

免疫表型分析及分子遗传学在急性早幼粒细胞白血病诊断中的应用

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【摘要】 目的 研究免疫表型分析及分子遗传学在急性早幼粒细胞白血病(APL)诊断中的应用价值。方法 对2012年5月-2017年12月门诊或住院的798例APL患者的流式细胞术(FCM)免疫分型、染色体核型及染色体荧光原位杂交(FISH)进行回顾性分析,并深入研究FCM免疫表型及分子遗传学在APL诊断中的应用价值。结果 FCM诊断APL敏感性为91.9%,特异性为98.7%。APL具有独特免疫表型特点:典型APL的表型为侧向(SSC)偏大,CD34和HLA-DR表达缺失,表达或强表达CD33,均一表达CD13、CD9、CD123,可伴有CD56、CD7、CD2的表达。约10%的患者为非典型APL表型,一般伴有CD34和(或)HLA-DR表达,SSC减小,经常伴有CD2表达,而FCM免疫分型很难明确诊断为APL,需要依赖遗传学或者分子生物学检查结果。约1/3的患者除存在t(15;17)(q22;q21)外,还存在额外染色体异常;伴有t(15;17)的复杂核型、变异易位或者t(11;17)、t(5;17)等变异型的APL,FCM表型与单纯t(15;17)APL差异无统计学意义($P>0.05$)。结论 FCM能够快速诊断APL,伴有额外染色体异常患者和单纯t(15;17)患者FCM免疫表型没有明显差异。遗传学是诊断APL的金标准,免疫分型中约10%的患者依赖于分子遗传学来确诊。

【关键词】 流式细胞术; 染色体核型; 荧光原位杂交; 白血病,早幼粒细胞,急性

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Application of immunophenotypic analysis and molecular genetics in the diagnosis of acute promyelocytic leukemia

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【Abstract】 Objective To investigate the application values of immunophenotypic analysis and molecular genetics in the diagnosis of acute promyelocytic leukemia (APL). **Methods** The retrospective analyses of flow cytometric (FCM) immunophenotypic analysis, chromosome karyotype and chromosome fluorescence in situ hybridization (FISH) of 798 outpatient or hospitalization APL patients referred to our hospital between May 2012 and December 2017 were performed to further study the application values of FCM and molecular genetics in the diagnosis of APL. **Results** The sensitivity and specificity of FCM were 91.9% and 98.7% respectively. The typical characteristic immunophenotype for APL was as follows: a high SSC, absence of expression of cluster differentiation (CD) CD34 and HLA-DR, and expression or stronger expression of CD33, consistent expression of CD13, CD9, CD123, expression of CD56, CD7, CD2 (sometimes). The rest 10% of the cases harbored atypical APL phenotypes, generally accompanied by CD34 and/or HLA-DR expression, decreased SSC and often accompanied by CD2 expression, it was difficult to definitively diagnose APL by this FCM phenotype, and their diagnoses depended on the results of genetics or molecular biology tests. Compared with normal individuals, complex karyotypes APL with t(15;17) translocation, other variant translocations and variant t(11;17), t(5;17) had no significant differences in terms of their FCM phenotypes. **Conclusions** FCM could rapidly and effectively diagnose APL. Despite the fact that complex karyotypes with various additional chromosomal abnormalities were detected in approximately one third of APL cases in addition to

the pathognomonic t(15;17)(q22;q21), they had no observable impact on the overall immunophenotype. Molecular and genetic criteria were the golden criteria for the diagnosis of APL. About 10% of immunophenotyping cases relied on molecular genetics for diagnosis.

【Key words】 Flow cytometry; Chromosome karyotype; Fluorescence in situ hybridization; Leukemia, promyelocytic, acute

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急性早幼粒细胞白血病(APL)是一种高侵袭性的急性髓系白血病(AML),疾病发展迅速,早期病死率高,早期诊断及治疗能够大大改善APL的预后。白血病的诊断依赖于细胞形态学、免疫学、遗传学和分子生物学(MICM)^[1]。APL常伴有t(15;17)(q22;q21),形成特征性融合基因PML-RAR α ,可以通过逆转录聚合酶链反应(RT-PCR)或荧光原位杂交(FISH)检测,遗传学是APL诊断的金标准。而以流式细胞术(FCM)为基础的免疫分型技术与细胞形态学相比更加客观、准确,与遗传学相比又具有快速的优势,因此在APL诊断中具有重要价值。与其他AML相比,APL具有独特的免疫表型特点,如SSC偏大,不表达CD34和HLA-DR等^[2-4]。而部分APL患者伴有CD34及HLA-DR的表达,FCM不能对其明确分型,需借助分子生物学及遗传学结果来确诊。同时多颗粒AML-M_{2a}及AML-M₅也偶尔被误诊为APL表型。现回顾性研究我院798例APL患者的表型特点,结合染色体核型和(或)FISH结果,研究其在APL中的应用价值。

病例与方法

一、研究对象

2012年5月至2017年12月于我院门诊或住院的798例初诊或者复发经PML-RAR α 融合基因或FISH及染色体核型检测确诊的APL患者纳入本研究,其中男450例,女348例,中位年龄43(2~87)岁,APL的临床诊断均符合文献[5]标准。对其免疫分型结果、融合基因、染色体核型和FISH结果进行了回顾性分析。为分析FCM诊断APL的特异性,对同期FCM免疫分型诊断为APL表型的743例连续患者进行分析。

二、免疫表型分析

1. 标本和材料:检测标本为初诊或者复发患者肝素抗凝的骨髓,所用单克隆抗体均购自美国BD公司、Beckman Coulter公司或丹麦DAKO公司,破膜剂产品及红细胞裂解液均购自美国BD公司。采用CD45/SSC设门法,标记的抗体包括CD34、

CD117、HLA-DR、CD38、CD33、CD15、CD7、CD64、CD13、CD11b、CD123、CD56、CD36、CD10、CD19、CD207、CD4、CD14、CD2、CD5、cCD79a、cCD3、mCD3、cMPO、TdT、CD9。采用美国Beckman Coulter公司FC-500和BD公司FACSCanto II流式细胞仪检测,分别采用Beckman Coulter CXP Software和BD FACSDiva Software进行分析。

2. FCM检测:采用单克隆抗体四色或八色直接荧光标记法,每管获取10 000个细胞,首先用FSC/SSC设门去除细胞碎片,随后用CD45/SSC设门,抗原表达将细胞分为红细胞、单核细胞、粒细胞、淋巴细胞、原始细胞或白血病细胞群,原始细胞或白血病细胞群命名为P3,分析P3门内的相关抗原表达情况。

3. 结果判定标准:CD45/SSC设门后的白血病细胞群膜表面抗原>20%判断为阳性^[6],胞内抗原>10%判断为阳性^[7]。以标本中成熟淋巴细胞和粒细胞群作为抗原表达阴、阳性的内对照,以正常髓系原始细胞抗原表达强度来判断白血病细胞阳性表达的强弱,表达强度低于正常髓系原始细胞的定义为弱阳性,反之定义为强阳性;淋系交叉抗原表达的强弱参照正常成熟淋巴细胞表达情况,阳性细胞比例>75%定义为阳性表达,>25%但<75%定义为部分阳性。

三、染色体核型分析

取患者骨髓(肝素抗凝),不加任何刺激剂经24 h短期培养(RPMI 1640培养基,20%小牛血清,美国Gibco公司产品)后,于收获前1 h加秋水仙酰胺(美国Sigma公司产品),终浓度为0.04 $\mu\text{g/ml}$ 终止分裂。常规收获后火焰法制片,R显带。通过全自动扫描分析Ikaros系统(德国Metasystems公司产品)进行分析,核型描述依据《人类细胞遗传学国际命名体制(ISCN2009)》。

四、FISH

收获细胞储存于-20 $^{\circ}\text{C}$,制片前新鲜固定液(甲醇:冰醋酸=3:1)固定10 min,在37 $^{\circ}\text{C}$ 、50%饱和度下滴片,系列乙醇(70%、80%、100%)梯度脱水,

气干过夜。选择双色双融合标记PML-RAR α 探针或双色分离RAR α 探针(美国Vysis公司产品),按说明书提供的方法进行变性及杂交。通过Isis分析系统(德国Metasystems公司产品)进行分析,每个标本分析500个间期细胞。

五、RQ-PCR

采用枸橼酸钠抗凝的骨髓标本经人淋巴细胞分离液分离获得单个核细胞。TRIzol试剂(上海生工生物工程技术服务有限公司)处理裂解细胞后,提取RNA;RNA逆转录为cDNA作为PCR模板,应用上海源奇生物医药科技有限公司的PML-RAR α 融合基因定量检测试剂盒,使用ABI 7500实时定量PCR仪进行条件扩增,RQ-PCR体系及条件参考文献[8]。SDS软件利用标本的CT根据标准曲线分别计算出目的基因及其内参基因ABL的拷贝数。目的基因mRNA水平(%)=(目的基因拷贝数/ABL拷贝数) \times 100%

六、统计学处理

采用SPSS17.0软件进行统计学分析,阳性率比较采用卡方检验, $P < 0.05$ 为差异有统计学意义。

结 果

1. 免疫分型结果:伴t(15;17)(q22;q21)和(或)PML-RAR α 或RAR α 阳性的798例APL患者的免疫表型结果见表1。CD33、MPO、CD123、CD9、CD13、CD117、CD64的阳性率分别为100%、100%、100%、99.4%、98.1%、97.5%和86.6%;其中强表达CD33和MPO的比例分别为83.1%和3.6%;CD34和HLA-DR多为阴性(87.6%和90.6%);CD38阴性和弱表达分别占29.9%和25.2%。798例确诊病例中,FCM免疫分型诊断为APL的病例为733例,敏感性为91.9%。大部分SSC偏大,不表达CD34、HLA-DR和CD15,强表达CD33,表达及强表达MPO,弱表达CD64等(图1)。其中65例(8.1%)未报APL表型患者中,有53例FCM免疫分型诊断为AML,未进行进一步亚型分类;余12例,其中6例为AML-M₅,5例为AML-M₂,1例为AML-M₁。经FCM未能诊断为APL表型的APL患者中,有50例(76.9%)伴有CD34和(或)HLA-DR的表达或部分表达,32例(49.2%)SSC不大,27例(41.5%)伴有CD2的表达,23例(35.4%)伴有CD56的表达。伴有CD34和HLA-DR表达的常SSC不偏大,MPO表达减弱,形态学提示为细颗粒型(图2A)。可见少量

病例SSC偏大,CD34、HLA-DR、CD117和CD15均不表达,弱表达CD64,表达CD33和MPO(图2B)。对FCM诊断APL组和FCM未能诊断APL表型组的CD34、HLA-DR、CD2、CD56、CD7和SSC表达进行了比较,两组差异均有统计学意义($P < 0.01$)(表2)。

表1 798例急性早幼粒细胞白血病患者抗原表达情况 [例(%)]

抗原	例数	强阳性	阳性	弱阳性或部分阳性	阴性
CD33	798	663(83.1)	13(16.9)	0(0)	0(0)
CD34	798	0(0)	18(2.2)	81(10.2)	699(87.6)
HLA-DR	798	0(0)	34(4.3)	41(5.1)	723(90.6)
CD117	798	0(0)	614(76.9)	164(20.6)	20(2.5)
CD123	754	2(0.3)	672(89.1)	80(10.6)	0(0)
CD9	698	4(0.5)	652(81.7)	42(5.3)	4(0.5)
CD13	798	8(1.0)	672(97.1)	42(1.9)	0(0)
CD38	798	0(0)	358(44.9)	201(25.2)	239(29.9)
CD15	798	0(0)	2(0.2)	46(5.8)	750(94.0)
MPO	660	24(3.6)	623(94.4)	13(2.0)	0(0)
CD64	798	0(0)	561(70.3)	228(28.6)	9(1.1)
CD2	798	0(0)	2(0.2)	59(7.4)	737(92.4)
CD7	798	0(0)	0(0)	17(2.1)	781(97.9)
CD56	798	0(0)	23(2.9)	50(6.3)	725(90.8)

在FCM免疫分型诊断为APL表型的743例患者中,727例RQ-PCR和(或)FISH检测PML-RAR α 为阳性;3例染色体核型为t(11;17),融合基因PLZF-RAR α 阳性;2例为t(5;17),融合基因NPM-RAR α 阳性;1例为t(7;17),FISH检测RAR α 为阳性;1例为t(8;21),RUNX1-RUNX1T1融合基因阳性;9例染色体核型正常且FISH(RAR α)及RQ-PCR检测均阴性,这9例患者中6例临床诊断为AML-M₅,3例诊断为多颗粒AML-M_{2a}(此10例患者未诊断APL,没有进行进一步研究),FCM免疫分型诊断APL的特异性为98.7%(733/743)。

2. 分子遗传学结果:618例患者进行了FISH检测,608例阳性,阳性率98.4%;790例患者(其中8例初诊只进行了FISH检测)有染色体核型分析结果,其中647例存在t(15;17)(q22;q21),3例t(11;17),2例t(5;17),1例t(7;17),检测阳性率82.7%。143例染色体核型未检测到t(15;17)的患者中FISH或RQ-PCR检测PML-RAR α 融合基因均阳性。201例(31.1%)除存在t(15;17)(q22;q21)外,还存在额

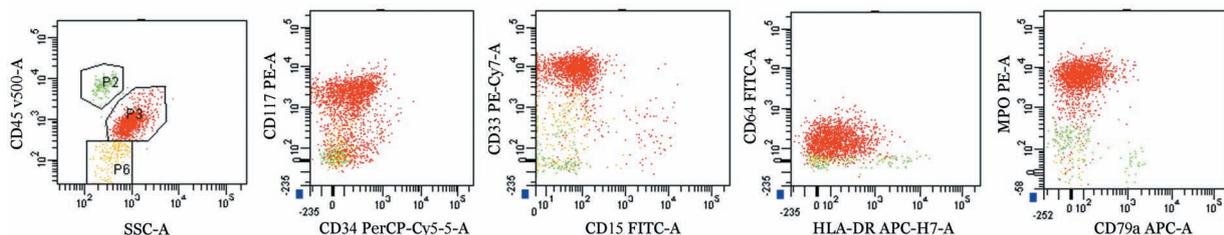
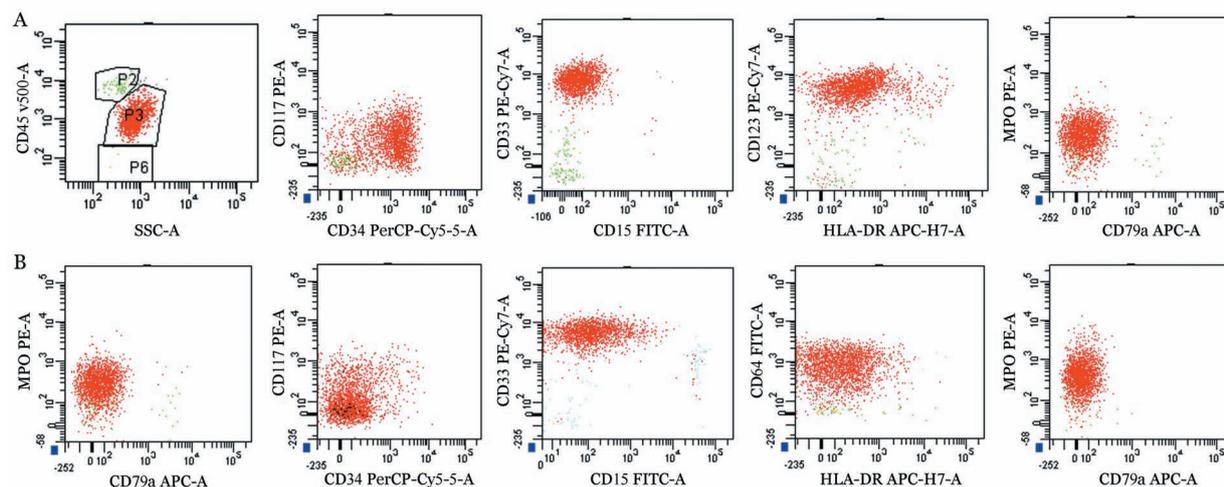


图1 经典急性早幼粒细胞白血病患者免疫表型:强表达CD33、MPO、CD117,弱表达CD64,不表达CD15、CD34和HLA-DR,SSC偏大



A:强表达CD33,表达CD117、CD123、CD34和HLA-DR,弱表达MPO,不表达CD15,SSC不大;B:强表达CD33,表达MPO,弱表达CD64,不表达CD34、HLA-DR、CD117和CD15,SSC偏大

图2 非典型急性早幼粒细胞白血病患者免疫表型

表2 FCM未能诊断APL组与FCM诊断APL表型组抗体表达比较[例(%)]

抗原表达	FCM未能诊断APL组 (65例)	FCM诊断APL表型组 (733例)	χ^2 值	P值
CD2(+)	27 (41.5)	34 (5.0)	0.000	0.000
CD56(+)	23 (35.4)	50 (7.3)	0.000	0.000
CD7(+)	10 (15.5)	7 (3.8)	0.000	0.000
CD117(±)	12 (18.5)	172 (25.2)	0.639	0.494
CD34和(或)HLA-DR(dim±)	50 (76.9)	66 (12.6)	0.000	0.000
SSC偏大	33 (50.8)	717 (97.8)	0.000	0.000

注:FCM:流式细胞术;APL:急性早幼粒细胞白血病

外染色体异常,包括染色体数目异常和(或)结构异常。其中t(15;17)(q22;q21),+8者58例,占伴有额外染色体异常组的28.9%;der(15)t(15;17)(q22;q21),ider(17)(q10)t(15;17)者25例,占12.4%;变异易位18例(9.0%),包括t(1;15;17)、t(3;15;17)、t(7;15;17)、t(8;15;17)、t(11;15;17)、t(15;15;17)、t(17;15;17)、t(15;17;20)等。除t(15;17)外存在3条或者3条以上异常(包括数目和结构异常)的复杂核型37例,占18.4%。201例伴有额外染色体异常组中,FCM免疫表型分析诊断为APL表型者182例

(90.5%),9例为AML表型;而单纯t(15;17)(q22;q21)组的446例患者中,FCM免疫表型分析为APL表型的为406例(91.0%),两组差异无统计学意义(P=0.775),所以FCM免疫分型在单纯t(15;17)组和伴有额外染色体异常组均具有较高的敏感性,可以筛查少见的复杂易位。

讨论

本研究中,FCM免疫分型诊断APL的敏感率为91.9%,约8.1%患者不能通过FCM免疫分型确诊

为APL表型,其中1.5%(12/798)的患者因CD34和(或)HLA-DR表达较强,SSC不大等而误诊断为AML-M₂、AML-M₁及AML-M₅表型。如果CD34和(或)HLA-DR表达较强,SSC不大时,常伴有CD2和CD56的表达,此类患者依赖FCM免疫表型分析很难区分APL表型与AML-M₅。因为AML-M₅免疫表型一般为CD33、HLA-DR及CD9的表达,此时需借助分子生物学及遗传学来确诊。FCM未能诊断APL表型组中有80.0%(56/65)伴有CD34和(或)HLA-DR表达,38.5%伴有CD2表达,35.4%伴有CD56表达^[9-10],明显高于FCM诊断APL组。这类伴有CD34和HLA-DR表达患者分化比较差,文献报道伴有CD34表达的APL常伴有CD2的表达^[11-12],伴有CD56表达的患者预后也差。

FCM细胞免疫表型分析在血液肿瘤诊断中的价值越来越高,能够快速诊断,且具有较高的敏感性和特异性^[13-14]。本研究显示APL具有独特的表型特点:①典型APL表型SSC偏大,CD34和HLA-DR表达缺失,表达或强表达CD33,均一表达CD13、CD9、CD123,均一表达CD64,可伴有CD56、CD7、CD2的表达或部分表达,与文献报道一致^[15-16]。②10%左右患者为非典型APL表型(细颗粒型),一般伴有CD34和(或)HLA-DR表达,SSC不大,伴有CD2或CD56的表达^[11-15]。Lee等^[17]报道20%~41%的APL患者伴有CD34的表达,Paietta等^[18]报道约9%的患者伴有HLA-DR的表达。约10%的非APL的AML中伴有CD34和HLA-DR的表达缺失^[19-21]。我院约8.0%的病例不能通过FCM免疫表型分析诊断为APL,需借助于分子生物学及遗传学结果来确诊。但是FCM诊断快速,几小时内就能出结果,且免疫分型分析诊断APL敏感性为91.9%,特异性达到98.7%,为临床早期治疗提供了可靠的依据。

遗传学是APL确诊的关键,染色体核型检测因中期分裂象少或者无分裂象等原因对APL的检测敏感率为82.7%,低于FCM结果,但FISH检测敏感率高达98.4%,因此FISH检测是APL确诊必不可少的手段,是对传统核型分析的重要补充。染色体核型结果中约31%的患者除t(15;17)外,还伴有其他染色体异常,最常见的为+8、ider(17)t(15;17)、变异易位及复杂核型等^[22-24]。文献报道伴有额外染色体异常对预后有一定影响,特别是伴有ider(17)t(15;17)^[25-28]。伴额外染色体异常组[包括t(11;17)、t(5;17)及t(7;17)易位变异型的APL]的FCM免疫表型

与单纯t(5;17)组差异无统计学意义,因此可以通过FCM表型分析和染色体核型发现罕见的如t(15;17:n)、t(11;17)、t(5;17)、t(3;17)等易位的APL。

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