. However, from historical data, it has been estimated that CIN3 incurs a probability of progressing to invasive cancer of 12–30%, depending on lesion size and follow-up time,[2,3] whereas for CIN2 this probability is substantially less. Moreover, treatment of CIN by excision is associated with obstetric morbidity. Therefore, women with minor cytological lesions require careful management balancing future cancer risk, adverse effects and costs.

## TOMBOLA trial

The TOMBOLA (Trial of Management of Borderline and Other Low-grade Abnormal smears) trial enrolled 4439 women with borderline ($n$ = 2863) or mild dyskaryosis ($n$ = 1576), aged 20–59 years, and was intended to evaluate several management options: cytological surveillance (trial arm 1), immediate referral to colposcopy (arm 2), followed by treatment based on the histological result of colposcopy-targeted biopsies (arm 2a) or, followed by immediate treatment of colposcopically visible lesions (arm 2b).[4] At randomisation, an endocervical sample was taken to identify the presence of DNA of 14 high-risk human papillomavirus types (hrHPV) using a polymerase chain reaction (PCR) with GP5+/6+ primers and enzyme immunoassay identification. The results of HPV testing did not interfere with management. The trial included final colposcopy assessment for all participants. Detection of histologically confirmed CIN2+ or CIN3+ was the main outcome.

Within arm 2, cross-sectional accuracy of hrHPV testing for presence of underlying CIN2/3+ was assessed. The cumulative diagnosis of CIN2/3+ over the whole study period, according to initial HPV status and trial arm, was the longitudinal end point.

## Results and comparison with other studies

### Cross-sectional accuracy

In TOMBOLA, the reported cross-sectional sensitivities of hrHPV triage of borderline and mild dyskaryosis were 69.9% and 75.2%, for detection of underlying CIN2+, and 74.3% and 80.6% for CIN3+, respectively. The corresponding specificity values were: 71.3%, 46.9%, 69.0% and 43.8%.

The large American ASCUS-LSIL Triage Study (ALTS) and meta-analyses of other triage studies using the Hybrid

 

**Table 1.** Cross-sectional accuracy of high-risk human papillomavirus testing to triage women with atypical squamous cells of undetermined significance or borderline dyskaryosis to detect underlying CIN2+ or CIN3+

| | Test | Outcome | Sensitivity (%) | Specificity (%) | Test + (%) | PPV (%) | NPV (%) |
|---|---|---|---|---|---|---|---|
| **Triage of ASCUS/borderline dyskaryosis** | | | | | | | |
| Sherman *et al.*, 2002[7] | HC2 | CIN2+ | 95.7 | 51.5 | 54.0 | 20.6 | 98.9 |
| (ALTS) | | CIN3+ | 96.1 | 48.6 | – | 10.4 | 99.5 |
| Meta-analysis (without ALTS) | HC2 | CIN2+ | 89.8 | 63.8 | 41.0 | 19.1 | 99.0 |
| Arbyn *et al.*, 2006[1] | | CIN3+ | 94.4 | 61.1 | – | 8.7 | 99.7 |
| TOMBOLA, 2010[4] | GP PCR | CIN2+ | 69.9 | 71.3 | 33.7 | 25.3 | 94.5 |
| | | CIN3+ | 74.3 | 69.0 | – | 13.9 | 97.6 |
| **Triage of LSIL/borderline dyskaryosis** | | | | | | | |
| Sherman *et al.*, 2002[7] | HC2 | CIN2+ | 97.8 | 18.8 | 84.8 | 24.8 | 96.9 |
| (ALTS) | | CIN3+ | 96.7 | 16.6 | – | 12.2 | 97.7 |
| Meta-analysis (without ALTS) | HC2 | CIN2+ | 95.2 | 32.1 | 76.1 | 25.8 | 98.2 |
| Arbyn *et al.*, 2006[1] | | CIN3+ | 97.6 | 23.8 | – | 10.7 | 98.9 |
| TOMBOLA, 2010[4] | GP PCR | CIN2+ | 75.2 | 46.9 | 60.1 | 39.3 | 80.5 |
| | | CIN3+ | 80.6 | 43.8 | – | 21.1 | 92.4 |

ALTS, ASCUS-LSIL Triage Study; ASCUS, atypical squamous cells of undetermined significance; HC, hybrid capture; LSIL, low-grade squamous intraepithelial lesion; NPV, negative predictive value; PPV, positive predictive value; TOMBOLA, Trial of Management of Borderline and Other Low-grade Abnormal smears.

Capture-2 assay ([HC2] Qiagen, Gaithersburg, MD, USA) showed substantially higher sensitivity and lower specificity values.[1,5–7] To verify whether the conclusions of previous meta-analyses were determined by this major American trial, we recomputed pooled accuracy estimates of HPV-based triage[1] after exclusion of ALTS (see Table 1 and Figure 1). Sensitivity estimates, pooled from other studies, were not statistically significantly lower than those from ALTS, but were still significantly higher than those observed in TOMBOLA. However, pooled specificity values were intermediate and differed significantly from ALTS and TOMBOLA. The hrHPV positivity rates were lowest in TOMBOLA. Specificity of hrHPV testing increased significantly with age in both ALTS and TOMBOLA. While sensitivity decreased with age in TOMBOLA, no age effect was observed in ALTS. Consistent findings were the higher hrHPV positivity rates[8] as well as higher prevalence rates of CIN2+ and CIN3+ in LSIL/mild dyskaryois compared with ASCUS/borderline dyskaryosis (ratio LSIL/ASCUS between 1.57 and 1.86 for hrHPV rates and between 1.83 and 2.58 for CIN2+ or CIN3+). These different rates indicate separate study outcomes for both triage groups.

### Longitudinal findings

The 3-year cumulative risks of CIN2+ and CIN3+, combined over the study arms among HPV-positive women, were 30.3% (532/1755) and 17.2% (302/1755), respectively, which were 3.14 and 4.30 times higher than among hrHPV-negative women. These relative risks (RR) did not vary significantly by study arm, age or history of prior lesions.

The GP5+/6+ PCR at enrolment identified 71% and 77% of the cumulative diagnosis of CIN2+ and CIN3+ (=longitudinal sensitivity). Within arm 1, repeated Papanicolaou smears revealed 77% of the cumulative diagnoses of CIN2+ and 87% of CIN3+.[9] The cumulative sensitivity of hrHPV testing once-only and repeat cytology were not compared directly and could not be computed from reported data because no paired cross-tables were presented. In ALTS, HC2 at enrolment and repeated cytology in case of ASCUS showed similar longitudinal sensitivities but the number of colposcopy referrals was substantially greater in the arm with cytological surveillance.[10] For LSIL, the ALTS data demonstrated very low specificity because of the high hrHPV-positivity rate.

A few other studies contained data on the longitudinal performance of HPV-based triage but no meta-analyses have been performed. Cuzick *et al.* did not find any high-grade CIN after 1 year of follow-up of women with borderline or mild dyskaryosis with an initial negative HC2 test.[11] Bais *et al.* followed women with two consecutive smears showing minor cytological abnormalities and used the same triage PCR test as in TOMBOLA. One case of high-grade CIN in an HPV-negative woman was found, but further follow-up with final colposcopy after 1 year did not identify other cases (cumulative sensitivity of 50/51 = 98%).[12]

## Comments

The cross-sectional and longitudinal results of TOMBOLA contrast with those from the ALTS study and with the majority of other studies. Most obviously, the sensitivity

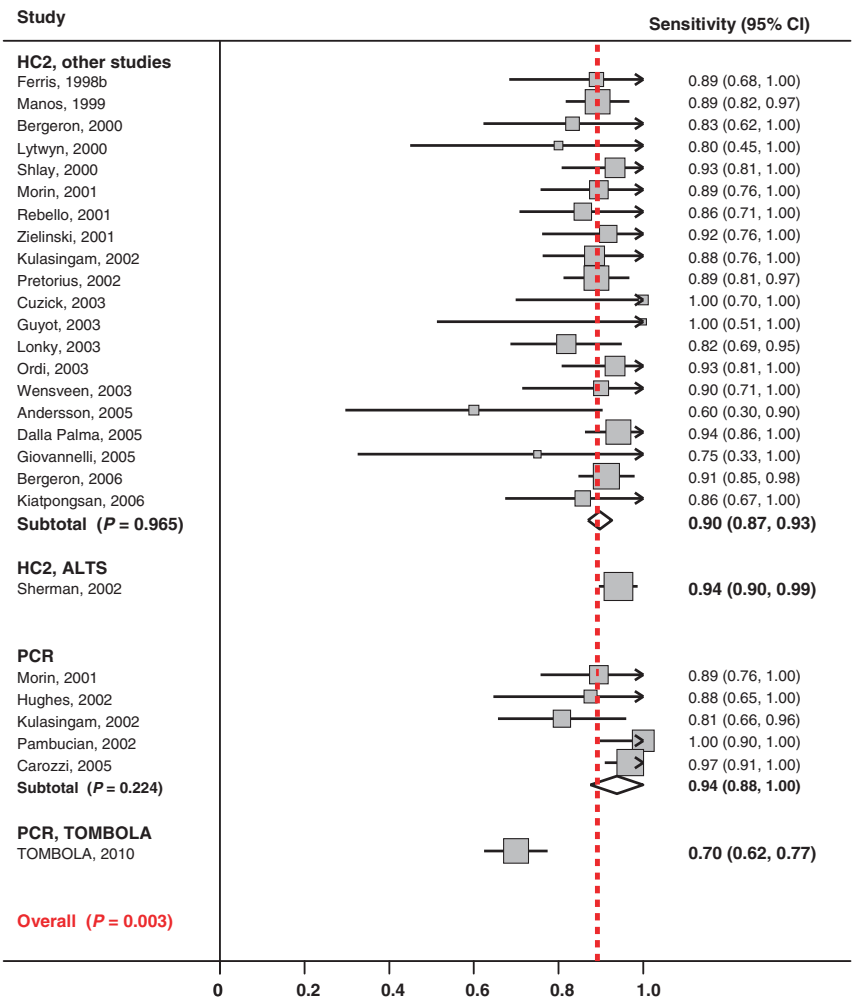


**Figure 1.** Meta-analysis of the sensitivity of high-risk human papillomavirus triage of women with atypical squamous cells of undetermined significance or borderline dyskaryosis to detect underlying CIN2 or worse, using Hybrid Capture 2 (HC2) or polymerase chain reaction (PCR). [Adapted from Arbyn *et al.*, *Vaccine* 2006;24(Suppl. 3):S78–S89 and Cotton *et al.*, *BJOG* 2010; DOI: 10.1111/j.1471-0528.2010.02519.x [E-pub ahead of print].]

for prevalent CIN2+ and CIN3+ was substantially lower in TOMBOLA compared with ALTS and the meta-analysis, which may be explained by differences in HPV test sensitivity or differences in outcome assessment. The GP5+/6+ PCR system used in TOMBOLA showed very good results in various studies, with similar sensitivity to the HC2 test used in ALTS (Figure 1). Differences in specificity between ALTS and TOMBOLA could be attributable, at least partially, to the inclusion of relatively more older women in the latter trial. Although we note some differences in the sampling protocol in TOMBOLA compared with other studies (sampling of the endocervix, sample storage in phosphate-buffered saline), we do not believe that these differences provide a plausible explanation for the rather high rate of false-negative results, given the robustness of the assay.[5] The pragmatic study design of TOMBOLA mainly relied on community-based histology outcomes whereas in ALTS, a stringent and intense quality control programme resulted in the reassessment of all histological outcomes by an independent panel. The lower sensitivity of HPV testing for prevalent CIN2+ in TOMBOLA could be partially explained by overcalling of low-grade abnormalities or atypical squamous metaplasia as CIN2 or CIN3.

In TOMBOLA, there was no significant interaction by study arm between CIN2+ detection in HPV-positive versus HPV-negative women (similar RRs). The authors interpreted this as evidence for not recommending more aggressive management for hrHPV-positive women. We believe that the interaction is not necessarily that important but we are concerned about the low RR which was the result of the rather frequent detection of CIN2+ among hrHPV-negative women, in all subgroups.

As a result of the lack of a triage-arm based on hrHPV testing and the incomplete reporting of end points

separated by baseline cytology, a full comparison between ALTS and TOMBOLA is difficult. In particular, a more straightforward comparison with the longitudinal outcomes of ALTS, which provided evidence for different management policies for ASCUS and LSIL, would be helpful. The inclusion of an arm with immediate excision of the transformation zone in case of borderline cytology could have been omitted from TOMBOLA because this option is uncommon practice and usually considered as overtreatment. Based on current knowledge, the inclusion of an alternative study arm assessing single versus multiple biopsy sampling or parallel testing for molecular markers would have been extremely useful.

To conclude, we believe that the HPV test results of TOMBOLA are not consistent with the wealth of evidence that supports guidelines recommending HPV-based triage of women with equivocal cervical cytology. For triage of LSIL the situation is less clear. More specific tests, addition of markers, or restriction to older women are options besides direct referral to colposcopy or cytology surveillance. Unfortunately TOMBOLA does not provide evidence on this. An independent review of HPV-negative versus HPV-positive CIN2+, possibly supported by immunohistochemical stains (indicative of HPV-related transformation and increasing reproducibility), might help to elucidate whether the differences between TOMBOLA and ALTS are indeed related to overcalling of pathology.

### Disclosure of interest
All authors declare not to have a conflict of interest regarding assays discussed in the paper.

### Contribution to authorship
None.

### Details of ethics approval
None.

### Funding
Financial support was received from: (1) the European Commission (Directorate of SANCO, Luxembourg, Grand-Duchy of Luxembourg), through the ECCG (European Co-operation on development and implementation of Cancer screening and prevention Guidelines, IARC, Lyon, France); (2) the Belgian Foundation Against Cancer; (3) IWT (Institute for the Promotion of Innovation by Science and Technology in Flanders, project number 060081), Brussels, Belgium; (4) the Belgian Cancer Centre; and (5) from the Intramural Research Program of the National Institutes of Health, National Cancer Institute, Bethesda, MD, USA.

### Acknowledgements
None. ■

## References

1 Arbyn M, Sasieni P, Meijer CJ, Clavel C, Koliopoulos G, Dillner J. Clinical applications of HPV testing: a summary of meta-analyses. *Vaccine* 2006;24(Suppl. 3):S78–S89.
2 Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. *Int J Gynecol Pathol* 1993;12:186–92.
3 McCredie MR, Sharples KJ, Paul C, Baranyai J, Medley G, Jones RW, *et al.* Natural history of cervical neoplasia and risk of invasive cancer in women with cervical intraepithelial neoplasia 3: a retrospective cohort study. *Lancet Oncol* 2008;9:425–34.
4 Cotton S, Sharp L, Little J, Cruickshank M, Seth R, Smart L, *et al.* The role of human papillomavirus testing in the management of women with low-grade abnormalities: multicentre randomised controlled trial. *BJOG* 2010; DOI: 10.1111/j.1471-0528.2010.02519.x [E-pub ahead of print].
5 Arbyn M, Buntinx F, Van Ranst M, Paraskevaidis E, Martin-Hirsch P, Dillner J. Virologic versus cytologic triage of women with equivocal Pap smears: a meta-analysis of the accuracy to detect high-grade intraepithelial neoplasia. *J Natl Cancer Inst* 2004;96:280–93.
6 Arbyn M, Paraskevaidis E, Martin-Hirsch P, Prendiville W, Dillner J. Clinical utility of HPV DNA detection: triage of minor cervical lesions, follow-up of women treated for high-grade CIN. An update of pooled evidence. *Gynecol Oncol* 2005;99(Suppl. 3):7–11.
7 Sherman ME, Schiffman MA, Cox JT. Effects of age and human papilloma viral load on colposcopy triage: data from the randomised atypical squamous cells of undetermined significance/low-grade intraepithelial lesion triage study (ALTS). *J Natl Cancer Inst* 2002;94:102–7.
8 Arbyn M, Martin-Hirsch P, Buntinx F, Van Ranst M, Paraskevaidis E, Dillner J. Triage of women with equivocal or low-grade cervical cytology results. A meta-analysis of the HPV test positivity rate. *J Cell Mol Med* 2009;13:648–59.
9 TOMBOLA Group. Cytological surveillance compared with immediate referral for colposcopy in management of women with low grade cervical abnormalities: multicentre randomised controlled trial. *BMJ* 2009;339:b2546–57.
10 ASCUS-LSIL Triage Study Group. Results of a randomized trial on the management of cytology interpretations of atypical squamous cells of undetermined significance. *Am J Obstet Gynecol* 2003;188:1383–92.
11 Cuzick J, Szarewski A, Cubie H, Hulman G, Kitchener HC, Luesley D, *et al.* Management of women who test positive for high-risk types of human papillomavirus: the HART study. *Lancet* 2003;362:1871–6.
12 Bais AG, Rebolj M, Snijders PJ, De Schipper FA, van der Meulen DA, Verheijen RH, *et al.* Triage using HPV-testing in persistent borderline and mildly dyskaryotic smears: proposal for new guidelines. *Int J Cancer* 2005;116:122–9.


 