# ORIGINAL ARTICLE

# Detection of telomerase, its components, and human papillomavirus in cervical scrapings as a tool for triage in women with cervical dysplasia

N Reesink-Peters, M N Helder, G B A Wisman, A J Knol, S Koopmans, H M Boezen, E Schuuring, H Hollema, E G E de Vries, S de Jong, A G J van der Zee

...................................................................................................................

See end of article for authors' affiliations
.......................

Correspondence to:
Dr A G J van der Zee,
University Hospital
Groningen, The
Netherlands;
a.g.j.van.der.zee@og.azg.nl

**Aim:** To examine whether the detection of either telomerase and its components or high risk human papillomavirus (HPV) are of value in predicting the presence of cervical intraepithelial neoplasia (CIN) grade II/III in women referred because of cervical cytology reports showing at most moderate dyskaryosis.

**Methods:** Cervical scrapings of 50 women referred with cytological borderline, mild, or moderate dyskaryosis were analysed. Telomerase activity was assessed by a commercially available telomere repeat amplification protocol assay and its components human telomerase RNA (hTR) and human telomerase reverse transcriptase (hTERT) were assessed by reverse transcriptase polymerase chain reaction (PCR). HPV was detected by GP5+/6+ PCR enzyme immunosassay. Histological findings on colposcopy guided biopsies or excised cervical tissue were regarded as the final pathological diagnosis. The sensitivity and specificity for detecting CIN II/III were calculated.

**Results:** Twenty eight women were diagnosed with CIN II/III. Telomerase activity was detected in none, hTR in 88%, hTERT in 23%, and high risk HPV was detected in 79% of these women. As a diagnostic test none of the described analyses combined a sensitivity of at least 90% with a specificity ≥ 90%. Despite the small numbers, calculation of the 95% confidence intervals excluded a combined sensitivity and specificity of at least 90% for all of the evaluated parameters.

**Conclusions:** Neither detection of telomerase or its components, nor detection of high risk HPV seem suitable for the triage of women with borderline, mild, and moderate cytological dyskaryosis.

C ervical cancer, which develops from cervical intraepithelial neoplasia (CIN), is an important cause of death in women worldwide.[1] A CIN lesion can either regress, persist, or progress towards (micro)invasive carcinoma. Most low grade CIN lesions (CIN I) will regress, whereas in the long term 12–40% of high grade CIN lesions (CIN II/III) progress to squamous cell carcinoma.[2][3] Because there are no markers to identify those lesions that will progress, clinicians have felt compelled to treat at least all CIN II/III lesions.

Cytomorphological examination of cervical smears is the most widely applied screening method for cervical cancer and its precursors. The disadvantages are the high numbers of false negative and false positive cervical smears. Cervical cytology alone is a good predictor for the presence of CIN II/III when it shows severe dyskaryosis or carcinoma in situ. CIN II/III or cancer was found in 89–93% of women with these severely abnormal smears.[4][5] In contrast, 51–58% of women with mild or moderate dyskaryosis on cytology are diagnosed with CIN II/III.[5][6] All of the women with cytological mild or moderate dyskaryosis are subjected to colposcopic evaluation, implying an overshoot of diagnostic procedures.[7][8] Thus, there is a need for parameters in cervical scrapings that could more accurately predict the presence of CIN II/III or cancer in women with borderline, mild, or moderate dyskaryotic smears.

> "There is a need for parameters in cervical scrapings that could more accurately predict the presence of cervical intraepithelial neoplasia II/III or cancer in women with borderline, mild, or moderate dyskaryotic smears"

Although it has been suggested that high risk human papillomavirus (HPV) testing may improve cervical cancer screening,[9][10] its role in the triage of women with cytological borderline, mild, or moderate dysplasia is less clear.

Another possible parameter that is reported on is telomerase activity assessment by the polymerase chain reaction (PCR) based telomere repeat amplification protocol (TRAP) assay.[11][12] Telomerase is an enzyme that replenishes short stretches of repeat nucleotides lost from the telomeric ends of chromosomes with each round of replication. Studies in both tumour cell lines and human tumour specimens have shown that, in contrast to normal somatic cells, most malignant cells (> 90%) are characterised by increased telomerase activity.[11][13] Therefore, the determination of telomerase activity has been suggested for early cancer detection. In a previous study by our group it was shown that although telomerase activity was associated with the severity of cervical neoplasia, it was only detected in 27% of scrapings from women with CIN II/III and cervical cancer.[14] In contrast, Reddy *et al* detected telomerase activity in 96.5% of cervical cancer samples and in 68.7% of premalignant cervical scrapings.[11] In a previous study by our group and in the study of Reddy *et al* telomerase activity was detected by a non-commercially available TRAP assay.

Low sensitivity has been found for telomerase activity assessment in urine and screening for bladder cancer. However, when the detection of the human telomerase RNA

...........................................................

component (hTR) was performed, sensitivity for detecting bladder cancer increased.[15] In another study, hTR detected by in situ hybridisation in frozen cervical samples was related to the grade of CIN.[16] We therefore speculate that the detection of hTR in cervical scrapings might be a more sensitive alternative to telomerase activity assessment in screening for CIN II/III. Another option could be the detection of human telomerase reverse transcriptase mRNA (hTERT) in cervical scrapings. hTERT is the catalytic subunit of telomerase, which is thought to be the rate limiting component in the formation of functional telomerase.[17–20]

The aim of our present study was to examine whether the detection of telomerase activity hTR, hTERT, and HPV in cervical scrapings has clinical value in the triage of women referred because of cytological borderline, mild, or moderate dyskaryosis.

## PATIENTS AND METHODS
### Patients
Patients were recruited from the outpatient clinic of the department of gynaecology, University Hospital Groningen, the Netherlands. All patients referred by their general practitioner in the period May 1999 to August 2000 because of a cervical cytology report showing borderline or mild dyskaryosis twice or with a single moderately dyskaryotic smear were eligible for participation in our study. In the Netherlands, cervical smears are classified according to a modified Papanicolaou system in which no atypical squamous cells of undetermined significance diagnosis exists.[21] Instead, a cervical smear can be classified as borderline dyskaryotic, but the two terms do not necessarily describe identical abnormalities. Exclusion criteria were previous colposcopic examination because of an abnormal cervical cytology report and pregnancy at the time of the diagnostic or therapeutic procedure.

All patients were asked to participate in our study during their initial visit to the outpatient clinic. All specimens used for our study were collected during the initial visit, before diagnostic procedures. All patients underwent colposcopically directed biopsies. CIN was diagnosed and graded according to international criteria.[22] If CIN II or III was diagnosed, the transformation zone was excised four to six weeks later by diathermic loop excision. Cervical neoplasia was classified according to the most severe lesion found on histological examination of the biopsy and loop excision specimen, and categorised into low grade lesions for no dysplasia (CIN 0) and CIN I and high grade lesions for CIN II and III. This categorisation corresponds well with the classification of low grade and high grade squamous intraepithelial lesions, which is also widely used. The study protocol was approved by the medical ethical committee of the University Hospital Groningen. All patients gave informed consent.

### Cervical scrapings
The cervix of all eligible women was scraped with the blunt or pointed end of an Ayre's spatula and with an endocervical brush. The scraped cells were suspended in 5 ml ice cold phosphate buffered saline (6.4mM $Na_2HPO_4$, 1.5mM $KH_2PO_4$, 0.14M NaCl, and 2.7mM KCl (pH 7.2)) and kept on ice until further processing. Of this cell suspension, 4 ml was centrifuged and washed with wash buffer (10mM Hepes/KOH (pH 7.5), 1.5mM $MgCl_2$, 10mM KCl, and 1mM dithiothreitol), as described previously,[14] after which half of the pellet was snap frozen in liquid nitrogen and then stored at −80°C until used for the telomerase activity assessment by means of the TRAP assay. The other half of the pellet was suspended in 500 μl guanidine isothiocyanate (GT) buffer, quickly frozen in liquid nitrogen, and stored at −80°C until analysed by reverse transcription (RT) PCR.

### TRAP assay
The TRAP assay was performed with the TRAPeze XL telomerase detection kit (Intergen Company, Purchase, New York, USA) in accordance with the manufacturer's instructions. Lysis of the pellet was performed as described previously.[16] All samples were tested in duplicate and average results were categorised as positive when peaks represented ≥ 10 GLC4 (a human small cell lung cancer cell line) cell equivalents (the measurement comparable to the normalised fluorescence of 10 GLC4 cell equivalents is 10 U/μg protein).

### HPV analysis
DNA was prepared from the pellets of scrapings, obtained after extraction of proteins with TRAP lysis buffer using the GT diatom procedure and dissolved in 100 μl of 10mM Tris/HCl and 1mM EDTA (pH 7.0). This procedure did not affect the HPV PCR results of cervical carcinoma cell lines with known HPV status, namely: Hela S3, Caski, SiHa, and C33a (results not shown). The GP5+/6+ PCR enzyme immunoassay for high risk HPV detection was performed as described previously.[23]

### RT-PCR for hTR and hTERT
The isolation of RNA and RT-PCR were performed as described previously by Wisman *et al*.[16] PCR was performed separately for hTERT mRNA (35 cycles), hTR (35 cycles), and a housekeeping mRNA, glyceraldehyde-3-phosphatase dehydrogenase (GAPDH; 30 cycles). GAPDH, hTR, and hTERT values in 1 μg total RNA of GLC4 cells were set at 100%. Expression in the cervical samples was related to the expression levels in GLC4 cells, after which the degree of expression was normalised to the housekeeping gene GAPDH. The expression of hTR and hTERT was categorised as follows: no expression, negative; expression < 10% of 1 μg total GLC4 RNA, very low; expression between 10% and 75% $GLC_4$, low; expression between 75% and 200% $GLC_4$, moderate; and expression between 200% and 1000% $GLC_4$, high.

### Statistics
The Mann-Whitney-U test was used to test for differences in the degree of expression of hTR and hTERT in women with no dysplasia/CIN I and CIN II/III. Independent associations of HPV and age with the two diagnostic categories were estimated using a multiple logistic regression model. A difference associated with a p value ≤ 0.05 was considered to be significant. Diagnostic test characteristics were calculated by using the proportion of women with CIN 0/I and CIN II/III. The 95% confidence intervals were calculated using the CIA software (©Gardner and British Medical Journal, London, UK). All other analyses were performed using the statistical analysis program SPSS (©SPSS Inc, Chicago, Illinois, USA).

## RESULTS
In the period May 1999 to August 2000, 50 consecutive patients participated in our study. The median age was 37 years (interquartile range (IQR), 31–45). Of the 50 women, 22 were diagnosed with CIN 0/I (no dysplasia, n = 11; CIN I, n = 11) and 28 with CIN II/III (CIN II, n = 17; CIN III, n = 11). The median age of women with CIN 0/I was 43.5 years (IQR, 34.8–48.8) and the median age of women with CIN II/III was 35.5 years (IQR, 30–42.5; p = 0.01). The association between age and the diagnostic categories remained borderline significant (p = 0.065) when adjusted for the presence of HPV. Table 1 shows the final histological diagnosis in relation to the cytology results of the cervical smear at referral.

Table 2 shows the proportion of women with CIN 0/I or CIN II/III positive for telomerase components or high risk HPV. All scrapings could be analysed for the presence of telomerase activity and HPV, although the quality of three cervical scrapings was too poor to analyse with RT-PCR for hTR and hTERT.

**Table 1** Histological diagnosis related to cytology results of cervical smears at referral

| Cytological dysplasia | N | Histology | |
| | | CIN 0/I | CIN II/III |
| --- | --- | --- | --- |
| Borderline | 4 | 4 | |
| Mild | 24 | 12 | 12 |
| Moderate | 22 | 6 | 16 |
| Total | 50 | 22 | 28 |

CIN, cervical intraepithelial neoplasia.

**Table 2** Telomerase components and HPV in cervical scrapings of women referred for a borderline, mild, or moderate dyskaryotic smear

| | Histology | |
| | CIN 0/I n=22 | CIN II/III n=28 |
| --- | --- | --- |
| Telomerase activity | 1(4.5%) | 0 |
| hTR expression | 22(100%) | 22*(88%) |
| hTERT mRNA expression | 4(18%) | 7*(28%) |
| High risk HPV | 11(50%) | 22(79%) |

Telomerase activity was classed as present when 10 U/μg protein was found. *The quality of 3 samples was too poor to analyse. CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; hTERT, human telomerase reverse transcriptase mRNA; hTR, human telomerase RNA.

Telomerase activity was only detected once, in a cervical scraping from a woman with CIN 0/I. Because hTR was detected in 44 of 47 scrapings we analysed whether the frequency of low and high hTR expression levels differed between the two diagnostic categories. In women with CIN 0/I, hTR expression was negative in none, weak in 13, moderate in six, and high in three. In women with CIN II/III, hTR expression was negative in three, weak in eight, moderate in 11, and high in three (p = 0.74). For hTERT, most samples had very low or low expression, with no differences between CIN 0/I and CIN II/III. One sample was found to have moderate expression and one high expression. Both samples were taken from patients with CIN II/III.

Of the six women diagnosed with CIN II/III who were high risk HPV negative four were diagnosed with CIN II and two with CIN III. The 33 HPV positive women were younger (median age, 35 years; IQR, 30–43.5) than the 17 HPV negative women (median age, 44 years; IQR, 33.5–49.5; p = 0.017). The presence of HPV was independently associated with the severity of the histological diagnosis (p = 0.023), after adjusting for age.

We investigated whether the detection of one of the telomerase components or HPV analysis could be used as a diagnostic test by calculating sensitivity, specificity, positive predictive value, and negative predictive value for the detection of CIN II/III (table 3). A combination of high (90% or higher) sensitivity and high specificity was not seen in the tests analysed. The 95% confidence intervals excluded a combined sensitivity and specificity of at least 90% for all the evaluated parameters (table 3).

## DISCUSSION

Recently, there has been much debate in the literature about whether low grade cervical lesions should be diagnosed and treated.[24] Because CIN I is estimated to progress to cervical cancer in only approximately 1% of cases,[2] immediate treatment of these lesions seems too drastic an option to take. A major drawback of identifying women with CIN 0/I during screening is that such women may become worried that they are at risk of developing cervical cancer, when in fact most will never develop this disease.[25] In most cervical screening programmes, patients are referred for colposcopy when cytological atypia or borderline dyskaryosis is found more than once, or directly when cytological abnormalities are more severe. Using this policy, a large number of women with CIN I/0 are referred and need to undergo time consuming and invasive diagnostic procedures. Therefore, a diagnostic tool is needed that can sensitively detect CIN II/III in women with cytological borderline, mild, or moderate dyskaryosis, which also has high specificity, so that few women have to undergo invasive procedures unnecessarily. Such a diagnostic test would best be performed on cervical scrapings, as was done for all of the parameters analysed here.

In our study, CIN II/III was diagnosed in 46% of all patients. Others have found 4.7–26% in comparable groups of women.[4 8 26] The high percentage found in our study probably results from the fact that more than half of the patients were referred because of moderate dyskaryosis and only four women were referred because of a borderline dyskaryotic smear. Furthermore, in the Netherlands women are not referred with a single borderline or mildly dyskaryotic smear but only when such an abnormality is found twice, leading to a higher percentage of women being diagnosed with CIN II/III. Lanham et al found a similar proportion of histologically confirmed CIN II/III in women referred because of moderate dyskaryotic smears.[4]

TRAP assays are commercially available, so that telomerase activity could be assessed routinely. However, we found only one scraping with telomerase activity using a commercially available TRAP assay, indicating that the sensitivity of this assay is too low for the detection of CIN II/III in the clinical laboratory. In a previous study, we used an in house[14] TRAP assay that detected telomerase activity more frequently. Nonetheless, even this in house TRAP assay was not sensitive enough because telomerase activity was found in only seven cervical scrapings from 48 women with cytological borderline,

**Table 3** Test characteristics of telomerase components and HPV when used for the detection of CIN II/III in women referred because of cervical cytology reports showing at most moderate dyskaryosis

| | Test characteristics in % (95% confidence interval) | | | |
| | Sensitivity | Specificity | PPV | NPV |
| --- | --- | --- | --- | --- |
| Telomerase activity | 0 (0 to 12) | 95 (77 to 100) | 0 | 43 (29 to 58) |
| hTR expression | 88 (69 to 98) | 0 (0 to 15) | 50 (35 to 65) | 0 (0 to 71) |
| hTERT mRNA expression | 28 (12 to 49) | 78 (60 to 95) | 58 (31 to 89) | 49 (33 to 67) |
| High risk HPV | 79 (59 to 92) | 50 (28 to 72) | 67 (48 to 82) | 65 (38 to 86) |

Telomerase activity was classed as present when >10 U/μg protein was found. CIN, cervical intraepithelial neoplasia; HPV, human papillomavirus; hTERT, human telomerase reverse transcriptase mRNA; hTR, human telomerase RNA; NVP, negative predictive value; PPV, positive predictive value.

mild, or moderate dyskaryosis who had histologically confirmed CIN II/III. In a review of the recent literature, Nowak[27] showed that studies using cytology specimens of more than 10 women reported telomerase activity detection rates of 0–11% in normal women, 12–31% in low grade lesions, 6–66% in high grade lesions, and 31–100% in women with cervical cancer. In the same review, detection percentages in frozen tissue samples were reported to be between 0% and 92% for normal tissue, 17% and 96% for high grade lesions, and 82% and 100% for cervical cancer tissue. These widely varying numbers show that the assessment of telomerase activity is too variable and its sensitivity and specificity are too low to be clinically useful for the detection of CIN II/III or cervical cancer.

We found low specificities in predicting CIN II/III for both hTR (100% positive in CIN 0/I) and hTERT (18% positive in CIN 0/I) analyses in cervical scrapings. Low specificity of hTR assessment for the detection of CIN II/III has been reported previously.[4] Snijders et al found hTERT mRNA expression in 40% of CIN 0/I tissue samples, also demonstrating low specificity.[28] Lanham et al used cytology specimens for the detection of hTERT and found positivity in only 6% of CIN 0/I cases and in none of the 40 women with CIN II/III.[4]

It is known that the prevalence of HPV declines with age.[29] Therefore, our finding that HPV negative women were older than HPV positive women was not surprising. However, we did not expect women with CIN 0/I to be older than those with CIN II/III. This difference in age might be explained by the lower proportion of HPV positive women in the CIN 0/I group. The association between age and diagnosis was only borderline significant when adjusted for the presence of HPV. Another explanation for the difference in age between the two diagnostic categories might be that reactive changes in cervical smears, mistaken for cervical dyskaryosis, are more frequent in older women, leading to a high percentage of CIN 0/I in older women.

> "We found low specificities in predicting cervical intraepithelial neoplasia II/III for both human telomerase RNA and human telomerase reverse transcriptase mRNA analyses in cervical scrapings"

HPV studies in women with cervical smears showing more than mild dyskaryosis, as presented here, are rare. In a Dutch non-intervention study,[30] high risk HPV was found in 182 of 297 (61%) women referred for mild or moderate dyskaryosis. This corresponds well to the 66% of positives found in our study population. HPV testing as a screening tool for women at risk for CIN II/III has been studied extensively in women with cervical cytology showing atypical squamous cells of undetermined significance (this corresponds best with borderline dyskaryosis) or mild dyskaryosis. The ALTS trial (randomised atypical squamous cells of undetermined significance/low grade squamous intraepithelial lesion triage study) showed that HPV DNA testing represents a promising approach for colposcopy triage of atypical squamous cells of undetermined significance, but not for low grade squamous intraepithelial lesions.[10] High risk HPV testing in women with cervical cytology showing low grade squamous intraepithelial lesions had good sensitivity but low specificity for the detection of CIN III. It has been suggested that the specificity of high HPV viral load is higher than the specificity of HPV presence alone because viral load is associated with the severity of the histological diagnosis.[29] However, Sun et al reported that specificity remains low because high HPV viral load is also found in 12.5% of women without dysplasia and in 25% of women with low grade lesions.[31] Therefore, we think that HPV detection is not useful in the triage of women with cytological borderline, mild, or moderate dyskaryosis.

We realise that the number of patients in our study is low, leading to broad 95% confidence intervals for the calculated

## Take home messages

- In the 28 women diagnosed with cervical intraepithelial neoplasia (CIN) II/III, telomerase activity was detected in none, human telomerase RNA in 88%, human telomerase reverse transcriptase mRNA in 23%, and high risk human papillomavirus (HPV) in 79%
- As a diagnostic test none of these analyses combined a sensitivity of at least 90% with a specificity ⩾ 90%
- Despite the small numbers, calculation of the 95% confidence intervals excluded a combined sensitivity and specificity of at least 90% for all of the parameters evaluated
- Thus, neither detection of telomerase or its components, nor detection of high risk HPV are suitable for the triage of women with borderline, mild, and moderate cytological dyskaryosis

test characteristics (table 3). However, for all of the presented tests the calculated 95% confidence intervals exclude the possibility for a test to combine 90% sensitivity with 90% specificity. This shows that the chance that one of the tests will prove to be of clinical value in a larger study is low. Therefore, we conclude that other diagnostic tests are needed for the triage of women with cytological borderline, mild, or moderate dyskaryosis because high risk HPV detection by PCR and the detection of telomerase activity and telomerase components are not suitable for this purpose.

. . . . . . . . . . . . . . . . . . . . .

### Authors' affiliations
**N Reesink-Peters, M N Helder, G B A Wisman, A J Knol,**
**A G J van der Zee,** Department of Gynaecological Oncology, University Hospital Groningen, 9700 RB, Groningen, The Netherlands
**H M Boezen,** Department of Epidemiology and Biostatistics, University Hospital Groningen
**S Koopmans, E Schuuring, H Hollema,** Department of Pathology, University Hospital Groningen
**E G E de Vries, S de Jong,** Department of Medical Oncology, University Hospital Groningen

## REFERENCES
1 **Pisani P**, Parkin DM, Ferlay J. Estimates of the worldwide mortality from eighteen major cancers in 1985. Implications for prevention and projections of future burden. *Int J Cancer* 1993;**55**:891–903.
2 **Pinto AP**, Crum CP. Natural history of cervical neoplasia: defining progression and its consequence. *Clin Obstet Gynecol* 2000;**43**:352–62.
3 **Mitchell MF**, Tortolero-Luna G, Wright T, *et al.* Cervical human papillomavirus infection and intraepithelial neoplasia: a review. *J Natl Cancer Inst Monogr* 1996;**21**:17–25.
4 **Lanham S**, Herbert A, Watt P. HPV detection and measurement of HPV-16, telomerase, and survivin transcripts in colposcopy clinic patients. *J Clin Pathol* 2001;**54**:304–8.
5 **Burger MP**, Hollema H, Pieters WJ, *et al.* Predictive value of human papillomavirus type for histological diagnosis of women with cervical cytological abnormalities. *BMJ* (*Clin Res Ed*) 1995;**310**:94–5.
6 **Flannelly G**, Jiang G, Anderson D, *et al.* Serial quantitation of HPV-16 in the smears of women with mild and moderate dyskaryosis. *J Med Virol* 1995;**47**:6–9.
7 **Herbert A**. Is cervical screening working? A cytopathologist's view from the United Kingdom. *Hum Pathol* 1997;**28**:120–6.
8 **Schaffer P**, Sancho-Garnier H, Fender M, *et al.* Cervical cancer screening in France. *Eur J Cancer* 2000;**36**:2215–20.
9 **Bovicelli A**, Bristow RE, Montz FJ. HPV testing: where are we now? *Anticancer Res* 2000;**20**:4673–80.
10 **Sherman ME**, Schiffman M, Cox JT. Effects of age and human papilloma viral load on colposcopy triage: data from the randomized atypical squamous cells of undetermined significance/low-grade squamous intraepithelial lesion triage study (ALTS). *J Natl Cancer Inst* 2002;**94**:102–7.
11 **Reddy VG**, Khanna N, Jain SK, *et al.* Telomerase-A molecular marker for cervical cancer screening. *Int J Gynecol Cancer* 2001;**11**:100–6.
12 **Zheng PS**, Iwasaka T, Zhang ZM, *et al.* Telomerase activity in Papanicolaou smear-negative exfoliated cervical cells and its association with lesions and oncogenic human papillomaviruses. *Gynecol Oncol* 2000;**77**:394–8.
13 **Kyo S**, Kanaya T, Ishikawa H, *et al.* Telomerase activity in gynecological tumors. *Clin Cancer Res* 1996;**2**:2023–8.

14 **Wisman GB**, Hollema H, de Jong S, et al. Telomerase activity as a biomarker for (pre)neoplastic cervical disease in scrapings and frozen sections from patients with abnormal cervical smear. *J Clin Oncol* 1998;**16**:2238–45.

15 **Muller M**, Krause H, Heicappell R, et al. Comparison of human telomerase RNA and telomerase activity in urine for diagnosis of bladder cancer. *Clin Cancer Res* 1998;**4**:1949–54.

16 **Wisman GB**, de Jong S, Meersma GJ, et al. Telomerase in (pre)neoplastic cervical disease. *Hum Pathol* 2000;**31**:1304–12.

17 **Beattie TL**, Zhou W, Robinson MO, et al. Reconstitution of human telomerase activity in vitro. *Curr Biol* 1998;**8**:177–80.

18 **Bodnar AG**, Ouellette M, Frolkis M, et al. Extension of life-span by introduction of telomerase into normal human cells. *Science* 1998;**279**:349–52.

19 **Weinrich SL**, Pruzan R, Ma L, et al. Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. *Nat Genet* 1997;**17**:498–502.

20 **Counter CM**, Meyerson M, Eaton EN, et al. Telomerase activity is restored in human cells by ectopic expression of hTERT (hEST2), the catalytic subunit of telomerase. *Oncogene* 1998;**16**:1217–22.

21 **Hanselaar AG**. Kwaliteit van cytopathologisch onderzoek in het herziene bevolkingsonderzoek naar baarmoederhalskanker. *Nederlands Tijdschrift voor Obstetrie en Gynaecologie* 1996;**109**:207–10.

22 **Kurman RJ**, Norris HJ, Wilkinson E. Tumors of the cervix, vagina, and vulva; tumors of the cervix. In: Rosai J, ed. *Atlas of tumor pathology*. Washington DC: Armed Forces Institute of Pathology, 1992:37–139.

23 **Jacobs MV**, Snijders PJ, van den Brule AJ, et al. A general primer GP5+/GP6(+)-mediated PCR-enzyme immunoassay method for rapid detection of 14 high-risk and 6 low-risk human papillomavirus genotypes in cervical scrapings. *J Clin Microbiol* 1997;**35**:791–5.

24 **Flannelly G**, Kitchener H. Every woman with an abnormal cervical smear should be referred for treatment: debate. *Clin Obstet Gynecol* 1995;**38**:585–91.

25 **Raffle AE**, Alden B, Mackenzie EF. Detection rates for abnormal cervical smears: what are we screening for? *Lancet* 1995;**345**:1469–73.

26 **Hutchinson ML**, Zahniser DJ, Sherman ME, et al. Utility of liquid-based cytology for cervical carcinoma screening: results of a population-based study conducted in a region of Costa Rica with a high incidence of cervical carcinoma. *Cancer* 1999;**87**:48–55.

27 **Nowak JA**. Telomerase, cervical cancer, and human papillomavirus. *Clin Lab Med* 2000;**20**:369–82.

28 **Snijders PJ**, van Duin M, Walboomers JM, et al. Telomerase activity exclusively in cervical carcinomas and a subset of cervical intraepithelial neoplasia grade III lesions: strong association with elevated messenger RNA levels of its catalytic subunit and high-risk human papillomavirus DNA. *Cancer Res* 1998;**58**:3812–18.

29 **Peyton CL**, Gravitt PE, Hunt WC, et al. Determinants of genital human papillomavirus detection in a US population. *J Infect Dis* 2001;**183**:1554–64.

30 **Nobbenhuis MA**, Walboomers JM, Helmerhorst TJ, et al. Relation of human papillomavirus status to cervical lesions and consequences for cervical-cancer screening: a prospective study. *Lancet* 1999;**354**:20–5.

31 **Sun CA**, Lai HC, Chang CC, et al. The significance of human papillomavirus viral load in prediction of histologic severity and size of squamous intraepithelial lesions of uterine cervix. *Gynecol Oncol* 2001;**83**:95–9.

# ECHO ...................................................................................................

## Serum Ro52 antibody denotes connective tissue disease

A hitherto unrecognised subgroup of patients can now be identified, since the discovery of another, independent, serum marker for connective tissue diseases. Eighteen serum samples among more than 1700 tested routinely for antinuclear antibodies (ANAs), denoting connective tissue diseases, showed specificity solely for 52 kDa protein Ro52 and no cross reactivity with anti-SSA/Ro or SSB/La. None of them reacted with classic anti-SSA/Ro immunological methods—double immunodiffusion with thymus/spleen nuclear extract or natural Ro60, immunoblotting with natural or recombinant Ro60, and ELISA with natural SSA/Ro60 or recombinant Ro52 and Ro60 in unspecified proportions. Immunoblotting showed natural Ro60 as having just one 60 kDa protein band, which reacted exclusively with Ro60 monoclonal antibodies. However, 16 of the 18 sera reacted positively in ELISAs with equal amounts of recombinant Ro60 and Ro52, or Ro52 alone, or immunoblots with HeLa S100 substrate. Classic connective tissue disease was diagnosed for 12 of the 18 patients. The incidence of Ro 52 specific antibody was calculated at about 1% of serum samples positive for ANA.

In all, 1727 consecutive ANA positive serum samples were tested in parallel in double immunodiffusion against thymus/spleen nuclear extract and line immunoassay with recombinant Ro52, SSB/La, and natural Ro60. Samples that were positive for Ro52 alone were tested by a range of different methods to establish their specificity.

Anti-SS/Ro antibodies are the most prevalent ANAs, and they recognise Ro60 kDa protein; SSB/La antigen is also associated. Most anti-SS/Ro sera cross react with Ro52 kDa protein, but it was not known whether this protein was an independent marker.

▲ *Annals of the Rheumatic Diseases* 2002;**61**:929–933.

Please visit the Journal of Clinical Pathology website [www.jclinpath.com] for link to this full article.