

# FOCUS

PAPANICOLAOU SOCIETY OF CYTOPATHOLOGY

Companion Society of the United States and Canadian Academy of Pathology

*Dedicated to Clinical Practice, Clinical Education and Clinical Research*

## From the Editor's Desk

Vinod B. Shidham, MD, FRCPath, FIAC



PSC is continually growing strong. We thank our past president Martha Bishop Pitman, M.D. for her active role in reinvigorating Focus and welcome incoming president Lester Layfield, MD.

I am delighted to share with PSC membership the updated Publication Committee and Focus Editorial board. Andrew H. Fischer, M.D. is retiring as Associate editor and Adebowale Joel Adeniran, M.D. has agreed to take over from May 2011. Please join me in thanking Dr. Fischer for his service and Dr. Adeniran for accepting the role of Associate editor of Focus. Please welcome Oluwale Fadare, MD from Vanderbilt University Medical Center, Nashville, TN who is filling in the vacated PSC Publication Committee and Focus Editorial board membership position.

As a reminder members are encouraged to send the articles and other contributions (eg. interesting images in cytology, book reviews). The deadlines for submitting the contributions are flexible, generally for June issues April 15 and for Dec issues Oct 15.

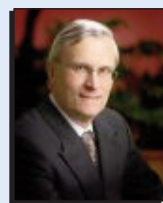
Please spread the word and encourage your colleagues to join PSC. The PSC membership details are elaborated on the last page of this Focus issue and the membership form is available at <http://www.papsociety.org/pscapp2009.pdf>

Sincerely,

Vinod B. Shidham, MD, FRCPath, FIAC  
Editor

## PSC President's Message

Lester Layfield, M.D.



The annual PCS meeting was held in San Antonio, TX during the 100th USCAP Annual Meeting. On behalf of the current PSC leadership and members, I would like to thank the officers and committee chairs and members for their 2009-2011 terms of service to the PSC. Special thanks go to the immediate past president, Dr. Martha Bishop Pitman. The officers for the 2011-2013 term include myself as President, Zubair Baloch as President-Elect, Dr. Eric Suba as treasurer and Dr. David Chhieng as Secretary. The Board of Directors is listed in Table I and the Committees and their membership are listed in Table II.

The 2011 PSC presentations began with the International Relations Committee Afternoon Session moderated by Drs. Matthew Zarka and Eric Suba. The presentations included, **Live Strong Programs in Mexico and South Africa** given by Rebekkah Schear of the

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### Membership Application

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# The Humanities Corner

By Manon Auger, MD, FRCP(C)  
Department of Pathology  
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## On mythology, medicine and snakes

As my 14 year-old son was recently easily reciting what seemed to be the entire genealogical tree of the Olympian gods for his upcoming Latin exam, I thought that this would be an opportune time for me to brush up on my very faded memories on the subject. What a better place to start than with Asklepios, the god of the medical art? What follows are a few interesting facts collected from my readings.

### Asklepios

Asklepios (also called Asclepius) is the god of medicine and healing in ancient Greece, whom the Romans called Aesculapius. He was worshipped from about 800BCE to 400CE.



Figure 1: Statue of Asklepios with his serpent-entwined staff (from public domain)

### • Birth and life

It is unclear whether Asklepios is pure mythology or inspired from a healing man who actually existed. According to the most common version of the history, he was the favorite son of Apollo and of a mortal, the princess Coronis. During her pregnancy, Coronis became enamoured with Ischys. Apollo, informed of her infidelity by a raven which he had sent to watch over her, asked his sister, Artemis, to kill Coronis. But as Coronis lay on the funeral pyre, Apollo felt remorse for the unborn child and cut him out of her womb. This is why the baby was given the name of "Asklepios" which means "to cut open".

Apollo then brought Asklepios to the centaur Chiron who raised him and instructed him in the art of medicine. When Asklepios was fully grown, he became so successful in the art of healing that reports spread that he could not only heal the sick, but also bring the dead back to life. This caused Hades, the god of the underworld, to become so troubled for fear no more dead spirits would come to him, that he asked his brother Zeus to kill Asklepios, which he did with a thunderbolt. In retaliation, Apollo killed the Cyclopes who had forged Zeus' thunderbolt. After Asklepios' death, Zeus placed him among the stars as the constellation Ophiuchus, the "serpent bearer".

### • Descendants

Asklepios was married to Epione and had nine children:

- three sons
  - o Telesphoros
  - o Machaon, and Podaleirios, both physicians during the Trojan war.

- six daughters

- o Panacea, the goddess of healing (from whose name originates "panacea" denoting a universal remedy),
- o Hygieia, the goddess of health (from whose name comes "hygiene"),
- o Meditrina, Aceso, Iaso, and Aglaea

### • Snakes and medical symbolism

Two stories clarify how Asklepios gained the power to bring the dead back to life. According to the first, he had received from Athena the blood that had flowed from the veins of Gorgo; indeed, the blood from the right side of her body possessed the power of restoring the dead to life. The second story is that one day, as Asklepios was attending to a patient, he killed a serpent wrapped around his staff. However, another serpent appeared with an herb in its mouth and revived the dead serpent with it. From then on, Asklepios used that same herb to restore life.

The last tale also explains why the staff or rod of Asklepios, with a single snake curled around it, has become the symbol of medicine, used by the American Medical Association and many other medical societies. Of note, some medical societies have mistakenly used the caduceus, emblem with two snakes instead of the traditional one, symmetrically twined around a wand, bearing a pair of wings on its top. Indeed, the wand bearing two snakes is not attributed to Asklepios, but rather to Hermes, messenger to the Olympian gods.

The snake itself has a long history of magical powers in many parts of the world, and its worship dates back to the Neolithic times. The snake represents both death by its poison, and renewal, convalescence and long life by its graceful movements and the annual shedding of its skin.

### • Cult of Asklepios

The followers of Asklepios established temples called asclepiions, temples of Asklepios and of healing. The sick spent a night at the temple and the proper remedies were revealed during a dream to the priests of the temple and the cured had to make a suitable sacrifice (usually a rooster or goat) to the god. The cult centers for Asklepios were at Epidauros, Kos, and Pergamon. Of note, in 2007, the name of the "archaeological site of Epidauros" was changed to "Sanctuary of Asklepios at Epidauros" and it is now a world heritage site by UNESCO.

### • Hymns to Asklepios

Many hymns refer to Asklepios, the most famous being The Hippocratic Oath, written by Hippocrates, a famous Greek physician (circa 5th to 4th B.C.E):

*"I swear by Apollo the physician, and Asklepios, and Hygieia, and Panakeia, and all the gods and goddesses, that, according to my ability and judgment, I will keep this Oath and this stipulation . . ."*

- **Link with cytopathology**

Of interest, from 1995 to 2001, the front of the Greek 10,000 drachmas banknote depicted Dr. George Papanicolaou, whereas the reverse represented Asklepios. This banknote was discontinued with the adoption of the euro in 2002

- **Latest news**

Today, the term asclepius refers to the genus of the milkweed pod.

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## President's Message

Lance Armstrong Foundation, Austin TX. Dr. Britt-Marie Ljung spoke on **Cytology in Ghana** followed by Dr. Ludwig Eric Gonzales from the Mexican Academy of Cytopathology in Mexico City, Mexico giving a lecture entitled, **Papanicolaou Screening in Mexico**. Finally, Dr. Andrew Field of St. Vincent's Hospital, Sydney, Australia gave a presentation entitled, **Fine-Needle Biopsy Training Programs in Africa**. These informative presentations were followed by the Annual Business Meeting and cocktail reception.

The PSC then presented its Companion Society Evening Session. Immediately prior to the session, a brief awards ceremony occurred in which Andrew Field, MD was honored with the **Yolanda Oertel Interventional Cytopathologist of the Year Award**, Fernando Schmitt, MD, PhD received the **L.C. Tao Educator of the Year Award** and Carlos Bedrossian, MD received the newly established **PSC Lifetime Achievement Award**. Thanks was given to Drs. Oertel, Tao and Abati for their support of these awards.

The Scientific Program commenced with moderation by Zubair W. Baloch, MD. Dr. Sylvia Asa gave the presentation entitled, **Practicing Morphology in the Era of Special Techniques**. This was followed by the presentation entitled, **How Much Molecular Pathology Does the Cytopathologist Need to Know?** given by Jennifer Hunt, MD. Dr. Dara Aisner then presented the presentation entitled, **Lessons Learned From Molecular Analysis of "Unbelievably Small" Cytology Specimens**. The Scientific Session was concluded with the presentation of **HPV Analysis of Head and Neck Fine-Needle Aspiration Specimens** given by William Westra, MD. The 2012 Scientific Program to be given at the 101st USCAP Annual Meeting will focus on pulmonary cytology. Presentations will be given by:

**Robert Viggiano, MD:** EBUS and Other Biopsy Techniques for Diagnosis of Lung Tumors

**Kevin Leslie, MD:** Histopathology of Lung Tumors: An Update

**Kim Geisinger, MD:** Cytologic Classification of Pulmonary Adenocarcinoma

**Neil Linderman, MD:** Molecular Aspects of EBUS/Transbronchial Biopsies

The Papanicolaou Society is developing guidelines/recommendations for pancreaticobiliary cytology. The guidelines will be developed with input from as broad a range of medical practitioners with interest in pancreaticobiliary cytology as possible. These guidelines will be developed in cooperation with multiple professional societies including the American Society of Cytopathology. Other professional organizations are invited to participate including those from the general pathology community, radiology societies, endoscopy societies and surgical societies with interest in pancreaticobiliary disease. Following the development of these pancreaticobiliary guidelines, further guidelines in the area of pulmonary cytopathology will be developed using a similar web based discussion forum with input from appropriate professional societies.

The Papanicolaou Society continues to reach out both nationally and globally. Dr. Andrew Field with his colleagues Drs. Britt-Marie Ljung, Eric Suba and Matthew Zarka continue to reach out internationally with particular emphasis in sub Saharan Africa. The Papanicolaou Society buddy system for distribution of Diagnostic Cytopathology journals is proceeding well with an expected distribution of the first set of journals to occur in June or July of 2011. This should facilitate distribution of medical literature to our international colleagues. The Papanicolaou Society continues to expand its educational material by participating in international conferences, FNA tutorials, guideline development and scientific session presentations. I invite all the members of the Papanicolaou Society to become involved in these projects. The updated Papanicolaou Society of Cytopathology website can aid in communication and keeping members informed of Papanicolaou Society benefits and educational programs. I look forward to working with all of you as well as reaching out to other societies so that the Papanicolaou Society remains a leader in the field of cytopathology education with special emphasis on bridging the gap between Surgical Pathology and Cytopathology.

**Table 1. 2011-2013 Board of Directors****Executive Board**

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### Research Article

## Teenage cervical screening in a high risk American population

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### Abstract

**Background:** The new 2009 ACOG guideline for cervical cytology screening changed the starting age to 21 years regardless of the age of onset of sexual intercourse. However, many recent studies have shown a dramatic increase in the incidence of cervical epithelial abnormalities among adolescents within the past two decades. **Materials and Methods:** For this study, the reports of 156,342 cervical cytology were available of which 12,226 (7.8%) were from teenagers. A total of 192 teenagers with high grade intraepithelial lesion (HSIL) cervical cytology were identified. The ages ranged from 13 to 19 years with a mean of 17.7 years and a median of 18 years. Among them, 31.3% were pregnant, 12.0% were postpartum, and 13.5% were on oral contraceptive. Ninety-eight had prior cervical cytology. **Results:** The teenagers had statistically significant higher detection rates of overall abnormal cervical cytology (23.6% vs. 6.6%,  $P = 0$ ), with 15.4% vs. 3.2% ( $P = 0$ ) of low grade intraepithelial lesion (LSIL) and 1.8% vs. 1.0% ( $P = 2.56 \times 10^{-13}$ ) of HSIL compared to women  $\geq 20$  years. The teenage group had the highest abnormal cytology among all age groups. The LSIL/HSIL ratio was 8.5:1 for teenagers and 3.1:1 for women  $\geq 20$  years. A total of 131 teenagers had cervical biopsies within 12 months of the HSIL cytology, with diagnoses of 39 CIN 3, 1 VAIN 3, 15 CIN 2, 62 CIN 1, and 14 had a negative histology (CIN 0). Only in 19 of these 39 women, the CIN 2/3 lesion proved to be persistent. **Conclusion:** We conclude that cytology screening of high risk teenagers is effective in detecting CIN 2/3 lesions. Moreover, treatment and careful follow-up can be realized.

**Key words:** Adolescents, cervical cytology, HSIL, long-term outcomes

### INTRODUCTION

The incidence and mortality of cervical cancer have decreased significantly in the past 30 years in the United States due to the widespread availability of cervical cytology screening, with the rates declining from 14.8 per 100,000 women in 1975 to 6.4 per 100,000 women in 2007.<sup>[1]</sup> When organized cervical cytology screening programs have been introduced into communities,

marked reductions in cervical cancer incidence have followed.<sup>[2,3]</sup> However, cervical cancer still remains a significant health problem worldwide with an estimated 500,000 new cases and 240,000 associated deaths annually.<sup>[4]</sup> Approximately, 11,270 new cases of cervical cancer were diagnosed in the United States in 2009 with 4070 cervical cancer-related deaths.<sup>[5]</sup> In the European Union (EU) approximately 34,000 new cases and more than 16,000 cervical cancer-related deaths are reported annu-



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ally.<sup>[6]</sup> It is estimated that 50% of women in whom cervical cancer is diagnosed each year have never had cervical cytology testing done, and another 10% have not been screened within the 5 years prior to diagnosis.<sup>[7]</sup> Therefore, any approach aiming to reduce the incidence and mortality due to cervical cancer would have to include increasing the coverage of unscreened or infrequently screened women. However, the concerns, with regards to adolescents and young women, have always been the risk of over screening and over treatment. The Council of EU recommended that cervical cytology screening should start between age 20 and 30 years, but preferentially not before age 25 or 30 years. The new 2009 guideline from the American College of Obstetricians and Gynecologists (ACOG) stated that cervical cancer screening should begin at age 21 years regardless of the age of onset of sexual intercourse. This replaces the 2003 guideline, which states that cervical cytology screening should start approximately 3 years after initiation of sexual intercourse, but no later than age 21 years.<sup>[8]</sup> The new 2009 guideline is based on the high prevalence of HPV infection, high rate of regression of low grade intraepithelial lesion (LSIL), and very low incidence of cervical cancer in adolescents and young women, in addition to the anxiety, morbidity, expenses, and likely overuse of follow-up procedures.

However, many recent studies have shown a dramatic increase in the incidence of cervical epithelial abnormalities among adolescents in the past two decades with the rates of atypical squamous cell of undermined significance (ASC-US), LSIL, and high grade intraepithelial lesion (HSIL) ranging between 7–16%, 3–13%, and 0.2–3%, respectively.<sup>[9–16]</sup> Unlike LSIL cytology, there are only limited studies on the follow-up and outcomes of HSIL cytology in this population. This retrospective study documents the long-term follow-up findings in a cohort of teenagers (<20 years) with HSIL cervical cytology, over a 54-month period.

## MATERIALS AND METHODS

This study was approved by the Institutional Review Board of Louisiana State University Health Sciences Center (LSUHSC), Shreveport. All cases with HSIL cervical cytology diagnosed between January 2003 and December 2007 were electronically retrieved from the database of the Department of Pathology, LSUHSC. The demographic data and clinical information including the use of oral contraceptives, pregnancy, and postpartum history were extracted from the pathology reports. The base population in this study was mainly low-income underinsured women of mixed ethnicity, and the cervical cytology screening in this study was part of an organized screening program performed mainly in a Women Clinic at LSUHSC. Results of follow-up cervical biopsy, loop electrosurgical excision procedure (LEEP), and repeat cervical cytology were collected until May 2010. Accessible records of cervi-

cal cytology test results prior to the HSIL cytology were also recorded. More than 99% of the cervical cytology specimens were processed using ThinPrep, liquid-based cytology. The corresponding cervical biopsies were defined as biopsies performed within 12 months from the dates of the HSIL cytology diagnosis, and biopsies performed after 12 months were considered as long-term follow-up. LEEP biopsies were similarly categorized. The long-term follow-up cervical cytology and histology was divided into four interval periods in months: 19–30, 31–42, 43–54, and >54 months. Statistical analysis was performed using the chi-square Fisher's exact *t*-test.

## RESULTS

There were 156,342 cervical cytology tests performed and screened during the study period, which included 12,226 (7.8%) from teenagers and 144,116 (92.2%) from women ≥20 years. Table 1 shows the summary of cervical cytology diagnoses with age breakdown. Teenagers had statistically significant higher detection rates of overall abnormal cervical cytology (23.6% vs. 6.6%, *P* = 0), with 15.4% vs. 3.2% (*P* = 0) of LSIL and 1.8% vs. 1.0% (*P* =  $2.56 \times 10^{-13}$ ) of HSIL compared to women ≥20 years. The LSIL/HSIL ratio was 8.5:1 for teenagers and 3.1:1 for women ≥20 years. The teenage group had the highest abnormal cervical cytology, HSIL, LSIL and ASC among all age groups, followed with the 20–29 age group.

A total of 192 teenagers with HSIL cervical cytology were identified. The ages ranged from 13 to 19 years with a mean of 17.7 years and a median of 18 years. Among 31.3% of the women were pregnant, 12.0% were postpartum, and 13.5% were on oral contraceptive. Ninety-eight women had prior cervical cytology before January 2003, and 57 of them (57/98) had abnormal cytology, which included HSIL (10 cases), LSIL (26 cases), ASC-H (2 cases), and ASC-US (19 cases). Twenty-three teenagers also had prior cervical biopsies, including 6 CIN 2/3 and 17 CIN 1.

**Table 1: Cervical cytologic diagnoses in women <20 years (teenagers) and in women ≥20 years with age breakdown**

	<20 (%)	20–29 (%)	30–39 (%)	≥ 40 (%)	≥ 20 overall (%)
HSIL	1.8	1.6	1.0	0.2	1.0
LSIL	15.4	7.8	3.1	0.7	3.2
ASC	6.4	6.2	4.2	1.5	2.4
Total	23.6	16.6	8.3	2.4	6.6
abnormal rate					

HSIL: High grade intraepithelial lesion; LSIL: low grade intraepithelial lesion; ASC: atypical squamous cells [including atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells—cannot exclude high grade lesion (ASC-H)]



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A total of 131 teenagers had follow-up cervical biopsies within 12 months. The histologic diagnoses were: 15 CIN 3, 1 VAIN 3, 39 CIN 2, and 62 CIN 1. The remaining 14 teenagers had a negative for dysplasia (CIN 0). Twenty-three teenagers had LEEP (17/23 within 12 months and 6/23 after 12 months). The diagnoses included 14 CIN 2/3, 1 endocervical glandular dysplasia, 5 CIN 1, and 3 CIN 0. Three teenagers had a second LEEP for recurrent HSIL. The youngest teenager with LEEP was 16 years with CIN 3 [Figure 1a] and positive margins. She had HSIL in follow-up cervical cytology and a second LEEP with CIN 2 [Figure 1b] at 15 months after the first LEEP.

Table 2 shows the cytologic diagnoses in the 198 teenagers with long-term follow-up with 142 ending up with negative cytology. Table 3 shows the histologic diagnoses of 39 teenagers with histologic diagnosis with 19 CIN 2/3 cases.

## DISCUSSION

Sexually active adolescent women are at a substantial risk for cervical intraepithelial neoplasia, including high-grade lesions especially when there are associated high-risk

factors. In this review of 12,226 cervical cytology smears from teenagers, the overall abnormal cervical cytology rate was 23.6% with HSIL, LSIL, and ASC rates of 1.8%, 15.4%, 6.4 %, respectively, which were significantly higher than that in women  $\geq 20$  years (6.6%, 1.0%, 3.2%, and 2.4%, respectively). These results are in accordance with previously published recent data.<sup>[10-16]</sup> It is significantly higher than the rate reported by Sadeghi *et al.* in 1984, who noted that the rate of abnormal cervical cytology among more than 190,000 adolescents aged 15–19 years was only 1.9%.<sup>[9]</sup> The reasons for these high rates of cervical epithelial abnormalities in teenagers are likely multifactorial, which probably includes high-risk sexual behaviors such as an early-onset of sexual intercourse and multiple sexual partners and HPV infection.<sup>[17-19]</sup> Recent studies confirmed the significant increased rates of CIN 2–3 and stage IA cancer in young patients.<sup>[20,21]</sup> Study from Iceland showed the CIN3/AIS was significantly high in age 20–24 and 25–29, and the increased incidence of invasive carcinoma was mainly due to increased rates in the 25–34 year groups.<sup>[20]</sup>

The overuse of cervical cytology screening may be quite significant among young women, thereby putting enormous economic burden on the health care system. A recent study estimated that potentially unnecessary cervical cytology could approach 659,000 among 5.7 million

**Table 2: The cytologic diagnoses in the teenagers with long-term cytology follow-up**

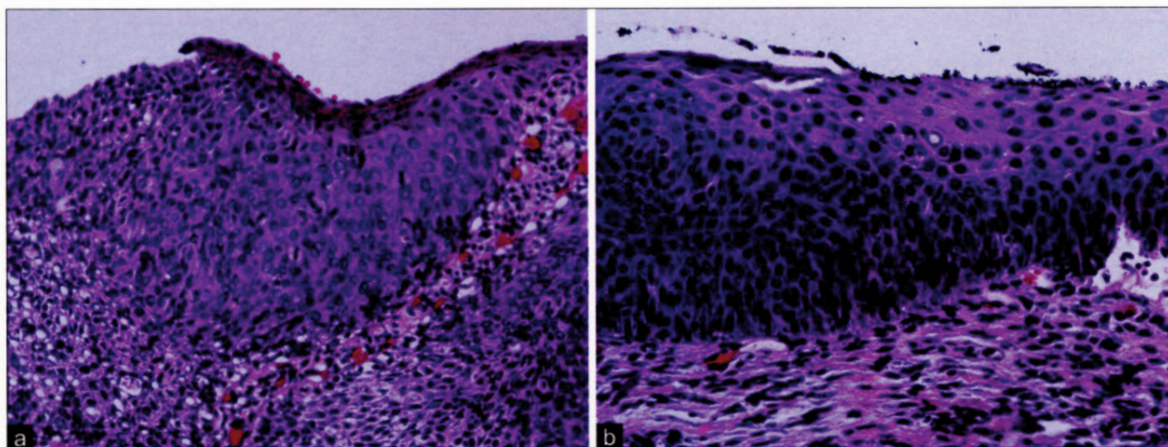
Cytology	Follow-up interval (by months)				Total
	19–30	31–42	43–54	>54	
HSIL	7	4	2	2	15
LSIL	12	6	3	2	23
ASC-H	0	1	1	1	3
ASC-US	9	2	2	2	15
Negative	55	38	30	19	142
Total	83	51	38	26	198

HSIL: High grade intraepithelial lesion; LSIL: low grade intraepithelial lesion; ASC-H: atypical squamous cells, cannot exclude high grade intraepithelial lesion; ASC-US: atypical squamous cells of undetermined significance

**Table 3: The histologic diagnoses in 39 teenagers with long-term follow-up for HSIL**

Histology	Follow-up interval (by months)				Total
	19–30	31–42	43–54	>54	
CIN 0	1	1	0	0	2
CIN I	9	4	4	1	18
CIN 2/3	9	4	2	4	19
Total	19	9	6	5	39

CIN: Cervical intraepithelial lesion; CIN 0: negative for dysplasia



**Figure 1:** a) A 16-year-old girl had high-grade squamous intraepithelial lesion (HSIL) on cervical cytology, and the cervical biopsy was severe squamous dysplasia (A, 200 $\times$ , H and E). b) She underwent LEEP but with positive resection margins. She had two times HSIL cervical cytology during 15-month follow-up, and underwent for the second LEEP for moderate squamous dysplasia (B, 200 $\times$ , H and E).



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women younger than 21 years, who are not sexually active.<sup>[22]</sup> This highlights the urgent need for the education of health care providers, to stress adherence to the guidelines, in order to reduce the economic burden and potentially unnecessary cervical screening cytology in young women. Overtreatment and the relative risk on future childbearing as well as the emotional impact of labeling an adolescent with a potential precancerous lesion are also significant, as adolescence is a time of heightened concern for self-image and emerging sexuality.<sup>[8]</sup> Arbyn *et al.* in a meta-analysis reported that LEEP had lower prenatal mortality, preterm delivery, and low birth weight compared to cold knife conization; however, LEEP was not completely free of adverse outcomes.<sup>[23]</sup> Significant increase in premature births in women previously treated with excisional procedures for dysplasia was also reported in a recent study by Kyrgiou *et al.*<sup>[24]</sup> Therefore, in teenagers the use of LEEP should be limited.

The regression rate of abnormal cytology in these young women should also be taken into account in planning treatment. Moscicki *et al.* demonstrated a regression rate of 61% and 91% for HPV/LSIL in adolescents after 1 and 3 years of follow-up, respectively, with only 3% progressing to CIN 3.<sup>[25]</sup> Two other studies in adolescents with biopsy confirmed CIN 2 showed 65% and 75% regression to negative after 18 months and 3 years, respectively.<sup>[14,26]</sup> The regression rate was much higher in adolescents than that of adults from a meta-analysis study by Melnikow *et al.*<sup>[27]</sup> The update of ASCCP treatment guidelines for CIN 2/3 lesions in adolescents are observation-colposcopy and cytology or treatment using excision or ablation of the T-zone. However, it also stated "when CIN 2 is specified, observation is preferred. When CIN 3 is specified, or colposcopy is unsatisfactory, treatment is recommended".<sup>[28]</sup> The reported high rates of regression support this guideline and immediate excision ("see and treat") is not recommended for teenagers. From the literature, the treatment of CIN 2 lesions in adolescents is not consistent, so we recommend to carefully follow the ASCCP guidelines and limit LEEP to CIN 3 or persistent CIN 2 lesions.

We conclude that cytology screening of high-risk teenagers is effective in detecting CIN 2/3 lesions. Moreover, treatment and careful follow-up of these young women can be realized.

## COMPETING INTEREST STATEMENT BY ALL AUTHORS

The authors declare that they have no competing interests.

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All authors of this article declare that we qualify for author-

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SZ participated in its design and coordination and helped to draft and finish the manuscript, and performed the statistical analysis. JT participated in its design and writing the manuscript. Jthibodeaux participated in the data collection and helped to draft the manuscript. AB participated in the data collection and helped to draft the manuscript. FA participated in its design and writing the manuscript.

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## ETHICS STATEMENT BY ALL AUTHORS

This study was conducted with approval from Institutional Review Board of LSUHSC-Shreveport and was conducted only at LSUHSC-Shreveport. Authors take responsibility to maintain relevant documentation in this respect.

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