

Accuracy of liquid based versus conventional cytology: overall results of new technologies for cervical cancer screening: randomised controlled trial

Guglielmo Ronco,¹ Jack Cuzick,¹² Paola Pierotti,⁸ Maria Paola Cariaggi,³ Paolo Dalla Palma,⁴ Carlo Naldoni,⁷ Bruno Ghiringhello,² Paolo Giorgi-Rossi,¹⁰ Daria Minucci,⁶ Franca Parisio,² Ada Pojer,⁴ Maria Luisa Schiboni,¹¹ Catia Sintoni,⁹ Manuel Zorzi,⁵ Nereo Segnan,¹ Massimo Confortini,³ and the New Technologies for Cervical Cancer Screening (NTCC) Working Group

EDITORIAL by Denton
RESEARCH pp 31, 35

¹Unit of Cancer Epidemiology, Centre for Cancer Prevention, Via S Francesco da Paola 31 10123 Turin, Italy

²S Anna Hospital, Turin, Italy

³Scientific Institute for Cancer Prevention of Tuscany Region, Florence, Italy

⁴S Chiara Hospital, Trento, Italy

⁵Venetian Tumour Registry, Istituto Oncologico Veneto, Padua, Italy

⁶Unit of Obstetrics and Gynaecology, University Hospital, Padua, Italy

⁷Emilia-Romagna Region, Bologna, Italy

⁸Maggiore Hospital, AU SL Bologna, Italy

⁹S Maria delle Croci Hospital, Ravenna, Italy

¹⁰Health Technologies Unit, Agency for Public Health Lazio Region, Rome, Italy

¹¹S Giovanni Hospital, Rome, Italy

¹²Cancer Research UK Clinical Centre, Epidemiology, Mathematics and Statistics, Queen Mary's School of Medicine and Dentistry, London

Correspondence to: G Ronco
guglielmo.ronco@cpo.it

BMJ 2007;335:28-31
doi:10.1136/bmj.39196.740995.BE

This article is an abridged version of a paper that was published on bmj.com on 21 May 2007. Cite this version as: *BMJ* 21 May 2007, doi: 10.1136/bmj.39196.740995.BE (abridged text, in print: *BMJ* 2007;335:28-31).

ABSTRACT

Objective To compare the accuracy of conventional cytology with liquid based cytology for primary screening of cervical cancer.

Design Randomised controlled trial.

Setting Nine screening programmes in Italy.

Participants Women aged 25-60 attending for a new screening round: 22 466 were assigned to the conventional arm and 22 708 were assigned to the experimental arm.

Interventions Conventional cytology compared with liquid based cytology and testing for human papillomavirus.

Main outcome measure Relative sensitivity for cervical intraepithelial neoplasia of grade 2 or more at blindly reviewed histology, with atypical cells of undetermined significance or more severe cytology considered a positive result.

Results In an intention to screen analysis liquid based cytology showed no significant increase in sensitivity for cervical intraepithelial neoplasia of grade 2 or more (relative sensitivity 1.17, 95% confidence interval 0.87 to 1.56) whereas the positive predictive value was reduced (relative positive predictive value v conventional cytology 0.58, 0.44 to 0.77). Liquid based cytology detected more lesions of grade 1 or more (relative sensitivity 1.68, 1.40 to 2.02), with a larger increase among women aged 25-34 (P for heterogeneity 0.0006), but did not detect more lesions of grade 3 or more (relative sensitivity 0.84, 0.56 to 1.25). Results were similar when only low grade intraepithelial lesions or more severe cytology were considered a positive result. No evidence was found of heterogeneity between centres or of improvement with increasing time from start of the study. The relative frequency of women with at least one unsatisfactory result was lower with liquid based cytology (0.62, 0.56 to 0.69).

Conclusion Liquid based cytology showed no statistically significant difference in sensitivity to conventional cytology for detection of cervical intraepithelial neoplasia of grade 2 or more. More positive results were found, however, leading to a lower positive predictive value. A large reduction in unsatisfactory smears was evident.

Trial registration Current Controlled Trials
ISRCTN81678807.

INTRODUCTION

Liquid based cytology is used widely for primary screening of cervical cancer but high quality studies on its accuracy are limited. We carried out a large randomised trial, on new technologies for cervical cancer screening, including liquid based cytology in women aged 25-60. We also studied the influence of women's age and interpreter's experience on the accuracy of liquid based cytology.

METHODS

We carried out a randomised controlled trial in nine cervical cancer screening centres in Italy, which routinely invite women aged 25-64 for tests every three years. The methods have been described.¹² Briefly, during 2002-3 women aged 25-60 attending for a new screening round were alternately randomised to conventional cytology or to liquid based cytology and testing for human papillomavirus (see bmj.com).

A standard slide was prepared for women in the conventional arm. Cervical cells from women in the experimental arm were put into PreservCyt solution (ThinPrep; Cytec, Boxborough, MA) and used to make liquid based slides and to test for human papillomavirus. The slides were read by cytologists in 14 laboratories, without knowledge of the virus results. Those showing abnormal results were reviewed before the women were informed.

Women in the experimental arm were referred to colposcopy if cytology showed atypical cells of undetermined significance or more severe results. Women with normal cytology but positive for human papillomavirus were referred directly to colposcopy if they were aged 35-60. Women in the conventional arms were referred for colposcopy if cytology showed atypical cells of undetermined significance or more severe results, except in two centres (see bmj.com).

In each centre the same colposcopists examined women in both arms and had access to the patient's results.

Our primary end point was histologically confirmed cervical intraepithelial neoplasia of grade 2 or more detected during the first phase as a result of abnormal cytology. Operationally we included lesions detected within one year from referral to colposcopy. The biopsy results for women with a histologically confirmed cervical intraepithelial neoplasia were reviewed independently and blindly to study arm and cytology result.¹

Statistical analysis

We calculated the relative frequency of cytology results, including unsatisfactory results in the experimental arm compared with the conventional arm. We estimated the relative sensitivity of liquid based cytology compared with conventional cytology as the relative detection rate compared with the conventional arm. We included all randomised eligible women in an intention to screen analysis. The relative positive predictive value of liquid based cytology compared with conventional cytology was calculated only for women who underwent colposcopy. We calculated confidence intervals using methods appropriate for ratios of independent proportions. We also calculated relative sensitivity and positive predictive value when considering only low grade intraepithelial lesions or more severe cytology as a positive result (cut-off point was low grade intraepithelial lesions).

We considered potential effect modifiers of relative sensitivity and relative positive predictive value: age,

centre, protocol for dealing with women with atypical cells of undetermined significance in the conventional arm, and experience with liquid based cytology. We tested for heterogeneity across different groups using the Breslow-Day test. In addition we studied the learning effect by considering the women's date of recruitment as a modifier with linear effect, using unconditional logistic regression and adjusting by centre. SAS software version 8.2 was used for analyses. P values are two sided.

RESULTS

Overall, 22 466 women were randomised to conventional cytology and 22 708 to liquid based cytology (see bmj.com). The median age was 41. In total, 49% of women in both arms (10 906 and 11 149) had a smear test registered in a programme within the past four years.

Three hundred and one women in the experimental arm (1.3%) had conventional cytology. At least one colposcopy was carried out in 93% (1998/2154) of women referred because of abnormal cytology results: 91% (661/724) in the conventional arm and 93% (1337/1430) in the experimental arm. Among women attending for colposcopy the mean number of colposcopies and mean number of biopsies in the conventional arm were 1.33 (SD 0.53) and 0.76 (0.90) and in the experimental arm were 1.33 (0.52) and 0.74 (0.94).

The overall proportion of women with at least one unsatisfactory cytology result was significantly reduced with liquid based cytology (see bmj.com). This reduction was larger in women aged 25-34

Relative sensitivity and relative positive predictive value of experimental (mainly liquid based cytology) compared with conventional arm (conventional cytology) for different histologically confirmed end points and with different cut-off points according to cytology result. Values are percentages (numbers) of women unless stated otherwise

	Histological cervical intraepithelial neoplasia end point		
	Grade 1 or more	Grade 2 or more	Grade 3 or more
Positive if cytology shows atypical cells of undetermined significance or more			
Detection rate:			
Conventional group	0.82 (184)	0.37 (84)	0.24 (53)
Liquid based group*	1.38 (313)	0.44 (99)	0.20 (45)
Relative sensitivity† (95% CI)	1.68 (1.40 to 2.02)	1.17 (0.87 to 1.56)	0.84 (0.56 to 1.25)
Positive predictive value:			
Conventional group	27.84	12.7	8.02
Liquid based group*	23.41	7.4	3.37
Relative positive predictive value† (95% CI)	0.84 (0.72 to 0.98)	0.58 (0.44 to 0.77)	0.42 (0.29 to 0.62)
Positive if cytology shows low grade intraepithelial lesions or more			
Detection rate:			
Conventional group	0.55 (123)	0.31 (70)	0.20 (44)
Liquid based group*	0.95 (211)	0.32 (73)	0.14 (32)
Relative sensitivity† (95% CI)	1.70 (1.36 to 2.12)	1.03 (0.74 to 1.43)	0.72 (0.46 to 1.13)
Positive predictive value:			
Conventional group	38.80	22.08	13.88
Liquid based group*	36.76	12.72	5.57
Relative positive predictive value† (95% CI)	0.95 (0.80 to 1.13)	0.58 (0.43 to 0.78)	0.40 (0.26 to 0.62)

For detection rate and relative sensitivity denominators are 22 466 women in conventional group and 22 708 in liquid based group.

For positive predictive values and relative positive predictive values denominators are women with positive cytology for atypical cells of undetermined significance who had had colposcopy: 661 in conventional group and 1337 in liquid based group.

*Only cases of cervical intraepithelial neoplasia detected by cytology considered.

†Ratio of percentages. Liquid based cytology compared with conventional cytology.

WHAT IS ALREADY KNOWN ON THIS TOPIC

Despite the widespread use of liquid based cytology only a few high quality studies and no large randomised trial have examined its diagnostic accuracy
Systematic reviews have produced conflicting conclusions

WHAT THIS STUDY ADDS

Liquid based cytology shows no significant difference in sensitivity to conventional cytology
A significant reduction in positive predictive value was, however, apparent

(relative frequency *v* conventional cytology 0.53, 95% confidence interval 0.44 to 0.63) than in women aged 35-60 (0.67, 0.59 to 0.76). The reduction was large for results considered unsatisfactory because of obscuring inflammation (see [bmj.com](#)).

The proportion of women with atypical squamous cells of undetermined significance or atypical glandular cells of undetermined significance and low and high grade squamous intraepithelial lesions was significantly increased in the experimental arm (see [bmj.com](#)). The increase in atypical squamous or atypical glandular cells of undetermined significance was larger in women aged 25-34 (relative frequency *v* conventional cytology 1.92, 1.56 to 2.36) than in women aged 35-60 (1.44, 1.27 to 1.64).

No significant increase was observed in sensitivity for cervical intraepithelial neoplasia of grade 2 or more for liquid based cytology compared with conventional cytology with either atypical cells or low grade lesions as cut-off points (table). The positive predictive value, however, was significantly reduced for liquid based cytology when using any end point or cut-off point (table). When the analysis was restricted to centres referring all women with atypical cells directly to colposcopy, using such cells as the cut-off point and cervical intraepithelial neoplasia of grade 2 or more as the end point the relative sensitivity was 1.11 and the relative positive predictive value was 0.65.

Liquid based cytology showed an increased sensitivity for cervical intraepithelial neoplasia of grade 1 or more when atypical cells of undetermined significance or more severe cytology were considered and when low grade lesions were the cut-off point (table). This increased sensitivity was larger in younger women ($P=0.0006$ with atypical cells as cut-off point and $P=0.02$ with low grade lesions as cut-off point): with atypical cells as the cut-off point the relative sensitivity was 2.21 (95% confidence interval 1.67 to 2.91) in women aged 25-34 and 1.33 (1.04 to 1.70) in women aged 35-60. The corresponding values with low grade lesions as the cut-off point were 2.23 (1.60 to 3.11) and 1.32 (0.98 to 1.78). No increased sensitivity was observed with cervical intraepithelial neoplasia of grade 3 or more as the end point.

No significant heterogeneity was observed between centres for relative sensitivity or relative positive predictive value when cervical intraepithelial neoplasia of grades 2 or more or 3 or more were used as end points. When centres were compared according to experience with liquid based cytology significant variability was not found for relative sensitivity or for relative positive

predictive values. In addition, after adjusting for centre no significant effect was observed for the interval from the start of the study either on relative sensitivity or relative positive predictive value with any end point or cut-off point. When the analysis was restricted to laboratories with experience of ThinPrep, the relative sensitivity and relative positive predictive value with atypical cells of undetermined significance as the cut-off point and cervical intraepithelial neoplasia of grade 2 or more as the end point were 1.12 and 0.63, respectively. When only the second half of enrolment in each centre was considered the corresponding values were 1.04 and 0.54.

DISCUSSION

Liquid based cytology for primary screening of cervical cancer showed a significantly increased sensitivity for cervical intraepithelial neoplasia of grade 1 but not for grade 3 or more. We observed a relevant reduction in unsatisfactory slides with liquid based cytology as a result of a decrease in obscuring inflammation. We observed a slight increase of the point estimate for relative sensitivity of liquid based cytology compared with conventional cytology for cervical intraepithelial neoplasia of grade 2 or more (1.17 with atypical cells of undetermined significance as the cut-off point, 1.03 with low grade intraepithelial lesions as the cut-off point) but this was far from significant. Values between 0.87 and 1.56 were included in the 95% confidence interval of relative sensitivity with atypical cells of undetermined significance as the cut-off point. Therefore we cannot exclude increases and decreases of sensitivity in this range. We carried out a large randomised trial nested in screening programmes, which was representative of routine activity. Colposcopy was carried out in a high proportion of women whose screening results were positive. Colposcopists were not blinded to type of cytology, but the number of biopsies per woman undergoing colposcopy was similar in the two arms. Histology was independently reviewed, with reviewers blinded to trial arm and cytology result. A few centres adopted a different protocol between study arms for the management of women with atypical cells of undetermined significance. However, the results were almost unchanged when the analysis was restricted to the centres that applied the same protocol in both arms and when low grade intraepithelial lesions were considered as the cut-off point for cytology.

We also found that liquid based cytology had a lower positive predictive value than conventional cytology. This reduction was the result of an increased frequency of abnormal findings (usually low grade) without an increase in high grade lesions on histology. An increased frequency of low grade lesions with liquid based cytology has already been observed.³ The relative frequency of atypical cells of undetermined significance varied between studies, but overall in high and medium quality studies more slides were classified as atypical cells by liquid based cytology.³

Our data do not show evidence of heterogeneity between the centres. We also did not observe any

difference in the performance of liquid based cytology compared with conventional cytology according to experience with liquid based cytology, nor with increasing experience in the study. Also, point estimates changed little when we restricted the analysis to centres with experience of ThinPrep, or to the second half of enrolment in each centre.

On the basis of this analysis, the main advantage of moving to liquid based cytology is a reduction in the rate of unsatisfactory slides. Other advantages are the shorter time needed for interpretation^{4,5} and using the same sample for testing for human papillomavirus and for other molecular tests.

We thank the staff who helped with the study and the women who participated in this study.

Contributors: See bmj.com.

Funding: European Union (Europe against cancer contracts SI.2.327046 and SPC.2002475), Italian Ministry of Health (applied research projects and L 138/2004) Compagnia di S Paolo FIRMS, Regione Piemonte, Regione Toscana, Regione Veneto, Regione Emilia-Romagna, Agenzia di Sanità Pubblica, Regione Lazio. Funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Competing interests: JC is a member of the speaker's bureau for Digene. His institution (Cancer Research UK) has received research funding from Roche Diagnostics for a different study. MC is the principal recipient of a grant to the Scientific Institute for Cancer Prevention of Tuscany Region from Menarini Diagnostics. This grant is for a different study.

Ethical approval: This study was approved by the local research ethics committees of the participating centres.

- 1 Ronco G, Segnan N, Giorgi-Rossi P, Zappa M, Casadei GP, Carozzi F, et al. Human papillomavirus testing and liquid-based cytology: results at recruitment from the New Technologies for Cervical Cancer randomized controlled trial. *J Natl Cancer Inst* 2006;98:765-74.
- 2 Ronco G, Giorgi-Rossi P, Carozzi F, Dalla Palma P, Del Mistro A, De Marco L, et al. Human papillomavirus testing and liquid-based cytology in primary screening of women younger than 35 years: results at recruitment for a randomised controlled trial. *Lancet Oncol* 2006;7:547-55.
- 3 Davey E, Barrat A, Irwig L, Chan SF, Macaskill P, Mannes P, et al. Effect of study design and quality on unsatisfactory rates, cytological classification, and accuracy in liquid-based versus conventional cervical cytology: a systematic review. *Lancet* 2006;367:122-32.
- 4 Sherman ME, Mendoza M, Lee KR, Ashfaq R, Birdsong GG, Corkill ME, et al. Performance of liquid-based, thin layer cervical cytology: correlation with reference diagnoses and human papillomavirus testing. *Mod Pathol* 1998;11:837-43.
- 5 Lavery CR, Farnsworth A, Thurloe JK, Grieves A, Bowditch R. Evaluation of the CytoRich slide preparation process. *Anal Quant Histol* 1997;19:239-45.

Accepted: 1 April 2007

Accuracy of reading liquid based cytology slides using the ThinPrep Imager compared with conventional cytology: prospective study

Elizabeth Davey,¹ Jefferson d'Assuncao,¹ Les Irwig,¹ Petra Macaskill,¹ Siew F Chan,¹ Adele Richards,² Annabelle Farnsworth²

EDITORIAL by Denton
RESEARCH pp 28, 35

¹Screening and Test Evaluation Program, School of Public Health, University of Sydney, NSW 2006, Australia

²Cytology Department, Douglass Hanly Moir Pathology, North Ryde, NSW, Australia

Correspondence to: E Davey
daveye@health.usyd.edu.au

BMJ 2007;335:31-5
doi:10.1136/bmj.39219.645475.55

This article is an abridged version of a paper that was published on bmj.com on 29 June 2007. Cite this version as: *BMJ* 2007;335:31-5.
doi: 10.1136/bmj.39219.645475.55 (abridged text, in print: *BMJ* 2007;335:31-5).

ABSTRACT

Objective To compare the accuracy of liquid based cytology using the computerised ThinPrep Imager with that of manually read conventional cytology.

Design Prospective study.

Setting Pathology laboratory in Sydney, Australia.

Participants 55 164 split sample pairs (liquid based sample collected after conventional sample from one collection) from consecutive samples of women choosing both types of cytology and whose specimens were examined between August 2004 and June 2005.

Main outcome measures Primary outcome was accuracy of slides for detecting squamous lesions. Secondary outcomes were rate of unsatisfactory slides, distribution of squamous cytological classifications, and accuracy of detecting glandular lesions.

Results Fewer unsatisfactory slides were found for imager read cytology than for conventional cytology (1.8% v 3.1%; P<0.001). More slides were classified as abnormal by imager read cytology (7.4% v 6.0% overall and 2.8% v 2.2% for cervical intraepithelial neoplasia of grade 1 or higher). Among 550 patients in whom imager read cytology was cervical intraepithelial neoplasia grade 1 or higher and conventional cytology was less

severe than grade 1, 133 of 380 biopsy samples taken were high grade histology. Among 294 patients in whom imager read cytology was less severe than cervical intraepithelial neoplasia grade 1 and conventional cytology was grade 1 or higher, 62 of 210 biopsy samples taken were high grade histology. Imager read cytology therefore detected 71 more cases of high grade histology than did conventional cytology, resulting from 170 more biopsies. Similar results were found when one pathologist reread the slides, masked to cytology results.

Conclusion The ThinPrep Imager detects 1.29 more cases of histological high grade squamous disease per 1000 women screened than conventional cytology, with cervical intraepithelial neoplasia grade 1 as the threshold for referral to colposcopy. More imager read slides than conventional slides were satisfactory for examination and more contained low grade cytological abnormalities.

INTRODUCTION

Liquid based cytology for cervical smears is replacing conventional cytology in many countries yet evidence is insufficient to confirm that it is more accurate than conventional cytology.¹