

白细胞形态学和中性粒细胞碱性磷酸酶 在手足口病合并感染患儿诊疗中的应用

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【摘要】目的 探讨白细胞(WBC)形态学和中性粒细胞碱性磷酸酶(NAP)染色积分值在儿童手足口病(HFMD)合并感染诊疗中的应用价值。**方法** 选取2017年1月至2017年10月于玉林市红十字会医院儿科门诊及住院的150例儿童作为研究对象,根据检查结果及病情分为正常对照组、单纯HFMD组及HFMD合并感染组,每组各50例。所有病例均采用一次性EDTA扩凝真空采血管,按常规静脉采血1~2 ml,同时做血涂片2张,1张用于瑞氏染色,另1张血片干燥后用10%甲醛固定30 s,按NAP试剂盒说明书进行染色,由细胞室工作人员在显微镜下鉴别WBC形态学变化并计数NAP阳性率和积分。**结果** 正常对照组患儿NAP阳性率为(23.58±11.89)%,积分为(28.18±13.82);单纯HFMD组患儿NAP阳性率为(22.8±10.49)%,积分为(26.92±11.9);HFMD合并感染组患儿NAP阳性率为(77.96±8.99)%,积分为(332.7±58.42);HFMD合并感染抗感染治疗后组NAP阳性率为(22.38±10.54)%,积分为(27.74±12.16);HFMD合并感染组分别与正常对照组、单纯HFMD组、HFMD合并感染抗感染治疗后NAP阳性率比较,差异均有统计学意义($t=25.80$ 、 28.23 、 28.37 , P 均 <0.001);HFMD合并感染组分别与正常对照组、单纯HFMD组、HFMD合并感染抗感染治疗后NAP积分比较,差异均有统计学意义($t=35.87$ 、 36.27 、 36.14 , P 均 <0.001);其余各组NAP阳性率和积分两两比较,差异均无统计学意义(P 均 >0.05)。正常对照组、单纯手足口病组、手足口病合并感染组患儿异型淋巴细胞、中毒颗粒、空泡变性细胞、杜勒小体比例差异均有统计学意义($H=81.9939$ 、 129.1737 、 117.5489 、 89.4793 , P 均 <0.001);手足口病合并感染抗感染治疗前后患儿异常淋巴细胞、中毒颗粒、空泡变性细胞和杜勒小体比例差异有统计学意义($U=8.2967$ 、 8.6138 、 8.6318 、 5.4355 , P 均 <0.001)。**结论** 白细胞形态学检查和NAP阳性率及积分观察HFMD合并感染患儿疾病发生发展及对疾病诊断、预后监测均具有重要临床意义。

【关键词】 手足口病; 白细胞形态学; 中性粒细胞碱性磷酸酶; 积分

Application of white blood cell morphology combined with neutrophil alkaline phosphatase in the diagnosis and treatment of children with hand, foot and mouth disease complicated with infection

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【Abstract】Objective To investigate the value of white blood cell (WBC) morphology and neutrophil alkaline phosphatase (NAP) staining score in the diagnosis and treatment of hand, foot and mouth disease (HFMD) complicated with infection. **Methods** From January 2017 to October 2017, a total of 150 outpatient and hospitalized children in Hospital of Yulin Red Cross Society Guangxi were selected. According to the examination results and the disease condition, all patients were divided into three groups: normal control group, simple HFMD group and HFMD with infection group, 50 cases in each group. All cases were treated with disposable vacuum blood coagulation EDTA expansion, and blood samples were collected by routine venous blood collection of 1-2 ml, while two blood smears were carried out, one was for Rayleigh staining, the other was fixed with 10% formaldehyde for 30 seconds after drying, and stained according to

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the instructions of NAP kit. The morphological changes of WBC were identified under microscope and the positive rate and integral of alkaline phosphatase (ALP) in neutrophils were counted by cell room workers.

Results The positive rate of NAP in children of the control group was $(23.58 \pm 11.89)\%$ and the score was (28.18 ± 13.82) ; the positive rate of NAP in children of simple HFMD group was $(22.8 \pm 10.49)\%$ and the score was (26.92 ± 11.9) ; the positive rate of NAP in children of HFMD with infection group was $(77.96 \pm 8.99)\%$ and the score was (332.7 ± 58.42) ; the positive rate of NAP in children of HFMD with infection for anti-infection treatment group was $(22.38 \pm 10.54)\%$ and the score was (27.74 ± 12.16) , respectively. The positive rate of NAP in HFMD with infection group was significantly higher than the other three groups, with significant differences ($t = 25.80, 28.23, 28.37$; all $P < 0.001$). The score of NAP in HFMD with infection group was significantly higher than the other three groups, with significant differences ($t = 35.87, 36.27, 36.1374$; all $P < 0.001$). There was no significant difference in positive rate and score of NAP between the other groups pairwise comparison (all $P > 0.05$). The rates of abnormal lymphocytes, toxic particles, vacuolar degeneration and Duller bodies among normal control group, simple HFMD group and HFMD with infection group were significantly different ($H = 81.9939, 129.1737, 117.5489, 89.4793$; all $P < 0.001$). The rates of abnormal lymphocytes, toxic particles, vacuolar degeneration and Duller bodies in children of HFMD with infection group before and after anti-infection treatment were significantly different ($U = 8.2967, 8.6138, 8.6318, 5.4355$; all $P < 0.001$). **Conclusions** WBC morphological examination, positive rate of neutrophil alkaline phosphoric acid staining and integral observation of the occurrence and development of HFMD children with infection were significantly important to disease diagnosis and prognosis monitoring.

【Key words】 Hand, foot and mouth disease; Leukocyte morphology; Neutrophil alkaline phosphatase; Score

手足口病(hand, foot and mouth disease, HFMD)是由肠道病毒感染引起的传染病。近年来,儿童手足口病是一种儿科常见病,部分医院尤其在基层单位,临床上多以白细胞总数及其分类比值作为诊断依据^[1-3]。但个体差异、昼夜差异以及情绪波动等均影响白细胞(white blood cells, WBC)计数及其比值,故上述检测结果不能正确反映患者感染状态及鉴别感染类型^[4-8]。而细菌培养费时,且易受药物、取样时段、取样部位及取样量等因素影响,阳性检出率低,远不能满足临床诊断的要求^[9-12]。

本研究旨在通过临床诊断确诊,并结合WBC形态、中性粒细胞碱性磷酸酶(neutrophil alkaline phosphatase, NAP)阳性率及积分检测结果,评价同时检测WBC形态、NAP阳性率及积分在诊断儿童手足口病合并感染中抗感染治疗的临床应用价值,为判断手足口病合并感染儿童抗感染治疗寻找快速、理想检测指标,现报道如下。

资料与方法

一、研究对象

收集于2017年1月至2017年10月于玉林市红十字会医院儿科门诊及住院就诊的150例患儿进行回顾性研究,年龄为0~5岁,平均年龄为 (2.5 ± 1.7) 岁,其

中男性患儿96例(64%),女性患儿54例(36%);根据检查结果及病情将研究对象分为正常对照组、单纯手足口病组及手足口病合并感染组共3组,每组50例,其中手足口病合并感染组治疗后复查定为手足口病合并感染抗感染治疗后组。正常对照组为健康体检儿童;单纯手足口病组为具有手足口病临床症状,经实验室检测结果确诊为手足口病患者,但未合并其他病原体感染的患儿;手足口病合并感染组为确诊手足口病的基础上有1种以上其他病原体感染的患儿。各组患儿年龄和性别差异均无统计学意义(P 均 > 0.05)。入组病例均经本单位医学伦理道德委员会和患儿家属同意,无任何法律纠纷。

二、方法

研究对象均采用一次性乙二胺四乙酸二钾(EDTA-K₂)抗凝真空采血管,按常规静脉采血1~2 ml,同时做血涂片2张,1张用于瑞氏染色,另1张血片干燥后,用10%甲醛固定30 s后,按NAP试剂盒说明书进行染色,血片经NAP染色后,在油镜下观察100个成熟中性粒细胞胞浆中颗粒,并根据颗粒大小、形状、多少及分布来计算积分,积分判读标准见表1, NAP阳性率为100个成熟中性粒细胞胞浆有阳性反应颗粒的细胞所占的百分率。瑞氏染色后的血片,油镜下观察100个白细胞的细胞形态,最后综合分析手足口病合并感染患儿白细胞形

态学和NAP阳性率及积分。

三、统计学处理

采用SPSS 19.0软件进行统计学分析, 各组患者间NAP阳性率、积分为计量资料, 均呈正态分布, 采用 $\bar{x} \pm s$ 表示, 多组间比较采用方差分析, 组间两两比较采用独立样本 t 检验; WBC形态学异常比例为计量资料, 呈非正态分布, 采用均数(四分位间距)[M (P25, P75)]表示, 多组间比较采用Kruskal-wallis H 检验, 两组间比较采用Mann-Whitney U 检验, 以 $P < 0.05$ 为差异有统计学意义。

结 果

一、各组患儿NAP阳性率和NAP积分

各组患儿NAP阳性率和NAP积分差异均有统计学意义(P 均 < 0.001), 见表2。手足口病合并感染组患儿分别与正常对照组、单纯手足口病组患者NAP阳性率比较, 差异均具有统计学意义($t = 25.80$ 、 28.23 , P 均 < 0.001); 手足口病合并感染组患儿分别与正常对照组、单纯手足口

病组NAP积分比较, 差异均具有统计学意义($t = 35.87$ 、 36.27 , P 均 < 0.001); 手足口病合并感染抗感染治疗前后NAP阳性率和NAP积分差异均有统计学意义($t = 28.37$ 、 $P < 0.001$, $t = 36.14$ 、 $P < 0.001$), 见表3。

二、各组患儿WBC形态学

正常对照组和单纯手足口病组患儿白细胞形态学检查偶见异型淋巴细胞和空泡变性, 未见中毒颗粒和杜勒小体异常。手足口病合并感染组及抗感染后组患儿则多数可见异型淋巴细胞、中毒颗粒、空泡变性细胞及杜勒小体。正常对照组、单纯手足口病组、手足口病合并感染组患儿异型淋巴细胞、中毒颗粒、空泡变性细胞、杜勒小体比例差异均有统计学意义($H = 81.9939$ 、 129.1737 、 117.5489 、 89.4793 , P 均 < 0.001); 手足口病合并感染抗感染治疗前后患儿异常淋巴细胞、中毒颗粒、空泡变性细胞和杜勒小体比例差异有统计学意义($U = 8.2967$ 、 8.6138 、 8.6318 、 5.4355 , P 均 < 0.001), 详见表4~5; 不同患儿WBC形态学表现见图1。

表1 外周血片 NAP 积分标准表

| 定性 | 积分标准 | 各类细胞计数(个) | 积分计算 |
|----|-----------------------------|-----------|---------------|
| — | 无阳性反应沉淀物 | n1 | $0 \times n1$ |
| + | 仅少数浅灰色颗粒 $< 1/4$ 浆面积 | n2 | $1 \times n2$ |
| 2+ | 反应较强, 灰棕色, 密片状颗粒占 $1/2$ 浆面积 | n3 | $2 \times n3$ |
| 3+ | 反应更强, 棕黑色密片状颗粒占 $3/4$ 浆面积 | n4 | $3 \times n4$ |
| 4+ | 反应最强, 棕黑色团块状充满甚至遮蔽细胞核上 | n5 | $4 \times n4$ |

注: $n1 + n2 + n3 + n4 + n5 = 100$, 100 为每张片要在油镜下计数的成熟中性粒细胞总数, 积分是把这 100 个成熟中性粒细胞的所有 NAP 积分累加所得。NAP 的阳性率 = $(n2 + n3 + n4 + n5) \div 100 \times 100\%$

表2 各组患儿 NAP 阳性率和 NAP 积分 ($\bar{x} \pm s$)

| 组别 | 例数 | NAP阳性率 (%) | NAP积分 (分) |
|-----------|----|-------------------|-------------------|
| 正常对照组 | 50 | 23.58 ± 11.89 | 28.18 ± 13.82 |
| 单纯手足口病组 | 50 | 22.8 ± 10.49 | 26.92 ± 11.9 |
| 手足口病合并感染组 | 50 | 77.96 ± 8.99 | 332.7 ± 58.42 |
| F 值 | | 215.362 | 664.210 |
| P 值 | | < 0.001 | < 0.001 |

表3 手足口病合并感染组患儿抗感染治疗前后 NAP 阳性率和积分 ($\bar{x} \pm s$)

| 组别 | 例数 | NAP阳性率 (%) | NAP积分 (分) |
|-------|----|-------------------|-------------------|
| 治疗前 | 50 | 77.96 ± 8.99 | 332.7 ± 58.42 |
| 治疗后 | 50 | 22.38 ± 10.54 | 27.74 ± 12.16 |
| t 值 | | 28.370 | 36.140 |
| P 值 | | < 0.001 | < 0.001 |

表4 各组患儿 WBC 形态学异常比例 [% , M (P25, P75)]

| 组别 | 例数 | 异型淋巴细胞 | 中毒颗粒 | 空泡变性 | 杜勒小体 |
|-----------|----|---------------|-------------------|-------------------|--------------|
| 正常对照组 | 50 | 0 (0, 3.75) | 0 (0, 0) | 0 (0, 1.75) | 0 (0, 0) |
| 单纯手足口病组 | 50 | 4 (2.0, 5.75) | 0 (0, 0) | 3 (2.0, 4.0) | 0 (0, 0) |
| 手足口病合并感染组 | 50 | 7 (6.0, 8.0) | 40 (34.25, 46.50) | 17.5 (15.0, 22.0) | 3 (2.0, 4.0) |
| H 值 | | 81.9939 | 129.1737 | 117.5489 | 89.4793 |
| P 值 | | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

表5 手足口病合并感染抗感染治疗前后 WBC 形态学异常比例 [%，M (P25，P75)]

| 组别 | 例数 | 异型淋巴细胞 | 中毒颗粒 | 空泡变性 | 杜勒小体 |
|-----|----|-------------|------------------|------------------|-------------|
| 治疗前 | 50 | 7 (6.0，8.0) | 40 (34.25，46.50) | 17.5 (15.0，22.0) | 3 (2.0，4.0) |
| 治疗后 | 50 | 2 (2.0，3.0) | 2 (2.0，3.0) | 2 (1.0，2.0) | 0 (0.0，1.0) |
| U值 | | 8.2967 | 8.6138 | 8.6138 | 5.4355 |
| P值 | | < 0.001 | < 0.001 | < 0.001 | < 0.001 |

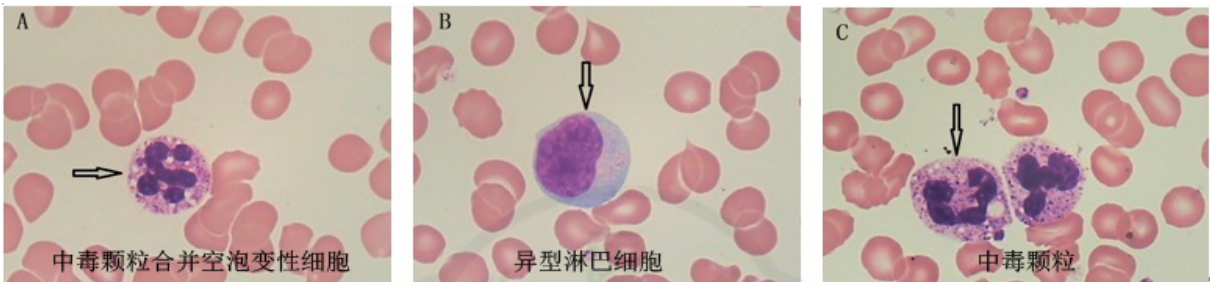


图1 油镜观察手足口病患者白细胞异常形态 (瑞氏染色，×100)

讨 论

手足口病作为一种由肠道病毒引起的传染病，严重危害儿童的身体健康。据报道，引起手足口病的肠道病毒毒株有20多种（型），其中以柯萨奇病毒A16型（CoxA16）和肠道病毒71型（EV71）最为多见^[13-15]。多发于5岁以下儿童，表现为低热、厌食、口腔疼痛，手、足、口腔等部位出现小疱疹或小溃疡物，多数患儿1周左右可自愈，少数患者并发肺水肿、无菌性脑膜脑炎或心肌炎等^[16-18]。个别重症患者病情进展迅速，引起心功能衰竭而死亡。目前尚无特效的药物进行治疗，多数仅能对症治疗^[19-20]。

NAP主要存在于成熟中性粒细胞胞浆内，当机体发生细菌感染时，NAP活性显著增高，随着疾病进展或加重，NAP阳性率及积分显著增高，如经治疗，患者趋向好转和恢复，NAP阳性率及积分逐渐下降或恢复正常水平^[3-4]。本研究结果显示，HFMD合并感染组患儿中性粒细胞碱性磷酸酶阳性率及积分显著高于正常对照组、单纯手足口病组；HFMD合并感染组患儿抗感染治疗后，其NAP阳性率及积分均显著下降，这与相关报道一致^[5-6]，提示NAP不但可作为细菌感染诊断指标，而且对监测疾病的发生、发展，疾病愈后、好转有重要意义，故NAP染色有助于儿童HFMD合并感染的鉴别诊断及抗感染治疗的疗效观察。提示临床HFMD合并感染者需尽快进行抗细菌感染治疗，以免病情加重，危及患者生命。

本研究亦提示，HFMD合并感染患儿外周血白细胞形态学改变较正常对照组、单纯HFMD组和抗感染后组变化显著，正常对照组和单纯HFMD组白细胞形态学检查偶见异型淋巴细胞和空泡变性，HFMD合并感染组及抗感染后组则多数可见异型淋巴细胞、中毒颗粒空泡变性细胞及杜勒小体。HFMD合并感染者与正常对照组、单纯HFMD组抗感染后组异常淋巴细胞阳性率、空泡变性细胞阳性率等差异均有统计学意义；正常对照组与单纯HFMD病组未见中毒颗粒和杜勒小体；HFMD合并感染组与抗感染后组中毒颗粒、杜勒小体数差异有统计学意义，与相关研究结果相一致^[10-15]，提示外周血白细胞胞浆中的中毒颗粒、空泡变性及杜勒小体与感染类型、感染严重程度密切相关，HFMD合并感染者白细胞形态学改变较为显著，故临床上可通过监测外周血白细胞形态学的变化来监测患者病情的进展和抗感染疗效观察。

综上，白细胞形态学检查和NAP阳性率及积分有助于HFMD合并感染患儿的鉴别诊断、疗效观察和预后监测。

参 考 文 献

[1] Huang Y, Deng T, Yu S, et al. Effect of meteorological variables on the incidence of hand, foot, and mouth disease in children: a time-series analysis in Guangzhou, China[J]. BMC Infect Dis,2013,13(1):134.

[2] Kashyap RR, Kashyap RS. Hand, foot and mouth disease--a short case report[J]. J Clin Exp Dent,2015,7(2):336-338.

[3] Long L, Xu L, Xiao Z, et al. Neurological complications and risk factors of cardiopulmonary failure of EV-A71-related hand, foot and mouth disease[J]. Epidemiol Infect,2016,6(1):23444.

- [4] Keawcharoen J. Hand, foot and mouth disease[J]. Thai J Vet Med,2012,42(3):225-257.
- [5] Banta J, Lenz B, Pawlak M, et al. Notes from the field: outbreak of hand, foot and mouth disease caused by Coxsackievirus A6 among basic military trainees--Texas, 2015[J]. MMWR Morb Mortal Wkly Rep,2016,65(26):678-680.
- [6] Feder HM, Bennett N, Modlin JF, et al. Atypical hand, foot, and mouth disease: a vesiculobullous eruption caused by Coxsackie virus A6[J]. Brit Dent J,2014,14(1):83-86.
- [7] Gaunt E, Harvala H, Osterback R, et al. Genetic characterization of human coxsackievirus A6 variants associated with atypical hand,foot and mouth disease: a potential role of recombination in emergence and pathogenicity[J]. J Gen Viro,2015,96(5):1067-1079.
- [8] Vuorinen T, Osterback R, Kuisma J, et al. Epididymitis caused by Coxsackievirus A6 in association with hand, foot, and mouth disease[J]. J Clin Microb,2014,52(12):4412-4413.
- [9] Li T, Yang Z, Liu X, et al. Hand-foot-and-mouth disease epidemiological status and relationship with meteorological variables in Guangzhou, Southern China, 2008-2012[J]. Revista do Instituto de Medicina Tropical de São Paulo,2014,56(6):533-539.
- [10] Huang X, Wei H, Wu S, et al. Epidemiological and etiological characteristics of hand, foot and mouth disease in Henan, China, 2008-2013[J]. Sci Rep,2015,5(1):8904.
- [11] Han J, Xu S, Zhang Y, et al. Hand, foot and mouth disease outbreak caused by Coxsackievirus A6, China, 2013[J]. J Infect,2014,69(3):303-305.
- [12] Yan X, Zhang ZZ, Yang ZH, et al. Clinical and etiological characteristics of atypical hand-foot-and-mouth disease in children from Chongqing, China: A retrospective study[J]. Bio Res Inter,2015,26(8):1-8.
- [13] Lin H, Sun L, Lin J, et al. Protective effect of exclusive breastfeeding against hand, foot and mouth disease[J]. BMC Infect Dis,2014,14(1):645.
- [14] Ventarola D, Bordone L, Silverberg NB, et al. Update on hand-foot-and-mouth disease[J]. Clin Dermatol,2015,33(3):340-346.
- [15] Zhang S, Zhao J. Spatio-temporal epidemiology of hand, foot and mouth disease in Liaocheng City, North China[J]. Exp Ther Med,2015,9(3):811-816.
- [16] Wang ZL, Xia A, Li Y, et al. Socioeconomic burden of hand, foot and mouth disease in children in Shanghai, China[J]. Epidemiol Infect,2016,144(1):138-143.
- [17] Li Y, Zhang J, Zhang X, et al. Modeling and preventive measures of hand, foot and mouth disease (HFMD) in China[J]. Int J Envir Res Pub Heal,2014,11(3):3108-3117.
- [18] Zhang J, Kang Y, Yang Y, et al. Statistical monitoring of the hand, foot and mouth disease in China[J]. Biometrics,2015,71(3):841-850.
- [19] Zhang X, Wang H, Ding S, et al. Prevalence of enteroviruses in children with and without hand, foot and mouth disease in China[J]. BMC Infect Dis,2013,13(1):606.
- [20] Mao Q, Wang Y, Bian L, et al. EV71 vaccine, a new tool to control outbreaks of hand, foot and mouth disease (HFMD)[J]. Expert Rev Vaccines,2016,15(5):599-606.

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