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The increased detection of cervical intraepithelial neoplasia when using a second biopsy at colposcopy

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HIGHLIGHTS

- A second lesion-directed biopsy increases in CIN2+ detection.
- A low threshold for abnormality of any acetowhitening should be used at colposcopy.

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ABSTRACT

Objective. It has been suggested that colposcopy can miss a significant percentage of high-grade cervical intraepithelial neoplasia (CIN2+). Improved disease ascertainment was evaluated by taking multiple lesion-directed biopsies.

Methods. In a cross-sectional multicenter study in the Netherlands and Spain, 610 women referred to colposcopy following abnormal cervical cytology results were included. Multiple directed biopsies were collected from lesions and ranked according to impression. A non-directed biopsy of normal-appearing tissue was added if fewer than four biopsies were collected. We evaluated the additional CIN2+ yield for one and two directed biopsies. Colposcopic images were reviewed for quality control.

Results. In women with at least two lesion-directed biopsies the yield for CIN2+ increased from 51.7% (95%CI; 45.7–57.7) for one directed biopsy to 60.4% (95%CI; 54.4–66.2, $p < 0.001$) for two biopsies. The highest CIN2+ yield was observed in women who were HPV16-positive, had high-grade squamous intraepithelial lesion (HSIL) cytology, and high-grade colposcopy impression. The yield increased from 83.1% (95%CI; 71.5–90.5) with one directed biopsy to 93.2% (95%CI; 83.8–97.3) with two directed biopsies. Only 4.5% additional CIN2+ were detected in biopsies not targeting abnormal areas on the cervix.

Conclusions. A second lesion-directed biopsy is associated with a significant increase in CIN2+ detection. Performing a second lesion-directed biopsy and using a low threshold for abnormality of any acetowhitening should become the standard clinical practice of colposcopy.

Introduction

Colposcopy with lesion-directed biopsy is currently a widely used standard for the evaluation of women referred with abnormal cervical cytology. The aims of colposcopy are to evaluate the cervix, with particular attention for the cervical squamocolumnar junction (SCJ), and to determine which abnormal areas should be biopsied. Current biopsy procedures rely on the colposcopic identification of the area on the

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cervix that most likely represents the worst lesion [1]. The biopsy result determines further management. Generally, if a CIN2 or worse (CIN2+) is found the woman receives treatment. Despite the central role of colposcopy in detecting CIN2+, it has been suggested that it can miss 30–55% of high-grade lesions [2–6]. In a study investigating women with atypical cells of undetermined significance (ASC-US) cytology and CIN3 or invasive cervical cancer (ICC), it was found that gains in detecting cervical precancer can be obtained by increasing the number of lesion-directed biopsies from one to two [6]. Moreover, in studies that include biopsies of regions without colposcopic abnormality in addition to lesion-directed biopsies, 12 to 37% of the overall CIN2+ lesions were detected in this biopsy only [5,7,8].

In this study, the benefit of collecting a second lesion-directed biopsy to detect CIN2+ in women with abnormal cytology from two European study sites was investigated. Furthermore, the benefit of collecting an additional biopsy of visual normal appearing tissue (non-directed biopsy) was examined. We studied CIN2+ yields taking into account the women's referral cytology grade, human papillomavirus (HPV) status, and colposcopic impression.

Methods

Study population

Between August 2010 and October 2012, 610 women aged 17 years and older, visiting the gynecological outpatient clinic of the Hospital Clínic in Barcelona, Spain or the Reinier de Graaf Groep in Voorburg, the Netherlands, were enrolled in a cross-sectional study. In Spain, there is opportunistic screening and all women with abnormal cytology are referred for colposcopy. In the Netherlands, there is an organized 5-yearly screening program starting at the age of 30. Cytology grading is done according to the CISOE-A (composition, inflammation, squamous epithelium, other and endocervical columnar epithelium, and adequacy of the smear) classification [9]. Women with Pap smears graded as borderline or mild dyskaryosis (BMD) are recalled for repeat cytology after 6 and 18 months and are referred for colposcopy if the repeat cytology result is abnormal (borderline dyskaryosis or worse). Outside the national screening program, women with clinical symptoms indicative of cervical pathology (e.g. post coital bleeding) also receive cytological examination. All women in this study were referred for colposcopic evaluation because of abnormal cytology, which was detected at local health centers between 1 and 6 months prior to the study visit. Inclusion and exclusion criteria and patient characteristics have been described previously [10]. This study was approved by the medical ethical boards of both hospitals. All women provided signed informed consent. This study is registered in the Dutch Trial register (NTR3464).

Cytology and high-risk HPV (hrHPV) detection

Before colposcopy, a liquid based cytology sample using a Cervex-Brush® (Rovers Medical Devices B.V., Oss, The Netherlands) was obtained. The Cervex-Brush was rinsed in ThinPrep® medium (Hologic, Marlborough, MA) in Spain, and in SurePath™ medium (Klinipath BV, Duiven, The Netherlands) in The Netherlands. Cytological examination and classification were performed at the local laboratory in Spain according to the Bethesda 2001 classification (negative, ASC-US, low-grade squamous intraepithelial lesion (LSIL), high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells of undetermined significance (AGUS), atypical squamous cells—cannot exclude HSIL (ASC-H), adenocarcinoma in situ (AIS) or ICC). In the Netherlands grading was done according to the CISOE-A classification, which was translated into the Bethesda 2001 classification as described earlier [9].

GP5+/6+ PCR based HPV genotyping was performed at both laboratories in The Netherlands and in Spain [10]. In brief, DNA extraction was performed using 250 μL of the cytology specimen to obtain 100 μL of eluate with the QIAamp MinElute Virus Spin kit (QIAgen Inc., Valencia, CA). In the Netherlands, 10 μL of isolated DNA was amplified and genotyped, using the LMNX HPV GP Genotyping kit (Labo Biomedical Products BV, Rijswijk, The Netherlands) [11]. In Spain, HPV detection was performed using the GP5+/6+-PCR-EIA (Diassay, Rijswijk, The Netherlands). The EIA-positive GP5+/6+ amplimers were genotyped by the Genotyping kit HPV GP (Diassay). The strip-based genotyping test targets the same 18 HPV types as the LMNX test.

Colposcopy procedure

Colposcopic examination was performed using a digital colposcopy imaging system (Boundary Marketing Tool) created by the National Cancer Institute (NCI) in collaboration with the National Library of Medicine (NLM), Bethesda, USA [12]. A traditional binocular colposcope was used to perform colposcopy. An image of the uterine cervix was captured with a digital camera fixed to the colposcope. Nine colposcopists were involved in this study. Acetic acid 5% was applied for eliciting the acetowhite epithelial response for at least 1 min. Up to four directed biopsies were collected from different lesions or different regions within 1 lesion. Distinct areas within a large complex lesion were biopsied separately. If fewer than 4 directed biopsies were taken, a biopsy from any normal appearing epithelium of the SCJ (non-directed biopsy) was added. If no acetowhitening was observed, only a non-directed biopsy was performed. An endocervical curettage (ECC) was collected if the SCJ was not or only partially visible, if there was suspicion of ICC, if the visualized lesion extended in the endocervical canal and if the SCJ was visible but no or marginal abnormalities were visualized. Each biopsy was individually sent for analysis in a separate specimen pot. Overall colposcopic impression was graded as normal (including acetowhitening suggestive for metaplastic changes), low-grade, high-grade or worse [13,14]. Biopsies were ranked by order of severity according to their colposcopic impression. When the impression was similar between two lesions, the two biopsies were ranked by order of collection. From women with an available digital colposcopic image a review of the total colposcopic impression and the impression of the location of the non-directed biopsy was conducted. In total 447/610 (73.3%) digital colposcopy images were available for review. Each colposcopist in Voorburg reviewed a subset of the Barcelona images in order to have all images reviewed and three of six colposcopists in Barcelona reviewed the Voorburg images. A low threshold for abnormality (any acetowhitening suggestive of metaplastic changes) was used in the reviewing process. For our main analyses, the original colposcopic impression was used. For an ancillary analysis of the non-directed biopsy impressions, the review impressions were also used.

Pathological diagnosis and grading

Biopsy specimens were fixed in 10% formalin and paraffin-embedded. Hematoxylin and eosin (H&E) sections were examined by a local pathologist and classified as normal, CIN1, CIN2, CIN3, including 2 women with adenocarcinoma in situ (AIS), or ICC. The overall histological diagnosis per woman was based on the worst diagnosis found in all specimens, including ECC, of each woman. All biopsies were independently reviewed by a second central gynecological pathologist. In case of disagreement between the original and review diagnosis, a third central pathologist reviewed the discordant cases independently. Consensus diagnosis was determined by the agreement of 2 of 3 interpretations. In case of 3 different diagnoses, the 2 central pathologists came to an agreement after reviewing the discordant case together. P16 immunohistochemical staining (Clone JC8, SantaCruz Biotechnology Inc.) was performed on an adjacent section and scoring was based on the extent of the staining. The scoring of p16 included nuclear and cytoplasmic staining and was graded as negative/patchy staining or positive staining.