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DNA 定量分析与液基细胞学在胆管良恶性病变中的应用价值

颜云

(扬州大学附属医院病理科, 江苏 扬州 225000)

[摘要] 目的: 对比内镜逆行胰胆管造影术(endoscopic retrograde cholangiopancreatography, ERCP)中胆管刷检液基细胞学、DNA定量分析结果, 探讨ERCP胆管刷检液基细胞学及DNA定量分析在胆管疾病诊断中的应用价值。方法: 选取ERCP胆管刷检病例同时做液基细胞学诊断及DNA定量分析, 将其结果与组织学活检进行对比。结果: 胆管刷检液基细胞学诊断恶性肿瘤41例, 疑癌10例, 核异质42例, 阴性91例; DNA定量分析诊断见异倍体细胞102例, 阴性82例; 病理活检诊断癌90例, 异型增生22例, 阴性72例。液基细胞学诊断灵敏度、特异度分别为66.1%与70.8%。DNA定量分析的灵敏度、特异度分别为84.8%与95.8%。液基细胞学的诊断准确性为67.9%, DNA定量分析的诊断准确性为88.6%。DNA定量分析诊断的准确性、灵敏度及特异度较液基细胞学诊断高, 二者比较差异有统计学意义($P < 0.05$)。结论: 液基细胞学及DNA定量分析联合检测能提高胆道疾病的检出率, 降低漏诊及误诊的风险。

[关键词] 内镜逆行胆管造影术; 刷检; 液基细胞学; DNA定量分析

Application value of DNA quantitative analysis and liquid based cytology in benign and malignant biliary duct lesions

YAN Yun

(Department of Pathology, Affiliated Hospital of Yangzhou University, Yangzhou Jiangsu 225000, China)

Abstract **Objective:** To compare the liquid-based cytology and DNA quantitative analysis of bile duct brushing in endoscopic retrograde cholangiopancreatography (ERCP), and to explore the value of ERCP liquid-based cytology and DNA ploidy analysis in the diagnosis of bile duct diseases. **Methods:** Cholangio-brushing cases of ERCP were selected for liquid-based cytological diagnosis and DNA quantitative analysis, and the results were compared with histological biopsy. **Results:** A total of 41 malignant tumors, 10 suspected cancers, 42 nuclear heterogeneity and 91 negative cases were diagnosed by liquid-based cytology of bile duct brush examination; 102 aneuploid cells and 82 negative cases were diagnosed by DNA quantitative analysis; 90 cancer cases were diagnosed by pathological biopsy, 22 dysplasia cases and 72 negative cases. The sensitivity and specificity of liquid-based cytology were 66.1% and 70.8%, respectively. The sensitivity and specificity of DNA quantitative analysis

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通信作者 (Corresponding author): 颜云, Email: baiyunzhou999@sina.cn

were 84.8% and 95.8%, respectively. The diagnostic accuracy of liquid-based cells was 67.9%, and that of DNA quantitative analysis was 88.6%. The diagnostic accuracy, sensitivity and specificity of DNA quantitative analysis were higher than those of liquid-based cytology ($P<0.05$). **Conclusion:** The combined detection of liquid-based cytology and DNA quantitative analysis can improve the detection rate of biliary tract diseases and reduce the risk of missed diagnosis and misdiagnosis.

Keywords endoscopic retrograde cholangiography; brushing; liquid-based cytology; DNA quantitative analysis

胆管癌发病隐匿^[1], 对于胆管癌非外科手术获得病理诊断的方法包括经皮经肝胆道镜活检、十二指肠镜细胞刷或活检钳在透视下活检^[2]。内镜逆行胰胆管造影术(endoscopic retrograde cholangiopancreatography, ERCP)是胆道恶性肿瘤和良性疾病的诊断方法之一, ERCP曾被视为胆管狭窄诊断的“金标准”, 但单靠ERCP影像对狭窄性质的判断效果并不好。文献[3]报道其准确率为73%。ERCP时通过细胞刷或活检钳在透视下取标本存在盲目性, 且敏感性不高^[4]。本研究对比ERCP中胆管刷检液基细胞学诊断、DNA定量分析和组织学诊断结果, 拟探讨联合胆道刷检液基细胞学和DNA定量分析在胆管疾病诊断中的应用价值。

1 材料与方法

1.1 材料

选取扬州大学附属医院病理科2014年1月至2018年8月ERCP中胆管刷检184例, 其中男98例, 女86例, 男女比例为1.14:1, 年龄17~82(中位56.3)岁。

1.2 方法

1.2.1 液基细胞学制片及诊断

采用美国BD公司沉降式液基薄层全自动制片系统, 同时制作2张细胞片, 1张做HE染色显微镜观察, 另1张采用Feul-gen染色, 干片后做DNA定量分析。液基细胞学诊断分为恶性肿瘤细胞、可疑恶性肿瘤细胞、核异质细胞及未找见肿瘤细胞。

1.2.2 细胞DNA定量分析

采用细胞DNA定量分析系统(武汉兰丁医学高科技有限公司), 阳性诊断为见异倍体细胞, 包括有3个以上DNA含量为 $>5c$ 为见大量异倍体细胞, 有1~2个DNA含量为 $>5c$ 为见少量异倍体细胞, 没有 $>5c$ 的为阴性诊断。

1.3 统计学处理

采用SPSS 19.0统计软件进行数据分析, 百分率比较采用 χ^2 检验, $P<0.05$ 为差异有统计学意义。

2 结果

2.1 液基细胞学与DNA定量分析结果对比

ERCP中胆管刷检液基细胞学诊断恶性肿瘤41例(22.30%), 疑癌10例(5.40%), 核异质42例(22.80%), 阴性91例(49.50%), DNA定量分析诊断见异倍体细胞102例(图1, 2), 阴性82例(图3), DNA定量分析阳性95例(51.60%), 阴性89例(48.40%)。病理活检诊断癌90例, 异型增生22例, 阴性72例。液基细胞学诊断灵敏度、特异度分别为66.1%(74/112)与70.8%(51/72), 液基细胞的诊断准确性67.9%(125/184), 阳性预测值79.6%(74/93), 阴性预测值56%(51/91)。DNA定量分析的灵敏度、特异度分别为84.8%(95/112)与95.8%(69/72), DNA定量分析的诊断准确性88.6%(163/184), 阳性预测, 93.1%(95/102), 阴性预测值84.1%(69/82)。液基细胞学诊断恶性肿瘤(图4)的病例DNA定量分析全部诊断阳性, 病理活检均为癌。DNA定量分析诊断阴性而液基细胞学诊断阳性的8例中病理活检诊断异型增生2例, 良性6例。DNA定量分析诊断阳性而液基细胞学诊断阴性的22例中病理活检诊断恶性13例, 异型9例。DNA定量分析灵敏度、特异度均高于液基细胞学诊断, 二者比较差异有统计学意义($P<0.05$)。

2.2 胆管刷检液基细胞学、DNA定量分析及两者联合诊断结果比较

胆管刷检液基细胞学、DNA定量分析与两者联合3种诊断方法比较, 差异有统计学意义($\chi^2=7.8936$, $P<0.05$; 表1)。液基细胞学联合DNA定量分析的诊断准确性、灵敏度和特异度均高于单一液基细胞学或DNA定量分析。

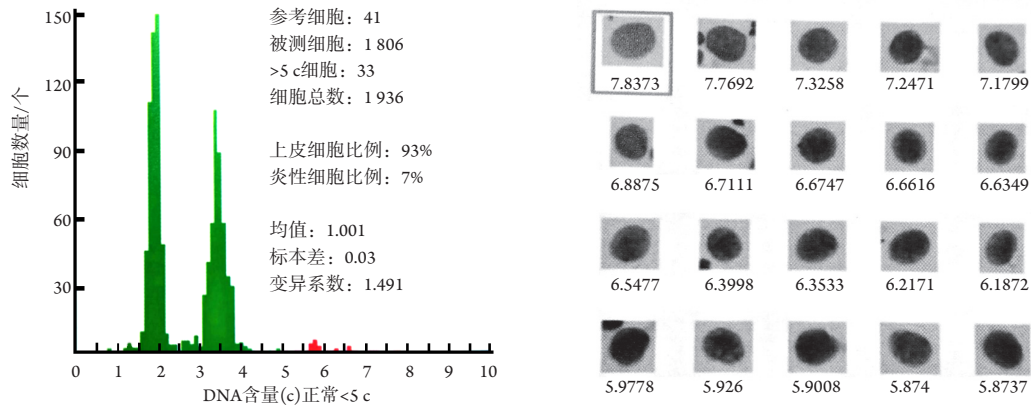


图1 胆管刷检细胞DNA定量分析(阳性), 大量异倍体细胞

Figure 1 Quantitative analysis of DNA in bile duct brush cells (positive), a large number of aneuploid cells

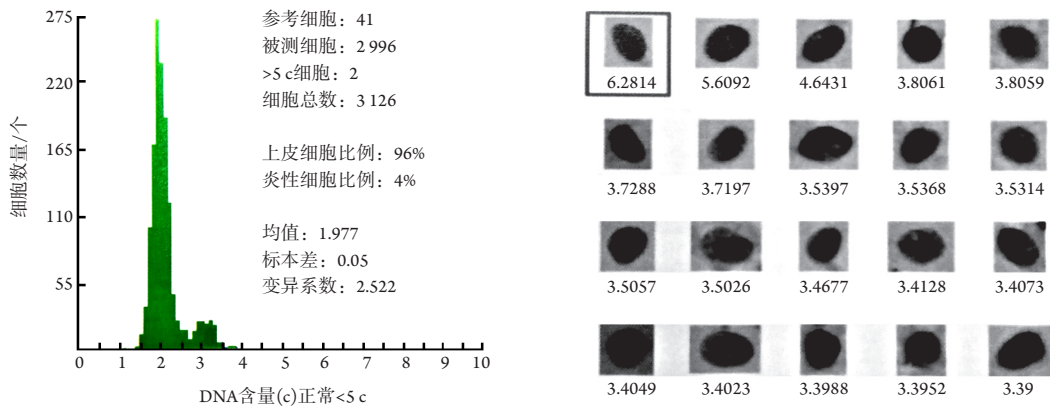


图2 胆管刷检细胞DNA定量分析(阳性), 少量异倍体细胞

Figure 2 Quantitative analysis of DNA in bile duct brush cells (positive), a small number of aneuploid cells

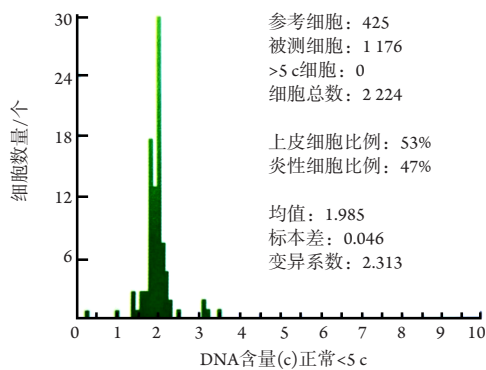


图3 胆管刷检细胞DNA定量分析(阴性), 未见异倍体细胞
Figure 3 Quantitative analysis of DNA in biliary brushing cells (negative), with no aneuploid cells

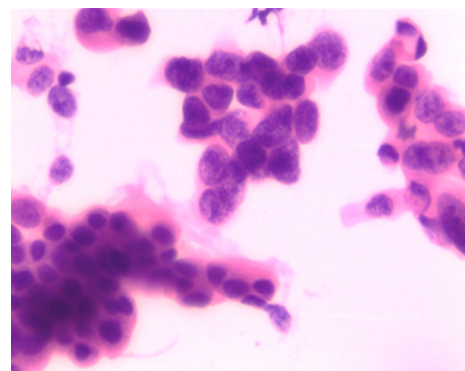


图4 胆管刷检液基细胞学诊断恶性肿瘤细胞(腺癌; HE, $\times 40$)
Figure 4 Liquid-based cytological diagnosis of malignant tumor cells (adenocarcinoma; HE, $\times 40$)

表1 胆管刷检液基细胞学、DNA定量分析及两者联合诊断结果

Table 1 Liquid-based cytology, DNA quantitative analysis and combined diagnosis results of bile duct brushing

检查方法	n	阳性	阴性	阳性率/%
液基细胞诊断	184	74	110	40.2
DNA定量分析	184	95	89	51.6
液基细胞+DNA定量分析	184	99	85	53.8
合计	552	268	284	48.6

3 讨论

胆管癌发病率较低，且缺乏有效的治疗手段。近年来胆管癌的发病率有逐渐增高的趋势^[5]。ERCP是目前诊断胆总管梗阻病因的一线方案^[6]，具有相对安全、有效、微创、并发症少等优点，手术时间短，深受患者欢迎，已成为当今诊治胰胆疾病、恶性胆管狭窄的重要手段^[7-8]；治疗晚期恶性梗阻性黄疸疗效显著^[3]，也是胆道结石的首选诊治手段^[9]。

胆管的良性和恶性疾病均可导致狭窄发生，恶性胆道狭窄被发现时常过了最佳治疗时机，预后较差^[10]。ERCP下细胞刷检操作简单对胆管狭窄诊断的特异性达100%，但敏感性波动较大，在胆管狭窄的定性诊断上，单靠一种诊断措施很难作出正确诊断^[11]。胆管刷检细胞学局限于显微镜下观察细胞形态，对细胞异型性不明显的病变诊断率不高，导致漏诊和误诊。有报道^[12]显示：诊断敏感度仅23%~56%，加长刷子刷取细胞也仅提高灵敏度到27%^[13]。近年来，DNA定量分析已在宫颈细胞^[14]、胸腔积液^[15]、支气管肺泡灌洗液^[16]等细胞学诊断中有很好的应用。DNA定量分析采用静态图像分析术，通过对细胞DNA进行特异染色(Feul-gen染色)，而后进行显微镜下图像定量分析。研究^[17]显示：DNA定量分析诊断恶性肿瘤比病理学诊断可以提早多达15个月，对诊断细胞有无病变具有极大优势，但不能具体分辨出病变种类及程度，而液基细胞学诊断对细胞种类辨别具有优势，ERCP胆管刷检液基细胞学联合细胞DNA定量分析可以较好的解决此问题，提高肿瘤检出率。本研究DNA定量分析的诊断准确性、灵敏度、特异度等均高于液基细胞学，两者联合诊断准确率更高，三者比较，差异有统计学意义

($P < 0.05$)。DNA定量分析的假阳性多与细胞反应性增生或其他如病毒或真菌感染有关，而导致假阴性因素有：1)有些肿瘤细胞本身就是二倍体导致仪器不能检出；2)肿瘤DNA异常但仪器测值正常；3)刷检标本细胞量太少。细胞量获取不足是胆管细胞刷检病理学敏感度低下的主要原因，在临床工作中，增加刷检细胞量是可行的减少假阴性的有效方法，多次重复取样可提高胆管细胞刷检病理学诊断的准确性^[18]。

综上所述，ERCP胆管刷检液基细胞学联合DNA定量分析可以提高胆管肿瘤的检出率，减少漏诊和误诊，在胆管疾病诊断中有较好的应用价值。

参考文献

- Razumilava N, Gores GJ. Cholangiocarcinoma[J]. Lancet, 2014, 383(9935): 2168-2179.
- 金浩, 刘会春, 李宗狂, 等. 超细胆道镜在经皮经肝途径胆管肿瘤活检中的应用[J]. 中国微创外科杂志, 2017(8): 698-700.
JIN Hao, LIU Huichun, LI Zongkuang, et al. Ultrafine choledochoscope in percutaneous through liver bile duct tumor biopsies[J]. Chinese Journal of Minimally Invasive Surgery, 2017(8): 698-700.
- Vazquez-Sequeiros E, Baron TH, Clain JE, et al. Evaluation of indeterminate bile duct strictures by intraductal US[J]. Gastrointest Endosc, 2002, 56(3): 372-379.
- 刘素丽, 王鼎鑫, 邢国璋, 等. 内镜下胆管癌组织活检方法研究[J]. 中国内镜杂志, 2009, 15(5): 456-457.
LIU Suli, WANG Dingxin, XING Guozhang, et al. Endoscopic bile duct carcinoma tissue biopsy method research[J]. China Journal of Endoscopy, 2009, 15(5): 456-457.
- 陈昆仑, 翟文龙. 胆管癌的治疗现状[J]. 中华肝脏外科手术学电子杂志, 2017, 6(6): 447-449.
CHEN Kunlun, ZHAI Wenlong. The treatment of bile duct carcinoma[J]. Chinese Journal of Hepatic Surgery. Electronic Edition, 2017, 6(6): 447-449.
- 孙力祺, 金震东. 内镜新技术在胆胰疾病诊断中的应用[J]. 临床肝胆病杂志, 2018, 34(3): 467-472.
SUN Liqi, JIN Zhendong. New technology of endoscopy in the diagnosis of biliary pancreatic diseases[J]. Chinese Journal of Clinical Hepatology, 2018, 34(3): 467-472.
- 谢雨佳, 张俊文. 经内镜逆行胰胆管造影术并发症的防治研究进展[J]. 现代医药卫生. 2018, 34(16): 2496-2499.
XIE Yujia, ZHANG Junwen. Advances in the prevention and treatment of complications of retrograde endoscopic cholangiopancreatography[J]. Modern Medicine Health, 2018(16):

- 2496-2499.
8. 王伟, 黄晓俊, 王祥, 等. ERCP联合胆管腔内超声及胆管活检对胆管良恶性狭窄的诊断价值[J]. 中国微创外科杂志, 2018, 18(8): 677-681.
WANG Wei, HUANG Xiaojun, WANG Xiang, et al. ERCP combined bile ducts cavity ultrasound and bile duct biopsy in the diagnosis of benign and malignancy narrowness of bile duct value[J]. Chinese Journal of Minimally Invasive Surgery, 2018, 18(8): 677-681.
 9. 金晓昇, 陈璐茜, 季婷婷. ERCP胆管支架置入治疗晚期恶性梗阻性黄疸的疗效研究[J]. 中华全科医学, 2018, 16(10): 1643-1645.
JIN Xiaosheng, CHEN Luqian, JI Tingting. ERCP bile duct stenting treatment the curative effect of late malignant obstructive jaundice study[J]. Applied Journal of General Practice, 2018(10): 1643-1645.
 10. Zhou Y, Liu S, Wu L, et al. Survival after surgical resection of distal cholangiocarcinoma: a systematic review and meta-analysis of prognostic factors[J]. Asian J Surg, 2017, 40(2): 129-138.
 11. 黄平, 张筱风, 张啸, 等. 经内镜逆行胰胆管造影途径下腔内超声及细胞刷对胆管恶性狭窄的早期诊断价值[J]. 中华肝胆外科杂志, 2013, 19(9): 661-664.
HUANG Ping, ZHANG Xiaofeng, ZHANG Xiao, et al. Through ercp inferior vena brush ultrasound and cells within the early diagnostic value of malignant bile duct stenosis[J]. Chinese Journal of Hepatobiliary Surgery, 2013, 19(9): 661-664.
 12. Burnett AS, Calvert TJ, Chokshi RJ. Sensitivity of endoscopic retrograde cholangiopancreatography standard cytology: 10-y review of the literature[J]. J Surg Res, 2013, 184(1): 304-311.
 13. Fogel EL, deBellis M, McHenry L, et al. Effectiveness of a new long cytology brush in the evaluation of malignant biliary obstruction: a prospective study[J]. Gastrointest Endosc, 2006, 63(1): 71-77.
 14. Macey R. DAN-image cytometry and computer-assisted brush biopsy have potential as diagnostic tools for clinically suspected oral precancer and oral cancer[J]. J Evid Based Dent Pract, 2016, 16(2): 113-114.
 15. 张素霞, 周红, 宇小婷, 等. 全自动DNA定量分析检测对恶性胸腔积液诊断的价值[J]. 中华病理学杂志, 2015, 44(9): 653-654.
ZHANG Suxia, ZHOU Hong, YU Xiaoting, et al. Automatic DNA quantitative analysis the value of detection of malignant pleural effusion diagnosis[J]. Chinese Journal of Pathology, 2015, 44(9): 653-654.
 16. 石安琪, 江涛. DNA定量分析在肺癌诊断中的应用价值[J]. 现代医药卫生, 2018, 34(10): 1502-1504.
SHI Anqi, JIANG Tao. DNA ploidy analysis value in the diagnosis of lung cancer[J]. Journal of Modern Medicine & Health, 2018, 34(10): 1502-1504.
 17. Maraki D, Becker J, Boecking A. Cytologic and DNA-cytometric very early diagnosis of oral cancer[J]. J Oral Pathol Med, 2004, 33(7): 398-404.
 18. 黄平, 张筱风, 张啸, 等. 胆管腔内超声联合细胞刷检病理、基因突变检测对胆管恶性狭窄的早期定性诊断价值[J]. 中华消化内镜杂志, 2014, 31(12): 685-689.
HUANG Ping, ZHANG Xiaofeng, ZHANG Xiao, et al. Bile duct intracavitary ultrasonic joint brush cell pathology, gene mutation detection early qualitative diagnostic value of malignant bile duct stenosis[J]. Chinese Journal of Digestive Endoscopy, 2014, 31(12): 685-689.

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