TITLE PAGE

Risk of persistent or recurrent cervical neoplasia in patients with 'pure' adenocarcinoma-in-situ (AIS) or mixed AIS and high-grade cervical squamous neoplasia (cervical intraepithelial neoplasia grades 2 and 3 [CIN 2/3])

Running title: Comparison of health outcomes of 'Pure' and 'mixed' AIS

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ABSTRACT

Objective.
To compare outcomes of patients with pure AIS and mixed AIS/CIN 2/3 lesions including the incidence of AIS persistence, recurrence and progression to adenocarcinoma.

Design.
Retrospective cohort study.

Setting
Statewide population in Western Australia.

Population
Women diagnosed with AIS between 2001 and 2012.

Main Outcome Measures
De-identified linked data were utilized to ascertain the association between patient age at excisional treatment, margin status, lesion type, lesion size, and risk of persistent AIS (defined as the presence of AIS <12 months from treatment), recurrent AIS (≥12 months post treatment), and adenocarcinoma.

Results.
Six hundred thirty-six patients were eligible for analysis. The mean age was 32.3 years and median follow-up interval was 2.5 years. Within the study cohort, 266 (41.8%) patients had pure AIS and 370 (58.2%) had mixed AIS/CIN 2/3. Overall, 47 (7.4%) patients had AIS persistence/recurrence and 12 (1.9%) had adenocarcinoma. Factors associated with persistence/recurrence were pure AIS (HR 2.3; 95%CI 1.28 – 3.94; p = 0.005), age >30 years (HR 2.1; 95%CI 1.16 – 3.81; p = 0.015), positive endocervical margins (HR 5.8; 95%CI 3.05 – 10.92; p = <0.001) and AIS lesions >8mm (HR 2.5; 95%CI 1.00 – 6.20; p = 0.049). A histologically positive AIS ectocervical margin was not associated with persistence/recurrence.

Conclusion.
In this study, pure AIS was associated with greater risk of persistence/recurrence compared to mixed AIS/CIN 2/3. AIS lesions >8mm and positive endocervical margins were significant predictors for persistent or recurrent disease.

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Keywords:
Adenocarcinoma-in-situ; cervical; neoplasia; pure AIS; mixed AIS; adenocarcinoma.

1. Introduction

Adenocarcinoma in situ (AIS) of the uterine cervix is a precursor to cervical adenocarcinoma and may coexist with both high-grade squamous dysplasia (cervical intraepithelial neoplasia grades 2 and 3, [CIN 2/3]) and invasive carcinoma. In contrast to high grade CIN the incidence of AIS is increasing in absolute and relative terms[1,2]. Following the diagnosis of AIS on cervical cytology or colposcopic biopsy it is essential that patients undergo an excisional biopsy such as a cold knife cone (CKC) biopsy or loop electrosurgical excision procedure (LEEP) to exclude a coexistent invasive malignancy[3]. In young women desiring fertility preservation, excisional biopsy may be adequate treatment providing the margins are clear[4].

To date, data comparing the clinical outcomes of pure AIS and mixed AIS/CIN 2/3 lesions are lacking. Our objective was to investigate the risks of persistent, recurrent or progressive cervical neoplasia following the diagnosis of AIS, stratifying by lesion type ('pure' vs. AIS/ CIN2/3), in a large population-based cohort.

2. Methods

2.1 Data sources and linkage procedure

The Western Australian Data Linkage System provided a de-identified extraction of linked data from the Cervical Cancer Registry (CSR) of Western Australia (WA) (2001-2012) and the WA Death Registry (2001-2012) for all women with biopsy confirmed AIS. The CSR of WA is a legislatively mandated register of all cervical test results (HPV, cytology and histology) for WA residents(1). Less than 0.05% of women request the removal of their demographic information and test results[5]. Data fields in the CSR of WA include cervical screening information, the type of procedure (e.g. punch biopsy, LEEP, CKC biopsy or hysterectomy), patient age, margin status, presence of

coexisting CIN2/3, lesion size, and length of the surgical specimen (measured macroscopic extent along the cervical canal).

Study data were obtained following approval from the Curtin University Human Research Ethics Committee (HREC) (project number: HR 86/2012), the Western Australian Department of Health HREC (project number: 2012/49) and the University of Notre Dame Australia HREC (project number: 016031F).

2.2 Selection of patients

Patients aged 18 years or older who had histologically confirmed AIS recorded in the CSR of WA from 2001 to 2012 were identified. Patients were then excluded if:

- There was a history of previous treatment for high-grade CIN or cervical carcinoma.
- AIS was initially treated with hysterectomy.
- Follow-up data were not available.

Cervical cytology findings were classified according to the Australian Modified Bethesda System 2004(2). Patient age at the time of excisional treatment was classified as ≤30 years or >30 years. The patients underwent excisional treatment procedures including CKC biopsy, LEEP or hysterectomy at the recommendation and advice provided by the treating specialist.

2.3 Histopathology findings

All histopathology reports were reviewed to confirm the type of excisional biopsy (CKC or LEEP), the number of tissue specimens in each procedure, the length of the specimen, the presence of concurrent high-grade CIN, and the resection margin

status. The length of the surgical specimen was classified as i) Type I (< 10 mm), ii) Type II (> 10mm and < 15mm), or Type III (> 15mm). The number of specimens was classified as 1 or >1, the latter including both intentional two-stage procedures (LEEP followed by 'top hat' endocervical sampling) and technically difficult procedures which resulted in multiple, fragmented or incomplete specimens. Margin status was considered positive if any margin (ectocervical, endocervical or deep/ circumferential) was involved by AIS only, negative if all margins were histologically clear of neoplasia, and 'indeterminate' if margins could not be assessed or were not documented.

The extent of AIS was determined whenever possible from the initial histopathology reports in one of two ways. In some specimens, the maximal extent of AIS was specifically documented while in other cases the number of tissue blocks demonstrating AIS was recorded; in the latter situation, AIS extent was estimated assuming a 'standard' block width of 2.5mm multiplied by the total number of involved blocks[6]. In the event that the lesion size and/or number of positive blocks was not reported, the specimens were recorded as 'lesion size not reported'. For those cases with a documented AIS extent, the lesion size was categorized as i) ≤2.5mm, iii) >2.5 to ≤8mm iv) and >8 to ≤30mm.

2.4 Follow-up

Follow-up of patients potentially included cytological review, repeat CKC or LEEP biopsy and/or hysterectomy. The follow-up period was defined as the interval from the initial excisional procedure to the last follow-up procedure (e.g. cervical cytology, biopsy or hysterectomy) or death.

2.5 Principal outcomes

The principle outcomes investigated in this study were time from initial AIS excisional treatment to the patients first case of AIS or adenocarcinoma within 12 months (classified as 'persistence' in the follow-up period) or > 12 months (classified as 'recurrence' in the surveillance period). The outcomes were mutually exclusive as we only investigated the first event of interest that occurred for each woman: single failure time-to-event model.

2.6 Statistics

Kaplan–Meier graphs were constructed to investigate the survivorship function (time from initial excisional procedure until a second case of biopsy-confirmed AIS and adenocarcinoma, and log-rank tests were used to assess equality of the survivorship function. Proportional hazards models were constructed after simultaneously adjusting for multiple factors to investigate the relative rate (hazard ratio) of having, or subsequently, developing a high-grade cervical lesion post initial treatment. Models were constructed using purposeful selection of covariates including: presence of CIN 2/3), age at first excisional treatment, location of margin involved by AIS lesion, number of excised specimens and depth of excised tissue.

Statistical significance was determined as a p-value <0.05 and the 95% confidence intervals (CI) for hazard rate ratios were calculated. Violation of the proportional-hazard assumptions was assessed and biologically plausible interaction terms between variables were tested. STATA/IC 13.0 (STATA Corporation, College Station, TX, USA) was used for statistical analysis.

3. Results

3.1 Study cohort

An overview of the study cohort is presented in Figure 1. During the study period 707 patients underwent excisional treatment for AIS (following cervical cytology and/or a colposcopic biopsy reporting AIS, or AIS diagnosed incidentally following an excision biopsy for high grade CIN). 71 patients were excluded from further analysis because follow-up data were not available or because hysterectomy was performed as the initial treatment (Figure 1). For the remaining 636 patients, the mean age was 32.3 years (range 18 to 76 years) and the median follow-up interval was 2.5 years (range 0.2 months to 12.2 years). Of the 636 patients, 338 (53.1%) cases of AIS were confirmed with either punch biopsy or cervical cytology prior to excisional treatment. The remaining 298 (46.9%) incidentally detected cases that occurred for patients that were initial treated for CIN2/3, however, AIS was confirmed on the excisional biopsy specimens. CKC and LEEP biopsies were performed in 301 (47.3%) and 335 (52.7%) cases, respectively. The majority of surgical specimens were submitted as a single pass excision (81.9%). AIS size could be ascertained in 369 (58.0%) cases of which 206 were directly measured and 163 determined from the number of involved blocks (Table 1). The median lesion size was 4mm (range 0.4 to 31 mm).

3.2 Follow-up period (< 12 months' post treatment)

In the 12 months following the initial CKC or LEEP biopsy, 190 (29.9%) patients had a second excisional procedure including 83 (43.7%) who had a further CKC or LEEP, and 107 (56.3%) who underwent hysterectomy. The indications for further excision included positive or indeterminate margins in the initial excision specimen, subsequent

high grade cervical cytology, and/or biopsy, or abnormal/unsatisfactory colposcopy during follow-up. Of the 190 patients who underwent a second procedure, 121 (63.7%) had negative findings, 13 (6.8%) had low-grade changes (atypia, HPV effect, mild dysplasia (CIN 1), endometrial atypical hyperplasia), 7 (3.7%) had CIN 2/3, 39 (20.5%) had AIS, and 10 (5.3%) had invasive adenocarcinoma. Of the 39 cases with persistent AIS, 24 (61.5%) had positive pathological margins for AIS in the original excisional biopsy specimen.

3.3 Surveillance period (≥ 12 months' post treatment)

After exclusion of the 107 women who underwent hysterectomy and/or patients censored due to documented AIS or carcinoma during the follow-up period, a total of 529 patients entered the surveillance period. Of these, 55 (10.4%) underwent a second excisional procedure or hysterectomy. Negative findings were recorded in 40 patients (72.7%), while 4 (7.3%) had low-grade changes, 2 (3.6%) had CIN 2/3 and 8 (14.5%) had recurrent AIS and 1 (1.8%) had invasive adenocarcinoma. One patient underwent a further excisional procedure for recurrent AIS and was subsequently diagnosed with adenocarcinoma. Hysterectomy was performed in a further 32 patients (29 cases were negative, 2 showed low-grade changes and 1 had CIN2/3).

Of the 636 patients who entered the follow-up and surveillance periods, and underwent a further excisional procedure, a total of 47 (7.4%) had AIS and 12 (1.9%) had adenocarcinoma.

3.4 Adenocarcinoma

Overall there were 12 (1.9%) cases of invasive cervical adenocarcinoma detected in the follow-up and surveillance study periods. Of these, 6 patients had a LEEP performed initially with the majority (N=4) of specimens submitted in multiple fragments making pathological assessment difficult. In each case, extensive AIS involving the biopsy margins was reported. Ten of the cases were pure AIS lesions.

3.5 Factors associated with disease persistence and recurrence

At 18 months after initial treatment, 8.8% (95% CI: 0.07 – 0.11) of the study cohort had confirmed persistence and/or recurrence of endocervical neoplasia. This varied significantly by the original lesion type, with persistent or recurrent neoplasia observed in 14.6% (95% CI: 0.10 – 0.20) of women with pure AIS compared to 4.6% (95% CI: 0.03 – 0.08) of women with mixed AIS/CIN 2/3 (Figure 2). The Kaplan–Meier curve and log rank tests indicate a significant difference between the survival curves for pure AIS and mixed AIS/CIN 2/3 lesions (p-value < 0.001).

Multivariate time-to-event analysis confirmed the association of lesion type with disease persistence and recurrence after adjusting for potential confounders and other factors (Table 2). Women with an initial pure AIS lesion were 2.3 times more likely to have persistent or recurrent endocervical neoplasia compared to women presenting with a mixed AIS/ CIN 2/3 lesion. Other factors associated with disease persistence or recurrence were age >30 years, positive endocervical or indeterminate margin involvement, and AIS lesion size >8mm.

4. Discussion

Main Findings

To our knowledge this is the largest cohort study comparing the outcomes of women with pure AIS and mixed AIS/CIN 2/3 lesions. Overall, persistent or recurrent disease was observed in 7.4% of the cohort and 1.9% were diagnosed with cervical adenocarcinoma during follow up which is consistent with the findings of a 2014 systematic review[7]. In the current study factors associated with AIS persistence and recurrence were 'pure AIS', age >30 years, positive endocervical margins and AIS lesions >8mm. Positive ectocervical margin involvement was not associated with persistent or recurrent disease.

Strengths and Limitations

Our study has acknowledged limitations including the relatively short median follow up (2.5 years) and the potential for selection bias. Caution needs to be exercised when interpreting the statistical significance of the AIS lesion size, as the single imputation method was used for estimation of the AIS lesion size when the number of blocks were counted. This could induce a measurement error in this covariate. It may be the case that the size is overestimated in this group if the entire block was not affected. The absence of HPV status and colposcopy data are additional limitations. Additionally, the findings on disease persistence/recurrence are conditional upon women remaining AIS free for a period of 12 months. Strengths of the study include the population based nature and size of the patient cohort. Case ascertainment was conducted through the CSR of WA which follows quality-assurance and reporting processes[1, 3]. Furthermore, legislative requirements for the reporting of cervical histopathology to the CSR of WA would have facilitated the capture of almost all AIS cases presenting during the study period[5].

Interpretation in light of other evidence

Older age and positive endocervical margin status are recognised risk factors for persistent/recurrent AIS[8-13]. Lesion size >8mm has been shown to correlate strongly with persistence/recurrence in women with incidental AIS[14] and the findings of the current study are consistent with this observation. It is notable that pure AIS was associated with more than twice the risk of disease persistence/recurrence compared to mixed AIS/CIN 2/3 lesions[5]. This observation could not be explained by differences in AIS extent since these were comparable between the two groups. It may be that pure AIS and mixed AIS/CIN 2/3 lesions differ biologically, or that the presence of concurrent CIN leads to earlier diagnosis and treatment, since it is recognized that the cytological detection of AIS is less accurate than that of high-grade CIN. We were not able to determine whether there might have been differences in HPV subtypes between the groups.

Conclusion

In this study, pure AIS, age >30 years, positive endocervical margins and AIS lesions >8mm were associated with higher risk of AIS persistence/recurrence. These findings require validation in prospective trials.

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Disclosure of Interests Statement
N. Steel is employed by the WA Cervical Cancer Prevention Program that is responsible for maintaining and operating the Cervical Screening Register of WA.
All other authors declare that there are no conflicts of interest.

Contribution to Authorship
EC, AM, CJRS and PAC conceived and planned the study. EC and AM were responsible for carrying out the study. EC, AM, CJRS, KS, SB, JC, NS, YL, JT, SGS, GRM and PAC were responsible for data analysis and writing and approving the final manuscript.

Details of Ethical Approval
Ethical approval for the study was granted by the Curtin University Human Research Ethics Committee (HREC) (project number: HR 86/2012), the Western Australian Department of Health HREC (project number: 2012/49) and the University of Notre Dame Australia HREC (project number: 016031F).

Funding Source
There is no funding source to declare

Legends

Figure 1. Overview of study cohort.

Figure 2. Kaplan Meier failure function of overall endocervical dysplasia persistence and recurrence (AIS or adenocarcinoma) according to type of *in-situ* lesion (pure AIS vs AIS/CIN 2/3 lesions).

Table 1. Baseline patient demographic and clinicopathological summary.

Table 2. Factors associated with relative hazard rate of persistent or recurrent AIS or adenocarcinoma.

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Table 1. Baseline patient demographic and clinicopathological summary.

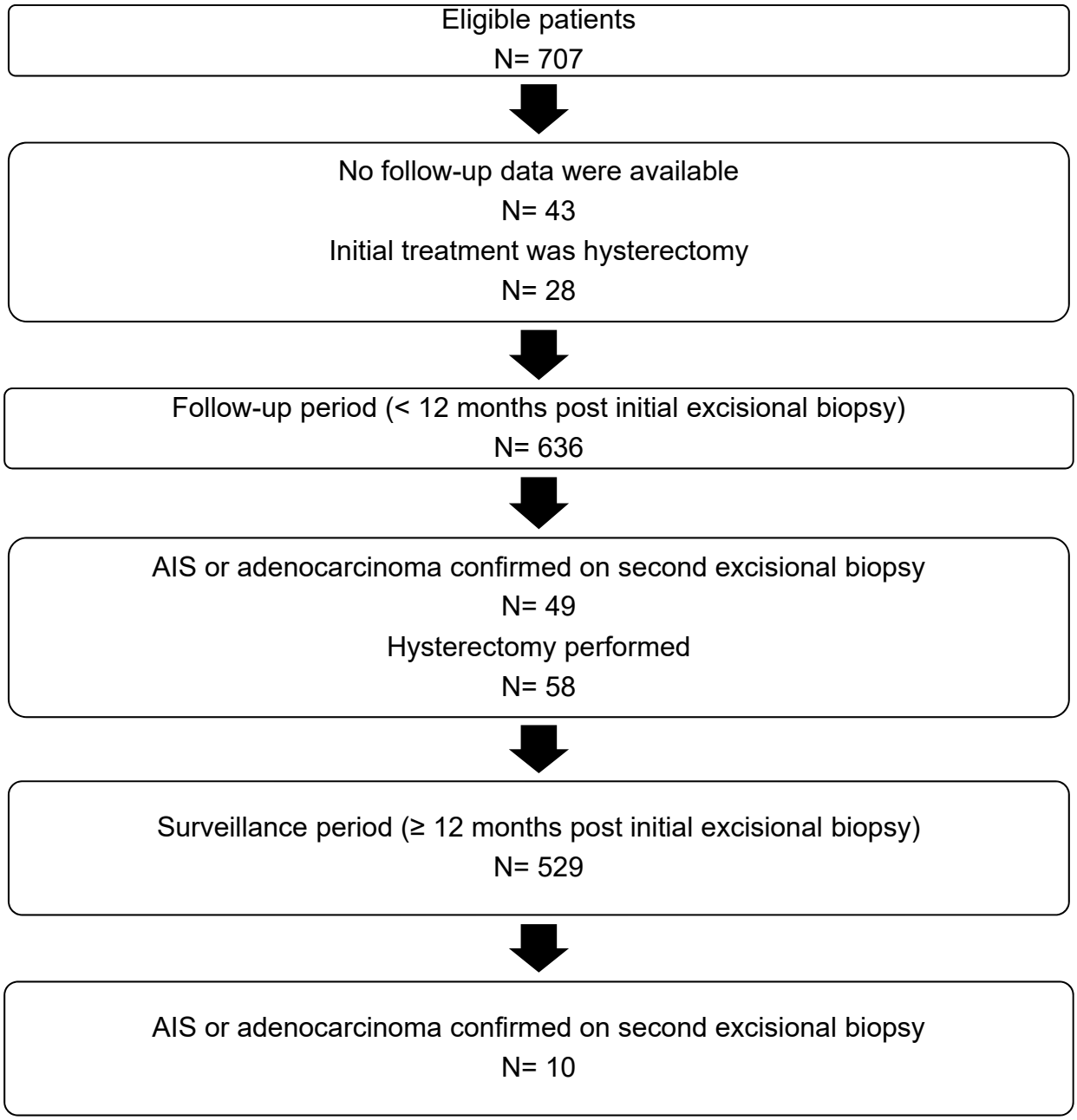
Characteristic	AIS only (N = 266)		AIS with CIN2/3 (N = 370)		p-value
	N	%	N	%	
Age (years)					
≤ 30	92	34.6	207	55.9	0.000
> 30	174	65.4	163	44.1	
Excisional biopsy specimen					
CKC	168	63.2	133	35.9	0.000
LEEP	98	36.8	237	64.1	
Number of surgical specimens					
1	221	83.1	300	81.1	0.518
> 1	45	16.9	70	18.9	
AIS lesion size (mm)					
Documented					
≤ 2.5	55	20.7	94	25.4	0.204
> 2.5 to < 8	67	25.2	77	20.8	
> 8 to ≤ 31	37	13.9	39	10.5	
Not documented	107	40.2	160	43.3	
Surgical specimen depth (mm)					
Mean (range)	13.4 (2 – 40)		11.2 (2 – 30)		
AIS involved at margin					
No involvement	162	60.9	247	66.8	0.085
Endocervical	71	26.7	70	18.9	
Ectocervical	13	4.9	23	6.2	
Enodcervical and Ectocervical	8	3.0	19	5.1	
Indeterminate	12	4.5	11	3.0	

CKC — cold knife cone, LEEP — loop electrosurgical excision procedure

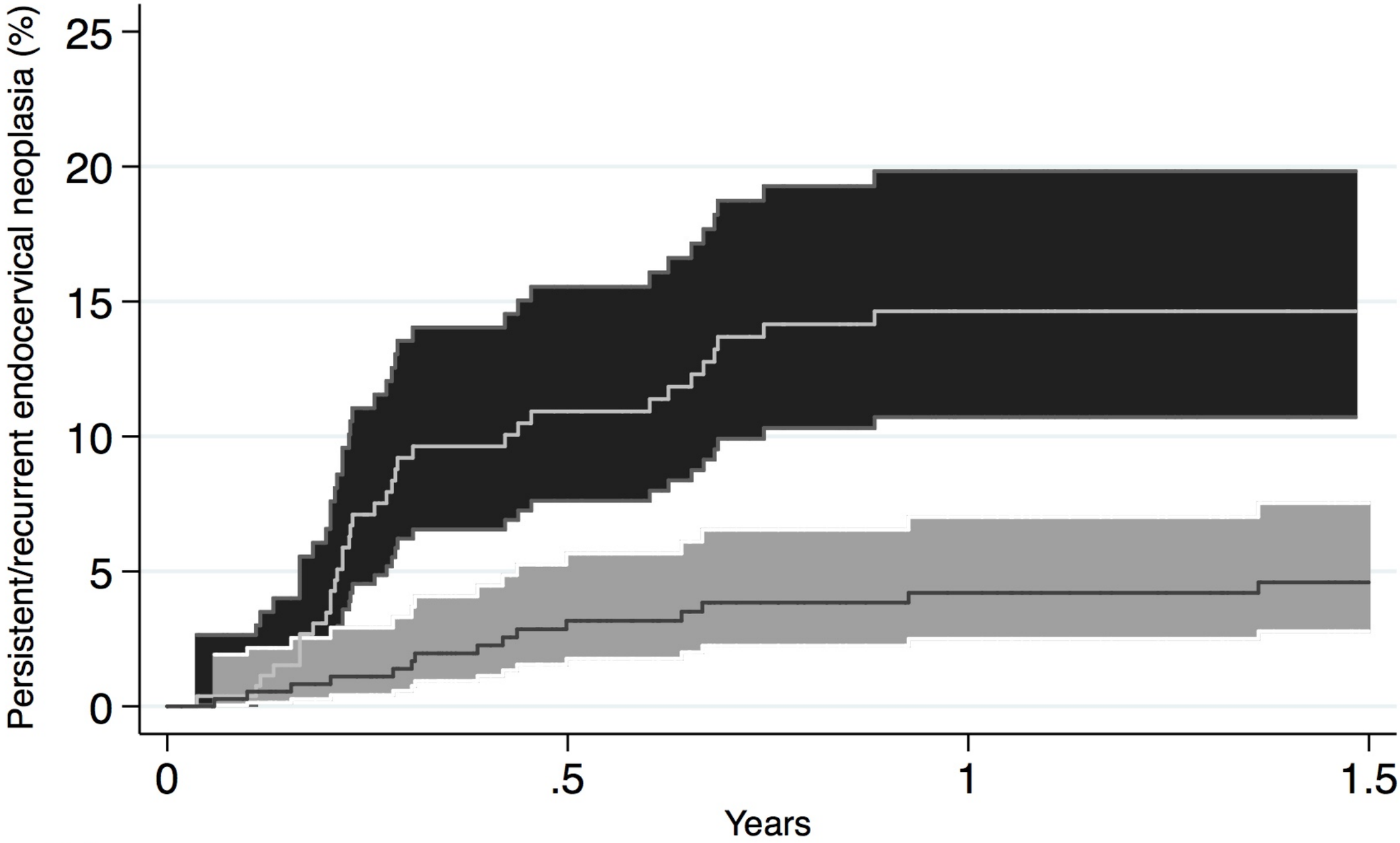
Table 2. Factors associated with relative hazard rate of persistent or recurrent AIS or adenocarcinoma (N=636).

Variable (N= 636)	Hazard Rate	95% Confidence Interval	p-Value
Age (years)			
≤ 30	1.00	ref	-
>30	2.10	1.16 – 3.81	0.015
Number of surgical specimens			
1	1.00	ref	-
> 1	0.97	0.50 – 1.86	0.924
Concurrent CIN			
Yes	1.00	ref	-
No	2.25	1.28 – 3.94	0.005
Specimen length (mm)			
Type 1 ≤ 10	1.00	ref	-
Type 2 10 - 15	0.68	0.31 – 1.48	0.325
Type 3 > 15	1.50	0.78 -2.86	0.222
AIS lesion size (mm)			
≤ 2.5	1.00	ref	-
> 2.5 to < 8	0.79	0.28 – 2.19	0.645
> 8 to ≤ 31	2.49	1.00 – 6.20	0.049
Not reported	1.59	0.68 – 3.69	0.282
Margin status			
No involvement	1.00	ref	-
Endocervical	5.78	3.05 – 10.92	0.000
Ectocervical	2.04	0.58 – 7.18	0.266
Enodcervical and Ectocervical	8.62	3.11 – 23.88	0.000
Indeterminate	3.89	1.08 – 14.06	0.038

Figure 1. Overview of study cohort.



AIS - adenocarcinoma-*in-situ,* excisional biopsy - loop electrosurgical procedure or cold knife cone biopsy.



Number at risk

Pure AIS	266	202	169	148
AIS with CIN2/3	370	306	265	238

95% CI
Pure AIS
95% CI
AIS with CIN2/3