

慢性粒细胞白血病加速期及急变期的形态学与免疫学分型的相关研究

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[摘要] **目的** 探讨慢性粒细胞白血病(CML)加速期及急变期的细胞形态学和免疫学分型特征。**方法** 对 73 例慢性粒细胞白血病急变期(CML-BP)患者的骨髓标本分别进行光镜细胞形态学及相关细胞化学染色观察,确定其 FAB 类型;应用流式细胞仪检测免疫学一系列相关抗原。**结果** 73 例 CML-BP 患者的 FAB 分型为慢性粒细胞白血病急变(CML-AML)44 例,急性淋巴细胞白血病(ALL)21 例,急性混合细胞白血病(HAL)8 例。流式细胞术行免疫表型分析,21 例 CML 向急性淋巴细胞白血病转变(CML-ALL)患者中,其免疫表型 B-ALL 19 例,T-ALL 2 例;且 12 例含髓系标志。8 例 CML 向急性混合细胞白血病(HAL)转变(CML-HAL)患者中,免疫表型为 B+My 6 例;T+My 2 例。44 例 CML-AML 患者中,15 例含 T 细胞标志,2 例含 B 细胞标志,其他未见跨系表达。73 例 CML-BP 患者中有 29 例在 CML 加速期行流式检查,其中 16 例急变为急性髓细胞白血病(AML),13 例急变为非 AML(ALL 9 例,HAL 4 例)。非 AML 中 2 例加速期查见髓系原始细胞和前体淋巴细胞同时存在,余 9 例为髓系原始细胞或伴淋巴细胞标志。**结论** 流式细胞术对 CML-BP 的方向及鉴别诊断有一定的提示作用。

[关键词] 白血病,髓系,慢性,BCR-ABL 阳性;白血病,髓系,加速期;细胞形态学;免疫表型分型

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Correlation research of morphology and immunological typing in acceleration phase
and blastic phase of chronic myelogenous leukemia

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[Abstract] **Objective** To explore the characteristics of morphology and immunology in acceleration phase and blastic phase of chronic myelogenous leukemia(CML). **Methods** Seventy-three cases of CML-BP bone marrow specimens were respectively conducted the morphology and related cell chemical dyeing observation for determining the FAB type. Flow cytometry was used to detect series immunological related antigens. **Results** Among 73 cases of FAB typing, there were 44 cases of CML-AML, 21 cases of ALL and 8 cases. 21 cases CML-ALL patients In the immunophenotyping by flow cytometry, among 21 cases of CML-ALL, there were 19 cases of B-ALL, 2 cases of T-ALL, moreover 12 cases contained myeloid marker. Among 8 cases CML-HAL, the immunophenotypes were 6 cases of B+My and 2 cases of T+My. Among 44 cases CML-AML, 15 cases contained T cell marker, and 2 cases contained B cell marker, other cases had no cross-lineage expression. Among 73 cases of CML-BP, 29 cases conducted the flow cytometry detection in the acceleration phase, in which 16 cases urgently changed to AML, and 13 cases to non-AML(9 cases of ALL and 4 cases of HAL). Among non-AML cases, 2 cases had the simultaneous existence of myeloid primitive cells and precursor lymphocyte in the acceleration phase and other 9 cases were myeloid primitive cell or accompanied by lymphocyte marker. **Conclusion** Flow cytometry has a certain implication role for the direction and differentiation diagnosis of CML-BP.

[Key words] leukemia, myelogenous, chronic, BCR-ABL positive; leukemia, myeloid, accelerated phase; cellular morphology; immunophenotyping

慢性粒细胞白血病(chronic myelogenous leukemia, CML)是一种起源于骨髓多能干细胞的克隆性增殖性肿瘤,主要累及粒细胞系。其自然病程由慢性期(chronic phase, CP)进展为加速期(accelerated phase, AP),最后发展为急变期(blast phase, BP)。CML 的 CP 预后相对良好,而 BP 预后极差,一旦急变,患者大多数在 3~5 个月内死亡。CML 急变的发生机制及急变后免疫表型较复杂。本研究主要对 73 例 CML 急变后的形态学和免疫学分型进行相关分析。

1 资料与方法

1.1 一般资料 选择 2009 年 1 月至 2015 年 12 月本院血液科诊断为慢性粒细胞白血病急变期(CML-BP)的 73 例患者,其中 3 例 CML 向急性淋巴细胞白血病(ALL)转变(CML-

ALL)患者病程仅 1 周至半个月。男 53 例,女 20 例,年龄 15~75 岁,平均(44.0±9.5)岁。诊断均依据张之南主编的《血液学诊断标准》^[1],并根据患者的临床表现、体征及相关实验室检查数据,如血象、骨髓象、免疫表型及细胞遗传学等检查,确诊为 CML-BP。

1.2 方法

1.2.1 主要仪器与试剂 流式细胞仪(FACS Calibur、FACS-canto II、流式抗体(美国 BD 公司),BX51 荧光显微镜(日本奥林巴斯公司),瑞氏染液(自制)。

1.2.2 骨髓细胞形态学及细胞化学染色 取患者骨髓液 0.1~0.3 mL 涂片 7 张,并附 2 张外周血涂片送检。取 2 张骨髓片和 1 张外周血片行瑞氏染色,显微镜(1 000 倍)下分类计

数200个骨髓细胞和100个外周血细胞,参照2008版世界卫生组织分型标准^[2]。并做过氧化物酶(peroxidase,POX)、酯酶双染[氯醋酸萘酚酯酶(chloro-naphthol acetate esterase,CAE)、丁酸萘酚酯酶(naphthol butyrate esterase,NBE)]、过碘酸雪夫反应(即糖原染色)等细胞化学染色。

1.2.3 流式细胞仪检测 取化疗前患者的乙二胺四乙酸(EDTA)抗凝骨髓液2 mL,全血标记,检测10 000~30 000个细胞,所有标本以SSC/CD45percp 设门法识别异常细胞群,做多标记检测。所用单抗包括:干/祖细胞为CD34、CD117、HLA-DR、CD38、CD123;髓系为CD13、CD33、CD11c、CD11b、CD36、CD16、CD14、CD15、CD64;T系为CD10、CD3、CD5、CD7、CD2、CD4、CD8、CD99、TdT;B系为CD10、CD19、CD22、CD20、CD79a。按欧洲白血病免疫分型协作组(European Group for the Immunological Characterization of Leukemia,EGIL)积分系统^[3]划分T、B、髓系。

2 结 果

2.1 细胞形态学分型结果 按FAB分型标准,联合骨髓和外周血涂片镜检结果显示:73例患者骨髓增生活跃至极度活跃;21例患者细胞形态学诊断为ALL,但均未进行FAB分型。44例细胞形态学诊断为急性髓细胞白血病(acute myeloid leukemia,AML),其中AML-M1 3例;AML-M2 17例;AML-M4 6例;AML-M5 10例;AML-M6 8例。8例细胞形态学诊断为急性白血病(acute leukemia,AL),未进行分型。

2.2 FAB分型结果与免疫分型结果的关系 FAB分型结果

与免疫分型结果之间的关系,见表1。

表 1 FAB 分型结果与免疫分型结果的关系(n)			
项目	ALL(n=21)	AL(n=8)	AML(n=44)
B-ALL	9	0	0
My+B-ALL	10	0	0
My+T-ALL	2	0	0
HAL(B+My)	0	6	0
HAL(T+My)	0	2	0
T+AML	0	0	15
B+AML	0	0	2
AML	0	0	27

2.3 CML加速期与急变期的原始细胞免疫表型的比较 73例CML-BP期患者仅有29例患者观察到疾病加速期,行骨髓穿刺涂片检查及流式细胞分析。骨髓检查结果为骨髓增生明显活跃,粒细胞系异常增生,原始细胞占6.8%~17.4%,并伴有嗜碱性显著增多;流式检查结果:所有病例原始细胞表达髓系标志,比例为8.0%~16.3%,主要表达CD34、CD117、CD33、CD13、CD38、CD123、CD7,少部分病例MPO弱阳性或阴性。29例CML-BP患者加速期原始细胞类型与急变期免疫分型的关系见表2。

表 2 29 例 CML-BP 患者加速期原始细胞类型与急变期免疫分型的比较(n)				
项目	n	加速期原始细胞类型		
		髓系原始细胞伴 T 细胞标志(n=7)	髓系原始细胞和前体 B 淋巴细胞共存(n=2)	髓系原始细胞无 T、B 标志(n=20)
B-ALL	4	0	1	3
My+B-ALL	5	0	1	4
HAL(B+My)	4	2	0	2
T+AML	7	5	0	2
AML	9	0	0	9

3 讨 论

CML是一类源于造血干细胞的恶性克隆性增殖性疾病,且伴t(9;22)(q34;q11)染色体(Ph染色体)异常^[4]。CML大约占成人白血病的30%,其临床发病率仅次于AML和ALL^[5-6],临床表现为外周血白细胞升高,脾脏变大,中位生存期一般为3~4年^[7-8],病程进展缓慢,死亡患者大多以慢性转为急性病变而死亡。CML急变期的原始细胞可以向多系转化,其细胞类型各种各样,最常见为CML向AML转变(CML-AML),占50%~60%;其次为CML-ALL,占20%~30%^[9-11]。此外还可见到的急变细胞类型有单核细胞、巨核细胞、粒-单细胞、红细胞;少见早幼粒细胞、嗜碱或嗜酸性粒细胞等^[12-14]。

从本组病例应用流式细胞术行免疫表型分析结果中发现,CML细胞急性变主要突变为前体B细胞和前体髓系细胞,病理表现为B-ALL和AML。然而有部分CML急变方向不明,非B非髓,流式检测结果表现出两类杂合细胞(髓系原始细胞

和前体淋巴细胞),类似于急性混合细胞白血病(HAL)改变^[15-17]。B-ALL变患者常伴有髓系标志,而AML变患者却常伴有T细胞标志(约1/3表达CD7),这可能与CML为干细胞疾病,肿瘤细胞突变较早有关系^[18-20]。而类似HAL这部分病例的髓系原始细胞可能是CML转化过程中残存下来的,治疗后微小残留检测往往会出现仅存在前体淋巴细胞现象。

本研究有3例CML-ALL患者的病程仅1周到半月,其是否从CML急变而来需要与Ph染色体阳性的ALL(Ph⁺ALL)相鉴别。两者除了BCR/ABL基因阳性以外,CML-ALL一般有CML病史、脾脏肿大、骨髓涂片常残留有病态的髓系后期细胞及嗜酸嗜碱粒细胞^[21-23]。在这3例病例流式散点图中发现除了异常前体淋巴(主要细胞群)外,还存在一群CD34⁺CD117⁺的异常髓系原始(<5%),且均有不同程度的脾脏肿大,治疗缓解后FISH及PCR仍可以检测到高比例的BCR/ABL融合基因及p210蛋白,因此考虑其从CML急变而来。故临床上初诊Ph⁺ALL的病例有可能是CML急变所致,这时

应注意病史、体征,观察有无脾脏肿大,并注意骨髓细胞形态学的改变和流式原始细胞的异常,并在治疗期间检测 BCR/ABL 融合基因下降与原始细胞比例下降是否吻合。

CML 患者加速期在细胞形态学主要呈现出髓系原始细胞及嗜碱性粒细胞增多,骨髓中异常前体淋巴细胞进行分类很困难。因此将 CML 加速期行流式细胞术检查的患者结果(原始细胞类型)与其急变期免疫分型结果比较,发现大多数病例 CML 加速期原始细胞类型均为髓系原始细胞,说明在加速期时肿瘤细胞可能还没有出现新的突变,病变的主体细胞群仍然是髓系各阶段细胞,酪氨酸激酶抑制剂(TKI)治疗可能依然有效,甚至部分病例治疗后又可能回到慢性期^[24-25]。

虽然 CML 加速期以髓系原始细胞为主,但是其急变期的免疫分型结果不一,表明加速期原始细胞类型不能判定其急变方向^[26-28]。然而本组数据中有 2 例病例加速期同时查见了髓系原始细胞和前体 B 淋巴细胞共存现象,且最终转变为 ALL,这说明加速期前体 B 淋巴细胞的出现对 B-ALL 转变有一定的提示作用。CML 加速期出现前体 B 淋巴细胞这种现象可能提示新的克隆突变已经出现了,而这个时候是否可以采取治疗措施仍还值得探讨。

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