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临床经验 CLINICAL PRACTICE

T淋巴细胞亚群和DNA倍体检测在腹水鉴别诊断中的价值

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■背景资料
腹水检测对疾病的判断有着非常重要的意义. 目前, 大多数医院仍然采用一般性状、生化、细胞及细菌检查等传统检测手段. 对于鉴别感染性与非感染性腹水方面的敏感性、特异性等远远不能满足临床的需要. 需要寻找新的检测方法而流式细胞仪检测相当准确、快捷, 有必要在这方面进行探讨.

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Value of T lymphocyte subset determination and DNA ploidy analysis for differential diagnosis of ascites

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Abstract
AIM: To investigate the value of T lymphocyte subset determination and DNA ploidy analysis for differential diagnosis of ascites.

METHODS: In 74 cases of ascites, 24 with tuberculous peritonitis, 21 with liver cirrhosis and 29 with carcinomatous ascites, T lymphocyte subsets and DNA ploidy in ascitic fluid were detected by flow cytometry.

RESULTS: In descending order of T lymphocyte ($CD3^+$), helper/inducer T lymphocyte ($CD4^+$), and helper T lymphocyte/suppressor T lymphocyte ($CD4^+/CD8^+$) in ascitic fluid, tuberculous peritonitis ($CD3^+$: 86.2% ± 5.1%, $CD4^+$: 64.3% ± 6.4%, $CD4^+/CD8^+$: 3.20% ± 0.30%), carcinomatous ascites (65.7% ± 4.6%, 32.5% ± 2.2%, 1.04 % ± 0.11%) and liver cirrhosis (15.1% ± 2.7%, 3.6% ± 0.5%, 0.36% ± 0.05%) ($P < 0.01$) were determined. In ascending order, $CD8^+$, liver cirrhosis (10.1% ± 3.2%), tuberculous peritonitis (20.1% ± 4.3%) and carcinomatous ascites (31.3% ± 5.2%) ($P < 0.01$) were determined. The positive rate from DNA ploidy analysis in carcinomatous ascites (89.7%, 26/29) was significantly higher than that in tuberculous peritonitis (4.2%, 1/21) or liver cirrhosis (4.7%, 1/27) ($P < 0.01$).

CONCLUSION: T lymphocyte subsets and DNA ploidy in ascitic fluid show obvious differences among patients with tuberculous peritonitis, liver cirrhosis and carcinomatous ascites. T lymphocyte subset determination and DNA ploidy analysis are thus useful for the differential diagnosis of ascites.

Key Words: T lymphocyte subsets; DNA ploidy; Ascites; Flow cytometry; Differential diagnosis

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摘要
目的: 探讨T淋巴细胞亚群和DNA倍体检测在腹水鉴别诊断中的价值.

方法: 腹水患者74例, 其中结核性腹膜炎24例, 肝硬化21例, 癌性腹水29例, 流式细胞仪测定腹水T淋巴细胞亚群和DNA倍体.

结果: 腹水中T淋巴细胞($CD3^+$)、T辅助/诱导细胞亚群($CD4^+$)、T辅助细胞亚群/ T抑制细胞亚群($CD4^+/CD8^+$)所占比例从大到小依次为结核性腹膜炎($CD3^+$: 86.2%±5.1%, $CD4^+$: 64.3%±6.4%, $CD4^+/CD8^+$: 3.20%±0.30%)、癌性腹水(65.7%±4.6%, 32.5%±2.2%, 1.04 %±0.11%)、肝硬化腹水(15.1%±2.7%, 3.6%±0.5%, 0.36%±0.05%)(三组间$P<0.01$), $CD8^+$比例从小到大依次为肝硬化腹水(10.1%±3.2%)、结核性腹膜炎(20.1%±4.3%)、癌性腹水(31.3%±5.2%)(三组间$P<0.01$). 腹水DNA倍体阳性率癌性腹水达89.7%(26/29), 与结核性腹膜炎4.2%(1/24)和肝硬化4.7%(1/21)具有显著性差异($P<0.01$).

结论: T淋巴细胞亚群和DNA倍体在结核性

腹膜炎、肝硬化及癌性腹水存在显著差异, 其检测可用于腹水的鉴别诊断.

关键词: T淋巴细胞亚群; DNA倍体; 腹水; 流式细胞; 鉴别诊断

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0 引言

腹水可由多种疾病产生, 腹水检查在其病因诊断中具有重要意义. 传统检测手段包括一般性状、生化、细胞及细菌检查, 近年肿瘤标志物、细胞因子等检测也逐渐步入临床[1-11], 由于敏感性、特异性、操作复杂程度的原因, 目前的检测方法仍不能满足临床的需要. 本文采用流式细胞仪对几种不同病因腹水中T淋巴细胞亚群和DNA倍体进行检测, 探讨其在腹水鉴别诊断中的价值.

1 材料和方法

1.1 材料 74例腹水患者为我院2001-01/2006-04住院患者, 其中结核性腹膜炎24例, 男14例, 女10例, 平均年龄36岁; 肝炎后肝硬化21例, 男12例, 女9例, 平均年龄38岁; 癌性腹水29例, 男17例, 女12例, 平均年龄41岁; 癌性腹水中胃癌14例; 结肠癌10例; 卵巢癌3例; 胰腺癌2例. 流式细胞仪为Beckman coulter公司Epics XL型号. 荧光mAb CD3-FITC、CD4-FITC、CD8-PE和红细胞溶解剂(IMMUNO PREPTM A)、白细胞稳定剂(IMMUNO PREPTM B)、细胞膜固定剂(IMMUNO PREPTM C)均购自法国Immunothch公司.

1.2 方法

收集3组患者肝素抗凝的新鲜腹水100 mL, 2000 r/min离心5 min, 弃上清, PBS洗涤, 制备成细胞悬液, 调整细胞数为10^6/mL. 取100 μL细胞悬液, 加入CD3-FITC、CD4-FITC、CD8-PE, 室温避光20 min, 分别加入IMMUNO PREPTM A、IMMUNO PREPTM B、IMMUNO PREPTM C, 置入流式细胞仪检测, 并用SYSTEMⅡTM software处理数据, 测定腹水T淋巴细胞亚群. 另取100 μL细胞悬液, 按试剂盒说明进行溶血、固定、PI染色, 置入流式细胞仪检测, 以腹水中二倍体DNA含量细胞为内标, 分析样品细胞的DNA指数(DNA index, DI)、增殖指数(proliferation index, PI)和S期细胞百分比(S phase fraction, SPF). DI = 肿瘤细胞G0/G1峰道数/正常二倍体细胞G0/G1期峰道数. DI<0.9或DI>1.1可判为异倍体, 且按照国际标准DNA异倍体必须在组方图上出现两个相分离的峰.

统计学处理 数据以均数±标准差(mean±SD)表示, 统计学处理采用方差分析及q检验.

2 讨论

2.1 不同病因腹水T淋巴细胞亚群变化 结核性腹膜炎、癌性腹水及肝硬化患者腹水$CD3^+$(T淋巴细胞)、$CD4^+$(T辅助/诱导细胞亚群)、$CD8^+$(T抑制/毒性细胞亚群)、$CD4^+/CD8^+$均存在显著差异(P<0.01), $CD3^+$、$CD4^+$、$CD4^+/CD8^+$由高到低依次为结核性腹膜炎>癌性腹水>肝硬化腹水(P<0.01), $CD8^+$由高到低依次为癌性腹水>结核性腹膜炎>肝硬化腹水(P<0.01), 肝硬化腹水$CD4^+/CD8^+$明显倒置(表1).

2.2 不同病因腹水DNA倍体结果 24例结核性腹膜炎和21例肝硬化患者中各有1例腹水DNA倍体为阳性, 阳性率分别为4.2%和4.7%, 而29例癌性腹水26例呈阳性, 阳性率达89.7%, 与前二组比较具有显著性差异(P<0.01).

3 讨论

腹水的病因在临床上以肝硬化、结核性腹膜炎和癌性腹水最为常见[12-22], 常需联合多种检测手段方能确诊, 甚至试验性治疗来进行鉴别诊断, 而腹水的直接检测对病因的诊断至关重要. 我们采用流式细胞仪对腹水的T淋巴细胞亚群和DNA倍体进行检测, 发现肝硬化腹水$CD3^+$、$CD4^+$、$CD8^+$、$CD4^+/CD8^+$较结核性腹膜炎和癌性腹水显著低下, 且$CD4^+/CD8^+$明显倒置, 3种腹水中$CD3^+$、$CD4^+$、$CD4^+/CD8^+$以结核性腹膜炎最高, 癌性腹水居中, 而$CD8^+$则以癌性腹水最高, 结核性腹膜炎次之. 腹水T淋巴细胞亚群反应了腹腔局部的免疫功能, T淋巴细胞由$CD4^+$和$CD8^+$两大亚群组成[23], $CD4^+$主要介导细胞和体液免疫, $CD8^+$则参与抗病毒、移植排斥反应及对非己抗原诱发的免疫应答的抑制[24-28], 免疫功能的改变往往敏感地表现在$CD4^+/CD8^+$比值改变上, $CD4^+/CD8^+$比值的下降被认为是疾病严重和预后不良的重要指标之一. 我们的研究提示, (1)肝硬化患者腹腔局部的免疫功能明显低下, 这可能是患者易并发自发性腹炎的一个原

■同行评价
本文采用流式细胞仪对几种不同病因腹水中T淋巴细胞亚群和DNA倍体进行检测, 探讨其在腹水鉴别诊断中的价值有一定的临床意义. 具有一定的创新性.

表 1 不同病因腹水T淋巴细胞亚群变化(mean ± SD)

分组	$CD3^+$(%)	$CD4^+$(%)	$CD8^+$(%)	$CD4^+/CD8^+$
结核性腹膜炎	86.2 ± 5.1	64.3 ± 6.4	20.1 ± 4.3	3.20 ± 0.30
癌性腹水	65.7 ± 4.6	32.5 ± 2.2	31.3 ± 5.2	1.04 ± 0.11
肝硬化	15.1 ± 2.7	3.6 ± 0.5	10.1 ± 3.2	0.36 ± 0.05

三组之间比较$P<0.01$.

因; (2)结核性腹膜炎患者腹膜腔局部免疫力增强. 当结核杆菌感染时, 首先致敏T细胞, 当机体再次受到结核杆菌或抗原物质入侵时与致敏的T细胞互相作用, 释放出一系列细胞因子(IL-1、TNF等)及黏附分子(P-选择素等), 使得淋巴细胞、单核细胞定向腹膜腔内募集, 聚集于结核杆菌或抗原所在部位周围; (3)癌性腹水中的$CD8^+$增高. 机体对肿瘤细胞的免疫监控过程中, 抗原提呈细胞将肿瘤抗原有效地提呈给T淋巴细胞, 激活肿瘤抗原特异性细胞毒性T淋巴细胞(CTL)是抗肿瘤免疫的关键环节[29-30], $CD8^+$增高致$CD4^+/CD8^+$降低, 降低了患者局部对肿瘤的免疫力, 可导致肿瘤细胞不断增殖与扩散.

正常细胞DNA倍体为二倍体, 具有较恒定的DNA含量, 而细胞癌变过程中DNA含量和(或)染色体结构异常是较普遍的, 尤其是分化程度很低的恶性肿瘤更为常见, 并以DNA指数的形式表现出来, 出现非整倍体细胞峰. 联合DNA倍体检测更有助于将癌性腹水鉴别开来, 本研究中癌性腹水DNA倍体阳性率达89.7%, 结核性腹膜炎、肝硬化患者腹水阳性率不足5%. 同时研究发现, 有些恶性腹水DNA倍体出现假阴性结果, 可能是: (1)原发肿瘤为二倍体肿瘤, 且转移灶与原发灶有同源性; (2)少量恶性细胞的异倍体峰被大量组织细胞所形成的二倍体细胞峰掩盖; (3)少量的染色体变异不能被流式细胞仪所识别; (4)大量间皮细胞稀释恶性细胞, 导致异倍体峰缺如; (5)染色体的机械缺失和复制中的错误相平衡, 而表现为二倍体. 另外良性腹水DNA倍体出现假阳性结果可能是因为细胞破坏、叠连而不能被流式细胞仪所识别, 反应性间皮细胞增生、染色体一过性畸变和数据分析也可造成假阳性结果.

我们的研究表明, T淋巴细胞亚群和DNA倍体在结核性腹膜炎、肝硬化及癌性腹水存在显著差异, 其检测可用于腹水的鉴别诊断, 而流式细胞仪检测又相当准确、快捷, 值得推广.

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