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Relative Accuracy of Cervical and Anal Cytology for Detection of High Grade Lesions by Colposcope Guided Biopsy: A Cut-Point Meta-Analytic Comparison

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Abstract

Background: We recently reported, using a receiver operating characteristic area metric, the first meta-analytic comparison of the relative accuracy of cervical and anal cytology in detecting moderate or severe histopathologic lesions by magnification directed punch biopsy. The aim of the present research was to meta-analytically examine cut-point specific operating characteristics (sensitivity, specificity) of cervical and anal cytology in detecting high grade squamous intraepithelial lesion (HSIL) histopathology by colposcope directed punch biopsy.

Methodology/Principal Findings: The primary eligibility requirement was availability of tabulated cytology (normal, atypical cells of unclear significance [ASCUS], low grade squamous intraepithelial lesion, HSIL or atypical squamous cells cannot rule out high grade [ASC-H]) and biopsy (<HSIL, ≥ HSIL) counts. Meta-analysis and meta-regression of diagnostic accuracy was performed with examination of study quality criteria and heterogeneity. Thirty-three cervical and 11 anal publications were eligible between 1990 and 2010. Meta-analytically cut-point analysis showed that using a cut-point of ASCUS the sensitivity in both settings is similar while anal cytology is less specific than cervical cytology (specificity [95% confidence interval] 0.33 [0.20–0.49] vs. 0.53[0.40–0.66], p = 0.04) for the detection of HSIL histopathology by colposcope directed punch biopsy.

Conclusions/Significance: Using a cytology cut-point of HSIL or ASC-H, anal cytology is less sensitive but comparably specific to cervical cytology. However, using a cut-point of ASCUS, differences in accuracy were of borderline significance.

Introduction

In response to increasing rates of invasive anal cancer among HIV-infected persons [1,2], a growing number of HIV clinics are implementing screening programs for anal cancer and its precursors modeled on procedures used in cervical cancer screening [3]. We recently reported, using a *receiver operating characteristic (ROC)* area metric, the first meta-analytic comparison of the relative accuracy of cervical and anal cytology in detecting moderate or severe (high grade squamous intraepithelial lesion - HSIL) histopathologic lesions by magnification directed punch biopsy [4]. While ROC area is a useful summary measure of test discrimination, a more clinically useful metric would be based on test sensitivity and specificity at varying cytology cut-points. We therefore conducted a secondary meta-analytic comparison of the previously published summary data tables. The aim of the present research was to meta-analytically examine *cut-point specific operating characteristics* (sensitivity [SE], specificity [SP]) of cervical and anal cytology in detecting HSIL histopathology by colposcopic and high resolution anoscopic (HRA) directed punch biopsy.

Methods

Eligible studies were identified by MEDLINE citation of relevant publications between 1990 and 2010 published in the English literature as described in flowchart of included studies in our original publication [4]. Briefly, the *index test* was cytologic sampling of cervicovaginal or anal canal tissues. The *reference standard* was defined as colposcope magnified and directed punch biopsy of the uterine cervix or anal canal, respectively. The addition of endocervical curettage sampling was allowed for colposcopy studies. Cytology diagnostic categories include negative ("no atypical or malignant cells" [NAMC]), atypical squamous cells of uncertain significance (ASCUS), atypical squamous cells can't rule out high grade (ASC-H), low grade squamous intraepithelial lesion (LSIL), and HSIL. "Cases" are defined as those with histopathologic evidence of HSIL or greater by colposcope directed punch biopsy. Cases included cervical or anal intraepithelial neoplasia 2 (CIN 2 or AIN 2), CIN/AIN 3, Carcinoma in situ or invasive carcinoma. A primary study eligibility requirement was availability of cross- tabulated cytology (normal, ASCUS, LSIL, HSIL or ASC-H) and biopsy (<HSIL,
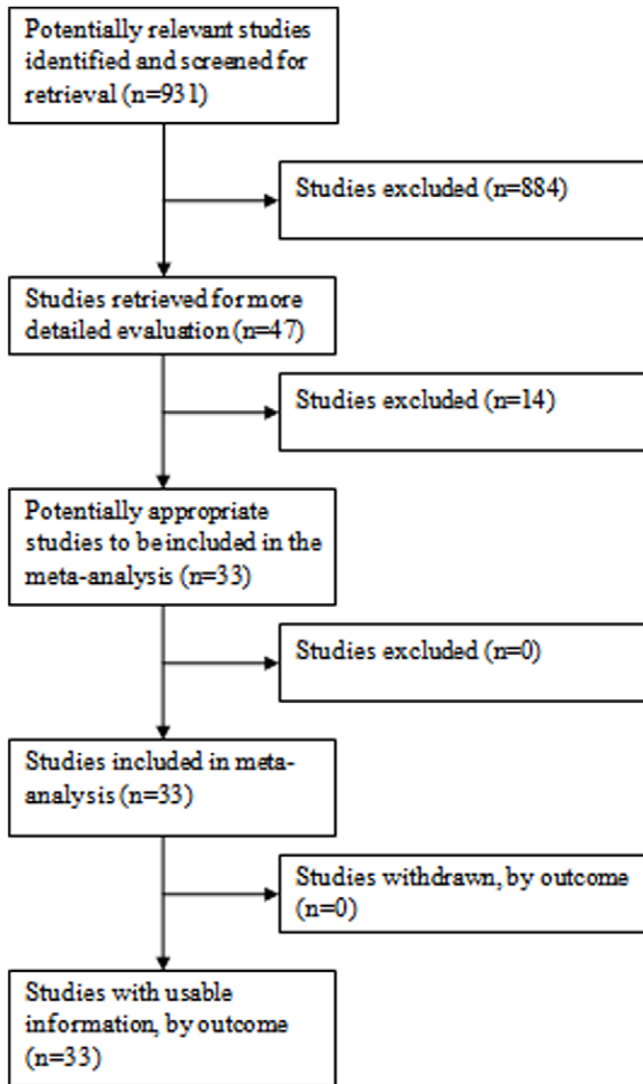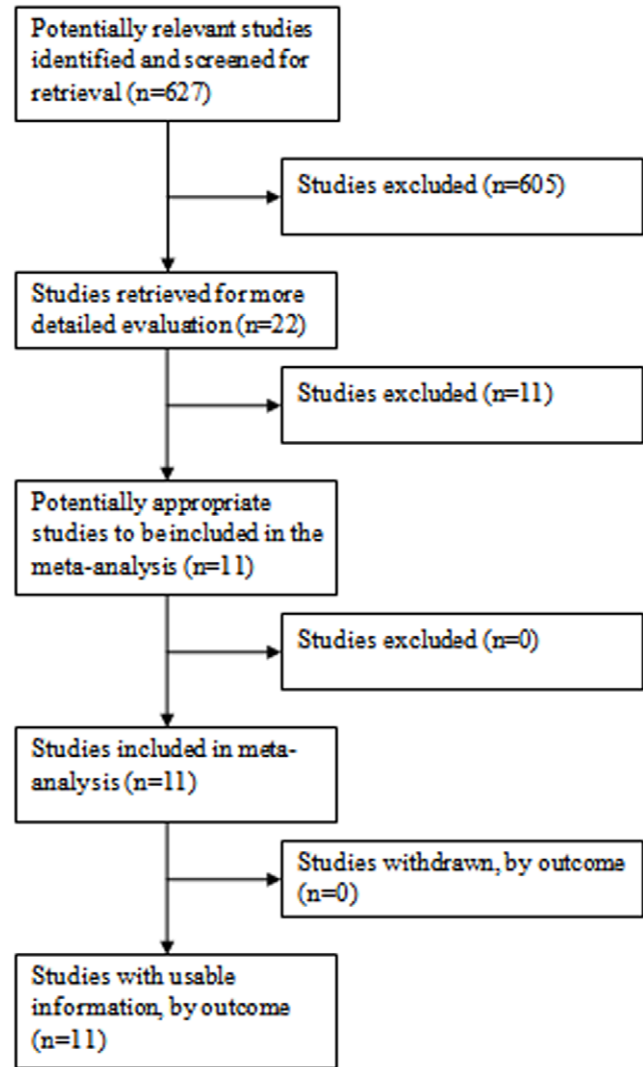
Figure 1. Flow of Included Studies: Cervical Screening.
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Figure 2. Flow of Included Studies: Anal Screening.
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≥ HSIL) counts. Source study quality was rated using QUADAS criteria as previously published [4].

We meta-analytically compared the joint sensitivity and specificity of cervical and anal cytology for biopsy confirmed high grade dysplasia or carcinoma. In this analysis, cytology as the index test was treated as a four-level ordinal measure (NAMC, ASCUS, LSIL, ASC-H or HSIL or carcinoma) and histopathology as the reference standard, as a dichotomous outcome (<HSIL, ≥ HSIL). Meta-analysis and meta-regression of diagnostic accuracy was performed using *metandi* and *midas*, respectively, implemented in Stata 11.2. Heterogeneity was examined using the I^2 statistic [5]. To illustrate the cut-point dependent tradeoffs between sensitivity and specificity, we plotted the ROC curves for anal and cervical cytology and calculated the corresponding ROC areas using the trapezoidal rule implemented using the *integ* function in Stata [6].

Results

Thirty-three cervical and 11 anal publications were eligible according to the MEDLINE search algorithm and evaluation of

review papers, Figures 1 and 2. Table S1 (supplementary information) presents the data extraction results and summary metric (cytology-biopsy ROC area) organized by study type (cervical and anal [7–50]. Table 1 presents the principal meta-analytic comparisons of the ability of cervical and anal cytology to differentiate between high grade and non-high grade histology by colposcope directed biopsy of uterine cervix and anal canal respectively, using different cytology cut-points. Using a cytology cut-point of either HSIL or ASC-H, cervical cytology, compared to anal cytology, had better sensitivity but comparable specificity to correctly identify HSIL histological lesions. However, using a cut-point of ASCUS, differences in accuracy were of borderline significance (cervical vs. anal: p = 0.04; $I^2 = 68$). Study heterogeneity was large in both screening settings. Funnel plots (Figure 3), demonstrate that relatively more of the cervical screening studies fall outside the pseudo 95% confidence intervals than is observed for the anal screening studies, however, the relative symmetry of both funnel plots is supported by the non-significant Egger test for both. Figure 4 presents ROC curves based on alternative cytology cut-points and compares the ability of anal and cervical cytology to

Table 1. Meta-analytically cut-point comparison of the joint sensitivity and specificity of cervical and anal cytology for biopsy confirmed high grade dysplasia.

Cytology Cut-Point	Sensitivity (SE)				Specificity (SP)			
	Anal		Cervical		Anal		Cervical	
	SE	(95% CI)	SE	(95% CI)	SP	(95% CI)	SP	(95% CI)
(HSIL or ASC-H) vs. (LSIL, ASCUS, Normal)[1]	0.30	(0.19–0.44)	0.63	(0.56–0.69)	0.93	(0.90–0.95)	0.96	(0.95–0.98)
(HSIL or ASC-H, LSIL) vs. (ASCUS, Normal)[2]	0.73	(0.62–0.82)	0.80	(0.75–0.85)	0.55	(0.45–0.65)	0.76	(0.66–0.83)
(HSIL or ASC-H, LSIL, ASCUS) vs. (Normal)[3]	0.90	(0.76–0.96)	0.91	(0.88–0.94)	0.33	(0.20–0.49)	0.53	(0.40–0.66)

1. Joint model comparison (cervical vs. anal): p<0.001; I^2 = 92.
2. Joint model comparison (cervical vs. anal): p<0.001; I^2 = 82.
3. Joint model comparison (cervical vs. anal: p = 0.04; I^2 = 68.
CI = Confidence Interval, HSIL = High grade squamous intraepithelial lesion, ASC-H = Atypical squamous cells can't rule out high grade, LSIL = Low grade squamous intraepithelial lesion, ASCUS = Atypical squamous cells of uncertain significance.
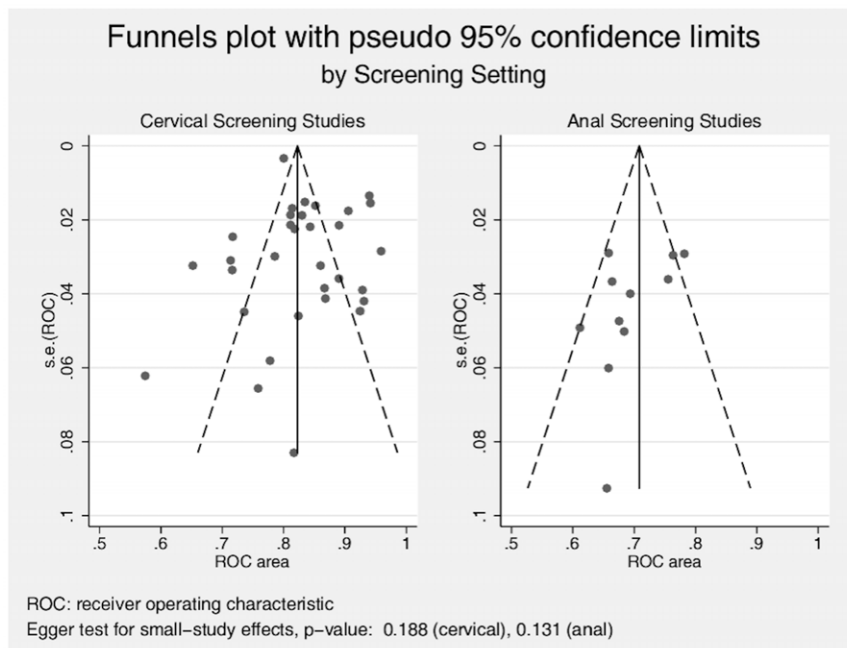doi:10.1371/journal.pone.0038956.t001

discriminate between histopathologic categories (<HSIL vs. ≥ HSIL).

Discussion

Anal cancer screening has not been recommended as standard of care in HIV clinics in the United States with the exception of the State of New York [51]. Our cytology cut-point specific meta-analytic comparison of the relative accuracy of cervical and anal cytology allows the following conclusions to be made: (1) anal cytology is overall less discriminating than cervical cytology using ROC area as the metric of test discrimination; (2) if a cytology cut-point of NAMC vs. ≥ ASCUS is used, the sensitivity in both settings is nearly identical while anal cytology is meaningfully less specific than cervical cytology; (3)at cytology cut-point of (HSIL or ASC-H) vs. ≤ LSIL, specificity of anal and cervical cytology is nearly identical while sensitivity of anal cytology is meaningfully less than that of cervical cytology. In the setting of screening for

anal cancer and its potentially modifiable precursor lesions, we believe that a selecting a cytology cut-point with maximal sensitivity is preferable to selecting a cut-point that maximizes specificity. The primary reason we argue this position is that, as we have previously shown [52], neither cervical nor anal histopathology obtained by colposcope magnified punch biopsy is a true "gold standard". Punch biopsy in both settings is subject to several sources of error including sampling error, operator error, and interpretation error [53,54]. Thus many false positive cytology results may in fact be erroneously classified because of the fallibility of the reference standard punch biopsy.

In summary, using a cytology cut-point of HSIL or ASC-H, anal cytology is less sensitive but comparably specific compared to cervical cytology. However, using a cut-point of ASCUS, differences in accuracy were of borderline significance. These results contribute to the evidence base regarding screening accuracy and might inform discussion regarding potential

ROC: receiver operating characteristic
Egger test for small–study effects, p–value: 0.188 (cervical), 0.131 (anal)

Figure 3. Funnel plots with pseudo 95% confidence limits, by Screening Setting.
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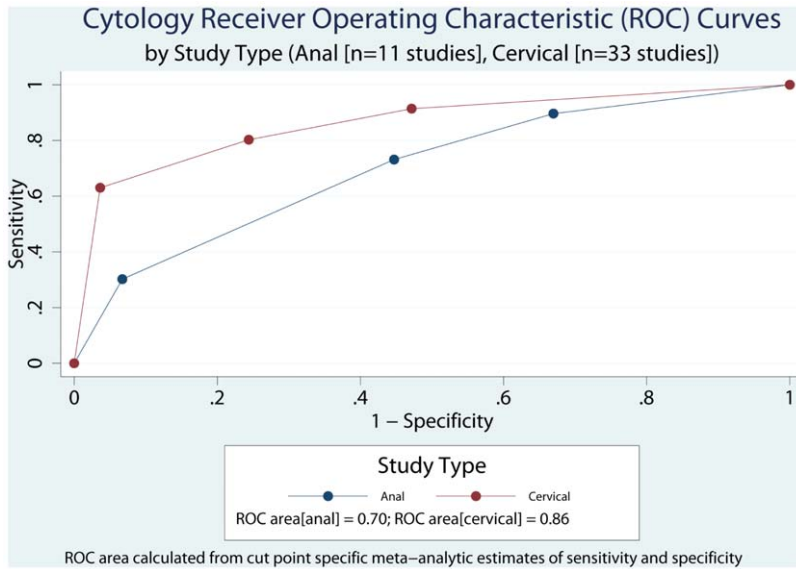
Figure 4. Performance of Diagnostic Receiver Operating Characteristic (ROC) areas by Screening Setting (Cervical, Anal) at different cut-points for identification of High grade squamous intraepithelial lesion histological lesions.
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guidelines for screening for anal cancer and its precursors in high risk populations.

Supporting Information

Table S1 Extracted Study Data and Outcome Metrics, by Study Type Cytology-Biopsy Joint Cell Frequencies. (DOC)

Author Contributions

Conceived and designed the experiments: ERC WCM. Analyzed the data: ERC WCM. Wrote the paper: ERC WCM WA. Reviewed significant number of manuscripts for meta-analysis: WA.

References

1. D'Souza G, Wiley DJ, Li X, Chmiel JS, Margolick JB, et al. (2008) Incidence and epidemiology of anal cancer in the multicenter AIDS cohort study. Journal of acquired immune deficiency syndromes 48: 491–9.
2. Piketty C, Selinger-Leneman H, Grabar S, Duvivier C, Bonmarchand M, et al. (2008) Marked increase in the incidence of invasive anal cancer among HIV-infected patients despite treatment with combination antiretroviral therapy. AIDS 22: 1203–11.
3. Palefsky J. (2012) Anal Cancer Info. San Francisco. University of California San Francisco. 2012:List of HRA providers. Available: http://id.medicine.ucsf.edu/analcancerinfo/all_providers.html#C. Accessed: 2012 February 21.
4. Mathews WC, Agmas W, Cachay E (2011) Comparative accuracy of anal and cervical cytology in screening for moderate to severe dysplasia by magnification guided punch biopsy: a meta-analysis. PLoS One 6: e24946.
5. Higgins JP, Thompson SG, Deeks JJ, Altman DG(2003) Measuring inconsistency in meta-analyses. BMJ 327: 557–60.
6. DeLong ER, DeLong DM, Clarke-Pearson DL (1998) Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. Biometrics 44: 837–45.
7. Adamopoulou M, Kalkani E, Charvalos E, Avgoustidis D, Haidopoulos D, et al. (2009) Comparison of cytology, colposcopy, HPV typing and biomarker analysis in cervical neoplasia. Anticancer Res 29: 3401–3409.
8. Alves RR, Rabelo-Santos SH, Ribeiro AA, Carneiro MA, Ximenes Y, et al. (2009) Usefulness of repeat cytology at the time of first colposcopy. Diagn Cytopathol 37: 68–73.
9. Andersson S, Sowjanya P, Wangsa D, Hjerpe A, Johansson B, et al. (2009) Detection of genomic amplification of the human telomerase gene TERC, a potential marker for triage of women with HPV-positive, abnormal Pap smears. Am J Pathol 175: 1831–1847.
10. Angstetra D, Tait T, Tan J, Symonds I (2009) Should liquid-based cytology be performed prior to colposcopy? A comparison of the accuracy, unsatisfactory rates and cost in a tertiary referral setting. Aust N Z J Obstet Gynaecol 49: 681–684.
11. Antonishyn NA, Horsman GB, Kelln RA, Severini A (2009) Human papillomavirus typing and viral gene expression analysis for the triage of women with abnormal results from papanicolaou test smears to colposcopy. Arch Pathol Lab Med 133: 1577–1586.

12. Baer A, Kiviat NB, Kulasingam S, Mao C, Kuypers J, et al. (2002) Liquid-based Papanicolaou smears without a transformation zone component: should clinicians worry? Obstet Gynecol 99: 1053–1059.
13. Belinson JL, Pan QJ, Biscotti C, Wu LY, Pretorius RG, et al. (2002) Primary screening with liquid-based cytology in an unscreened population in rural China, with an emphasis on reprocessing unsatisfactory samples. Acta Cytol 46: 470–474.
14. Benevolo M, Vocaturo A, Mottolese M, Mariani L, Vocaturo G, et al. (2008) Clinical role of p16INK4a expression in liquid-based cervical cytology: correlation with HPV testing and histologic diagnosis. Am J Clin Pathol 129: 606–612.
15. Bigras G, de Marval F (2005) The probability for a Pap test to be abnormal is directly proportional to HPV viral load: results from a Swiss study comparing HPV testing and liquid-based cytology to detect cervical cancer precursors in 13,842 women. Br J Cancer 93: 575–581.
16. Carns B, Fadare O (2008) Papanicolaou test in the detection of high-grade cervical lesions: a re-evaluation based on cytohistologic non-correlation rates in 356 concurrently obtained samples. Int J Clin Exp Pathol 1: 285–290.
17. Chung JH, Park EJ, Choi YD, Kim HS, Lee YJ, et al. (2005) Efficacy assessment of CellSlide in liquid-based gynecologic cytology. Gynecol Oncol 99: 597–602.
18. Cohn JA, Gagnon S, Spence MR, Harrison DD, Kluzak TR, et al. (2001) The role of human papillomavirus deoxyribonucleic acid assay and repeated cervical cytologic examination in the detection of cervical intraepithelial neoplasia among human immunodeficiency virus-infected women. Cervical Disease Study Group of the American Foundation for AIDS Research Community Based Clinical Trials Network. Am J Obstet Gynecol 184: 322–330.
19. DiBonito L, Falconieri G, Tomasic G, Colautti I, Bonifacio D, et al. (1993) Cervical cytopathology. An evaluation of its accuracy based on cytohistologic comparison. Cancer 72: 3002–3006.
20. Guerra B, De Simone P, Gabrielli S, Falco P, Montanari G, et al. (1998) Combined cytology and colposcopy to screen for cervical cancer in pregnancy. J Reprod Med 43: 647–653.
21. Guo M, Hu L, Martin L, Liu S, Baliga M, et al. (2005) Accuracy of liquid-based Pap tests: comparison of concurrent liquid-based tests and cervical biopsies on 782 women with previously abnormal Pap smears. Acta Cytol 49: 132–138.

22. Guo M, Patel SJ, Chovanec M, Jan YJ, Tarco E, et al. (2007) A human papillomavirus testing system in women with abnormal Pap results: a comparison study with follow-up biopsies. Acta Cytol 51: 749–754.

23. Harkness CB, Theofrastous JP, Ibrahim SN, Galvin SL, Lawrence HC (2003) Papanicolaou and thin-layer cervical cytology with colposcopic biopsy control. A comparison. J Reprod Med 48: 681–686.

24. Howard M, Sellors J, Kaczorowski J (2002) Optimizing the hybrid capture II human papillomavirus test to detect cervical intraepithelial neoplasia. Obstet Gynecol 100: 972–980.

25. Jones BA, Novis DA (1996) Cervical biopsy-cytology correlation. A College of American Pathologists Q-Probes study of 22 439 correlations in 348 laboratories. Arch Pathol Lab Med 120: 523–531.

26. Kumar K, Iyer VK, Bhatla N, Kriplani A, Verma K (2007) Comparative evaluation of smear cytology & hybrid capture II for the diagnosis of cervical cancer. Indian J Med Res 126: 39–44.

27. Lee GY, Kim SM, Rim SY, Choi HS, Park CS, et al. (2005) Human papillomavirus (HPV) genotyping by HPV DNA chip in cervical cancer and precancerous lesions. Int J Gynecol Cancer 15: 81–87.

28. Lorenzato F, Ho L, Terry G, Singer A, Santos LC, et al. (2000) The use of human papillomavirus typing in detection of cervical neoplasia in Recife (Brazil). Int J Gynecol Cancer 10: 143–150.

29. Mao C, Balasubramanian A, Koutsky LA (2005) Should liquid-based cytology be repeated at the time of colposcopy? J Low Genit Tract Dis 9: 82–88.

30. Mattosinho de Castro Ferraz Mda G, Nicolau SM, Stavale JN, Focchi J, Castelo A, et al. (2004) Cervical biopsy-based comparison of a new liquid-based thin-layer preparation with conventional Pap smears. Diagn Cytopathol 30: 220–226.

31. Pan Q, Belinson JL, Li L, Pretorius RG, Qiao YL, et al. (2003) A thin-layer, liquid-based pap test for mass screening in an area of China with a high incidence of cervical carcinoma. A cross-sectional, comparative study. Acta Cytol 47: 45–50.

32. Pimple SA, Amin G, Goswami S, Shastri SS (2010) Evaluation of colposcopy vs cytology as secondary test to triage women found positive on visual inspection test. Indian J Cancer 47: 308–313.

33. Ratnam S, Franco EL, Ferenczy A (2000) Human papillomavirus testing for primary screening of cervical cancer precursors. Cancer Epidemiol Biomarkers Prev 9: 945–951.

34. Sangwa-Lugoma G, Mahmud S, Nasr SH, Liaras J, Kayembe PK, et al. (2006) Visual inspection as a cervical cancer screening method in a primary health care setting in Africa. Int J Cancer 119: 1389–1395.

35. Simsir A, Ioffe OB, Bourquin P, Brooks SE, Henry M (2001) Repeat cervical cytology at the time of colposcopy. Is there an added benefit? Acta Cytol 45: 23–27.

36. Sun XR, Wang J, Garner D, Palcic B (2005) Detection of cervical cancer and high grade neoplastic lesions by a combination of liquid-based sampling preparation and DNA measurements using automated image cytometry. Cell Oncol 27: 33–41.

37. Taylor S, Kuhn L, Dupree W, Denny L, De Souza M, et al. (2006) Direct comparison of liquid-based and conventional cytology in a South African screening trial. Int J Cancer 118: 957–962.

38. Witt A, Hudelist G, Gregor H, Kucera E, Walchetseder C, et al. (2003) The detection of HPV DNA improves the recognition of cervical intraepithelial lesions. Arch Gynecol Obstet. 268: 29–34.

39. Yu BK, Kuo BI, Yen MS, Twu NF, Lai CR, et al. (2003) Improved early detection of cervical intraepithelial lesions by combination of conventional Pap smear and speculoscopy. Eur J Gynaecol Oncol. 24: 495–499.

40. Berry JM, Palefsky JM, Jay N, Cheng SC, Darragh TM, et al. (2009) Performance characteristics of anal cytology and human papillomavirus testing in patients with high-resolution anoscopy-guided biopsy of high-grade anal intraepithelial neoplasia. Diseases of the colon and rectum 52: 239–247.

41. Cranston RD, Darragh TM, Holly EA, Jay N, Berry JM, et al. (2004) Self-collected versus clinician-collected anal cytology specimens to diagnose anal intraepithelial neoplasia in HIV-positive men. Journal of acquired immune deficiency syndromes 36: 915–920.

42. Mathews WC, Sitapati A, Caperna JC, Barber RE, Tugend A, et al. (2004) Measurement characteristics of anal cytology, histopathology, and high-resolution anoscopic visual impression in an anal dysplasia screening program. Journal of acquired immune deficiency syndromes 37: 1610–1615.

43. Mathews WC, Cachay ER, Caperna J, Sitapati A, Cosman B, et al. (2010) Estimating the accuracy of anal cytology in the presence of an imperfect reference standard. PloS one 5: e12284.

44. Nahas CS, da Silva Filho EV, Segurado AA, Genevcius RF, Gerhard R, etal. (2009) Screening anal dysplasia in HIV-infected patients: is there an agreement between anal pap smear and high-resolution anoscopy-guided biopsy? Diseases of the colon and rectum 52: 1854–1860.

45. Nathan M, Singh N, Garrett N, Hickey N, Prevost T, et al. (2010) Performance of anal cytology in a clinical setting when measured against histology and high-resolution anoscopy findings. AIDS 24: 373–379.

46. Palefsky JM, Holly EA, Hogeboom CJ, Berry JM, Jay N, et al. (1997) Anal cytology as a screening tool for anal squamous intraepithelial lesions. Journal of acquired immune deficiency syndromes and human retrovirology 14: 415–422.

47. Panther LA, Wagner K, Proper J, Fugelso DK, Chatis PA, et al. (2004) High resolution anoscopy findings for men who have sex with men: inaccuracy of anal cytology as a predictor of histologic high-grade anal intraepithelial neoplasia and the impact of HIV serostatus. Clinical infectious diseases 38: 1490–1492.

48. Salit IE, Lytwyn A, Raboud J, Sano M, Chong S, et al. (2010) The role of cytology (Pap tests) and human papillomavirus testing in anal cancer screening. AIDS 24: 1307–1313.

49. Tramujas da Costa ESI, Coelho Ribeiro M, Santos Gimenez F, Dutra Ferreira JR, Galvao RS, et al. (2011) Performance of p16INK4a immunocytochemistry as a marker of anal squamous intraepithelial lesions. Cancer cytopathology 119: 167–76.

50. Williams VM, Metcalf C, French MA, McCloskey JC (2010) Audit of paired anal cytology and histopathology outcomes in patients referred to a public sexual health clinic. Sexual health 7: 346–351.

51. AIDS Institute New York Department of Health. (2007) New York State Guidelines recommendations on anal dysplasia and cancer screening. Available: http://www.hivguidelines.org/clinical-guidelines/adults/neoplastic-complications-of-hiv-infection/#V. Accessed: 2012 February 22.

52. Mathews WC, Cachay ER, Caperna J, Sitapati A, Cosman B, Abramson I (2010) Estimating the accuracy of anal cytology in the presence of an imperfect reference standard. PLoS One 5: e12284.

53. Lytwyn A, Salit IE, Raboud J (2005) Interobserver agreement in the interpretation of anal intraepithelial neoplasia. Cancer 103: 1447–56.

54. Mathews WC, Sitapati A, Caperna JC, Barber RE, Tugend A, et al. (2004) Measurement characteristics of anal cytology, histopathology, and high-resolution anoscopic visual impression in an anal dysplasia screening program. Journal of acquired immune deficiency syndromes 37: 1610–5.