

# P16/Ki-67 蛋白双染在未明确意义的非典型鳞状上皮细胞人群中分流作用

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**摘 要:** [目的] 探讨 p16/Ki-67 免疫细胞双染法对宫颈细胞学为未明确意义的非典型鳞状上皮细胞(ASCUS)人群的分流效果。[方法] 以 2016 年 4 月至 12 月在郑州大学第二附属医院妇科门诊就诊且被诊断为 ASCUS 的 135 例妇女为研究对象, 收集其宫颈脱落细胞标本, 进行 14 种高危型 HPV DNA 及 p16/Ki-67 蛋白检测, 所有妇女均进行阴道镜活检和病理学检查。以组织病理学诊断为金标准, 分别计算 p16/Ki-67 双染、高危型 HPV(HR-HPV)和 HPV16/18 检测的敏感性、特异性、阳性预测值(PPV)、阴性预测值(NPV)、转诊率及其 95%CI。[结果] 135 例 ASCUS 妇女平均年龄为(46.48±10.19)岁(23~64 岁), 其中诊断为宫颈上皮内瘤变(CIN)1 级者 7 例, 诊断为 CIN2 级及以上(CIN2+)者 22 例。随异常病理结果的严重性增加, p16/Ki-67 双染、HR-HPV 和 HPV16/18 检出阳性率均升高( $P<0.001$ )。以 CIN2+作为疾病终点指标时, p16/Ki-67 双染对 ASCUS 人群分流的敏感性、特异性、PPV、NPV 和转诊率分别为 86.4%(95%CI: 66.7%~95.3%)、85.8%(95%CI: 78.2%~91.1%)、54.3%(95%CI: 38.2%~69.5%)、97.0%(95%CI: 91.6%~99.0%)和 25.9%, 与之相比, HR-HPV 检测敏感性稍高[95.5%(95%CI: 78.2%~99.2%)], 但特异性较低[68.1%(95%CI: 59.1%~76.0%)], 转诊率较高(42.2%), 且差异均有统计学意义( $P<0.05$ )。与 p16/Ki-67 双染法相比, HPV16/18 的特异性较高[92.9%(95%CI: 86.7%~96.4%)], 但敏感性很低[59.1%(95%CI: 38.7%~76.7%)]. 按照 45 岁进行年龄分层后, p16/Ki-67 双染在 ≥45 岁组 ASCUS 人群中分流效果要更好, 敏感性为 81.8%(95%CI: 52.3%~94.9%), 特异性为 95.5%(95%CI: 87.5%~98.4%)。[结论] P16/Ki-67 双染检测在保持高敏感性的同时, 有更高的特异性, 因此对 ASCUS 人群的分流效果优于 HR-HPV 和 HPV16/18。P16/Ki-67 双染检测具有简便、客观、高效、易于重复的特点, 可为 ASCUS 人群提供一种新的分流方法。

**关键词:** 人乳头瘤病毒; p16; Ki-67; 未明确意义的非典型鳞状上皮细胞; 分流

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## Dual Staining of P16/Ki-67 Proteins in Triaging Women with Atypical Squamous Cells of Undetermined Significance

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**Abstract:** [Purpose] To evaluate the immunocytochemistry dual staining of p16/Ki-67 proteins in triaging women with atypical squamous cells of undetermined significance(ASCUS). [Methods] A total of 135 women diagnosed as ASCUS in the Second Affiliated Hospital of Zhengzhou University from April to December 2016 were enrolled in the study. HPV DNA detection and p16/Ki-67 dual staining were performed respectively. All patients underwent colposcopy biopsy and pathological examination. The histopathological result was used as the gold standard, the sensitivity, specificity, positive predictive value(PPV), negative predictive value(NPV), referral rate of p16/Ki-67 dual staining, high risk HPV(HR-HPV) and HPV 16/18 were calculated. [Results] The mean age of patients was (46.48±10.19) years old (23~64). Seven women were diagnosed as cervical intraepithelial neoplasia 1(CIN1), 20 were diagnosed CIN2+. The positive rates of p16/Ki-67 dual staining,

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HR-HPV DNA and HPV16/18 increased with the grade of pathologic diagnosis ( $P<0.001$ ). The sensitivity, specificity, PPV, NPV and referral rate of p16/Ki-67 dual staining for predicting CIN2+ lesion in women with ASCUS were 86.4%(95%CI:66.7%~95.3%), 85.8%(95%CI:78.2%~91.1%), 54.3%(95%CI:38.2%~69.5%), 97.0%(95%CI:91.6%~99.0%), 25.9%, respectively. To compared with p16/Ki-67 dual staining, HR-HPV DNA had higher sensitivity [95.5% (95% CI:78.2%~99.2%)] but lower specificity [68.1%(95%CI:59.1%~76.0%)] and higher referral rate (42.2%); HPV 16/18 had higher specificity[92.9%(95%CI:86.7%~96.4%)] and lower sensitivity[59.1%(95%CI:38.7%~76.7%)]. After age stratification, p16/Ki-67 dual staining had a better triage effect in ASCUS patients with age  $\geq 45$  years old; the corresponding sensitivity and specificity was 81.8%(95%CI:52.3%~94.9%) and 95.5%(95%CI:87.5%~98.4%), respectively.[Conclusion] P16/Ki-67 dual staining is better than HR-HPV DNA and HPV16/18 detection for triaging ASCUS woman under certain conditions, which is simple, objective, efficient and reproductive and can be a new method for triaging ASCUS women.

**Key words:** human papilloma virus;p16;Ki-67;atypical squamous cells of undetermined significance;triage

液基细胞学作为宫颈癌筛查最重要的方法,其发明和应用使宫颈癌的发生率和死亡率都明显下降<sup>[1]</sup>。在宫颈癌筛查中,约 3%~10%的妇女细胞学诊断为具有未明确意义的非典型鳞状上皮细胞 (atypical squamous cells of undetermined signification,ASCUS)<sup>[2-3]</sup>。许多年来高危型 HPV (high-risk human papillomavirus,HR-HPV)DNA 检测一直作为分流 ASCUS 人群的一个较好方法<sup>[4]</sup>,但研究显示 HPV DNA 在发现高级别宫颈上皮内瘤变的特异性较低<sup>[5]</sup>。目前一些细胞生物标志物被认为在提高宫颈癌筛查特异性方面具有一定的潜力<sup>[6]</sup>。其中,周期蛋白依赖性激酶抑制剂 p16INK4a(p16)被认为是最具潜力的细胞蛋白标志物之一<sup>[7-8]</sup>。P16 过表达被认为是 HPV E7 介导的 E2F 功能激活信号<sup>[9]</sup>。Ki-67 是细胞增殖标志物<sup>[10]</sup>,其与 p16 在正常情况下不能同时表达<sup>[11]</sup>。P16 和 Ki-67 同时表达则表示 HPV 转化细胞正在进行增殖,该指标为区分潜在高级别病变妇女提供了客观的检测依据,并且不依赖于细胞形态学检查的结果,简单易用,提高了诊断效率<sup>[12-13]</sup>。本次研究目的是通过对 p16/Ki-67 双染、HR-HPV DNA、HPV16/18 检测在 ASCUS 人群中的分流效果进行比较,探讨 p16/Ki-67 双染法用于 ASCUS 人群分流的临床价值。

## 1 资料与方法

### 1.1 研究对象

以 2016 年 4 月至 12 月在郑州大学第二附属医院妇科就诊的 20~65 岁且细胞学检测结果为 AS-

CUS 的妇女作为研究对象,在签署《知情同意书》后,采集其宫颈上皮脱落细胞和宫颈组织以进行相关检测。要求入选者没有临床怀孕的可疑症状,同时无宫颈外科手术史。该研究已获得郑州大学生命科学伦理审查委员会的批准(20150305)。

### 1.2 标本收集

首先,纳入研究的妇女由专科医生进行妇科检查,同时采用扫帚样刷子收集宫颈上皮脱落细胞置于转移介质 PreservCyt® 液体(美国 Hologic 公司)中,以进行相关实验室检测。其次,所有研究对象由有经验的阴道镜医生对其进行阴道镜检查,镜下暴露充分且存在病变部位者则异常处取活检,无病变者不取活检;镜下暴露不充分者则进行宫颈管搔刮术(endocervical curettage,ECC)。

### 1.3 实验室检测

以下所有检测和诊断过程均严格遵守盲法原则。

#### 1.3.1 液基细胞学检测(LBC,ThinPrep;Hologic-Cytte,Marlborough,Mass)

将采集的宫颈脱落细胞标本制片染色后按照 Bethesda 分类系统进行阅片诊断。液基细胞学片子由两名诊断经验丰富的细胞学医师独立阅片,不一致的片子由第三名高年资医师进行判读。

#### 1.3.2 HPV DNA 检测

从保存宫颈脱落细胞的样本混合液中分出 400 $\mu$ l 液体,采用 cobas 4800 进行 HPV DNA 检测。Cobas 4800 是一种通过聚合酶链反应和核酸杂交技术扩增检测 HR-HPV DNA 的方法。每次试验均设有阴性和阳性对照。检测 CT 值 $>40$ ,表示结果为阴性,

反之为阳性。该技术能够同时检测 14 种高危型 HPV, 包括 HPV 16、18、31、33、35、39、45、51、52、56、58、59、66 和 68 型,并能对 HPV16 和 18 进行分型。

### 1.3.3 P16/Ki-67 检测

采用德国 MTM 实验室研发的一项名为 CINtec PLUS 的新技术,使用抗 P16(E6H4)/Ki67(274-11 AC3) 单克隆抗体鸡尾酒试剂 (CINtec® Plus Cytology Kit, 厂家:Ventana Medical Systems, Inc.), 利用免疫细胞化学双染法原理进行 p16/Ki-67 检测。将采集的宫颈脱落细胞标本制片后,显微镜下观察,若视野中出现至少 1 个宫颈细胞的细胞质呈红色(p16),细胞核呈黄色或棕色(Ki-67)则判读为阳性;其他为阴性<sup>[14]</sup>。每次试验均设有阳性和阴性对照,同时对实验所有参与人员进行统一培训,内容包括实验操作流程、过程注意事项、试验结果判读等。

### 1.3.4 组织病理学诊断

组织病理学诊断在郑州大学第二附属医院病理科进行,阴道镜下所取的活检组织或 ECC 标本进行制片后,采用 CIN(宫颈上皮内瘤样病变)分级报告系统进行诊断,同时,由郑州大学附属肿瘤医院有经验的病理医师对诊断结果进行复核。

### 1.4 统计学处理

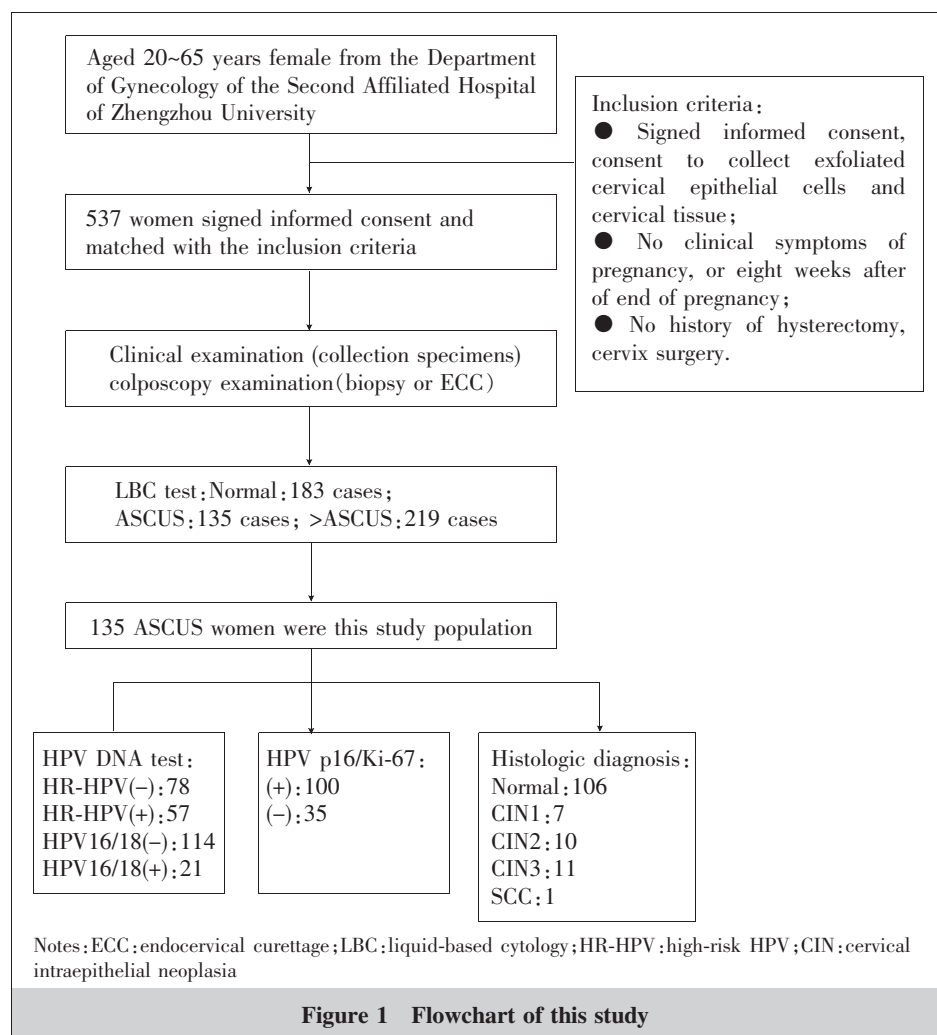
以最终病理学诊断结果为金标准,计算 p16/Ki-67 双染法、HR-HPV、HPV 16/18 对 CIN2 级及以上病变(CIN2+)(包括 CIN2、CIN3、鳞状细胞癌、腺癌)检出的敏感性、特异性、阳性预测值(positive predictive value, PPV)、阴性预测值(negative predictive value, NPV) 及其 95%CI 值、转诊率。各指标间的差异比较采用 McNemar 法进行分析。转诊率是指 ASCUS 人群中 p16/Ki-67、HR-HPV、HPV16/18 分别阳

性者被转诊阴道镜的比例。一项纳入 17 个人群研究共 30 207 名女性的综合分析研究显示,CIN3+在 45~49 岁年龄组人群中阳性率最高<sup>[15]</sup>,同时,考虑到女性一般在 45 岁后将会受到围绝经期的影响,故以 45 岁为界值条件进行年龄分层,计算各筛查方法对 ASCUS 人群的分流效果(Figure 1)。P<0.05 表示差异有统计学意义。

## 2 结 果

### 2.1 P16/Ki-67 蛋白、HR-HPV 和 HPV16/18 检出阳性率

共 537 位女性签署《知情同意书》,纳入本次研究的 ASCUS 妇女共 135 例(比例为 25.1%),平均年龄为(46.48±10.19)岁(23~64 岁),106 人(78.5%)病理学结果正常,7 例(5.2%)被诊断为 CIN1,22 例



(16.3%)被诊断为 CIN2+。随组织病理结果的严重性增加,p16/Ki-67、HR-HPV、HPV16/18 的阳性率均升高( $P<0.001$ )(Table 1)。

## 2.2 不同筛查方法对 ASCUS 人群的分流效果评价

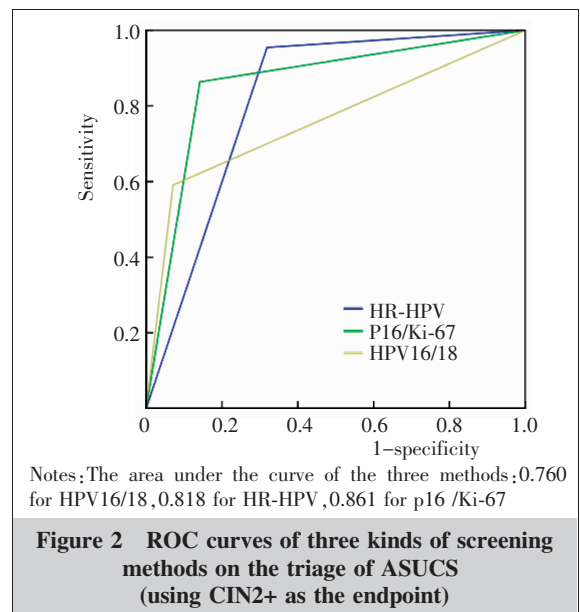
以 CIN2+作为疾病终点指标,计算不同筛查方法分流 ASCUS 人群的敏感性、特异性、PPV 和 NPV。结果显示,p16/Ki-67 双染法的敏感性、特异性、PPV 和 NPV 分别为:86.4%(95%CI:66.7%~95.3%)、85.8%(95%CI:78.2%~91.1%)、54.3%(95%CI:38.2%~69.5%)、97.0%(95%CI:91.6%~99.0%)。HR-HPV 与 p16/Ki-67 双染法相比,分流 ASCUS 人群的敏感性 (95.5%,95%CI:78.2%~99.2%)稍高,但差异无统计学意义( $P>0.05$ ),特异性(68.1%,95%CI:59.1%~76.0%)较低,转诊率也高(42.2%),差异有统计学意义 ( $P<0.05$ )。HPV 16/18 的分流效果虽有高的特异性(92.9%,95%CI:86.7%~96.4%),但敏感性很低 (59.1%,38.7%~76.7%)(Table 2;Figure 2)。

按照 45 岁进行年龄分层后,在年龄<45 岁(58 例)组和≥45 岁(77 例)组,被诊断为 CIN2+者分别为 11 例。P16/Ki-67 双染法在年龄<45 岁的 ASCUS 人群中分流效果的敏感性为 90.9%(95%CI:62.3%~98.4%),高于≥45 岁组的 81.8%(95%CI:52.3%~94.9%),但差异无统计学意义,然而 p16/Ki-67 双染法在年龄<45 岁组的特异性 72.3%(95%CI:58.2%~83.1%)明显低于≥45 岁组 95.5%(95%CI:87.5%~98.4%),且差异有统计学意义( $P<0.05$ )(Table 3)。

**Table 1 The positive rates of p16/Ki-67 protein,HR-HPV and HPV16/18 for 135 women with ASCUS**

| Histopathology |     | P16/Ki-67 |                  | HR-HPV   |                  | HPV 16/18 |                  |
|----------------|-----|-----------|------------------|----------|------------------|-----------|------------------|
|                |     | Positive  | Positive rate(%) | Positive | Positive rate(%) | Positive  | Positive rate(%) |
| Normal         | 106 | 14        | 21.7             | 30       | 21.7             | 7         | 6.6              |
| CIN1           | 7   | 2         | 28.6             | 6        | 85.7             | 1         | 14.3             |
| CIN2           | 10  | 9         | 80.0             | 9        | 90.0             | 4         | 40.0             |
| CIN3           | 11  | 9         | 90.9             | 11       | 100.0            | 9         | 81.8             |
| SCC            | 1   | 1         | 100.0            | 1        | 100.0            | 0         | 0.0              |
| Total          | 135 | 35        | 32.6             | 57       | 42.2             | 21        | 15.6             |
| $\chi^2$       |     |           | 46.475           |          | 35.679           |           | 38.931           |
| $P$            |     |           | <0.001           |          | <0.001           |           | <0.001           |

Notes: HR-HPV: high-risk HPV; CIN: cervical intraepithelial neoplasia



**Table 2 Triage effect of different screening methods for women with ASCUS(95%CI)**

| Screening methods | Positive | Sensitivity(%)   | Specificity(%)   | PPV(%)           | NPV(%)           | Referral rate(%) |
|-------------------|----------|------------------|------------------|------------------|------------------|------------------|
| HR-HPV            | 57       | 95.5(78.2~99.2)  | 68.1(59.1~76.0)  | 36.8(25.5~49.8)  | 98.7(93.1~99.8)  | 42.2             |
| P16/Ki-67         | 35       | 86.4(66.7~95.3)  | 85.8(78.2~91.1)* | 54.3(38.2~69.5)  | 97.0(91.6~99.0)  | 25.9*            |
| HPV16/18          | 21       | 59.1(38.7~76.7)* | 92.9(86.7~96.4)* | 61.9(40.9~79.3)* | 92.1(85.7~95.8)* | 15.6*            |

Notes: HR-HPV: High-risk HPV; ASCUS: atypical squamous cells of undetermined significance; CIN: cervical intraepithelial neoplasia; PPV: positive predictive value; NPV: negative predictive value; \*: compared with HR-HPV,  $P<0.05$

**Table 3 Triage effect of different screening methods after age stratification for women with ASCUS, using CIN2+ as the endpoint(95%CI)**

| Age(years) | Positive | Sensitivity(%)    | Specificity(%)   | PPV(%)           | NPV(%)            | Referral rate(%) |
|------------|----------|-------------------|------------------|------------------|-------------------|------------------|
| <45        |          |                   |                  |                  |                   |                  |
| HR-HPV     | 30       | 100.0(74.1~100.0) | 59.6(45.3~72.4)  | 36.7(21.9~54.5)  | 100.0(87.9~100.0) | 51.7             |
| P16/Ki-67  | 23       | 90.9(62.3~98.4)   | 72.3(58.2~83.1)  | 43.5(25.6~63.2)  | 97.1(85.5~99.5)   | 39.7             |
| HPV16/18   | 10       | 45.5(21.3~72.0)*  | 89.4(77.4~95.4)* | 50.0(23.7~76.3)  | 87.5(75.3~94.1)   | 17.2*            |
| ≥45        |          |                   |                  |                  |                   |                  |
| HR-HPV     | 27       | 90.9(62.3~98.4)   | 74.2(62.6~83.3)  | 37.0(21.5~55.8)  | 98.0(89.5~99.7)   | 35.1             |
| P16/Ki-67  | 12       | 81.8(52.3~94.9)   | 95.5(87.5~98.4)* | 75.0(46.8~91.1)* | 96.9(89.5~99.2)   | 15.6*            |
| HPV16/18   | 11       | 72.7(43.4~90.3)   | 95.5(87.5~98.4)* | 72.7(43.4~90.3)* | 95.5(87.5~98.4)   | 14.3*            |

Note: \*: compared with HR-HPV,  $P<0.05$

### 3 讨论

参加宫颈癌筛查的妇女中,细胞学检查结果为 ASCUS 者最为常见,而其组织病理学结果从炎症改变到宫颈癌均可见,如全部进行阴道镜转诊,势必造成大量的过度诊断,因此,迫切需要探寻一种可有效分流该类人群的新方法。近年来,HPV DNA 检测在许多国家已作为宫颈癌筛查的方法<sup>[16-18]</sup>,且用于 ASCUS 人群的分流管理,该方法虽有较高的敏感性,但研究表明 HPV DNA 阳性者中绝大多数是 HPV 一过性感染,有 90% 以上的感染者在 8~24 个月内可以自然转归<sup>[19]</sup>,因此,HPV DNA 检测的特异性较低,其用于 ASCUS 人群分流时容易造成过度转诊阴道镜。

本研究结果显示,细胞学检查结果为 ASCUS 的比例为 25.1%,高于宫颈癌人群筛查结果<sup>[20-21]</sup>。ASCUS 人群中 CIN2+ 的比例为 16.3%,高于 Watson 等<sup>[22]</sup>以宫颈筛查人群为研究对象的结果(11.8%),与同选择医院就诊患者为研究对象的 Zhu 等<sup>[23]</sup>研究结果相近(18.0%)。P16/Ki-67 双染的阳性率随异常病理结果的严重性增加而升高,这与相关研究一致<sup>[24-25]</sup>。Peeters 等<sup>[26]</sup>对 p16/Ki-67 双染法与 HR-HPV DNA 分流 ASCUS 的效果比较进行了 Meta 分析,结果显示 p16/Ki-67 双染对 ASCUS 的分流效果,与 HR-HPV DNA 有一样的敏感性,但有更高的特异性。White 等<sup>[27]</sup>为比较 p16/Ki-67 双染法与 HR-HPV DNA 对 LSIL/ASCUS 的分流效果进行了 2 年的前瞻性研究,结果显示 HR-HPV DNA 比 p16/Ki-67 双染法有更高的敏感性(95.8% vs 79.2%),但 p16/Ki-67 双染法有更高的特异性(75.2% vs 40.4%),提示 p16/Ki-67 双染与 HR-HPV DNA 联合对 ASCUS 人群会有更好的分流效果。本研究中,以 CIN2+ 作为疾病终点指标时,p16/Ki-67 双染法分流 ASCUS 人群的敏感性稍低于 HR-HPV DNA,但特异性高于 HR-HPV,HPV16/18 虽有高的特异性,但敏感性较低。按照 45 岁进行年龄分层后,p16/Ki-67 双染法在两个年龄组间的敏感性差异无统计学意义。在年龄 ≥45 岁的 ASCUS 人群中,分流效果的特异性明显要高于 <45 岁组。因此,p16/Ki-67 双染法在 ≥45 岁的 ASCUS 人群中分流效果要更好。年龄分层后,各层诊断为 CIN2+ 者比例均较低,双染法在不同年龄层的分流

效果还需通过增加样本量进一步验证。

综上所述,p16/Ki-67 双染检测在识别 ASCUS 人群的宫颈癌前病变及宫颈癌时,与 HR-HPV 和 HPV 16/18 检测相比,在保持高敏感性的同时,实现了更高的特异性,可以将转诊阴道镜的人数减少约 40%,同时,其检测不依赖于细胞的形态特征,具有客观性和重复性好的特点,因此,p16/Ki-67 双染法可作为 ASCUS 人群分流的有效方法。

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