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ORIGINAL ARTICLE

Invasive cervical cancer after treatment of CIN

Amanda Herbert, MB BS, FRCPath[a,*], Giuseppe Culora, BM, FRCPath[a], Emma McLean, MB BCh, FRCPath[a], Ali A. Kubba, MB ChB, FRCOG, FFSRH[b], Guy's & St Thomas' NHS Foundation Trust

[a] *Department of Cellular Pathology, St Thomas' Hospital, London, UK*
[b] *Colposcopy Unit, McNair Centre, Guy's Hospital, London, UK*

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Introduction A historical audit of 30 post-treatment cervical cancers (10% of 289 cancers, 1999-2016) compared with a one-year-equivalent control group treated for cervical intraepithelial neoplasia (CIN) grade 3 (n = 164).
Materials and methods We compared history and follow up of cancer patients and controls and reviewed initial excision biopsies preceding cancer and, in 41% of controls, high-grade recurrence (n = 17) or consistently negative follow-up (n = 51).
Results Either abnormal post-excision cytology without high-risk human papillomavirus (hrHPV) tests or immediate re-excision was recorded in 70% (19 of 27) of patients with squamous cell carcinoma (SCC). Negative investigations including cytology, colposcopy, re-excision, hysteroscopy, hrHPV, and/or treatment default were recorded in 83% (25 of 30) of all cancers. The mean interval between initial excision and cancer diagnosis was 79.8 ± 30.1 months versus 11.2 ± 30.1 months for CIN3 recurrence.

Eight, 13, and 9 patients with cancer had initial excision at age 20-34, 35-49, and 50+ years, respectively, compared with 71%, 23%, and 5% of controls. CIN3 more often preceded SCC than CIN2 (22:1); 5 of 30 initial excisions were originally reported as negative after severe dyskaryosis. No SCC or CIN3 recurrence followed complete excision. Depth of CIN3 2+ mm (20 of 82 reviewed) was strongly associated with cancer/high-grade recurrence or early stromal invasion on review (18 of 20; 90%). Discrepancies were found on review in 10% of biopsies and as occasional abnormal cells in 9 of 34 cytology slides.
Conclusions Residual disease may be inconspicuous or absent on cytology, colposcopy, and/or histology. Management taking account of risk of recurrence (age, CIN3 depth, incomplete initial excision) could avoid some post-treatment cancers.

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*Corresponding author: Amanda Herbert, MB BS, FRCPath; Department of Cellular Pathology, St Thomas' Hospital, Second Floor North Wing, London SE1 7EH, UK; Tel.: (020) 7188 2953; Fax: (44) 0 7188 8989.

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Introduction

In 2 invasive cervical cancer audits in Southampton and at Guy's & St Thomas' NHS Foundation Trust (GSTT) we reported potentially avoidable reasons for cancers not being prevented—such as lapses in screening, false negative cytology, defaults in follow-up or referrals—and a final category of around 10% of women who had been previously treated for cervical intraepithelial neoplasia (CIN).[1-3]

Although treatment of CIN is the essential mechanism for preventing cervical cancer, incidence remains higher in previously treated women than in the screened population.[4-8] Soutter et al.[4] showed that the rate declined in the first years after treatment but persisted at a constant rate (~5.8 per 1000) after 8-10 years. Our audit at GSTT was consistent with this rate: 16 post-treatment cancers were diagnosed during the same 9 years as 3027 cases of CIN2+ (0.5%).[2]

Women with cancer tended to be older than routine practice when first treated, as has also been described for high-grade CIN recurrence.[1-3,5,9] Incomplete excision is a known risk factor for treatment failure but high-risk human papillomavirus (hrHPV) positivity has been shown to be a better predictor of that outcome.[10-13] Although the sensitivity of hrHPV as a test of cure is higher than cytology, its specificity is lower: ~20% are hrHPV-positive although recurrence is found in less than half of those.[14-16] Similarly, a study of test of cure published in this journal reported a 32% HPV-positive rate during 3 years' follow up and CIN2-3 recurrence in 6.8%.[17]

It is uncertain whether post-treatment cancer results from persistent or recurrent high-grade CIN.[4] However, Herfs et al. showed that high-grade CIN cannot recur if specialized cells at the squamo-columnar junction (SCJ) have been completely excised and that HPV infection with low-grade changes after excision of the SCJ is likely to be non-progressive.[18-20] We have revisited and extended our previous GSTT audit to investigate post-treatment cancers, particularly with respect to adequacy of excision and reasons why persistent or recurrent CIN was not detected earlier.[2] We included a control group of women treated for CIN3—this group was restricted to CIN3 because post-treatment cancers had rarely followed CIN2,[2] which carries a lesser risk of progression.[21,22]

Materials and methods

Between 1999 and 2016, 289 invasive cervical cancers were diagnosed at GSTT, excluding tertiary referrals. Thirty cancers (10%), 27 squamous cell carcinomas (SCC) and 3 adenocarcinomas (AC), followed previous excision biopsies (1983 to 2016) for CIN, cervical glandular intraepithelial neoplasia (CGIN), or high-grade dyskaryosis.

Control cases (n = 164) equivalent to the number of histologic CIN3 diagnoses each year were taken from the GSTT register of outcome of colposcopy referrals (half referred in 2002 and half in 2004), giving time for at least 10 years' history and follow up: 1 (0.6%) had developed cancer and was already included in the study cases. Details of follow-up categories (groups A-D), based on outcome, of control cases are shown in Table 1. In the control group, 150 had CIN3 (alone or with concomitant CIN2) on large loop excision of the transformation zone (LLETZ) and 37 also had CIN3 on prior biopsy; 14 with CIN2 or less on LLETZ had CIN3 on prior biopsy.

Details of the original diagnoses of initial excision biopsies are shown in Table 1. Sections of the biopsies (when available) of women with cancer, high-grade recurrence (group A) and at least 4 negative follow-up cytology tests (group C) were subjected to "blinded" review (by G.C.). The size of excision biopsy was measured on the slide, as were dimensions of CIN and the number of blocks involved. Volume of CIN was calculated from depth by width multiplied by 3 times the number of blocks involved (assuming 3 mm as their average thickness). Depth of CIN was measured from the surface to the lowest level of crypt involvement. Excision biopsies reported as no CIN or CIN2 were also reviewed.

Discrepancies were reviewed and decided between authors G.C. and A.H. in light of the original report and its macroscopic description. The study was registered as a clinical audit at GSTT. Details of cancer cases were shared with the local Quality Assurance Reference Centre for the audit routinely carried out for the NHS Cervical Screening Programme (NHSCSP).[23]

Terminology of cytology used by the NHSCSP correlates to The Bethesda System as follows[24,25]: *dyskaryosis* equates to "high-grade squamous intraepithelial lesion" or "low-grade squamous intraepithelial lesion"; high-grade may be moderate (which includes mild-moderate and ungraded) or severe; SCC and AC are included with "severe" and described as ?invasive cancer and ?glandular neoplasia, respectively. *Borderline* equates to "atypical": Borderline changes in squamous cells may be "not otherwise specified" (BNOS) or "high-grade not excluded" (B?HG) equating to "atypical squamous cells of undetermined significance" and "atypical squamous cells, cannot exclude a high-grade lesion", respectively. Borderline glandular cells (BGC) equates to "atypical glandular cells".

Statistics

Statistical analysis (χ^2 test of association, t tests for independent samples and F tests for variance) was carried out using the statistical package available on the VassarStats Statistical Computation web site (www.vassarstats.net; Accessed 2.6.19). Significance was defined as $P < 0.05$.

Results

Age of women at first cytology test and initial excision biopsy

The mean age at which the first cytology test (a) and initial excision (b) was recorded was a decade later in life

Table 1 Initial excision biopsies with original histological diagnosis preceding cancer and 4 outcome groups in control cases.

	Initial excision biopsy as originally reported (17 LLETZ, 9 cone, 1 partial cone, 1 loop cone, 2 NR)				
	CGIN	CIN3	CIN2	CIN1	No CIN
Squamous cell carcinoma (n = 27)					
14 screen-detected (9 stage IA1, 5 IB1)	0	12	1	0	1
13 symptomatic (9 stage II+, 2 IB1, 2 IA1)	0	7	2	1[a]	3
Adenocarcinoma (n = 3)					
3 symptomatic (2 stage II+, 1 IB1)	1	0	0	1	1
All cancers (n = 30)	1	19	3	2	5[b]
Control group (all excisions LLETZ)					
A: High-grade recurrence (n = 17) (8 CIN3, 6 CIN2, 3 HG cytology < CIN2)	0	17	0	0	0
B: Low-grade recurrence (n = 37) (Low-grade/borderline/CIN1 ± negative)	0	35	0	0	2
C: 4+ negative cytology tests (n = 51) (48/51 had 5-14 years follow up)	0	43	6	0	2
D: Other (n=59) (47 1-3 negative, 2 hysterectomy for unrelated cause, 10 no follow up)	0	55	0	1	3
All CIN3 controls (n = 164) All control cases had CIN3 either on LLETZ, prior biopsy or both	0	150	6	1	7

Abbreviations: CGIN, cervical glandular intraepithelial neoplasia; CIN, cervical intraepithelial neoplasia; HG, high-grade; LLETZ, large loop excision of transformation zone; NR, not recorded.
[a]Referred for severe dyskaryosis; no prior biopsy.
[b]All referred for severe dyskaryosis: 3/5 had CIN3+ on prior biopsy, 2/5 had no CIN at any stage.

preceding cancer/high-grade recurrence than low-grade/negative/unknown outcome: (a) 35.8 ± 3.2 versus 25.3 ± 1.2 years; (b) 42.1 ± 3.8 versus 31.1 ± 2.0 years ($P < 0.0001$). The risk of post-treatment cancer increased with age at initial excision (Table 2) with respect to the steep decline of CIN3 in the control group as a whole, which was consistent with Office for National Statistics registrations for England.[26]

Screening history before initial excision

The mean interval between referral and initial excision biopsy was greater preceding cancer/high-grade recurrence than low-grade/negative/unknown outcome (8.3 versus 4.6 months; $P = 0.001$): 5 of 47 of the former and 4 of 147 of the latter group had delays of 12 to 60 months before initial excision due in 2 cases to prior negative colposcopy,

Table 2 Age bands at initial excision for women with cancer and control groups compared with national registrations.

	Age band (years) at initial excision			
	20-34	35-49	50-64	65+
Cancer (n = 30; 1.7 per year)	8 (27%)	13 (43%)	7 (30%)	2
A: High-grade recurrence (n = 17)				
CIN3 (n = 8)	1	3	4	0
CIN2 (n = 6)	3	2	1	0
High-grade cytology ≤ CIN1 (n = 3)	3	0	0	0
B: Low-grade ± negative (n = 37)	28	8	1	0
C: 4+ negative cytology tests (n = 51)	38	13	0	0
D: Other controls (n = 59)	44	12	3	0
Total in control group (n = 164)	117	38	9	0
% of all controls in each age band	71%	23%	5%	
% ONS registrations of CIS[a] (1999-2016)	69%	26%	5%	

Abbreviations: CIN, cervical intraepithelial neoplasia; CIS, carcinoma in situ; ONS, Office of National Statistics.
[a]Office of National Statistics records: CIS includes CIN3 and adenocarcinoma in situ.[26]

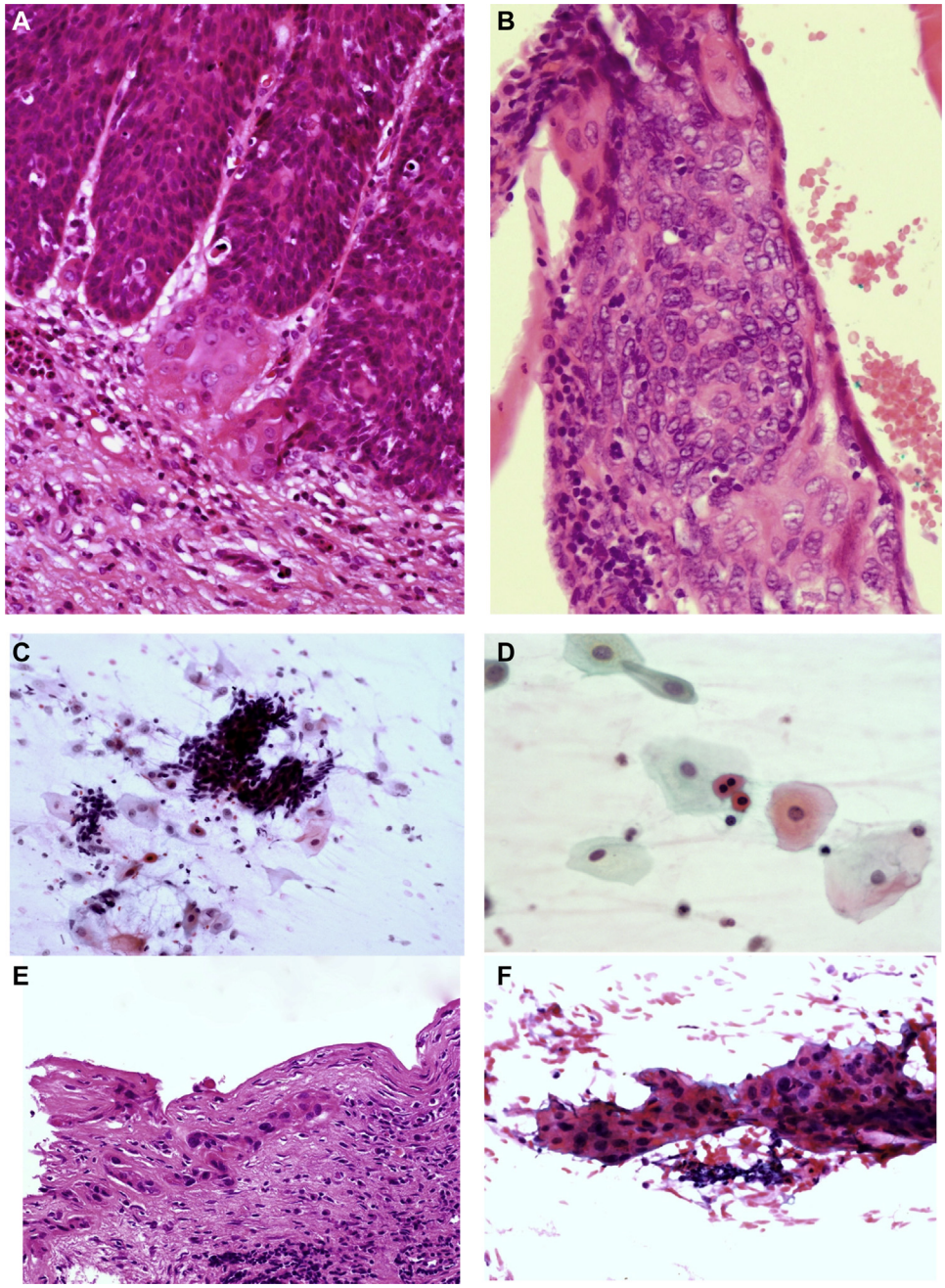

Figure 1 A, Early stromal invasion on review of initial excision of CIN3 2.5 mm in depth; stage IA1 squamous cell carcinoma on subsequent hysterectomy. B, Single focus suspicious of invasive carcinoma on review in an area of ulceration remote from all resection margins. C, Isolated hyperchromatic crowded cell group of high-grade dyskaryosis with adjacent endocervical cells and small keratinizing cells on conventional cytology reported as negative from a patient who refused hysterectomy. D, Small keratinized squamous cells from the same patient as C. E, Islands of invasive cancer below the surface on review of a colposopic biopsy reported as suspicious of invasion. F, Cytology reported as borderline ?high-grade after negative excision biopsy from the same patient as E; high-grade HPV was negative on this sample. (A: hematoxylin and eosin stain 100×; B, E: hematoxylin and eosin stain 200×; C: Papanicolaou stain 200×; D, F: Papanicolaou stain 400×).

in 3 to intervening pregnancy, and in 4 for unknown reason.

Referral cytology for initial excision in cancer cases (excluding 3 of 30 unknown) was high-grade in 93% (25 of 27); 1 with SCC was referred for B?HG and 1 for repeated BNOS. Referral cytology in control cases was high-grade in 86% (141 of 164); 3 were referred for B?HG, 14 for mild dyskaryosis, and 6 for BNOS.

Slide review of initial excision biopsies

Twenty-three of 30 initial excision biopsies (1991 to 2014, median 2003) in cancer cases were available for review and

Table 3 Margin status as reported, confirmed, clarified, or (in 1 case) revised on review.

Margins involved	Cancer 27 SCC, 3 AC	Group A High-grade Recurrence	Groups C 4 + negative cytology
CGIN (n = 1)			
No margins involved (n = 1)	1 AC	0	0
CIN3 (n = 81)	21[a]	17	43
All margins or endocervical and/or deep (n = 40)	15 (1 ESI; 1 AC)	11	14
Ectocervical margin alone (n = 17)	1 (ESI)	5	11 (2 ESI)
No margins involved (n = 19)	0	1[b]	18
'Incomplete'/uncertain (n = 1)	1	0	0
No full report/not reviewed (n = 4)	4	0	0
CIN2 (n = 8)	2	0	6
Endocervical and/or deep (n = 3)	2 (1 CIN3 in polyp)	0	1
Ectocervical margin alone (n = 1)	0	0	1
No margins involved (n = 4)	0	0	4
CIN1 (n = 2)	2	0	0
Ectocervical margin alone (n = 1)	1 AC	0	0
No margins involved (n = 1)	1	0	0
No CIN (n = 6)	4 (2 ?invasive)[c]	0	2
Total (n = 98)	30	17	51
% Endocervical and/or deep+ (excluding 11 no CIN/CIN1/uncertain)	28/38 (74%)		15/49 (31%)

Abbreviations: AC, adenocarcinoma; CGIN, cervical glandular intraepithelial neoplasia; CIN, cervical intraepithelial neoplasia; ESI, early stromal invasion on review.
Diagnoses changed on review are noted.
[a]One reported as no CIN (AC) and 1 as CIN2 (SCC) had CIN3 on review.
[b]High-grade cytology recurrence with CIN1 on histology.
[c]Large loop excision of the transformation zone and prior biopsy respectively suspicious of invasion (Fig. 1B, E).

significant discrepancies found in 5: two reported as CIN3 had early stromal invasion (Fig. 1A); 1 CIN2 was revised to CIN3. Two of 5 reported as no CIN had abnormalities: one had a single focus of high-grade CIN suspicious of invasion in an ulcerated transformation zone (TZ) away from all excision margins (Fig. 1B) and 1 (preceding AC) had a single focus of CIN3 at an endocervical margin. One of 3 confirmed as negative had inflammatory granulation tissue obscuring the TZ thought to represent the site of a colposcopic biopsy showing high-grade CIN suspicious of invasion (Fig. 1E).

In the control group, 16 of 17 initial excision biopsies (2002 to 2005, median 2004) preceding high-grade recurrence and 6 of 7 reported as no CIN were available for review and the diagnosis confirmed. Discrepancies were

Table 4 Depth of first excision biopsy (A) and CIN2+ (B) according to outcome.

	Cancer	HG recurrence (group A)	4 + negatives (group C)
A. Depth of biopsy			
Mean 9.1 mm ± 0.7[a] (range 3-22 mm)	9.5 ± 1.6	9.8 ± 1.7	8.9 ± 0.9
3-7 mm (n = 25)	7	2	16
8-9 mm (n = 25)	6	6	13
10-14 mm (n = 35)	8	7	20
15 mm+ (n = 5)	2	1	2
All biopsies (n = 90)	23	16	51
B. Depth of CIN2+			
Mean 1.5 mm ± 0.3[a]	2.3 ± 1.1	2.2 ± 0.9	1.0 ± 0.2
Range 0.1-10.0 mm			
<1.0 mm (n = 32)	4	5	23
1.0-1.9 mm (n = 30)	6	3	21
2.0-10.0 mm (n = 20)	8[b]	8	4[b]
All with CIN2+ (n = 82)	18	16	48

Abbreviations: CIN, cervical intraepithelial neoplasia; HG, high-grade.
[a]95% confidence limits.
[b]Includes all four with early stromal invasion and excludes all six with CIN2.

Table 5 Post-treatment cytology and interval from initial excision to diagnosis.

Pathway to diagnosis	Post-excision cytology	Interval in months, excision to diagnosis
SCC (n = 27)		Range (mean)
1. At least probably invasive at time of initial excision (n = 6: 4 SY, 2 SD)	3 HG (2 severe, 1 moderate) 1 B?HG; 1 Re-excision, no cytology 1 Negative cytology/colposcopy	3-120 (29.7)
2. Prompt re-excision (n = 5: all SD)	4 HG (severe) 1 Re-excision, no cytology	2-26 (14.6)
3. Low-grade changes (n = 1: SY)	1 LG x4 (later HG)	96
4. Refused treatment (n = 4: 1 SY, 3 SD)	4 HG (3 had 1, 7 and 7 negative cytology tests and later HG)	21-120 (77.3)
5. Negative investigations (n = 6: 3 SY, 3 SD)	3 HG (all moderate); 1 Negative 1 B?HG; 1 Re-excision, no cytology	16-228 (108)
6. Negative cytology alone (n = 4: 4 SY)	4 Negative (2, 4, 4, and 6 tests)	108-264 (102)
7. No follow-up (n = 1: SY)	Nil	54
Adenocarcinoma (n = 3: all SY)	2 Negative ×4 1 LG (10 negative tests later)	39-180 (91)
Total cancer (n = 30)	14 HG; 2 B?HG; 8 Negative 3 Re-excision, no cytology	2-264 (79.8 ± 30.1[a])
Control group A, high-grade recurrence		
CIN3 (n = 8)	5 HG; 3 Re-excision, no cytology	5-23 (11.2)
CIN2 (n = 6)	2 HG (severe); 2 LG; 2 Negative	3-19 (9.5)
HG cytology (n = 3)	1 HG (severe); 2 Negative (later HG)	5-68 (43)
High-grade recurrence (n = 17)	8 HG; 2 LG; 4 Negative 3 Re-excision, no cytology	3-68 (16.2 ± 9.4[a])

Abbreviations: B?HG, borderline ?high-grade (=ASC-H); CIN, cervical intraepithelial neoplasia; HG, high-grade; Hx, hysterectomy; LG, low-grade; Rx, treatment SD, screen-detected; SY, symptomatic.
[a]95% confidence intervals.

found in 2 of 51, with at least 4 negative follow-up tests (group C): early stromal invasion (with negative colposcopy followed by 6 and 7 negative cytology tests so far).

Table 3 records diagnoses after review and shows that 22 with SCC had CIN3 on LLETZ (n = 20), adjacent (n = 1), or prior (n = 1) biopsy and one had CIN2 on LLETZ and prior biopsy. (The CIN2 case was not available for review.)

Margin status of initial excision biopsies

Table 3 shows details of margin status in the 3 categories reviewed (cancer and control groups A and C) as reported, confirmed, clarified, or (in 1 case) revised on review. Endocervical and/or deep margins were involved in more cancer/high-grade recurrence cases than negative outcome: 74% versus 31%; $P < 0.0001$. (Diagnoses changed on review are noted in Table 3.)

Dimensions and extent of CIN2+ in initial excision biopsies

Differences in the dimensions of 90 initial excision biopsies were not significant: Depth of biopsy is shown in Table 4. Extent of CIN2+ was measured in 82 initial excision biopsies. Differences in width of CIN2+ were not significant and volume could not be calculated accurately in retrospect because of variability in thickness of blocks; depth of CIN2+ (Table 4) was 2.2 mm ± 0.7 mm preceding cancer/high-grade recurrence compared with 1.0 mm ± 0.2 with negative follow-up ($P = 0.001$). CIN3 depth of at least 2 mm was more frequent in patients older than 35 years: 16 of 34 (47%) versus 4 of 48 (8%); $P < 0.0001$. Early stromal invasion or subsequent high-grade recurrence/cancer involved 18 of 20 (90%) of those with CIN3 of at least 2 mm depth.

Slide review of negative follow-up cytology in patients with cancer

Thirty-five slides reported as negative in cancer patients were available for review (cervical cytology slides are discarded after 10 years) and 26 (72%) confirmed as negative. These slides had been subject to review as part of the NHSCSP procedures for cancer audit.[23] Small numbers of cells with high-grade or probable high-grade dyskaryosis were found in 9 slides from 7 patients: 2 with AC had features suggesting glandular neoplasia; 3 with SCC had hyperchromatic crowded cell groups and/or small keratinizing cells (Fig. 1C, D): 2 of 3 with SCC also had high-grade and confirmed negative cytology during follow-up delayed by treatment default; 2 with SCC and 2 with AC had negative cytology alone.

Human papillomavirus tests during follow-up

hrHPV tests were available for 4 patients: 2 with SCC had hrHPV-positive borderline and negative cytology tests during follow-up and 1 had hrHPV-negative B?HG and ungraded dyskaryosis at colposcopy before and after a negative initial LLETZ (Fig. 1E, F). One patient had hrHPV-negative/negative cytology after re-excision with recurrent CIN3 involving endocervical margins.

Diagnostic pathways to cancer and high-grade recurrence

Post-excision cytology and intervals between initial excision and diagnosis are categorized in Table 5 with supplementary information in the following paragraphs. Mean age and range refers to age (in years) at initial excision.

Squamous cell carcinoma (n = 27)

1. At least probably invasive at time of initial excision (n = 6: mean age 44, range 34-53).
   - *Early stromal invasion on review* ($n = 2$). Delay of 19 months before initial excision in 1 case; default from follow-up colposcopy in the other.
   - *Symptomatic at initial excision shortly before diagnosis* ($n = 2$). Delay of 30 months (after previous negative colposcopy) before initial excision in 1 case; negative colposcopy and cytology before clinical cancer diagnosis in the other.
   - *Initial excision biopsies reported as negative* ($n = 2$). Focus of probable invasion seen on review of the LLETZ in 1; prior biopsy suspicious of invasion in the other: the latter had negative hrHPV before and after negative LLETZ.
2. Prompt re-excision (n = 5: mean age 38, range 34-47).
   - At least one re-excision and hysterectomy (similar to CIN3 recurrence).
3. Low-grade changes during follow-up (n = 1: age 38).
   - Delay 40 months before initial excision (elsewhere, reason unknown).
   - Post-treatment low-grade/borderline cytology and genital warts; subsequent high-grade dyskaryosis and IA1 SCC on hysterectomy.
4. Refused treatment (n = 4: mean age 42.5, range 34-51).
   - Three patients refused hysterectomy: 1 had no CIN (with CIN3 in curettings) and CIN1 on re-excision (cone biopsies), 1 had negative colposcopy, biopsy (twice), curettings and, as did the third, 7 negative cytology tests. All 3 had recurrent high-grade dyskaryosis before final diagnosis.
   - One refused treatment for IA1 SCC on punch biopsy: stage IV SCC diagnosed 21 months later after severe dyskaryosis and negative punch biopsy.
5. Negative investigations (n = 6: mean age 49.7, range 25-66)
   - Two patients (with stage III SCC) had no CIN on initial excision after severe dyskaryosis: 1 had prior colposcopy (×2) for low-grade cytology and was diagnosed after no CIN on re-excision; the other was diagnosed after negative colposcopy and cytology (×2) and moderate dyskaryosis (×3).
   - Three patients (with post-treatment moderate dyskaryosis) had, respectively, negative colposcopy, no CIN on re-excision, and negative examination under anaesthesia: 2 had IA1 SCC on hysterectomy and the third had stage IV SCC.
   - One patient was managed in a menopause clinic after negative and then borderline changes thought to be endometrial before severe dyskaryosis, no CIN on re-excision (with CIN3 on curettings) and IA1 SCC on hysterectomy.
6. Negative cytology alone (n = 4: mean age 42.5, range 20-61).
   - Two patients were ~60 years of age at initial excision, had negative cytology up to age 65, and SCC at 70+ (1 had false-negative cytology).
   - One had no record of screening for the first 5 years after treatment (outside the UK); 1 (with false negative cytology) defaulted 5 years after treatment.
7. No follow-up (n = 1: age 29)
   - Presented with bleeding throughout pregnancy; cone biopsy for CIN3 outside the UK without follow-up.

Adenocarcinoma (n = 3: mean age 43, range 31-58)

- Initial excision showed, respectively, CIN1, focal CIN3 at the endocervical margin, and CGIN; the latter completely excised followed by negative colposcopy, cytology, and hysteroscopy on 4 occasions.
- Two with false-negative cytology also had simultaneous negative colposcopy.

High-grade recurrence, control group A (n = 17: mean age 39.9, range 24-64)

- *CIN3* ($n = 8$). All had at least 1 re-excision and/or (5 of 8) hysterectomy. One had no CIN on re-excision (cone biopsy) followed by a focus of CIN3 on hysterectomy. One patient had 5 years' delay before initial excision.
- *CIN2* ($n = 6$). Four of 6 with CIN2 on biopsy had re-excision: 2 CIN2, 1 CIN1, 1 no CIN; 2 had negative cytology without further biopsy.
- *High-grade cytology* ($n = 3$). One with severe dyskaryosis had no CIN on re-excision and negative follow up. One of 2 with negative cytology followed by moderate dyskaryosis had CIN1 on biopsy and re-excision, and the second had negative cytology only.

In summary, Table 5 shows 19 of 27 (70%) patients with SCC (and 8 of 8 with CIN3 recurrence) either had abnormal

post-treatment cytology or re-excision without cytology on the basis of colposcopic appearances and the history of incompletely excised CIN3. As described above, 25 (83%) of 30 patients with cancer defaulted from treatment or follow-up and/or had negative investigations compared with 2 of 8 control cases with CIN3 (and 7 of 9 with ≤CIN2). The mean interval between initial excision and diagnosis was significantly longer for cancer than high-grade recurrence: 79.8 ± 30.1 months versus 16.2 ± 9.4; $P = 0.0002$, and was shortest for CIN3 recurrence: 11.2 ± 5.1 months.

Discussion

As post-treatment cervical cancer is rare, this study is limited by small numbers but covers 18 years in a laboratory reporting more than 35,000 cytology tests per year. Thirty (10%) of 289 cancers affected previously treated women. As expected, cancer and high-grade recurrence were related to incomplete excision and higher age at initial excision.[1–3,5,9] The risk of post-treatment cancer increased with age at initial excision with respect to steeply declining percentages in equivalent age groups of CIN3 controls and national registrations.[26] We also found that cancer and high-grade recurrence were related to increasing age at first screening record—and to CIN3 being at least 2 mm deep, at which depth all except 2 of 20 had early stromal invasion or subsequent cancer/high-grade recurrence. Depth of CIN3 would be easy to measure in routine practice. Cancer was much more frequent after CIN3 than CIN2 (22:1).

Seventy percent (19 of 27) of women with SCC (and 8 of 8 with CIN3 recurrence) had abnormal post-excision cytology or were treated on the basis of colposcopic appearances and the history of incompletely excised CIN3—without the benefit of hrHPV testing. hrHPV status might help focus the mind when cytology, colposcopy, or histology is unexpectedly negative, but is not perfect.[14-16] Negative hrHPV tests should not negate abnormal cytology (as in 1 of our cases) and post-treatment HrHPV-positivity (~20%) is far more frequent than recurrence (~10%) or cancer (~1%).[15-17] Finding rare small foci of recurrence is an added challenge to colposcopists.

In contrast to post-excision findings described here, 83% (25 of 30) of patients with cancer had at least 1 negative investigation including cytology (post-excision or later during follow up), colposcopy, re-excision, punch biopsy, examination under anesthesia, hysteroscopy, hrHPV—and/or defaults from treatment/follow-up or inappropriate management. This led to a mean interval of 79.8 months before cancer diagnosis compared with 11.2 months for CIN3 recurrence.

Review of histology slides was as important as cytology. Missed abnormalities on histology slides were inconspicuous: foci of early stromal invasion, a small focus of CIN3 at an excision margin indicating a non-representative biopsy and probable invasive cancer in an area of ulceration. Discrepancies were found in 9% (7 of 82) of excision biopsies, which is consistent with a larger NHSCSP study.[27] Similarly, abnormalities found on review of cytology slides tended to involve small numbers of cells of the types known to be at risk for false-negative reports.[28-30] These abnormalities were seen relatively late in the history: In our experience small keratinizing cells (Fig. 1C, D) are more often seen in "cancer audit" cases, suggesting that they may signify invasive cancer.

No instance of SCC or CIN2+ recurrence followed known complete excision and most of the SCCs (and all with CIN3 recurrence) appeared to result from persistent CIN3 rather than reinfection with hrHPV, as signified by the following: i) incomplete excision of CIN3, ii) negative re-excision with adjacent CIN3, iii) initial excision at age 60+ years after which reinfection was unlikely, iv) high-grade rather than low-grade false-negative cytology, and v) the difficulty in finding small foci of recurrence. Two SCCs without CIN at any stage may have developed spontaneously: Cytology was persistently abnormal preceding both these cancers without a histologically or colposcopically recognizable precursor; unfortunately, their HPV profile is not known. Only one of 27 patients with SCC had low-grade/borderline cytology along with genital warts during follow-up compared with 23% of negative controls with CIN1 and/or low-grade/borderline cytology.

SCC was rare after CIN2 treatment (only 1 case without concomitant CIN3), suggesting a far lower risk when similar numbers of CIN2 and CIN3 are reported in routine practice.[2] (Also, 4 of 6 of our controls with CIN2 recurrence on biopsy had negative follow-up.) This supports evidence that CIN2 is more likely than not to regress spontaneously and requires treatment or close surveillance primarily because of its risk of progression to CIN3 and much lesser risk of invasion.[21,22]

Post-treatment adenocarcinoma was uncommon but so is CGIN—its ratio to CIN3 was 1:30 in our previous audit, suggesting a risk similar to CIN3.[2] One patient developed adenocarcinoma after completely excised CGIN and was unusual in having had a single excision biopsy whereas most have a further excision. Two of 3 patients with adenocarcinoma had non-glandular lesions on initial excision.

In conclusion, persistent CIN3 after treatment may be inconspicuous on biopsy, cytology, and at colposcopy and may lead to cancer many years later. Negative investigations after incomplete excision should be reviewed critically with multidisciplinary discussion taking account of the patient's age and depth of CIN3.

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References

1. Herbert A, Anshu GM, Gupta SS, Singh N. Invasive cervical cancer audit: a relative increase in interval cancers while coverage increased and incidence declined. *BJOG*. 2009;116:845–853.
2. Herbert A, Anshu, Culora G, et al. Invasive cancer audit: why cancers developed in a high-risk population with an organised screening programme. *BJOG*. 2010;117:736–745.
3. Gornall RJ, Boyd IE, Manolitsas T, Herbert A. Interval cervical cancer following treatment for cervical intraepithelial neoplasia. *Int J Gynecol Cancer*. 2000;10:198–202.
4. Soutter WP, Sasieni P, Panoskaltsis T. Long-term risk of invasive cervical cancer after treatment of squamous cervical intraepithelial neoplasia. *Int J Cancer*. 2006;118:2048–2055.
5. Strander B, Andersson-Ellström A, Milson I, Sparén P. Long term risk of invasive cancer after treatment for cervical intraepithelial neoplasia grade 3: population based cohort study. *BMJ*. 2007;335:1077.
6. Rebolj M, Helmerhorst T, Habbema D, et al. Risk of cervical cancer after completed post-treatment follow up of cervical intraepithelial neoplasia: population based cohort study. *BMJ*. 2012;345:e6855.
7. Melnikow J, McGahan C, Sawaya GF, Ehlen T, Coldman A. Cervical intraepithelial neoplasia outcomes after treatment: long-term follow-up from the British Columbia cohort study. *J Natl Cancer Inst*. 2009;101:721–728.
8. Del Mistro A, Matteucci M, Insacco EA, et al. Long-term clinical outcome after treatment for high-grade cervical lesions: a retrospective monoinstitutional cohort study. *Biomed Res Int*. 2015;2015:984528.
9. Flannelly G, Bolger B, Fawzi H, De Lopes AB, Monaghan JM. Follow up after LLETZ: could schedules be modified according to risk of recurrence? *BJOG*. 2001;108:1025–1030.
10. Gardeil F, Barry-Walsh C, Prendiville W, Clinch J, Turner MJ. Persistent intraepithelial neoplasia after excision for cervical intraepithelial neoplasia grade III. *Obset Gynecol*. 1997;89:419–422.
11. Skjeldestad FE, Hagen B, Lie AK, Isaksen C. Residual and recurrent disease after laser conisation for cervical intraepithelial neoplasia. *Obstet Gynecol*. 1997;90:428–433.
12. Zaitoun AM, McKee G, Coppen MJ, Thomas SM, Wilson POG. Completeness of excision and follow up cytology in patients treated with loop excision biopsy. *J Clin Pathol*. 2000;53:191–196.
13. Arbyn M, Redman CWE, Verdoodt F, et al. Incomplete excision of cervical precancer as a predictor of treatment failure: a systemic review and meta-analysis. *Lancet Oncol*. 2017;18:1665–1679.
14. Zielinski GD, Bais AG, Helmerhorst TJ, et al. HPV testing and monitoring of women after treatment of CIN3: review of the literature and meta-analysis. *Obstet Gynecol Surv*. 2004;59:543–553.
15. Cuschieri K, Bhatia R, Cruikshank M, Hillemanns P, Arbyn M. HPV testing in the context of post-treatment follow up (test of cure). *J Clin Virol*. 2016;76:S56–S61.
16. Katki HA, Schiffman M, Castle PE, et al. Five-year risk of recurrence following treatment of CIN2, CIN3 or AIS: performance of HPV and Pap cotesting in post-treatment management. *J Low Genit Tract Dis*. 2013;17:S78–S84.
17. Zhou C, Hong W, Li Z, et al. Human papillomavirus testing and cytologic/histopathologic "test of cure" follow-up results after excisional treatment for high-grade cervical intraepithelial neoplasia. *J Am Soc Cytopathol*. 2014;3:15–20.
18. Herfs M, Somja J, Howitt BE, et al. Unique recurrence patterns of cervical intraepithelial neoplasia after excision of the squamocolumnar junction. *Int J Cancer*. 2015;136:1043–1052.
19. Herfs M, Vargas SO, Yamamoto Y, et al. A novel blueprint for 'top down' differentiation defines the cervical squamocolumnar junction during development, reproductive life, and neoplasia. *J Pathol*. 2013;229:460–468.
20. Herfs M, Parra-Herran C, Howitt B, et al. Cervical squamocolumnar junction-specific markers define distinct, clinically relevant subsets of low-grade squamous intraepithelial lesions. *Am J Surg Pathol*. 2013;37:1311–1318.
21. Castle PE, Schiffman M, Wheeler CM, Solomon D. Evidence for frequent regression of cervical intraepithelial neoplasia-grade 2. *Obstet Gynecol*. 2009;113:18–25.
22. Tainio K, Athanasiou A, Tikkenen KAO, et al. Clinical course of untreated cervical intraepithelial neoplasia grade 2 under active surveillance: systematic review and meta-analysis. *BMJ*. 2018;360:k499.
23. NHS Cervical Screening Programme. Audit of invasive cervical cancer: protocol changes for 2012-2013. Available at: www.gov.uk/government/uploads/system/uploads/attachment_data/file/437903/nhscsp28-protocol-changes-2012-13.pdf. Accessed September 5, 2019.
24. Denton KJ, Herbert A, Turnbull LS, et al. The revised BSCC terminology for abnormal cervical cytology. *Cytopathology*. 2008;19:137–157.
25. Nayar R, Wilbur DC, eds. *The Bethesda System for Reporting Cervical Cytology: Definitions, Criteria and Explanatory Notes*. 3nd ed. New York: Springer; 2015.
26. Office for National Statistics (2011-2017). Available at: www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/bulletins/cancerregistrationstatisticsengland/previousReleases. [Registrations before 2011 are now available from National Archives.]. Accessed September 5, 2019.
27. Mitchell H, Medley G. Differences between Papanicolaou smears with correct and incorrect diagnosis. *Cytopathology*. 1995;6:368–375.
28. Leung KM, Lam KK, Tse PY, Yeog GP, Chan KW. Characteristics of false-negative ThinPrep cervical smears in women with high-grade squamous intraepithelial lesions. *Hong Kong Med J*. 2008;14:292–295.
29. Gupta N, John D, Dudding N, Crossley J, Smith JH. Factors contributing to false-negative and potential false-negative cytology reports in SurePath™ liquid-based cytology. *Cytopathology*. 2013;24:39–43.
30. Castanon A, Ferryman S, Patnick J, Sasieni P. Review of cytology and histopathology as part of the NHS Cervical Screening Programme audit of invasive cervical cancer. *Cytopathology*. 2012;23:13–22.