

# Inter observer variability in the interpretation of atypical squamous cells in Pap smear examination

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## ABSTRACT

**Introduction :** The incidence of cervical cancer has decreased due to wide spread use of Papanicolaou test (Pap) as a screening method to detect early cervical lesions. According to The Bethesda System 2001, atypical squamous cells are divided into two groups: Atypical squamous cells of undetermined significance (ASC-US), atypical squamous cells cannot exclude HSIL (ASC-H). As it is known, the microscopic interpretation of cytologic specimens is a subjective procedure highly dependent on skills and experience of the investigator. ASC reflects a diagnosis of uncertainty and is used as tool to measure interlaboratory and intralaboratory control for quality control purposes.

**Aims and Objectives:** To determine the inter observer variation in interpreting ASC variants in conventional pap smears based on the Bethesda system of reporting cervical cytology (2001).

**Materials & Methods:** A total of 833 cervical smears were sent for Pap test during the period July 2013 to –June 2014. Slides of all the cases of ASC and appropriate number of smears diagnosed as negative for intraepithelial lesion or malignancy were collected with relevant history. An observational study was performed with two evaluators blinded to the original reports. The review reports from the evaluators were compared with original reports to detect accuracy of reporting atypical squamous cells.

**Results :** Review of data showed 36 low-grade squamous intraepithelial lesion (LSIL), 8 high-grade squamous intraepithelial lesion (HSIL) and 17 cases coded as atypical squamous cells (ASCUS and ASC-H). ASCUS/SIL ratio was 0.33. All the agreements were found out to be statistically significant and the agreement between original diagnosis and observer 1 was found out to be stronger compared to others.

**Conclusion:** Interobserver reproducibility in reporting cervical smears according to Bethesda system 2001 is not 100%, even after following strict Bethesda guidelines. Rescreening of smears is one of the best ways to improve quality control of

the cytopathology laboratory.

**Keywords:** Pap smear, ASCUS, cervix

## INTRODUCTION

Screening for cervical cancers has been the most successful and one of the best implemented programs in the previous century. It is performed worldwide in order to detect cervical cancer in its earliest stage.

The incidence of cervical cancer has decreased due to the wide spread use of Papanicolaou test (Pap) as a screening method to detect early cervical lesions. But in the interpretation there is significant of inter observer variability noted, especially in ASC category. According to The Bethesda System 2001, atypical squamous cells thus detected are divided into two groups viz., atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells cannot exclude HSIL (ASC-H)<sup>1</sup>. It is known that the microscopic interpretation of cytologic specimens is a subjective procedure highly dependent on skills and experience of the investigator and the time spent on the sample. ASC reflects a diagnosis of uncertainty and is used as tool to measure interlaboratory and intralaboratory control for quality control purposes<sup>2</sup>.

## MATERIALS AND METHODS

The present study was carried out over a period of 1 year (July 2013 to June 2014). It is a reproducibility study which was conducted to compare the interobserver variability in reporting already reported ASC cases in cervical cytology smears.

A total 833 conventional Pap smear slides sent for Pap test during the study period were reviewed. Of all the cases diagnosed as ASC, appropriate number of smears diagnosed as negative for intraepithelial lesion or malignancy were collected with relevant history. Two evaluators blinded to the original reports reviewed these slides. The reports were reviewed and reported by using 2001 Bethesda system terminology. The review reports from the evaluators were

compared with original reports to detect accuracy of reporting atypical squamous cells. The inter observer variability in the interpretation of ASC was documented..

The cases in which the slides and/or the clinical details were unavailable were excluded.

**RESULTS**

A total of 833 Pap smears were examined. Of these 36 were reported as low-grade squamous intraepithelial lesion (LSIL), 8 as High-grade squamous intraepithelial lesion (HSIL), 17-ASC of which 12 were ASCUS and 5 were ASC-H. In these 17 ASC cases, 2 cases were excluded from the study. As the slides could not be retrieved.ASC/SIL ratio was 0.38. 7/10 ASC-US patients were post menopausal and 2/5 ASC–H patients were post menopausal. 10 /15 patients presented with pain abdomen and white discharge per vagina. Inflammation and atrophy was also noted in many patients.

Statistical analysis was performed by Cohen’s Kappa.

Interpretation of Kappa value Kappa statistics:

- If kappa is less than 0, "No agreement",
- If 0-0.2, "Slight agreement",
- If 0.2-0.4, "Fair agreement",
- If 0.4-0.6, "Moderate agreement",
- If 0.6-0.8, "Substantial agreement",
- If 0.8-1.0, "Almost perfect agreement"

The reproducibility of the diagnosis between observer 1 and observer 2 are depicted in Tables 1 and 2. The interobserver variability in the diagnosis is depicted in Table 3. The original diagnosis and the diagnosis rendered by the two observers is depicted in Figure 1.

**Table1: ORIGINAL DIAGNOSIS VS DIAGNOSIS OF OBSERVER 1**

		Diagnosis			Kappa value	P value
		NILM	ASC-US	ASC-H		
Observer 1	NILM	11	1	0	0.313	0.001
	ASC-US	4	7	2		
	ASC-H	0	1	2		
	LSIL	0	1	1		

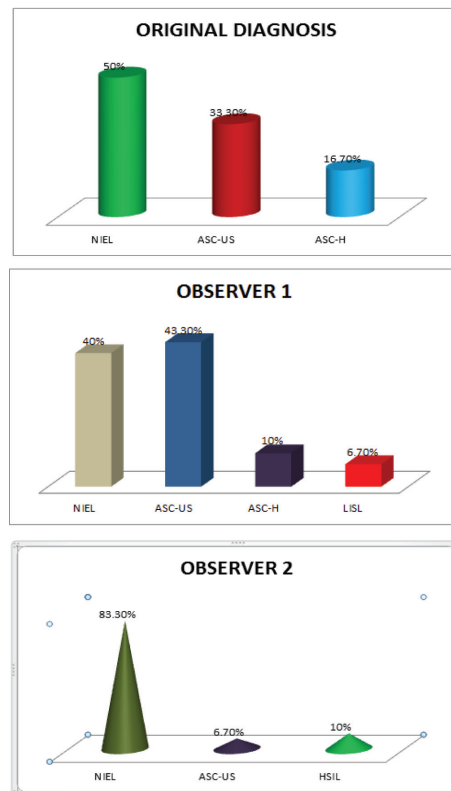
**Table2: ORIGINAL DIAGNOSIS VS DIAGNOSIS OF OBSERVER 2**

		Diagnosis			Kappa value	P value
		NILM	ASC-US	ASC-H		
Observer 2	NILM	15	6	4	0.189	0.017
	ASC-US	0	2	0		
	HSIL	0	2	1		

**Table3: DIAGNOSIS OF OBSERVER 1 VS DIAGNOSIS OF OBSERVER 2**

		Observer 2			Kappa value	P value
		NILM	ASC-US	HSIL		
Observer 1	NILM	12	0	0	0.130	0.030
	ASC-US	10	2	1		
	ASC-H	2	0	1		
	LSIL	1	0	1		

[NILM: negative for intraepithelial lesion or malignancy, HSIL: high grade intraepithelial lesion, LSIL:lowgrade intraepithelial lesion]



**Figure 1: Graphical depiction of the diagnosis rendered originally and subsequently by both observers.**

All the agreements were found out to be statistically significant and the agreement between original diagnosis and observer 1 is found out to be stronger compared to others. (k value= .313)

A comparison of the diagnosis rendered by both the observers for the cases diagnosed initially as ASC-US and ASC-H is depicted in Table 4 and 5 respectively

**Table 4: Evaluation of cases reported initially as ASCUS**

	OBSERVER1	OBSERVER2
NILM	1	6
ATROPHY	6	3
INFLAMMATION	3	0
ASC-US	7	2
ASC-H	1	0
HSIL	0	2
LSIL	1	0

**Table5: Evaluation of cases reported initially as ASC-H**

	OBSERVER1	OBSERVER2
REACTIVE CHANGES	0	2
ATROPHY	1	0
INFLAMMATION	5	3
ASC-US	2	0
ASC-H	2	0
HSIL	0	1
LSIL	1	0
NILM	0	4

**DISCUSSION**

According to the 2001 Bethesda system of reporting Pap smears, ASC is a distinct category. In 1991 ASC was diagnosis of exclusion and in 2001 ASC was a diagnostic category which was conveying a suspicion of SIL. Atypical squamous cells-ASC<sup>1</sup> in PAP smears are sub divided as atypical squamous cells of undetermined significance (ASC US) and atypical squamous cells that cannot exclude HSIL(ASC H). These cytologic changes are suggestive of squamous intraepithelial lesions (SIL) and these are qualitatively or quantitatively insufficient for a definitive interpretation. These designate squamous cells appears normal with few features suggestive but not fully diagnostic of squamous cell dysplasia. It is an important quality determining factor. It causes inter and intra observer variation<sup>3,4</sup>.

Assessment of observer variability represents a part of measurement system analysis and is a necessary task for any research that evaluates new method. Three features required to call it as ASC. They are squamous differentiation, with normal cells on the same slide to compare, increased nuclear cytoplasmic ratio, minimal nuclear changes and cytoplasmic changes like parakeratosis and koilocytes. Eliminating ASC results in increased reporting of LSIL and decreased reporting of HSIL<sup>5,6</sup>.

According to many studies ASC is the main category which shows maximum variations in reporting(6). It causes inter and intra observer variation.<sup>3,4</sup> Many features confound the diagnosis of ASC like drying artifact, degenerative changes, atrophic vaginitis, inflammation, prominent perinuclear halos, nuclear size, hyperchromasia, binucleations and menopausal atrophy.<sup>11</sup> We should be aware of these to reduce misinterpretation. Clinical details are important to determine ASC<sup>7,8,9</sup>.

Irrespective of the methodology, discrepancies are noted in reporting ASC lesions<sup>8</sup>. In 1996, a national survey of reporting practices in 768 laboratories was done, in which diagnosis of ASCUS accounted for a mean of 5.2% of all cervical cytology reports. It means that it was difficult to maintain ASC US less than 5%. ASC-US accounted for 90% in ASC interpretations. ASC-H was 10% of ASC interpretations in many laboratories<sup>1</sup>. ASCUS and ASC-H needs careful follow up<sup>9</sup>. Potential for overuse of ASCUS usually happens. ASC/SIL ratio should not be greater than 3:1<sup>10</sup>. In one study, out of 6 cytopathologists, ASC/SIL ratio was decreased by 4 pathologists and increased by 2 pathologists. It all depends on the experience of the reporting pathologist<sup>11-14</sup>.

The variations in the present study could be possible due to the varying experience of the two observers. Also, as was noted, confounding factors like inflammation and post menopausal atrophy can lead to smears being categorized as ASC.

**CONCLUSION**

Here we conclude that interobserver reproducibility in reporting cervical smears according to Bethesda system 2001 is not 100%, even after following strict Bethesda guidelines. Rescreening of smears is one of the best way to improve quality control of the cytopathology lab. In our laboratory ASC is maintained at 1.4% and ASC/SIL ratio is 0.38 and inter observer variability in this study is statistically significant. Atrophy and inflammation could be the associated factors leading to variation in interpretation.. Category of ASC is highly subjective in interpretation.

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