RESEARCH ARTICLE

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Clinical Significance of Atypical Squamous Cells of Undetermined Significance (ASC-US) and Atypical Squamous Cells-Cannot Exclude High-Grade Squamous Intraepithelial Lesion (ASC-H) in a Low-Income Clinical Setting: A Retrospective Analysis

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Abstract

Background: Atypical squamous cells represent a significant category in cervical cytology screening, with a varying degree of malignant potential between ASC-US and ASC-H. In low-resource settings, challenges in follow-up and testing necessitate a clear understanding of the risk associated with these diagnoses. **Objective:** To determine the Positive Predictive Value (PPV) for premalignant and malignant cervical lesions (CIN2+) in patients with ASC cytology results, and to evaluate management challenges, particularly the high loss to follow-up, in a low-resource clinical setting. **Methods:** This retrospective study analyzed 276 patients with ASC cytology results from February 2019 to December 2024 at a tertiary care center. Patients were categorized into ASC-US (n=210) and ASC-H (n=66) groups. Demographics, histological outcomes, HPV testing results, and management patterns were analyzed. Statistical analysis was performed using Pearson's Chi-Square and Fisher's Exact Tests. The primary outcome was the PPV for CIN2+ among the 85 (30.8%) patients who underwent histological verification. **Results:** Among 1,360 cervical cytology specimens, ASC prevalence was 20.3% (ASC-US: 15.4%, ASC-H: 4.8%). Biopsy was performed in 30.8% of patients (n=85). The Positive Predictive Value (PPV) for CIN2+ was significantly higher for ASC-H compared to ASC-US (ASC-H: 40% [16/40] vs. ASC-US: 4.4% [2/45]; $p<0.001$). One case of endometrial carcinoma was identified in the ASC-H group but was excluded from the PPV calculation for cervical CIN2+ lesions. HPV testing was performed in only 13.8% of cases, with a 68.4% positivity rate, reflecting significant selection bias. Loss to follow-up (LTFU), defined as no follow-up within 12 months, occurred in 75.7% of all cases. **Conclusions:** ASC-H carries a substantially higher risk for significant cervical pathology compared to ASC-US, supporting differential management approaches. The extremely high rate of loss to follow-up (75.7%), strictly defined as no record of colposcopy, biopsy, or repeat cytology within 12 months of the index ASC finding, is a critical programmatic failure that severely limits the generalizability of these findings and underscores the urgent need for systematic patient tracking, enhanced patient education, and the integration of costeffective screening technologies like self-collection HPV testing to improve patient outcomes in low-resource settings.

Keywords: Atypical squamous cells- cervical cytology- ASC-US- ASC-H- cervical cancer screening- HPV testing

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Introduction

Cervical cancer remains a significant global health challenge, ranking as the fourth most common malignancy among women worldwide [1, 2]. The disease disproportionately affects low- and middle-income countries, where 85% of deaths occur, highlighting persistent global health disparities [3, 4]. The Papanicolaou (Pap) test, introduced in the late 1940s, revolutionized cervical cancer prevention [5]. The Bethesda System for Reporting Cervical Cytology standardized cytological interpretation, introducing the category of atypical squamous cells (ASC), further subdivided into ASC of undetermined significance (ASC-US) and ASC-cannot exclude high-grade squamous intraepithelial lesion (ASC-H) [6, 7]. ASC-US is the most common abnormal cytological finding (6.5% of all cervical cytology results) and is associated with underlying CIN in 10-20% of cases [8, 9]. ASC-H represents approximately 5% to 10% of all ASC diagnoses. In contrast, ASC-H carries a substantially higher risk for underlying high-grade CIN, with approximately 25-40% of cases harboring CIN 2 or higher lesions upon histological evaluation [10, 11]. The management of ASC cytology results has evolved with the integration of high-risk human papillomavirus (HPV) testing, as high-risk HPV types, particularly HPV 16 and

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18, are responsible for over 90% of cervical cancers. Recent guidelines from major professional organizations have increasingly emphasized HPV-based screening strategies [12-14]. Despite these technological advances, significant challenges persist in ASC management, particularly in low-resource settings, including high rates of loss to follow-up (LTFU) and inadequate HPV testing utilization [15].

The present study aims to evaluate the clinical significance of ASC cytology results in a hospital-based population, examining the Positive Predictive Value (PPV) for premalignant and malignant lesions associated with different ASC categories among patients who underwent histological verification. By analyzing demographic characteristics, histological outcomes, HPV testing patterns, and, critically, the high rate of loss to follow-up (LTFU), this research seeks to provide contemporary insights into ASC management while identifying opportunities for improvement in light of recent technological and guideline developments. Furthermore, this study focuses on the differential risk profiles of ASC-US versus ASC-H and the impact of programmatic failures, such as high LTFU rates, on disease progression.

Materials and Methods

Study Design and Setting

This study was conducted at a tertiary care academic medical center in Baku, an urban setting in Azerbaijan. The study protocol was designed to evaluate the clinical significance and management patterns of atypical squamous cells detected through cervical cytological screening over a five-year period from February 2019 to December 2024.

Patient Population and Selection Criteria

The study population comprised women aged 21-65 years who underwent cervical cytological screening and received ASC results (ASC-US or ASC-H) according to the Bethesda System. Exclusion criteria included women outside the specified age range, pregnant women, patients with incomplete medical records, and those with a prior history of cervical cancer or hysterectomy. Comprehensive data collection was performed through a systematic review of electronic medical records, pathology reports, and clinical databases. Variables recorded included:

Demographic characteristics: age, gravidity, parity, number of abortions, infertility history, and contraceptive method utilization.

Clinical History

Systemic diseases, history of sexually transmitted infections, previous surgical procedures, and smoking history.

Cytological and Histological Results

Initial cytology classification, HPV testing results, colposcopic findings, and final pathological classification (CIN grading system).

Cytology Methods and Quality Control

All cytology slides were prepared using the Conventional Papanicolaou smear (Pap smear) method and stained with the Papanicolaou stain. Adequacy criteria followed the Bethesda 2014 system. All ASC-H diagnoses and a random 10% of negative cases were subject to double-reading by a second senior cytopathologist as part of the laboratory's internal quality control (QC) procedure. The laboratory employs three full-time cytopathologists, each with over 10 years of experience in gynecologic cytology.

Management and Follow-up include diagnostic procedures performed (colposcopy, cervical biopsy, endocervical curettage), treatment interventions (cryotherapy, LEEP, conization, hysterectomy), follow-up compliance, duration, and loss to follow-up rates and timing. Loss to follow-up (LTFU) was strictly defined as no record of colposcopy, biopsy, or repeat cytology within 12 months of the index ASC finding.

Outcome definitions

The primary outcome was the PPV for CIN2+ (CIN 2, CIN 3/carcinoma in situ, or invasive carcinoma). For the primary outcome, the Positive Predictive Value (PPV) for CIN2+ (CIN 2, CIN 3/CIS, or invasive carcinoma) was calculated using only the patients who underwent histological verification as the denominator. The PPV for CIN2+ was calculated as the number of CIN2+ diagnoses divided by the total number of patients who underwent histological verification (biopsy, LEEP, or hysterectomy) in each ASC category. Statistical analysis was performed using Pearson's Chi-Square and Fisher's Exact tests. A p-value < 0.05 was considered statistically significant.

HPV DNA testing was performed using commercially available assays capable of detecting high-risk HPV genotypes. When performed, testing utilized either Hybrid Capture 2 (HC2) or polymerase chain reaction (PCR)-based methods for high-risk HPV detection. Genotyping was performed to identify specific HPV types, with particular attention to HPV 16 and 18 as the most oncogenic variants.

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics Version 22.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated for all variables, with categorical variables presented as frequencies and percentages, and continuous variables presented as medians with interquartile ranges (IQR) due to non-normal distribution patterns.

Comparative Analysis included categorical variables that were compared between ASC-US and ASC-H groups using the Pearson Chi-Square test or Fisher's Exact test where cell counts were small. Parity was re-categorized into 0, 1-2, and ≥3 for comparative analysis. Continuous variables were compared using the Mann-Whitney U test. A two-sided p-value of <0.05 was considered statistically significant. A correlation analysis (Fisher's Exact test) was performed between HPV status and CIN2+ outcome within the biopsied subset to assess the predictive value of HPV testing in this cohort. Univariate analysis was

performed to identify demographic and clinical factors associated with the presence of premalignant or malignant lesions. Given the limited number of CIN2+ outcomes (n=18), multivariate analysis using Firth's penalized logistic regression was performed to account for small sample bias. Variables with $p < 0.10$ in univariate analysis (ASC category and parity) were included in the model.

Ethical approval. This study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. Patient confidentiality was maintained throughout the study period, with all data de-identified prior to analysis. The study protocol was reviewed and approved by the Institutional Review Board at the Azerbaijan Medical University (Decision number: No198, date 22 April 2024).

Results

During the five-year study period from February 2019 to December 2024, a total of 1,360 cervical cytology specimens were evaluated at our institution.

Patient Characteristics

A total of 276 patients with ASC results were included (ASC-US: n=210; ASC-H: n=66). Table 1 summarizes the demographic and clinical characteristics. The median age was 38.0 years (IQR: 32.0–45.0) for the ASC-US group and 39.0 years (IQR: 33.0–46.0) for the ASC-H group (p=0.452). Comparative analysis of demographic factors between the ASC-US and ASC-H groups showed no statistically significant difference in median age and gravidity. Parity was analyzed categorically (when re-categorized as 0, 1-2, and ≥3) and showed a statistically significant difference between the two groups (p=0.037), with the ASC-H group having a higher proportion of nulliparous women. Multivariate analysis using Firth's penalized logistic regression was performed to identify independent predictors of CIN2+. ASC-H category remained strongly associated with CIN2+ (OR 15.4, 95% CI 3.2-74.1, p<0.001), while parity category was not independently associated (OR 1.1, 95% CI 0.8-1.5, p=0.562) after adjusting for ASC category.

Histological Outcomes and Positive Predictive Value (PPV)

Biopsy was performed in 85 patients (30.8% of the total cohort). The PPV for CIN2+ was calculated based on these 85 histologically verified cases. Biopsy was performed in 30.8% of patients (n=85), with a significant disparity between the ASC-US (21.4%, n=45) and ASC-H (60.6%, n=40) groups (Figure 1). To accurately reflect the risk of underlying disease, the Positive Predictive Value (PPV) for CIN2+ was calculated using only the biopsied cases as the denominator (Table 2). The PPV for CIN2+ was significantly higher for ASC-H compared to ASC-US (p<0.001).

ASC-US (n=45 biopsied): PPV for CIN2+ was 4.4% (2 cases of CIN2).

ASC-H (n=40 biopsied): PPV for CIN2+ was 40.0% (16 cases of CIN2/CIN3/CIS/Invasive Carcinoma).

The PPV for CIN2+ was significantly higher for ASC-H compared to ASC-US (ASC-H: 40.0% [16/40] vs. ASC-US: 4.4% [2/45]; p<0.001). One case of endometrial carcinoma was identified in the ASC-H group during follow-up. This case was excluded from the PPV calculation for cervical CIN2+ lesions but is reported as a clinically relevant finding.

HPV Testing Results

HPV DNA testing was performed in only 38 of 276 ASC patients (13.8%), reflecting limited utilization of molecular diagnostics during the study period in our nation. Among tested patients, 26 (68.4%) were HPV positive, while 12 (31.6%) were negative (Table 3). The HPV test used was a broad-spectrum assay that detects 14 high-risk HPV types and low-risk types (6 and 11). A Chi-square test comparing HPV positivity between ASC-US and ASC-H groups showed no statistically significant difference (p=0.121). HPV testing was performed in only 13.8% of cases (n=38), with a 68.4% positivity rate, reflecting significant selection bias. A correlation analysis between HPV status and CIN2+ outcome within this small biopsied subset was performed but did not yield a statistically significant association, likely due to the limited sample size.

Loss to Follow-up (LTFU)

A critical finding of this study was the high rate of loss to follow-up across all ASC categories. Overall, 209 of 276 ASC patients (75.7%) were lost to follow-up, representing

Table 1. Patient Demographics and Clinical Characteristics

Characteristic	Overall ASC (n=276)	ASC-US (n=210)	ASC-H (n=66)	p-value
Median Age (years)	41.0	41.0	43.0	0.069
Median Gravidity	2.0	2.0	2.0	0.892
Median Parity	0	1-2	≥3	0.037*
Median Abortions	0.0	0.0	0.0	0.615
Infertility History (%)	11.4	14.8	4.1	0.058
IUD Use (%)	18.6	21.5	11.3	0.089
COC Use (%)	5.4	5.8	4.5	0.712
Systemic Disease (%)	67.7	71.0	58.8	0.098
Previous Surgery (%)	36.6	32.6	47.1	0.058

*(p < 0.05)

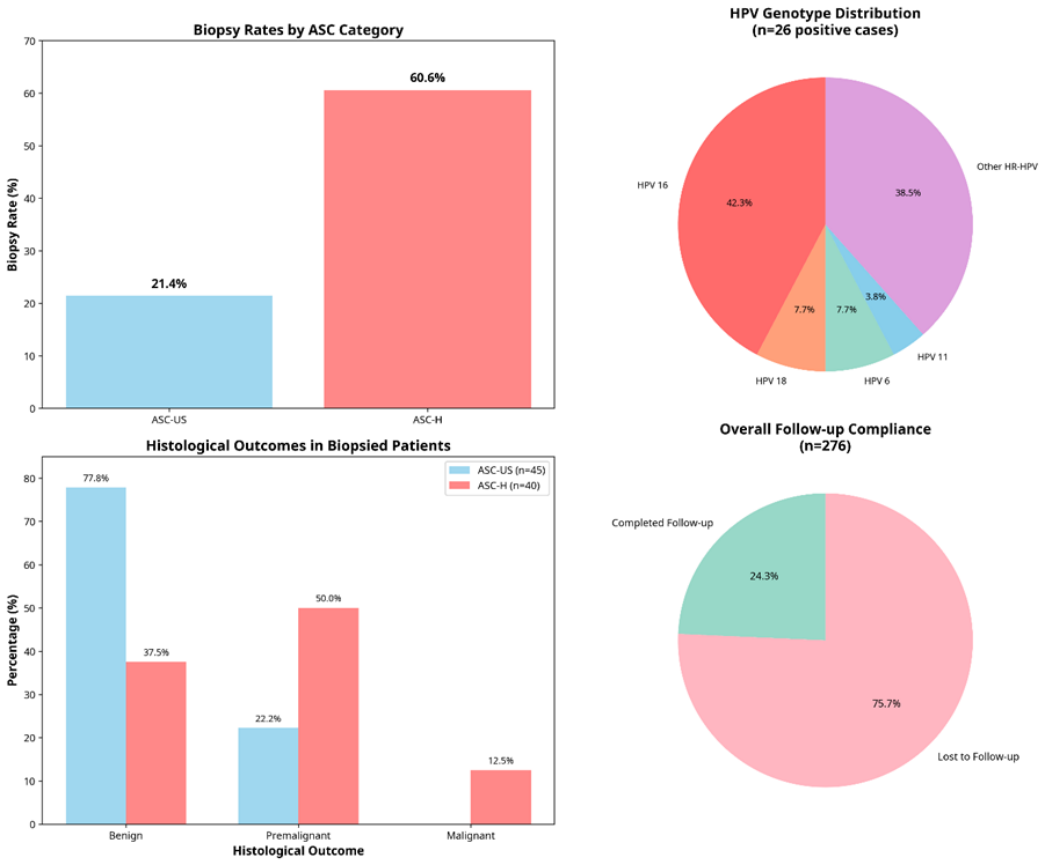

Figure 1. Management Patterns and Clinical Outcomes. A) Biopsy rates by ASC category showing appropriate risk-stratified management. B) HPV genotype distribution among positive cases (n=26) with HPV 16 predominance. C) Histological outcomes in biopsied patients demonstrating higher rates of significant pathology in ASC-H cases. D) Overall follow-up compliance showing high loss to follow-up rates (75.7%).

Table 2. ASC Prevalence and Histological Outcomes

Group	Total Cases	Biopsied (%)	Premalignant Lesions (%)	Malignant Lesions (%)	Combined Significant Rate (%)
ASC-US	210	45 (21.4%)	10 (4.7%)	0 (0%)	10 (4.7%)
ASC-H	66	40 (60.6%)	19 (28.7%)	6 (9.1%)	25 (37.8%)
Overall	276	85 (30.8%)	29 (10.5%)	6 (2.2%)	35 (12.7%)

$p < 0.001$ for comparison between ASC-US and ASC-H significant lesion rates

a substantial challenge for optimal patient management. Loss to Follow-up by ASC Category: ASC-US: 167 of 210 patients (79.5%) and ASC-H: 42 of 66 patients (63.6%). While ASC-H patients showed better follow-up compliance, the overall rates remain concerning and likely contribute to suboptimal patient outcomes

Table 3. HPV Testing Results and Genotype Distribution

Parameter	Value
Total HPV Tests Performed	38/276 (13.8%)
HPV Positive Cases	26/38 (68.4%)
HPV Negative Cases	12/38 (31.6%)
HPV Genotype Distribution (n=26)	
HPV 16	11 (42.3%)
HPV 18	2 (7.7%)
HPV 6	2 (7.7%)
HPV 11	1 (3.9%)
Other HR-HPV	10 (38.4%)

Discussion

This retrospective analysis of 276 patients with atypical squamous cells provides important insights into the clinical significance and management challenges associated with ASC cytology results. Our study confirms that ASC-H carries a substantially higher risk for significant cervical pathology (CIN2+) compared to ASC-US, even in a low-resource setting. By recalculating the risk as the Positive Predictive Value (PPV) among the biopsied cohort, we provide a more methodologically sound estimate of the true risk (Table 4). The PPV for CIN2+ in our ASC-H group was 40.0%, which is at the higher end of the 25-40% range reported in international literature [13]. This high PPV strongly supports the

Table 4. Comparison with Recent Literature (2020-2025)

Study/Source	ASC-US Prevalence	ASC-H Risk CIN2+	HPV Positivity	Management Approach
Current Study (2019-2024)	15.4%	28.7%	68.4%	Risk-stratified
IMPACT Trial (2021)	6.5%	N/A	15.1% (HRHPV)	HPV co-testing
WHO Guidelines (2024)	N/A	N/A	N/A	Dual-stain cytology
NCBI StatPearls (2025)	N/A	25-40%	N/A	HPV-based triage
Cubaka et al. (2024)	Variable	25-40%	Variable	Conservative <30y

current guidelines that mandate immediate colposcopy for ASC-H. Conversely, the PPV for CIN2+ in our ASC-US group was 4.4%, which is lower than the 10-20% range often cited, suggesting that the ASC-US diagnosis in our setting may be less predictive of high-grade disease, or that the high LTFU rate has skewed the observed outcome.

The findings of this study confirm that ASC-H carries a substantially higher risk for significant cervical pathology (CIN2+) compared to ASC-US in our low-resource setting, with a PPV of 40.0% versus 4.4% ($p<0.001$). This differential risk supports the current guideline-based approach of more aggressive management for ASC-H. The PPV for ASC-H is consistent with findings from other high-risk populations.

The most striking and concerning finding of our study is the extremely high rate of Loss to Follow-Up (LTFU), which reached 75.7% of all ASC cases. This programmatic failure is a critical limitation that severely compromises the validity and generalizability of our results. This high LTFU rate likely introduces a significant selection bias, as patients who fail to return for follow-up are often those with the highest barriers to care and potentially the highest risk of disease progression [14, 15]. Our reported PPV, while methodologically corrected, is based only on the 30.8% of patients who complied with follow-up. The true burden of CIN2+ in the entire cohort is likely much higher, masked by the high rate of non-compliance. This high LTFU rate severely limits the generalizability of our PPV findings, as the true prevalence of CIN2+ in the unverified 69.2% of the cohort is unknown. Our findings are consistent with studies from other low-resource settings, which also report high LTFU rates and highlight the critical need to address the care cascade for cervical cancer [15].

Our study was conducted using primarily Conventional Papanicolaou smears, which, along with the lack of systematic quality control, likely contributes to the high overall ASC prevalence of 20.3% observed in our cohort, a rate higher than the 6.5% reported in large-scale studies utilizing Liquid-Based Cytology (LBC) [15, 16]. The under-utilization of HPV testing (only 13.8% of cases) further limited our ability to triage ASC-US effectively, as the small, biased sample prevented a statistically significant correlation between HPV status and CIN2+ outcome. To overcome these critical limitations, future programmatic efforts in our setting must focus on: 1) Implementing systematic patient tracking and recall systems to reduce LTFU; 2) Transitioning to LBC or HPV-based primary screening to reduce the high ASC rate; and 3) Integrating cost-effective, novel technologies such as self-collection HPV testing and Artificial Intelligence (AI)-assisted screening to expand access and improve diagnostic accuracy in resource-limited areas [17, 18]. These technological and logistical improvements are essential to ensure that patients with ASC, particularly ASC-H, complete the diagnostic and treatment pathway. These technologies, while not measured in our study, are discussed only as potential programmatic solutions to the critical challenges identified. Our study provides baseline data to inform such programmatic changes in Azerbaijan.

In conclusion, ASC-H carries a substantially higher Positive Predictive Value (PPV) for CIN2+ (40.0%) compared to ASC-US, strongly supporting aggressive management. However, the extremely high rate of Loss to Follow-Up (75.7%) is the most critical finding, representing a major programmatic barrier to effective cervical cancer prevention in our low-resource setting. Enhanced patient education, systematic follow-up protocols, and the strategic integration of cost-effective screening technologies are essential to ensure the completion of the care cascade and optimize outcomes for women with ASC diagnoses. While new technologies such as self-collection for HPV testing and AI-assisted screening are promising, their implementation must be carefully considered within the context of a low-income setting.

Author Contribution Statement

Conceptualization: [Akbar Ibrahimov (A.I.)]; Methodology: [A.I., F.N.]; Formal analysis and investigation: [F.N.]; Writing - original draft preparation: [A.I.]; Writing - review and editing: [A.I.]; Supervision: [A.I.]. All authors have read and agreed to the published version of the manuscript.

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Ethics approval and informed consent statement

The study was conducted in accordance with the Declaration of Helsinki. Since the study was retrospective and observational, the Clinical Research Ethics Committee of Azerbaijan Medical University waived the need for written informed consent (Decision number: №198, date 22 April 2024)

Availability of data and materials

The data that support the findings of this study are

available from the corresponding author upon reasonable request.

Registration

This type of retrospective observational study is not required to be registered in a clinical trial registry.

Conflicts of interest

The authors have no conflicts of interest in this study

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