# HISTOLOGICAL OUTCOME OF ATYPICAL SQUAMOUS CELLS CANNOT EXCLUDE HIGH-GRADE SQUAMOUS INTRAEPITHELIAL LESION (ASC-H) PAPANICOLAOU SMEARS DIAGNOSED AT GROOTE SCHUUR HOSPITAL


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# DECLARATION PAGE

I, ***Raisa Wessels*** hereby declare that the work on which this dissertation/thesis is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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# DEDICATION

To my family.

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A histological outcome of atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H) Papanicolaou smears diagnosed in Groote Schuur Hospital.

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Nadia M. Ikumi: Data analysis and supervision.

Mmaphuti J Chokoe Maluleke: Conceptualization, project administration and supervision.

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# ABBREVIATIONS

| | |
|---|---|
| AIS: | Adenocarcinoma in situ |
| ASC: | Atypical squamous cells |
| ASC-H: | Atypical Squamous Cells, cannot exclude High grade squamous intraepithelial lesion |
| ASC-US: | Atypical Squamous Cells of Uncertain Significance |
| ATLS: | Atypical Squamous Cells of Undetermined Significance Low-Grade SIL Triage Study |
| CIN: | Cervical intraepithelial neoplasia |
| HPV: | Human papillomavirus (HPV) |
| HSIL: | High grade Squamous Intraepithelial neoplasia |
| IQR: | Interquartile range |
| LLETZ: | Large loop excision of the transformation zone |
| LIS: | Laboratory Information Systems |
| LSIL: | Low grade Squamous Intraepithelial Lesion |
| NHLS: | National Health Laboratory Service |
| NILM: | Negative for intraepithelial lesion or malignancy |
| SCC: | Squamous cell carcinoma |
| SIL: | Squamous Intraepithelial Lesions |
| SQMET: | Squamous metaplasia |
| SUBOPT: | Suboptimal |

MANUSCRIPT

# *Histological outcome of atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion (ASC-H) Papanicolaou smears diagnosed at Groote Schuur Hospital.*

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**Keywords:** ASC-H, Pap smear, Cervical cytology, Cyto-histological correlation, Atypical squamous cells

## Abstract

**Background** - Cervical cancer imparts a huge burden on South African women and the healthcare system, making up 17.33% of all cancers in females. Cervical cytology screening, assessed under the Bethesda system, forms an integral part of this effort. Within this system, the Atypical Squamous Cells, cannot exclude High grade squamous intraepithelial lesion (ASC-H) category is said to carry a positive predictive value for High grade Squamous Intraepithelial neoplasia (HSIL) that lies between Atypical Squamous Cells of Uncertain Significance (ASC-US) and HSIL.

**Aim**: The aim of this study was to assess the histological outcome of the ASC-H Papanicolaou smears diagnosed between January 2009 and December 2019 and to compare the yield of high grade histology with the literature.

**Methods**: A computerized search of the Laboratory Information Systems (LIS) at Groote Schuur Hospital yielded 1694 ASC-H cases.

**Results**: Of the 1694 cases identified, 699 cases had data on histology. Of the 699 cases with histology data, the majority of cases showed High Grade Squamous Intraepithelial Lesion (HSIL) on histology (n=271, 38.8%) . There were 225 (32.1%) cases of Low grade Squamous Intraepithelial Lesion (LSIL), 75 (10.7%) showed normal histology, 40 (5.7%) cases showed cervicitis, 26 (3.7%) cases showed squamous cell carcinoma, 25 (3.6%) cases were suboptimal for assessment, 21(3.1%) cases showed squamous metaplasia and 16(2.3%) cases reflected diagnoses falling into an “other” category (which includes entities such as benign polyps and glandular lesions).

**Conclusion**: With the larger proportion of cases showing HSIL, the intention of the ASC-H category to identify likely high risk cervical changes and expedite follow-up colposcopy, biopsy and management has been proven.

## Introduction

Cervical cancer is caused by persistent infection by high risk types of Human papillomavirus (HPV) [1]. According to the National Institute for Communicable Disease in South Africa, cervical cancer ranks as the second most prevalent cancer in South African women, making up 17.33% of all cancers in females according to the 2016 National Cancer Registry report [2]. In addition to the morbidity and mortality suffered by affected patients, an incredible burden is placed on our resource-limited healthcare system.

The approach to cervical cancer in the South African healthcare context involves screening, triage/secondary tests and treatment as appropriate [1]. A cervical cancer screening guideline developed by the South African HPV Advisory Board recommends two methods of primary screening for cervical cancer: cervical cytology (Papanicolaou or "pap" smear) or HPV testing [1]. Currently, in the public sector, cervical cytology remains the mainstay of cervical cancer screening, with pap smears offered to HIV negative women at ten yearly intervals from the age of 30 years, provided the smears are normal. [5,6] Guidelines for HIV positive women, see more frequent smears based on the national guidelines. [5,6] Cervical cytology is merely a screening tool or a gateway towards a final diagnosis and management plan for the patient. In line with this thinking, the 2001 Bethesda system workshop has done away with utilizing the term "diagnosis" in favour of "interpretation" or "result" [3].

The aims of the cervical cancer screening program are in line with the fundamental principles of primary health care, in which a "prevention is better than cure" approach is foundational. Primary prevention is a means whereby disease is prevented before the precursor phase has begun. Secondary prevention refers to detection and management of an already established, but asymptomatic precursor phase. Tertiary prevention aims to diagnose and effectively treat established disease [1]. The cervical cancer screening program aims to address all levels of prevention whilst being particularly focussed on strengthening primary prevention.

Evaluation of the pap smear aims to place the result into a certain category: negative for intraepithelial lesion or malignancy (or "NILM", which would also allow for organisms, reactive changes, inflammation and atrophy), epithelial cell abnormalities (including squamous cell abnormalities and glandular cell abnormalities) and "other" (which could include endometrial cells in a woman over the age of 40 years [4].

The squamous cell abnormalities include atypical squamous cells or “ASC” (comprising atypical squamous cells of undetermined significance or ”ASCUS” and atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion or “ASC-H”), low-grade squamous intraepithelial lesion or “LSIL” (which includes HPV changes, mild dysplasia and cervical intraepithelial neoplasia 1 or “CIN 1”), high grade squamous intraepithelial lesion or “HSIL” (which includes moderate to severe dysplasia, carcinoma-in-situ, CIN 2 and CIN 3) and squamous cell carcinoma [3,4]. The glandular cell abnormalities include atypical glandular cells, atypical glandular cells favoring neoplastic, adenocarcinoma in situ (AIS), and adenocarcinoma [3,4].

The ASC category is one that can often create a degree of confusion amongst clinicians and pathologists alike, with the relevance of the category being brought into question. The purpose of the ASC category is to alert the treating healthcare practitioner to smears which are thought to be more indicative of squamous intraepithelial lesion( SIL) and would thus require colposcopy. Arguments were made to do away with the “atypical squamous cells” category altogether, but the need for an equivocal category remained in light of the number of women who had underlying CIN 2 and 3 detected as a result of follow up biopsies done as part of their workup for an equivocal pap smear [3]. The ASC smears are not a diagnosis in their own right, but are deemed to be suggestive of a SIL lesion [3].

ASC-H is thought to comprise between 5 - 10% of ASC cases and is thought to have a positive predictive value for a histology result of CIN 2 or 3 that lies between that of ASC-US and HSIL [3]. Therefore, the aim of the new delineation was to allow for more rapid detection of CIN 2 and 3 cases [3].

ASC-H is interpreted as a smear demonstrating cytological features suggestive of HSIL, but lacking the criteria for its definitive diagnosis (moderate to severe dysplasia, carcinoma in situ, CIN 2, CIN 3) [4]. Included in secondary prevention is the implementation of a reliable treatment programme. The initial step in management includes colposcopy, where the cervix is evaluated thoroughly and a biopsy is performed if necessary. Currently, pre-cancerous cervical lesions are managed with ablative techniques such as cryotherapy or thermal coagulation, or excisional techniques such as large loop excision of the transformation zone (LLETZ) [5].
Moreover, management strategies involve a “wait and see” approach to CIN 1 lesions, with rescreening pap smears conducted within 3 years. CIN 2, CIN 3 and adenocarcinoma in situ lesions are managed with ablative or excisional techniques [5,6].

Factors contributing to the high incidence of cervical cancer in the South African population include poor socioeconomic status, rural place of residence (due to the logistical difficulties incurred when referral to next level of care is required), low levels of education and the language barrier (due to the majority of healthcare communication being in English), economic reliance on male partners which affects health seeking behaviours, and the inequitable distribution of specialist services, especially in the field of oncology [5].

In the South African context, resource limitations do not allow for routine HPV testing on Papanicolaou smears as a manner of routine triage for colposcopy, and all cases with ASC-H results are sent for colposcopy.

The purpose of this study, therefore, was to determine whether the outcome of ASC-H smears at histology shows a comparable level of underlying HSIL in our center, as seen in the literature, and to thus serve as supporting evidence for the utility of the category and its management being referral for colposcopy.

## Methods

This study was a retrospective, cross-sectional study at the National Health Laboratory Service (NHLS) Anatomical Pathology Laboratory at Groote Schuur Hospital, Cape Town, South Africa.

A computerised database search of TrakCare and DisaLab, laboratory information systems utilised by the NHLS, was conducted to identify participants meeting the inclusion criteria.

The following inclusion criteria were established and utilized: women with an ASC-H result on a pap smear, adult women aged 18 years and older, cytology samples which were processed at the National Health Laboratory Service (NHLS) Anatomical Pathology Laboratory at Groote Schuur Hospital.

The period evaluated was January 2009 to December 2019 for the pap smear samples.

The exclusion criteria were defined as individuals under 18 years of age and cases with no follow up histology.

Once ASC-H cases were identified, corresponding histology was sought for each case using the same laboratory information systems. Corresponding histology results for certain pap smears fell beyond December 2019 in cases where follow up occurred at a later date. The data were compiled into spreadsheets and assessed according to histology result and age distribution.

*Statistical analyses*

Statistical analyses were performed using STATA (version 18.0, STATA Corporation, College Station, Texas, USA) and R (version 4.2.1, R Core Team, Vienna, Austria). Primary stratification was based on the availability of histology reports. The ASC-H pap smears with histology reports were then represented graphically based on the corresponding histology for each category. The data is reported in frequencies and, proportions are reported as medians and quartile ranges. The differences between the groups were compared using Wilcoxon rank-sum tests and a p value of $<0.05$ was considered statistically significant.

## Results

A total of 1694 ASC-H cases were identified between January 2009 and December 2019. Of these, 699 cases (41.3%) had reports on histology and 995 (58.7%) had no histology data reported (shown in Fig. 1).

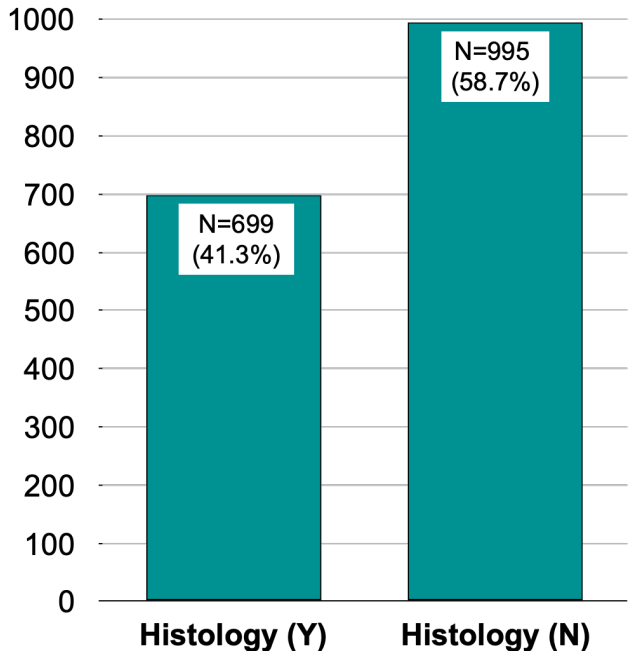


**Figure 1: Proportion of ASC-H cases with histology and without histology.**

*A total of 1694 ASC-H cases were identified. The bar graph depicts 699 cases (41.3%) with histology results and 995 cases (58.7%) without follow up histology.*

Of the 699 cases with histology data, most cases showed HSIL on histology (n=271, 38.8%). There were 225 (32.1%) cases of Low grade Squamous Intraepithelial Lesion (LSIL), 75 (10.7%) showed normal histology, 40 (5.7%) cases showed cervicitis, 26 (3.7%) cases showed squamous cell carcinoma (SCC), 25 (3.6%) cases were suboptimal for assessment, 21(3.1%) cases showed squamous metaplasia (SQMET) and 16 (2.3%) cases reflected diagnoses falling into an “other” category (which includes entities such as benign polyps and glandular lesions) (shown in Fig. 2).The ASC-H cytological result carries a positive predictive value of 42.49% in this study, with true positive values including histology results of HSIL and SCC.

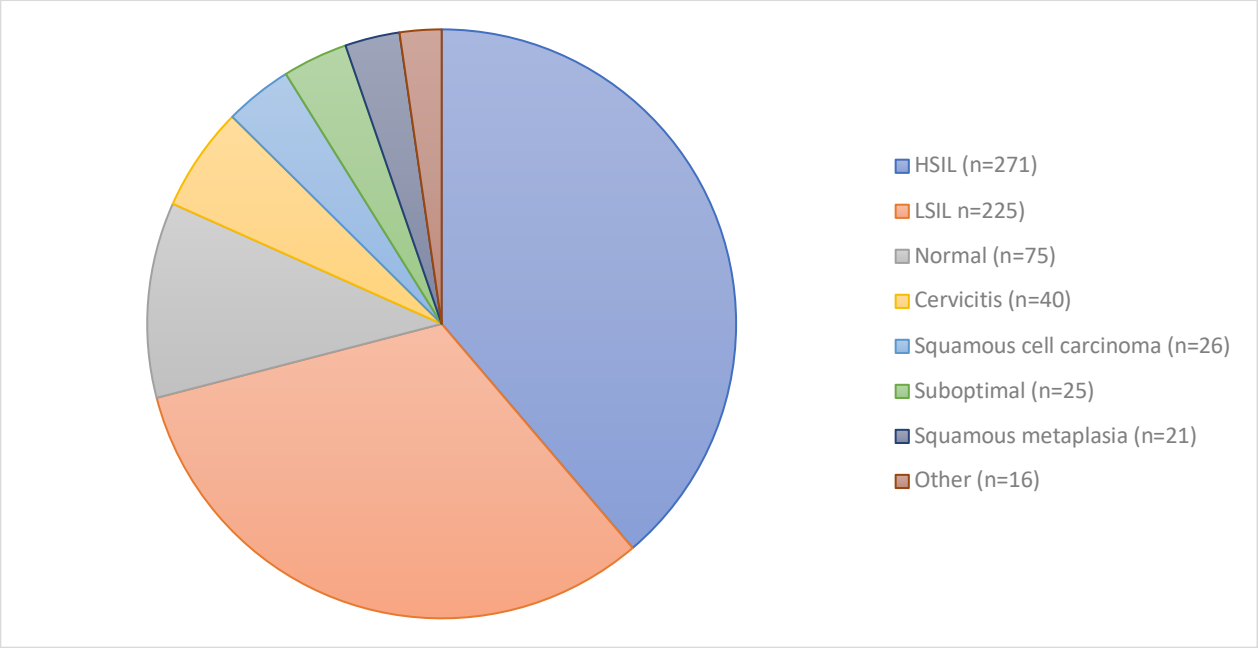


**Figure 2: Distribution of Histology Results of ASC-H pap smears**

*Among the cases with histology reports, there were 271 (38.8%) cases of High grade Squamous Intraepithelial Neoplasia (HSIL), 225 (32.1%) cases of Low grade Squamous Intraepithelial Lesion (LSIL), 75 (10.7%) showed normal histology, 40 (5.7%) cases showed cervicitis, 26 (3.7%) cases showed squamous cell carcinoma, 25 (3.6%) cases were suboptimal for assessment, 21(3.1%) cases showed squamous metaplasia and 16(2.3%) cases reflected diagnoses falling into an "other" category (which includes entities such as benign polyps and glandular lesions.*

**Age Distribution among Cases**

A total of 1238 (73.1%) of the 1694 cases had data on age (shown in Table 1). The mean age among these cases was 45 years (standard deviation, SD 13.4). The median age was 43 years (interquartile range, IQR, 34 - 55). Of the 699 cases with histology data, 493 cases reflected data on age. The minimum age was 18 years and the maximum age was 80 years. The mean age was found to be 44.2 (SD 12.5).

**TABLE 1: AGE DISTRIBUTION OF ASC-H PAP SMEARS**

| **Age (Years)** | | **Frequency (%)**<br>**Total = 1694** |
|---|---|---|
| | 18 – 29 | 116 (6.9) |
| | 30 – 39 | 369 (21.8) |
| | 40 – 49 | 277 (16.3) |
| | 50 – 59 | 269 (15.9) |
| | 60 – 69 | 147 (8.7) |
| | 70 – 79 | 51 (3.0) |
| | 80 – 89 | 9 (0.5) |
| | Missing | 456 (26.9) |
| | Mean (SD) | 45.3 (13.4) |
| | Median (IQR) | 43 (34 – 55) |

**Age distribution among cases stratified by histology**

When considering age as stratified by histology (Fig. 3), the median age of the patients in the normal group was 50 years (IQR, 39-59) and these patients were significantly older than patients in the LSIL group, median 42 years (IQR, 34 – 52) ($p=0.002$). In addition, the cases from the HSIL group were significantly younger than patients in the normal group ($p<0.0001$) and the cervicitis group ($p=0.01$). The median age in the HSIL group was 40 years (33-49) and in the cervicitis group, 48 years (40 – 46). Table 2 shows the distribution of age in cases with and without histology.

**Histological features**

ASC-H features identified on cytology include mild nuclear enlargement, moderate cytoplasm, mild nuclear membrane irregularity and a lack of nuclear hyperchromasia, as seen in Figure 4. The corresponding histology of cases showed normal squamous mucosa with no nuclear atypia identified (Fig. 4.1 ***b***), squamous metaplasia, where the unremarkable ectocervical squamous mucosa is seen overlying endocervical glands, beyond the transition zone, **r**eactive squamous epithelium with background inflammation, in keeping with a cervicitis (Fig. 4.1. ***f***), LSIL histology (Fig. 4.2. ***h***) with cervical mucosa showing koilocytic changes. Histology of High Grade Squamous Intraepithelial Lesion (HSIL), with nuclear enlargement, nuclear hyperchromasia, nuclear membrane irregularity and coarse chromatin was noted in the majority of cases (Fig. 4.2. ***g***). The corresponding histology of an invasive Squamous Cell Carcinoma (SCC) with paradoxical maturation, nuclear hyperchromasia, nuclear membrane irregularity, invasion into the surrounding stroma with features of desmoplasia is pictured in Figure 4.2. ***l*** and Figure 4.3. ***n, p, r*** and ***t***.

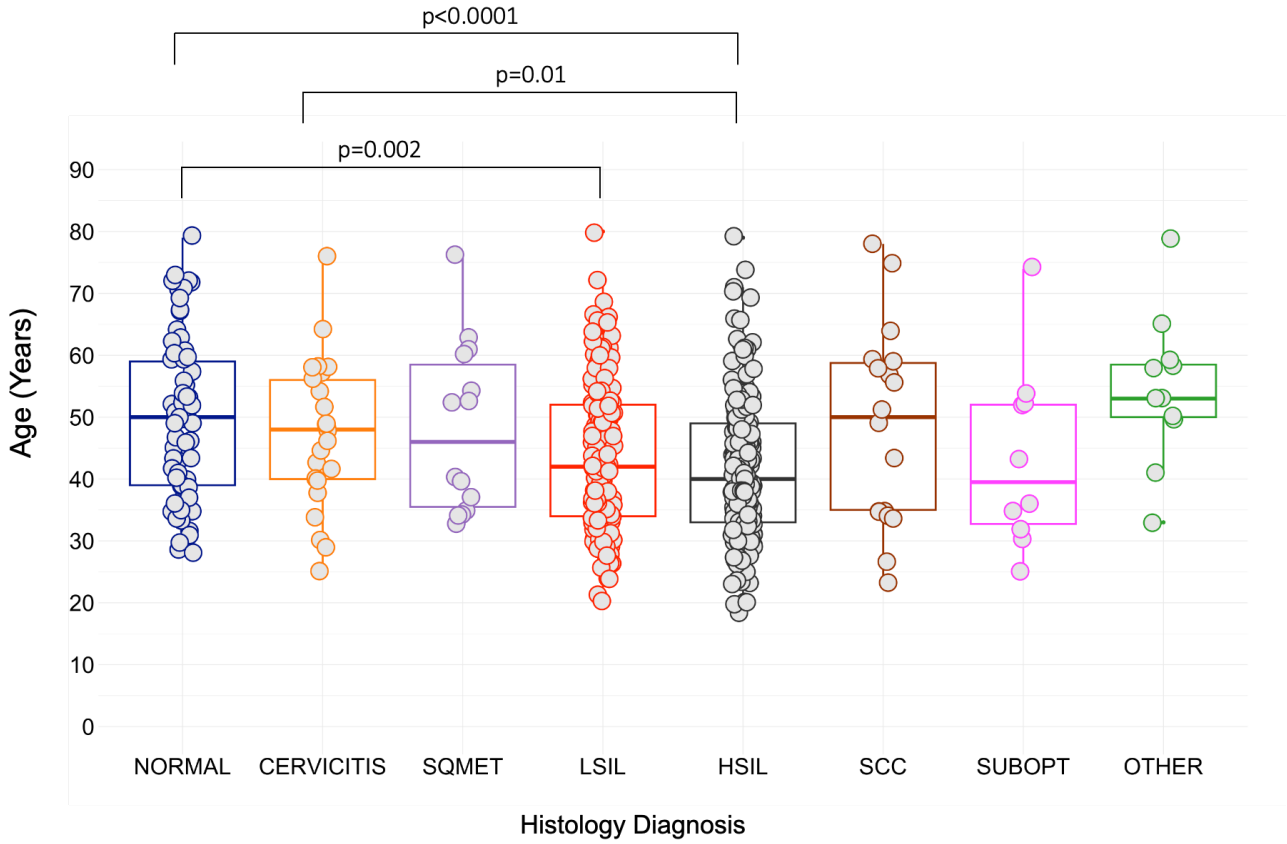


**Figure 3: Stratification of histology by age**

*The High Grade Squamous Intraepithelial Lesion (HSIL) group showed the youngest median age, whilst those in the "Other" and Squamous Cell Carcinoma (SCC) groups, showed the oldest median age.*

**Table 2: Age distribution among participants with ASC-H pap smears**

| Age (Years) | Frequency<br>Total (N=1238) | Min - Max | Mean (SD) | Median (IQR) |
|---|---|---|---|---|
| **ASC-H with histology** | | | | |
| Normal | 65 | 28 - 79 | 49.8 (13.1) | 50 (39 – 59) |
| Cervicitis | 25 | 25 - 76 | 47.2 (11.7) | 48 (40 – 56) |
| Squamous metaplasia | 14 | 33 – 76 | 48 (13.7) | 46 (35 – 60) |
| LSIL | 156 | 20 – 80 | 43.6 (11.9) | 42 (34 – 52) |
| HSIL | 194 | 18 - 79 | 41.3 (11.4) | 40 (33 – 49) |
| SCC | 18 | 23 - 78 | 48.4 (16.0) | 50 (35 – 59) |
| Suboptimal | 10 | 25 – 74 | 43.3 (14.8) | 39.5 (32 – 52) |
| Other | 11 | 33 - 79 | 54.4 (12.0) | 53 (50 – 59) |
| **Cases with no histology** | 745 | 18 - 84 | 46.1 (13.9) | 44 (34 – 56) |

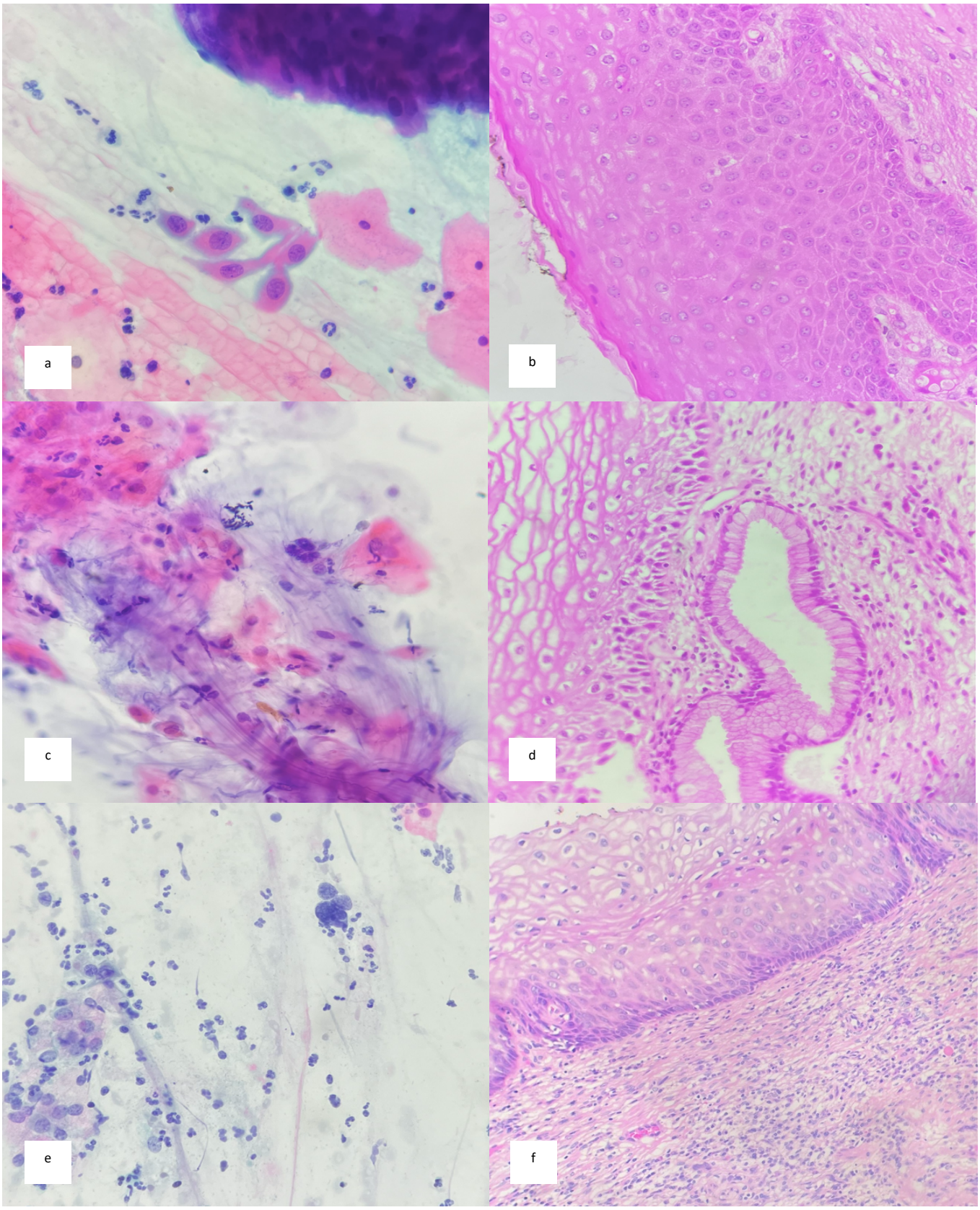
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**Figure 4.1 (a-f): Cytological and histological correlation of ASC-H cases**

*Representative images depicting in **(a)** Atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion (ASC-H) pap smear with mild nuclear enlargement, moderate cytoplasm, mild nuclear membrane irregularity and a lack of nuclear hyperchromasia. The corresponding histology of this case (**b**) shows normal cervical squamous mucosa with no nuclear atypia identified. **(c)** ASC-H with nuclear enlargement, nuclear membrane irregularity, anisochromatosis and moderate cytoplasm. **(d)** Corresponding histology with squamous metaplasia, where the unremarkable ectocervical squamous mucosa is seen overlying endocervical glands, beyond the transition zone (not pictured). **(e)** ASC-H with mild nuclear enlargement, moderate cytoplasm, mild nuclear membrane irregularity, a lack of nuclear hyperchromasia and background inflammatory cells. **(f)** Reactive squamous epithelium with background inflammation, in keeping with a cervicitis.*

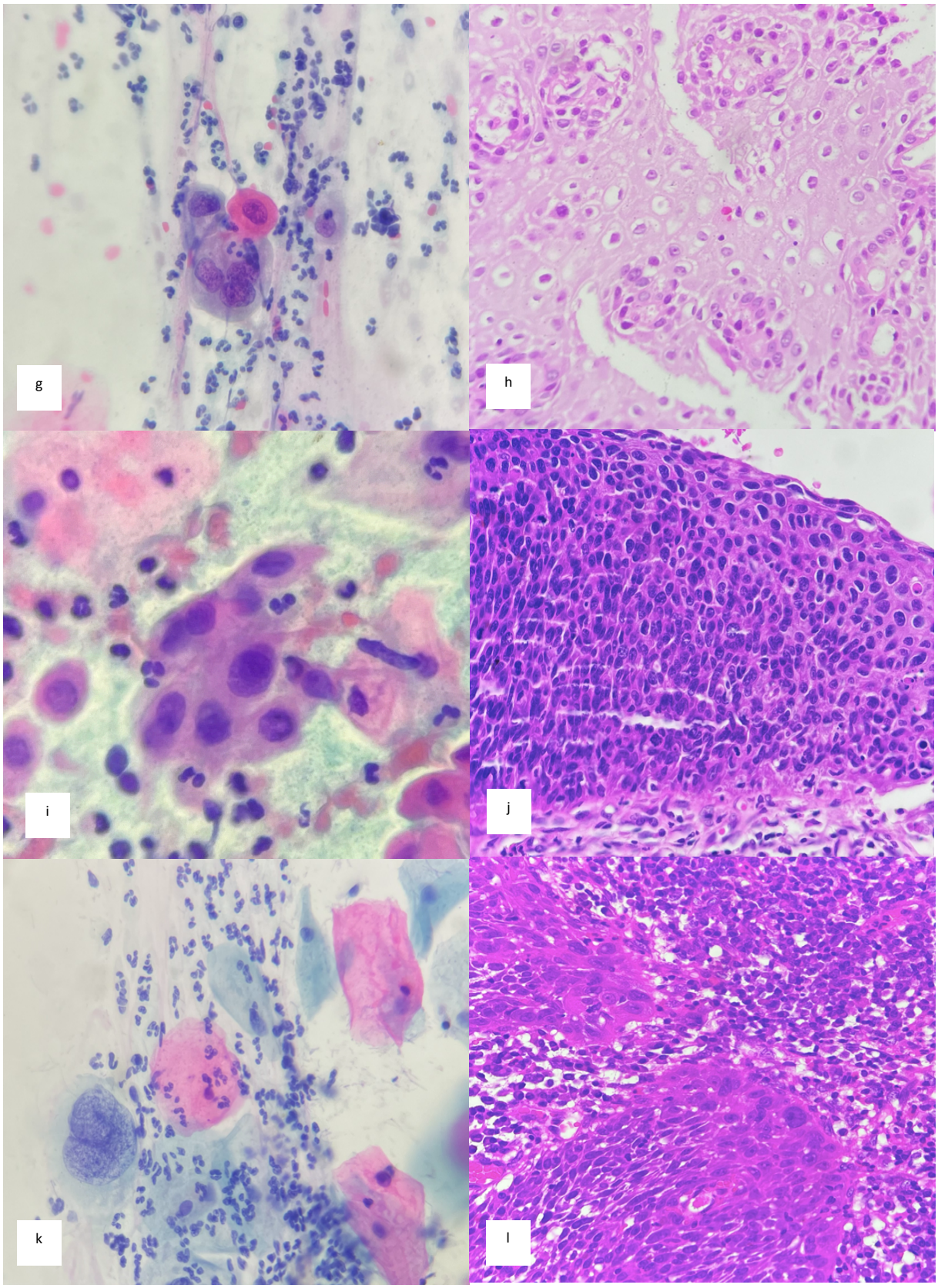
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**Figure 4.2 (g-l): Cytological and histological correlation of ASC-H cases**

***g*** *ASC-H pap smear with similar features seen in (a). The corresponding histology (**h**) shows cervical mucosa with koilocytic changes in keeping with a diagnosis of Low Grade Squamous Intraepithelial Lesion (LSIL).* ***i*** *ASC-H pap smear with features akin to those described in (c). The corresponding histology of High Grade Squamous Intraepithelial Lesion (HSIL), with nuclear enlargement, nuclear hyperchromasia, nuclear membrane irregularity and coarse chromatin (**g**).* ***k*** *ASC-H pap smear with a cluster of squamous cells showing nuclear enlargement, moderate cytoplasm and nuclear membrane irregularity. The corresponding histology of an invasive Squamous Cell Carcinoma (SCC)(**l**).*

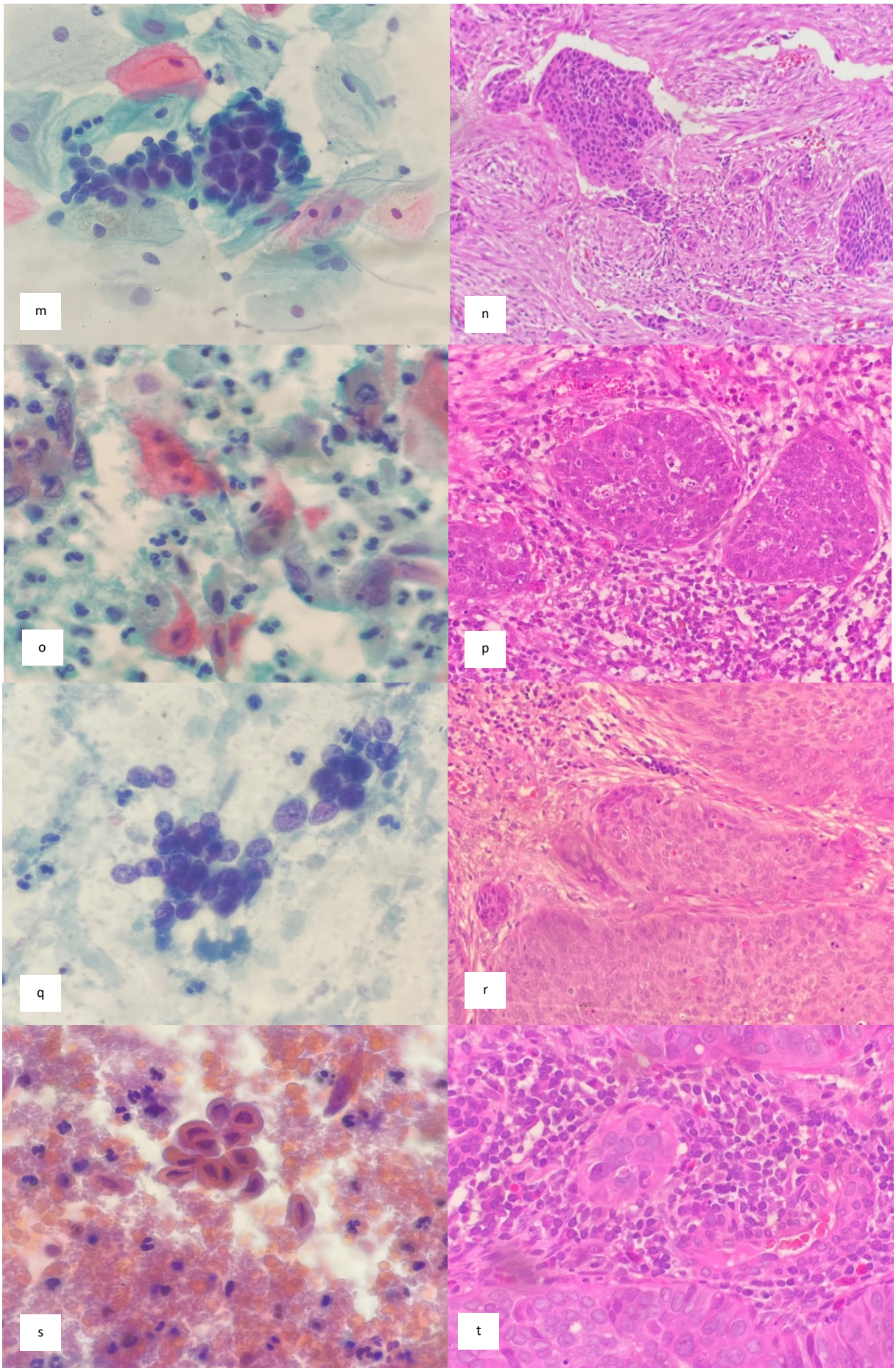
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**Figure 4.3 (m-t): Cytological and histological correlation of ASC-H cases**

***m, o, q, s*** *ASC-H pap smear with similar features seen in (a) with squamous cells showing nuclear enlargement, moderate cytoplasm and nuclear membrane irregularity. The corresponding histology of an invasive Squamous Cell Carcinoma (SCC)(**n, p, r, t**).*

## Discussion

Our study aimed to evaluate the histological outcomes of ASC-H pap smears conducted at Groote Schuur Hospital, and to compare these findings to the literature. The cases in the present study were from participants aged between 18 – 80 years. Interestingly, of the 1238 cases with data on age in our cohort, 753 (60.1%) were aged above 40 years. This finding may be indicative of the postmenopausal changes in cervical epithelium, causing an increase in inadequate smears and a higher proportion of atrophic cells and degenerative changes, which may hinder interpretation [7]. We found that the cases showing high grade histology on follow-up occurred in an overall younger age group (age range of 18 – 79 years with a mean age of 41.3 years). Squamous cell carcinoma results were noted in an older cohort, with a mean age of 48.4 years (SD 16.0) and an age range of 23-78 years, which is likely to reflect the time required for progression of HSIL lesions to invasive ones. Normal histology was also seen in an older age group, demonstrating a mean age of 49.8 years (SD 13.1).

ASC-H makes up only a small proportion of cervical cytological diagnoses, with a 2003 study by Suzanne and Selvaggi at the University of Wisconsin Hospital and Clinics finding only 25 (0.27%) of the 9214 cervical samples conducted between March 2002 and September 2002 being diagnosed as ASC-H [8]. The ASC-H category is thought to comprise a mixture of HSIL and its mimics [3], with characteristic features falling into one of two groups, those being either disorganized and crowded cell groups (hyperchromatic crowded groups) or single cells showing high nuclear-to-cytoplasmic ratios, hyperchromatic nuclei, nuclear membrane irregularity and variably prominent nucleoli. Additionally, atypical immature squamous metaplasia, atypical mature squamous metaplasia, small atypical cells with high nuclear-to-cytoplasmic ratios (nucleus 1.5-2.5 times the nucleus of a metaplastic squamous cell), atrophy associated atypical changes and atypical reparative changes may also be encountered [7,9,10]. ASC-H cells may also exist as small fragments within mucous [7,10]. In most cases, the cells lack abundance or definitive high grade features seen in HSIL interpretations [7,10]. These pitfalls add to the complexity in categorizing these pap smears into those delineated by the Bethesda System. The Selvaggi study found that cervical biopsy after a cytology result of ASC-H showed 15 cases (88%) with HSIL, 2 cases (12%) of LSIL [8].

A South African study conducted at the University of Witwatersrand in Johannesburg detailing the cytohistological correlation of ASC-H pap smears in a population of women living with Human Immunodeficiency Virus (HIV), found that 41 (1.94%) of 2111 smears of pap smears conducted in their target population were diagnosed as ASC-H [7]. Of these ASC-H smears, HSIL diagnoses (comprising CIN 2 and CIN 3) were made on 69.3% [7]. In addition, the study highlighted the

difficulties in servicing the population in a resource-limited setting. The issue raised was that ASC-H smears usually fell behind their HSIL counterparts in terms of priority cases for colposcopy but the authors concluded that current practices are still in accordance with national guidelines in that all ASC-H cases are referred for colposcopy.

With arguments having been made bringing into question the relevance of this category, one should consider the utility of the category in low- and middle-income countries (LMICs) particularly those which bear the brunt of the HIV epidemic. In countries such as South Africa, a high burden of HIV infection increases the risk of HPV infection, and this further drives the progression of cervical dysplasia to invasive squamous cell carcinoma. This is reflected in the age of diagnosis in this setting where women living with HIV have been found to present with cervical cancer around 10 years earlier than HIV negative women [11]. Compounded with the fact that access to healthcare for many women in South Africa remains a logistical challenge, it is imperative that cases carrying suspicion for high-risk dysplasia be prioritized and investigated as an integral part of preventative management in this setting.

A study conducted in Chile found that 82.6% of ASC-H cases had abnormal colposcopy results, with two out of every three women demonstrating HSIL lesions on histology [12]. The findings in our study followed a similar trend of predominance of HSIL on histology. However, the proportion of HSIL lesions did not match the greater than two thirds predominance seen in these studies. The proportions reflected greater similarity to the findings of the Atypical Squamous Cells of Undetermined Significance Low-Grade SIL Triage Study (ATLS) [13].

The ATLS study was a randomized trial of 5060 women that took place in the United States of America from November 1996 and concluded at the end of 2000 [13]. The aim of the study was to determine the best way to manage women with ASCUS and LSIL results on cervical cytology [13]. The study proposed three management options: immediate colposcopy, repeat Papanicolaou smear with colposcopy only if the initial smear showed a high grade lesion, and HPV testing with repeat cytology, which would occur in combination with colposcopy if the HPV test was positive or if the cytology showed a high grade lesion [13]. Cytology was repeated every six months with colposcopy referral if HSIL was found [13] .

Using the data and outcomes of the ALTS study, Sherman et al attempted to better define the ASC-H category [14]. As the ALTS study was conducted before the 2001 additions were made to the Bethesda system. These changes included the addition of the "atypical squamous cells" (ASC) category, which now replaces "atypical squamous cells of undetermined significance" (ASCUS) [3]. The ASC category contains ASC of "undetermined significance" (ASC-US) and ASC "cannot exclude high-grade squamous intraepithelial lesion (HSIL)," or (ASC-H) [3]. Sherman et al recategorized cases with ASCUS results to fit into the 2001 Bethesda classification. The study found that of the cases reclassified as ASC-H, 28% had subsequent CIN 2 on histology, and 17% had subsequent CIN 3 on histology [14]. This was comparable to cases that had HSIL detected on follow up smears which, at histology, showed 26% CIN 2 and 18% CIN 3 detection [14]. The study discussion argued that ASC-H is associated with higher rates of CIN 2 and CIN 3 at histology than ASC-US, but not as high as HSIL cytology results [14]. Suggestions were also made that ASC-H is equivocal for HSIL and ASC-US is equivalent to LSIL, and should likely be managed as such [14].

Gupta et al, in a study conducted between January 2006 and December 2008, found that out of the 25,203 cervical smears conducted during this period, 424 (1.7%) were ASC. Of the ASC group, only 11 (2.6%) were found to be ASC-H [15]. Of those in the ASC-H group, 5 (45.4%) were found to have a corresponding histological diagnosis of CIN 2 and higher (what the study had designated as "clinically significant") [15]. It should be noted, however, that only 155 (36.5%) of the ASC cases had corresponding follow-up histology available for evaluation [15]. The authors conclude that in instances where resources are limited, the referral threshold for colposcopy should be ASC, rather than a squamous intra-epithelial lesion (SIL) [15]. This takes into account the likely histological outcome of ASC-H smears and the resource constraints experienced by many countries where HPV testing, which is set to be used as an alternative primary screening method, is not readily available or possible. Such a sentiment is certainly applicable in the South African context, where routine HPV testing has not yet been implemented nationally and where socio-economic barriers to healthcare access exist.

The ALTS study discussed above, also found that ASC-H lesions were associated with a significantly greater frequency of high-risk HPV types and underlying HSIL, when compared to ASC-US [13,16]. In further exploration of these results, the 2005 study by Srodon and colleagues included an analysis of the ASC-H category in terms of association with high risk HPV subtypes and underlying HSIL diagnosis in comparison to the ASC-US category [16]. The study aimed to determine the potential for HPV

testing to be used as a triage for colposcopy and found that the ASC-H category had a higher rate of high risk HPV infections (66.7%) when compared to ASC-US (44.9%) [16]. At histological follow-up, 40% of the ASC-H cases that were HPV positive had HSIL lesions, which was higher than the 10.2% seen in the ASC-US category. HSIL lesions were detected in only 4.5% of the HPV-negative ASC-H cases [16]. The ALTS study had found a similar percentage of HSIL lesions in HPV-positive ASC-H (41%) [13,16]. Taking this into account, Srodon and colleagues concluded that it would be reasonable to use HPV testing to triage ASC-H cases for colposcopy, seeing that differences in the percentage of underlying HSIL in HPV-positive and HPV-negative ASC-H cases exist [16]. The histological finding of LSIL following an ASC-H smear was the second most prevalent diagnosis (36%), nearing those of the HSIL category (39%). As both categories show nuclear atypia related to HPV infection, it is understandable that these groups would show similar numbers [16].

When assessing pap smears, pathologists and cytology technicians should be aware of mimickers of cervical squamous epithelial dysplasia on pap smears. The appearance of reactive epithelial changes seen in cases of cervicitis are known to mimic features of dysplasia, which can account for the proportion of cervicitis cases reported on follow-up histology of ASC-H pap smears. The smears of cervicitis may show squamous cell streaming and cellular fragments in an inflammatory background which may be misinterpreted as the atypical syncytial groups seen in cervical dysplastic lesions [9]. When scanty or obscured in nature, a diagnosis of ASC-H may ensue.

Similarly, other mimics of HSIL such as squamous metaplasia, could be misinterpreted as dysplasia, with this category making up 3% (n=21) of cases. In their 2023 paper discussing HSIL and its mimics on cytology, Shin et al described the cytomorphology of the HSIL cell as a single, scattered and discrete cell or the appearance of tissue fragments [9]. These findings could be found in cases of immature squamous metaplasia, atrophic cervicitis, intrauterine device related changes, endocervical adenocarcinoma in situ and tubal metaplasia [9]. Additionally, the mild nuclear enlargement seen in squamous metaplasia may be difficult to differentiate from dysplasia [9].

Ratree et al examined the histological outcome of women with ASC-H pap smears in an effort to establish the rate of significant pathology detected by the category. Significant findings were defined as HSIL, SCC and adenocarcinoma in situ (AIS), which amounted to 30.8% [17]. This study found 43% normal cervical histology, 25.6% LSIL, 24.8% HSIL, 4.5% SCC and 1.5% adenocarcinoma in situ [17]. The proportion of normal cervical histology outcome is far greater than the 11% of normal histology showed on our observation, highlighting the difficulties in differentiating reactive cellular changes on pap smears from significant cellular changes indicative of dysplasia.

### Strengths:

Despite the lack of histological follow up data, our study identified 699 cases with corresponding histology, making the sample size adequate. The data assessed spanned a significant period of ten years. Few studies have been conducted in South Africa focused solely on the histological outcome of ASC-H pap smears.

### Limitations:

The lack of follow up histology was noted in the majority of ASC-H cases, raising concerns about patient follow-up and algorithmic management of these potentially high-grade lesions.

## Conclusion:

The burden of HPV-associated cervical dysplasia is prevalent in South Africa. Given the additional health-related and socio-economic factors affecting our population, surveillance measures to detect and expedite treatment of commonly occurring and potentially devastating conditions is essential. The use of the ASC-H category in identification of pap smears showing features concerning for HSIL, and thus requiring expedited clinical management has been shown to lead to identification of a larger proportion of HSIL histology in this study. These findings thus validate the intended purpose of the ASC-H category. The high burden of HIV infection places even greater risk on South African women having abnormal pap smears and significant pathology on histological evaluation. Until such a time as routine HPV testing is available as part of our national screening programs, algorithmic management of women based on pap smear results should continue. In future, HPV serological testing might be seen as available alternative to reduce numbers of women receiving colposcopy follow-ups and thus decrease the strain on our healthcare system.

## Statement of Ethics

The study protocol was reviewed and approved by the Human Research Ethics Committee at the University of Cape Town. The study was conducted without the requirement of informed consent from individual patients, as ethical approval was granted by the Human Research Ethics Committee at the University of Cape Town, with the reference number 677/2021. The study was based on archived laboratory results and thus had no impact on the treatment or follow up of patients. The study was conducted in accordance with the Declaration of Helsinki.

## Conflict of Interest Statement

The authors have no conflicts of interest to declare.


## Funding Sources

Funding was not required for this study.


## Author contributions

RW: conceptualization, data curation, methodology, data analysis, project administration, writing – original draft and editing.

NI: data analysis, review and editing of the manuscript,.

MJC: conceptualization, methodology, review and editing of the manuscript.

## Data Availability Statement

The data in this study was obtained from the Trakcare and DisaLab Laboratory Information Systems via the National Health Laboratory Service, where certain restrictions in line with the Protection of Personal Information Act 4 of 2013 (POPIA) may apply. The data that support the findings of this study are available from the corresponding author upon reasonable request.

## References


1 Botha MH, Dreyer G. Guidelines for cervical cancer screening in South Africa. South African J Gynaecol Oncol. 2017;9(1):8–12.

2 NICD. National Cancer Registry 2016 South AfricaAvailable from: https://www.nicd.ac.za/centres/national-cancer-registry/

3 Solomon D, Davey D, Kurman R, Moriarty A, O'Connor D, Prey M, et al. The 2001 Bethesda System: Terminology for reporting results of cervical cytology. J Am Med Assoc. 2002;287(16):2114–9.

4 Nayar, Ritu, and David C. Wilbur, eds. The Bethesda system for reporting cervical cytology: definitions, criteria, and explanatory notes. Springer, 2015.

5 Marañón CT, Mastrapa CK, Flores B, Vaillant LL, Landazuri LS. Cervical Cancer Prevention and Control. Cerv Cancer Prev Control Policy. 2017;National D(1):187–203.

6 Support I, Hpv FOR, Primary B. Guideline Document Cervical Cancer Screening in South Africa 2015 International Support for Hpv Based Primary. 2015;1–10.

7 Michelow P, Hartman I, Schulze D, Lamla-Hillie S, Williams S, Levin S, et al. Atypical squamous cells, cannot exclude high grade squamous intraepithelial (ASC-H) in HIV-positive women. Cytojournal. 2010;7(August 2009):1–13.

8 Selvaggi SM. Reporting of atypical squamous cells, cannot exclude a high-grade squamous intraepithelial lesion (ASC-H) on cervical samples: Is it significant? Diagn Cytopathol. 2003;29(1):38–41.

9 Shin E, Yu J, Hong SW. Trouble-makers in cytologic interpretation of the uterine cervix. J Pathol Transl Med. 2023;57(3):139–46.

10 Hata H, Okayama K, Iijima J, Teruya K, Shiina N, Caniz T, et al. A comparison of cytomorphological features of ASC-H cells based on histopathological results obtained from a colposcopic target biopsy immediately after pap smear sampling. Asian Pacific J Cancer Prev. 2019;20(7):2139–43.

11 Dhokotera T, Asangbeh S, Bohlius J, Singh E, Egger M, Rohner E, et al. Cervical cancer in women living in South Africa: a record linkage study of the National Health Laboratory Service and the National Cancer Registry. Ecancermedicalscience. 2022;16:1–11.

12 López-Alegría F, De Lorenzi DS, Quezada OP. Follow-up of women with atypical squamous cells cannot exclude high-grade squamous intraepithelial lesions (ASC-H). Sao Paulo Med J. 2014;132(1):15-22. doi: 10.1590/1516-3180.2014.1321597. PMID: 24474075; PMCID: PMC10889458

13 Schiffman M, Adrianza ME. ASCUS-LSIL Triage Study: Design, methods and characteristics of trial participants. Acta Cytol. 2000;441.(5):726–42.

14 Sherman ME, Castle PE, Solomon D. Cervical cytology of atypical squamous cells-cannot exclude high-grade squamous intraepithelial lesion (ASC-H): Characteristics and histologic outcomes. Cancer. 2006;108(5):298–305.

15 Gupta N, Srinivasan R, Nijhawan R, Rajwanshi A, Dey P, Suri V, et al. Atypical squamous cells and low-grade squamous intraepithelial lesion in cervical cytology: Cytohistological correlation and implication for management in a low-resource setting. Cytopathology. 2011;22(3):189–94.

16 Srodon M, Dilworth HP, Ronnett BM. Atypical squamous cells, cannot exclude high-grade squamous intraepithelial lesion: Diagnostic performance, human papillomavirus testing, and follow-up results. Cancer. 2006;108(1):32–8.

17 Ratree S, Kleebkaow P, Aue-Aungkul A, Temtanakitpaisan A, Chumworathayi B, Luanratanakorn S. Histopathology of women with “atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion” (ASC-H) smears. Asian Pacific J Cancer Prev. 2019;20(3):683–6.

## Figure Legends

Fig. 1. Proportion of ASC-H cases with histology and without histology. A total of 1694 ASC-H pap smears were identified. The bar graph depicts 699 cases (41.3%) with histology results and 995 cases (58.7%) without follow up histology.

Fig. 2. Histology Results of ASC-H pap smears. Among the cases with histology reports, there were 271 (38.8%) cases of High grade Squamous Intraepithelial Neoplasia (HSIL), 225 (32.1%) cases of Low grade Squamous Intraepithelial Lesion (LSIL), 75 (10.7%) showed normal histology, 40 (5.7%) cases showed cervicitis, 26 (3.7%) cases showed squamous cell carcinoma, 25 (3.6%) cases were suboptimal for assessment, 21(3.1%) cases showed squamous metaplasia and 16(2.3%) cases reflected diagnoses falling into an "other" category (which includes entities such as benign polyps and glandular lesions

Fig. 3. Stratification of histology by age. The High Grade Squamous Intraepithelial Lesion (HSIL) group showed the youngest median age, whilst those in the "Other" and Squamous Cell Carcinoma (SCC) groups, showed the oldest median age.

Fig. 4.1 Cytological and histological correlation of ASC-H cases**. a** Atypical squamous cells, cannot exclude high grade squamous intraepithelial lesion (ASC-H) pap smear with mild nuclear enlargement, moderate cytoplasm, mild nuclear membrane irregularity and a lack of nuclear hyperchromasia. The corresponding histology of this case (**b**), shows normal cervical squamous mucosa with no nuclear atypia identified. **c** ASC-H with nuclear enlargement, nuclear membrane irregularity, anisochromatosis and moderate cytoplasm. **d** Corresponding histology with squamous metaplasia, where the unremarkable ectocervical squamous mucosa is seen overlying endocervical glands, beyond the transition zone (not pictured). **e** ASC-H with mild nuclear enlargement, moderate cytoplasm, mild nuclear membrane irregularity, a lack of nuclear hyperchromasia and background inflammatory cells. **f** Reactive squamous epithelium with background inflammation, in keeping with a cervicitis.

Fig. 4.2 Cytological and histological correlation of ASC-H cases**. g** ASC-H pap smear with similar features seen in (a). The corresponding histology (**h**) shows cervical mucosa with koilocytic changes in keeping with a diagnosis of Low Grade Squamous Intraepithelial Lesion (LSIL). **i** ASC-H pap smear with features akin to those described in (c). The corresponding histology of High Grade Squamous Intraepithelial Lesion (HSIL), with nuclear enlargement, nuclear hyperchromasia, nuclear membrane

irregularity and coarse chromatin (**g**). **k** ASC-H pap smear with a cluster of squamous cells showing nuclear enlargement, moderate cytoplasm and nuclear membrane irregularity. The corresponding histology of an invasive Squamous Cell Carcinoma (SCC)(**l**).

Fig. 4.3 Cytological and histological correlation of ASC-H cases**.** ***m, o, q, s*** *ASC-H pap smear with similar features seen in (a) with squamous cells showing nuclear enlargement, moderate cytoplasm and nuclear membrane irregularity. The corresponding histology of an invasive Squamous Cell Carcinoma (SCC)(**n, p, r, t**).*

# APPENDICES

## Appendix 1: MMed Research Methods Course Attendance - Confirmation Letter

**Ms Annemie Stewart**
UCT Clinical Research Centre, L51, Old Main Building
Groote Schuur Hospital, Observatory, 7925, Cape Town
☎ 021 406 6498 / 072 408 0459
✉ annemie.stewart@uct.ac.za
**W** www.crc.uct.ac.za

01 Mar 2024

To Whom it May Concern

**Statement confirming MMeds Research Methods**

This letter confirms that **Dr Raisa Wessels atte**nded the **Clinical Research Centre's MMeds Research Methods Course on the 23rd Sep and 1st Oct 2021**

If you have any questions or concerns, please do not hesitate to contact me on **021 406 6498** or **072 408 0459**.

Best wishes

Annemie Stewart
**Operations Manager**
Clinical Research Centre (CRC)
UCT Faculty of Health Sciences

Appendix 2: Ethics Approval Letter

HREC Approval 2023 to 2024

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

## FHS016: Annual Progress Report / Renewal

| HREC office use only (FWA00001637; IRB00001938) | | | |
|---|---|---|---|
| This serves as notification of annual approval, including any documentation described below. | | | |
| ☑ Approved | Annual progress report | Approved until/next renewal date | 30.11.2024 |
| ☐ Not approved | See attached comments | | |
| Signature Chairperson of the HREC/ Designee | | Date Signed | 24/10/2023 |

HUMAN RESEARCH ETHICS COMMITTEE
23 OCT 2023
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

**Note:** Please email this form and supporting documents (if applicable) in a combined pdf file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
http://www.health.uct.ac.za/fhs/research/humanethics/forms

| Comments to PI from the HREC |
|---|
| |

**Principal Investigator to complete the following:**

**1. Protocol information**

| | | | |
|---|---|---|---|
| Date (when submitting this form) | 18 October 2023 | | |
| HREC REF Number | 677/2021 | Current Ethics Approval was granted until | 30/11/2023 |
| Protocol title | A histological outcome of atypical squamous cells cannot exclude high grade squamous intraepithelial lesion (ASC-H) Papanicolaou smears diagnosed at Groote Schuur Hospital | | |
| Protocol number (if applicable) | Not applicable | | |
| Are there any sub-studies linked to this study? | | ☐ Yes | √ No |
| If yes, could you please provide the HREC Reference number for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study. | Not applicable | | |



(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

HREC Approval 2022 to 2023

HUMAN RESEARCH ETHICS COMMITTEE
- 8 NOV 2022
HEALTH SCIENCES FACULTY
UNIVERSITY OF CAPE TOWN

UNIVERSITY OF CAPE TOWN FACULTY OF HEALTH SCIENCES
Human Research Ethics Committee

## FHS016: Annual Progress Report / Renewal

| HREC office use only (FWA00001637; IRB00001938) This serves as notification of annual approval, including any documentation described below. | | | |
|---|---|---|---|
| ☑ Approved | Annual progress report | Approved until/next renewal date | 30/11/2023 |
| ☐ Not approved | See attached comments | | |
| Signature Chairperson of the HREC/ Designee | | Date Signed | 14/11/2022 |

Note: Please email this form and supporting documents (if applicable) in a combined pdf-file to hrec-enquiries@uct.ac.za.
Please clarify your plan for research-related activities during COVID-19 lockdown.
Please use the latest form found on our website:
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| Comments to PI from the HREC |
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**Principal Investigator to complete the following:**

**1. Protocol information**

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| Date (when submitting this form) | 18 October 2022 | | |
| HREC REF Number | 677/2021 | Current Ethics Approval was granted until | 30 November 2022 |
| Protocol title | A histological outcome of atypical squamous cells cannot exclude high grade squamous intraepithelial lesion (ASC-H) Papanicolaou smears diagnosed in Groote Schuur Hospital | | |
| Protocol number (if applicable) | Not applicable | | |
| Are there any sub-studies linked to this study? | ☐ Yes | √ No | |



(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

## Appendix 3: Data Capture Instrument and List of Variables

The variables included patient age, Papanicolaou smear specimen number, cervical histology result, cervical histology specimen number, patient ethnicity (where available).

## Appendix 4: Instructions for Authors

### About the Journal: Aims and Scope

With articles offering an excellent balance between clinical cytology and cytopathology, Acta Cytologica fosters the understanding of the pathogenetic mechanisms behind cytomorphology and thus facilitates the translation of frontline research into clinical practice. As the official journal of the International Academy of Cytology and affiliated to over 50 national cytology societies around the world, Acta Cytologica evaluates new and existing diagnostic applications of scientific advances as well as their clinical correlations. Original papers, review articles, meta-analyses, novel insights from clinical practice, and letters to the editor cover topics from diagnostic cytopathology, gynecologic, and nongynecologic cytopathology to fine needle aspiration, molecular techniques, and their diagnostic applications. As the perfect reference for practical use, Acta Cytologica addresses a multidisciplinary audience practicing clinical cytopathology, cell biology, oncology, interventional radiology, otorhinolaryngology, gastroenterology, urology, pulmonology, and preventive medicine.

### Research Article

Research Articles report on primary research. They must describe significant and original observations. Consideration for publication is based on the article's originality, novelty, and scientific soundness, and the appropriateness of its analysis.

Research Articles are reports of original work. Authors are asked to follow the EQUATOR Network for Research Articles.

Prior approval from an Institutional Review Board (IRB) or an Ethics Review Committee is required for all investigations involving human subjects.

Manuscript Template

The section below provides the downloadable manuscript template for Original Research Articles in the Acta Cytologica Journal.

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***Manuscript Title***

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[a] Department, Institute/University/Hospital, City, (State,) Country

[b] Department, Institute/University/Hospital, City, (State,) Country

[c] Department, Institute/University/Hospital, City, (State,) Country

Short Title: to be used as running head

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Keywords: Please provide 3–5 keywords highlighting the most important points of your paper.

## Abstract

A short Abstract should summarize the main points and reflect the content of the article. It should be written in a clear and concise way and be structured using the following subheadings: Introduction, Methods, Results, and Conclusion. Abbreviations used in the main text may be introduced and used. Use neither bibliographic references nor references to figures or tables in the Abstract.

## Introduction

The Introduction should provide a summary of the background to the relevant field of research and the specific problems addressed and should state the hypotheses being explored as well as the main goal(s) of the study. Conclusions or findings should not appear in the Introduction.

## Methods

The Methods section should clearly list all inclusion and exclusion criteria, methods of research, and variables evaluated and should state how outcomes were assessed. All terms should be adequately defined and statistical information should be sufficiently detailed so that a study can be repeated. If your manuscript is a clinical trial, please provide the clinical trial number.

## Results

The Results section should describe the most important findings of the study, analysis, or experiment. The most important results should be indicated, and relevant trends and patterns should be described.

## Discussion

The Discussion should provide an evaluation of the results. There should be a clear discussion of the implications, significance, and novelty of the results presented and whether the data support or contradict previous studies. A final conclusion may be added.

## Statements

All papers must contain the following statements after the main body of the text and before the reference list. More detailed information can be found at Publication Ethics and Editorial Policies | Karger Publishers.

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In the Acknowledgement section, authors may include individuals, who are not listed as authors, and organizations that have made substantive contributions to the research or the manuscript. An exception is where funding was provided, which should be included in Funding Sources.

### Statement of Ethics

Please address the following aspects in your Statement of Ethics.

Study approval statement: Provide name and affiliation of the committee who approved the study and the decision reference number. An example statement can be found here: "*This study protocol was reviewed and approved by [committee name and affiliation], approval number [XXX]*." If ethics approval was not required, or if the study has been granted an exemption from requiring ethics approval, this should also be stated, including the name of the ethics committee who made that decision.

Consent to participate statement: For studies using human participants, state whether written informed consent was obtained from participants (or their parent/legal guardian/next of kin) to participate in the study. If written informed consent was not required, or if the study has been granted an exemption from requiring written informed consent, this should also be stated, including the name of the ethics committee who made that decision.

### Conflict of Interest Statement

Authors are required to disclose any possible conflicts of interest. All forms of support and financial involvement (e.g. employment, consultancies, honoraria, stock ownership and options, expert testimony, grants or patents received or pending, royalties) which took place in the previous three years should be listed, regardless of their potential relevance to the paper. Also the nonfinancial relationships (personal, political, or professional) that may potentially influence the writing of the manuscript should be declared. If there is no conflict of interest, please state: “The authors have no conflicts of interest to declare.”

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Authors must give full details about the funding of any research relevant to their study, including sponsor names and explanations of the roles of these sources in the study design, execution and analysis, and manuscript conception, planning, writing and decision to publish. If the sponsor or funder had no role in any of the above, please use the following statement: "The funder had no role in the design, data collection, data analysis, and reporting of this study." Please ensure to include any support that could be perceived as a potential conflict of interest in the Conflict of Interest Statement. It is strongly advised to write out the funding body in full and add the grant number in brackets. Multiple grant numbers should be separated by commas and spaces. If no funding was received for the study, please use the following statement: "This study was not supported by any sponsor or funder."

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