UNIVERSIDADE FEDERAL DE SERGIPE
CENTRO DE CIÊNCIAS BIOLÓGICAS E DA SAÚDE
DEPARTAMENTO DE FARMÁCIA

LOUISE DANTAS TRINDADE
LUCIO HENRIQUE SOUSA PINHEIRO

FENÓTIPOS ABERRANTES EM LEUCEMIA MIELOIDE AGUDA E SUA RELAÇÃO COM PROGNÓSTICO E SOBREVIDA: UMA REVISÃO SISTEMÁTICA

SÃO CRISTÓVÃO
2017

LOUISE DANTAS TRINDADE

LUCIO HENRIQUE SOUSA PINHEIRO

FENÓTIPOS ABERRANTES EM LEUCEMIA MIELOIDE AGUDA E SUA RELAÇÃO COM PROGNÓSTICO E SOBREVIDA: UMA REVISÃO SISTEMÁTICA

Trabalho de conclusão de curso, apresentado como requisito parcial à obtenção do grau de Bacharelado em Farmácia, pela Universidade Federal de Sergipe, sob a orientação da Prof.ª Dr.ª Dulce Marta Schimieguel Mascarenhas Lima

SÃO CRISTÓVÃO

2017

SUMÁRIO

1. REVISÃO DA LITERATURA 01

1.1. Neoplasias Hematológicas – Leucemias 01

1.2. Leucemia mieloide aguda (LMA) 02

1.3. Imunofenotipagem por citometria de fluxo.....04

1.4. Fenótipos Aberrantes.....06

REFERÊNCIAS 08

CAPÍTULO 1 14

Aberrant Phenotypes In Acute Myeloid Leukemia And Its Relationship With Prognosis And Survival: A Systematic Review …….15

APPENDIX ….....33

1. REVISÃO DA LITERATURA

1.1 NEOPLASIAS HEMATOLÓGICAS – LEUCEMIAS

As neoplasias hematológicas compreendem um grupo de doenças originárias das células hematopoéticas que possuem caráter bastante heterogêneo e uma grande variabilidade em relação a etiologia, incidência, prognóstico e sobrevida. As principais desordens hematológicas de caráter maligno são as leucemias, classificadas de acordo com a linhagem celular afetada (HASSAN, 2014).

As leucemias decorrem de uma série de mutações nas células pluripotentes da medula óssea durante a hematopoese comprometendo a maturação destas células que não são capazes de originar clones, e consequentemente células maduras normais. A proliferação descontrolada das células leucêmicas reduz o espaço na medula óssea, diminuindo a produção de células normais, caracterizando as leucemias como uma desordem hematológica clonal maligna. Essas mutações ocorrem em qualquer nível de maturação celular, causando anormalidades em especial,na linhagem mieloide, o que caracteriza sua heterogeneidade. Uma característica que todas as leucemias têm em comum é o fato de que todas se originaram de um progenitor celular anormal (ROSE-INMAN; KUEHL, 2014).

As leucemias podem ser classificadas em quatro principais categorias, levando em consideração a linhagem da célula progenitora que sofreu mutação e o estágio de evolução da doença: leucemia linfocítica crônica, leucemia linfoblástica aguda, leucemia mieloide crônica e leucemia mieloide aguda. Cada categoria possui seus respectivos subtipos com suas características próprias e influência no prognóstico e na sobrevida (WINTERS, 2015; ARBER, 2016).

As leucemias podem se manifestar em qualquer idade, porém cada tipo de leucemia afeta um tipo de população em especial. As leucemias mieloides agudas acometem geralmente os adultos, ao contrário das leucemias linfoblásticas agudas que são mais comuns na primeira infância e raras na fase adulta. (JULIUSSON, 2016).

Segundo o Instituto Nacional do Câncer (INCA) foi estimado para o ano de 2016, 5.540 novos casos de leucemia em homens e 4.530 novos casos em mulheres, no Brasil. De acordo com esses números, o risco estimado é de 5,63 casos novos a cada 100 mil homens e 4,38 casos novos para cada 100 mil mulheres. Dentre os tipos de câncer que afetam os indivíduos do sexo masculino, as leucemias ocupam a 9ª posição de maior frequência na região Nordeste, enquanto

para as mulheres, as leucemias são o 10°tipo de câncer mais frequente nesta região.

Pouco é conhecido sobre a etiologia das leucemias, mas vários fatores podem desencadear uma mutação genética levando ao desenvolvimento da doença. Sabe-se que a exposição à radiação ionizante e à solventes químicos como o benzeno são responsáveis por causar vários dos tipos de leucemias. Outros fatores de risco ao desenvolvimento das leucemias são os relacionados a genética e a hereditariedade. Obesidade, fumo, poluição e exposição ocupacional a pesticidas são mais alguns dos fatores de risco, porém que representam apenas 10% dos casos de leucemias (WINTERS, 2015; JULIUSSON, 2016).

As leucemias possuem aspectos clínicos não-específicos que se manifestam durante a evolução da doença, mas que estão correlacionados com o mal funcionamento da medula óssea que está comprometida devido a infiltração da medula óssea por blastos. As principais manifestações clínicas têm como base uma tríade de sintomas: anemia, trombocitopenia e leucopenia. A anemia ocorre devido a diminuição dos eritrócitos no sangue periférico, tendo como consequência os seguintes sintomas: fatiga, dispneia e dor de cabeça. A trombocitopenia é uma desordem hematológica quantitativa das plaquetas, que levam ao aparecimento de hematomas e sangramentos, em especial do nariz e gengivas. Por último, como resultado da diminuição do número de leucócitos, o paciente é mais susceptível a infecções, pois seu sistema imunológico está comprometido, aumentando o risco de infecções recorrentes (ROSE-INMAN; KUEHL, 2014; JAHEDI, 2014).

1.2 LEUCEMIA MIELOIDEAGUDA (LMA)

A leucemia mieloide aguda é uma desordem hematológica de caráter clonal que afeta as células progenitoras da linhagem mieloide por inibir a diferenciação celular, comprometendo a hematopoese normal (FERRARA, 2013). A fisiopatologia das leucemias mieloides agudas ainda não é completamente entendida, porém alterações citogenéticas estão bastante envolvidas e têm sido usadas como marcadores de prognóstico e diagnóstico para pacientes acometidos por leucemia mieloide aguda (LEY, 2013).

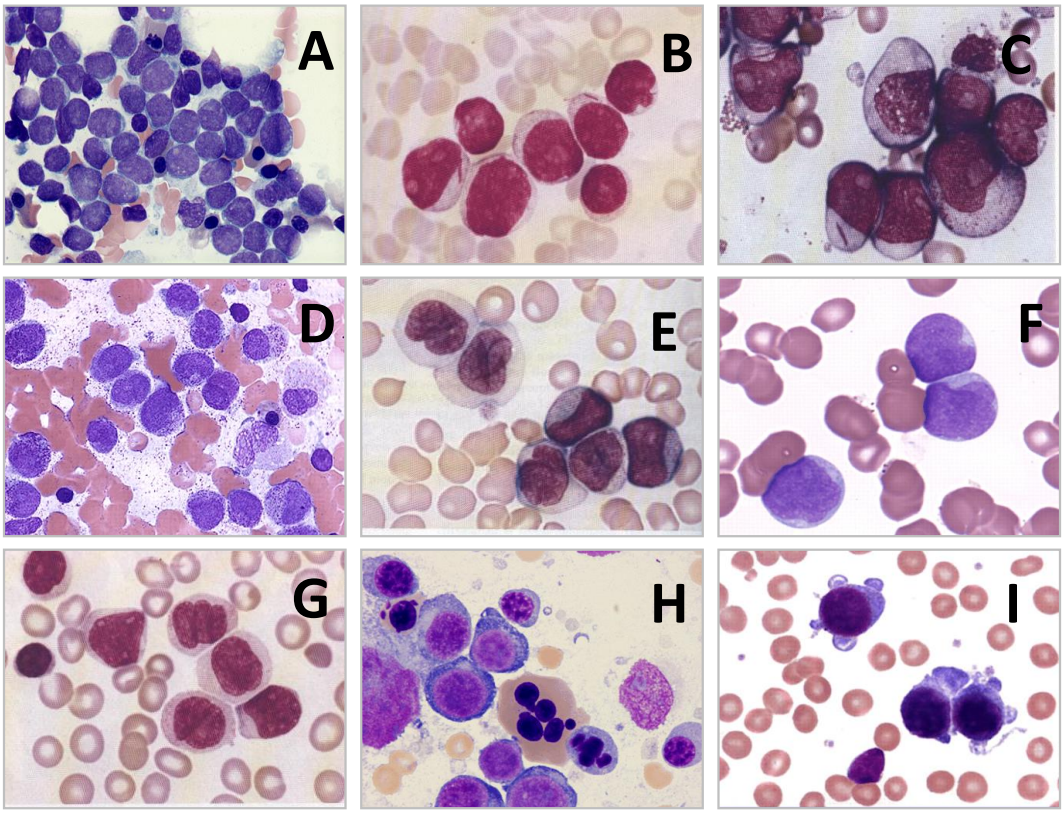

Figura1: Classificação FAB da leucemia mieloide aguda. (A) M0- Indiferenciada; (B) M1- Sem maturação; (C) M2-Com maturação; (D) M3-Promielocítica; (E) M4-Mielomonocítica; (F) M5a-Monoblástica; (G) M5b-Monocítica; (H) M6-Eritróide; (I) M7-Megacariocítica.
Fonte: Modificado de MASLAK, 2009; BART, et al., 2004

A leucemia mieloide aguda é uma doença que pode ocorrer em indivíduos de todas as idades, porém é mais comum em pacientes adultos mais idosos com idade média de 69 anos. Dentre todas as leucemias, a leucemia mieloide aguda é o segundo tipo mais comum nos Estados Unidos acometendo em média 3.6 a cada 100.000 indivíduos por ano (ORAN, 2012). Crianças diagnosticadas com LMA apresentam melhor prognóstico do que os adultos, pois a sobrevida diminui drasticamente com o aumento da idade, tornando-se a idade um fator de mau prognóstico (DESANTIS, 2014).

As taxas de sobrevida em pacientes acometidos por leucemia mieloide aguda é de três anos em 9-10% e de cinco anos em 3-8% dos pacientes com idade de 60 anos ou superior. Em mais de 50% dos pacientes mais jovens, a taxa de sobrevida é de cinco anos. As baixas taxas de sobrevida estão principalmente relacionadas a idade avançada dos pacientes, que também apresentam comorbidades que influenciam na remissão da doença (ORAN, 2012; JULIUSSON, 2009).

O tratamento para leucemia mieloide aguda tem sido o mesmo durante muitos anos, não havendo alterações significantes, dessa forma, a remissão completa da doença é obtida em 70-

80% dos pacientes com idade inferior a 60 anos. Contudo, em pacientes com idade mais avançada, as taxas de remissão são mais baixas e as taxas de recaída aumentam com os anos. Estes dados mostram que a idade é um fator de prognóstico bastante importante, tendo grande influência na sobrevida do paciente, e indicam que a resposta ao tratamento incluindo a presença de doença mínima residual também devem ser levados em consideração, tornando-se fatores de prognóstico essenciais (BOWER, 2016).

A heterogeneidade das leucemias mieloides agudas levou a criação de uma classificação que foi inicialmente estabelecida em 1976 pelo Grupo Cooperativo Franco-Americano-Britânico (FAB) que levou em consideração as características morfológicas e imunohistocitoquímicas das células. A classificação FAB tem como propósito providenciar a objetividade no diagnóstico das leucemias mieloides agudas, dividindo-as em oito subtipos (M0-M7),tendo sua última atualização sido feita em 2008. Contudo, anormalidades cromossômicas e genéticas tornaram-se importantes e imprescindíveis para o diagnóstico das LMAs, pois estas características proveem fatores prognósticos requeridos para um melhor direcionamento terapêutico (WALTER, 2013).

Em 2001, a Organização Mundial da Saúde classificou as leucemias mieloides agudas de acordo com características morfológicas, imunológicas, citogenéticas e clínicas, e teve sua última atualização em 2016. Além das diferenças das técnicas para realização do diagnóstico, a nova classificação também difere em outros aspectos, como a redução de 30% para 20% de blastos na medula óssea para o diagnóstico confirmatório de leucemia mieloide aguda (ARBER, 2016).

A leucemia mieloide aguda é uma doença bastante heterogênea que apresenta uma alta variedade de fenótipos, e além disso, possui um prognóstico bastante desfavorável. Mais de 95% dos casos de leucemia mieloide aguda pode ser facilmente distinguida de leucemia linfoblástica aguda por meio da análise de antígenos de superfície (JAHEDI, 2014; WASS, 2016).

1.3 IMUNOFENOTIPAGEM POR CITOMETRIA DE FLUXO

A imunofenotipagem se tornou uma técnica essencial para o diagnóstico de leucemias, pois por meio de marcadores celulares, essa técnica provê informações relevantes sobre a doença, sendo possível classificar o tipo de leucemia de acordo com a linhagem celular e o estágio maturativo das células (BÉNÉ, 2011).

Os marcadores biológicos, também chamados de *Cluster designation* (CD), são

expressos na membrana dos leucócitos, de acordo com os diferentes estágios de diferenciação de cada linhagem específica. Com o uso de diferentes combinações de anticorpos monoclonais, via imunofenotipagem, é possível detectar especificamente cada marcador biológico presente na superfície das células, identificando as subpopulações de leucócitos (AL-SAIMARY, 2013).

A imunofenotipagem, juntamente com outras técnicas, é utilizada para obter o diagnóstico do paciente. O uso da imunofenotipagem é essencial para a análise do caráter imunofenotípico de blastos leucêmicos, além de permitir a detecção de alterações na expressão de antígenos de superfície, sendo assim capaz de diferenciar células do tecido hematopoiético normais de células malignas, além de identificar os subtipos de leucemias (NOVOA, 2013; JAHEDI, 2014).

A citometria de fluxo é uma ferramenta tecnológica capaz de identificar componentes normais e anormais presentes nas células do sistema imune. O citômetro de fluxo tem sido bastante utilizado para o diagnóstico de leucemias e outras neoplasias hematológicas. A partir de antígenos de superfície presentes nas células do sistema imunológico é possível analisar características complexas presentes nas células em amostras de sangue periférico e outros líquidos corporais. Isto porque a imunofenotipagem por citometria de fluxo se tornou uma ferramenta essencial para a detecção e caracterização de células malignas, é possível prover dados de relevância com relação ao prognóstico e direcionamento terapêutico do paciente (FINAK, 2016; HAMAD, 2016).

A imunofenotipagem é capaz de coletar características tanto intrínsecas como extrínsecas das células. As características intrínsecas são relativas ao tamanho e/ou complexidade celular, enquanto as características extrínsecas dizem respeito as propriedades funcionais, conteúdo dos ácidos nucléicos e constituição antigênica (HAMAD, 2016).

A técnica da imunofenotipagem se baseia em uma suspensão celular que permite a passagem rápida das células uma a uma através de lasers, que consistem em um ou mais feixes de luz monocromática. No momento em que as células passam pelo feixe de luz, elas são capazes de desviar a incidência da luz (HAMAD, 2016). A porção de luz desviada que é coletada na mesma direção que a luz incide é conhecida como dispersão frontal da luz ou *Forward Scatter* (FSC). Esta fração da luz faz uma alusão a estimativa do tamanho da célula, desta forma, quanto maior a fração de luz desviada maior o tamanho da célula. A porção de luz que é desviada para a lateral é conhecida como dispersão lateral da luz ou *SideScatter* (SSC). Esta análise lateral da dispersão da luz está relacionada com o grau de complexidade celular que pode ser descrito por características morfológicas como rugosidade da superfície celular, membrana celular, núcleo, granulosidade e número de organelas (LÉONARD, 2016).

Em pesquisas, cada estudo tende a usar sua própria combinação de marcadores e fluorocromos mesmo quando são usados os mesmos tipos de células. Nesses estudos, o manuseio das amostras, o tipo e a configuração do instrumento, estratégias de análise, além do modo em que os dados são expressos, podem todos variar. Infelizmente, estas diferenças podem afetar os resultados e a maneira como os resultados são interpretados (FINAK, 2016).

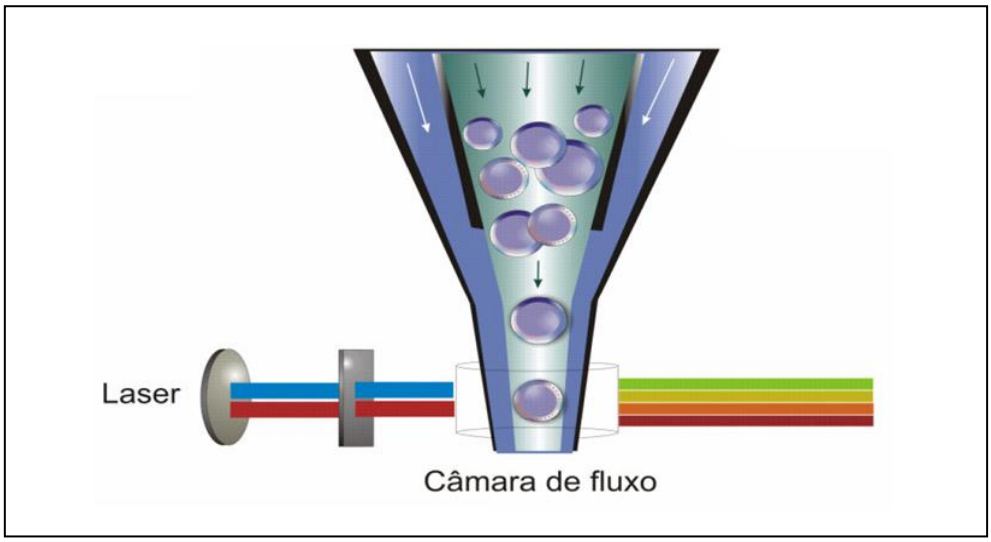

Figura 2: Esquema interno de um citômetro de fluxo indicando a câmara de fluxo contínuo e a fonte de laser. *Fonte: adaptado de* http://flow.csc.mrc.ac.uk/?page_id=852

1.4 FENÓTIPOS ABERRANTES

As células precursoras das linhagens mieloide e linfoide expressam antígenos (CD) específicos para suas populações celulares, e os diferentes tipos de leucemias são classificados de acordo com a expressão desses antígenos. Contudo, muitas células leucêmicas apresentam imunofenótipos que não são próprios do tipo de diferenciação celular normale, portanto, apresentam uma expressão anormal de marcadores imunofenotípicos chamados de fenótipos aberrantes ou expressão aberrante de marcadores (MAZHER, 2013).

Os fenótipos aberrantes ocorrem quando marcadores de linhagem mieloide encontram-se associados a marcadores da linhagem linfoide em mieloblastos ou quando marcadores da linhagem mieloide são expressos em linfoblastos. A incidência dos fenótipos aberrantes é notada tanto em leucemias mieloides agudas quanto em leucemias linfoides agudas, com frequência alta, atingindo até 88%. Dos casos de leucemias mieloides agudas, mais de 48% demostraram expressão de fenótipos aberrantes de pelo menos um único antígeno associado com células da linhagem linfoide (JAHEDI, 2014).

Atualmente, os fenótipos aberrantes presentes em leucemias mieloides agudas são

classificados dentro de diferentes tipos: co-expressão de antígenos associados a linhagem linfoide; expressão assincrônica de antígenos, que consiste na co-expressão de antígenos de linhagens mais jovens com antígenos de linhagens maduras; ausência de expressão de antígenos da linhagem mieloide, e por fim, a superexpressão de antígenos (JHA, 2013).

A presença de fenótipo aberrante é um indicador de mau prognóstico em casos de leucemia mieloide aguda. Para ser considerado um caso de fenótipo aberrante é necessário haver uma expressão acima de 20% de marcadores imunofenotípicos de caráter aberrante. A expressão dos fenótipos aberrantes em homens e mulheres acontece numa razão de 1.5/1.0 (JAHEDI, 2014).

A correlação de fenótipos aberrantes com fatores prognósticos é outro achado que facilitaria o diagnóstico mais preciso do subtipo de leucemia mieloide aguda, além de ajudar no direcionamento farmacoterapêutico mais adequado ao paciente. A expressão de antígenos aberrantes tem demonstrado uma frequência variável, sendo os mais comuns em leucemia mieloide aguda os CD7, CD9, CD19 e CD56. Nos casos de leucemia mieloide aguda, a expressão de determinados marcadores tem sido correlacionada com os subtipos da classificação FAB associada a anormalidades genéticas recorrentes. Um desses exemplos é aco-expressão do CD2 em casos de LMA M4E com inv(16) ou t(16;16), e a alta expressão do CD56 em LMA-M5 com t(9;11) (ABDULATEEF, 2014). Outro exemplo é a expressão aberrante dos marcadores de linhagem linfoide CD19 e CD56 que são expressos na LMA-M2 com t(8;21), sendo que a expressão do CD56 indica um pior curso clínico da doença com uma taxa de resposta completa ao tratamento mais baixa e consequentemente, uma baixa sobrevida global (YANG, 2007). O CD7, um marcador de linhagem linfoide, está relacionado com um mau prognóstico da doença, além de baixas taxas de remissão, uma sobrevida livre de doença mais curta e maior agressividade da doença (ELYAMANY, 2013).

Ainda são necessários muitos estudos para entender e descobrir as características clínicas e a significância da co-expressão de dois ou mais marcadores de linhagens distintas em células leucêmicas (ABDULATEEF, 2014). Contudo, alguns marcadores merecem maior atenção, pois sua expressão está relacionada com a sobrevida (OSSENKOPPELE, 2011).

A presença de marcadores imunofenotípicos aberrantes tem sido relatada concomitantemente com anormalidades genéticas. Contudo, devido a inconsistências e achados contraditórios junto com a forte associação desses marcadores aberrantes com a citogenética, esses marcadores ainda não foram incluídos em padrões de fatores de prognóstico (BASSO, 2007). Não obstante, essas associações servem como guia para pesquisas sobre alterações citogenéticas em leucemias mieloides agudas com aberrações imunofenotípicas conhecidas.

Desta forma, podendo oferecer informações a respeito da contribuição funcional desses fenótipos no prognóstico (OSSENKOPPELE, 2011).

Com base na co-expressão de diferentes tipos de linhagens celulares, baseada em antígenos, a citometria de fluxo multiparamétrica de alta resolução tem sido utilizada para identificar características de linhagens em leucemias. Por meio do estudo da expressão positiva ou negativa, alta ou baixa, e translinhagem de alguns antígenos em células malignas, tem-se conseguido informações a respeito do imunofenótipo de diferentes subtipos de leucemias. A ampla aplicação de testes imunofenotípicos em casos de leucemia mieloide aguda tem-se, portanto, levado a um melhor entendimento da relação entre os imunofenótipos celulares e a morfologia das células e sua citogenética (ZHENG, 2008).

REFERÊNCIAS

AALBERS, A. M. et. al. Telomere Length and Telomerase Complex Mutations in Pediatric Acute Myeloid Leukemia. *Leukemia*, v. 27, n. 8, p. 1786–1789, 2013.

ABDULATEEF, N. A. B. et. al. Clinical Significance of Co-Expression of Aberrant Antigens in Acute Leukemia: A Retrospective Cohort Study in Makah Al Mukaramah. *Asian Pacific Journal Cancer Prevention*, v. 15, n. 1, p. 221-227, 2014.

AL-SAIMARY, I. E. et. al. Immunophenotyping Related to Cluster of Differentiation (CD) Markers in Patients with Atopic Dermatitis. *Medical Journal of Islamic World Academy of Sciences*, v. 21, n. 2, p. 57-60, 2013.

ARBER, D. A. et al. The 2016 Revision to the World Health Organization (WHO) Classification of Myeloid Neoplasms and Acute Leukemia. *Blood*, v. 127, n. 20, p. 2391-2405, 2016.

BAIN, B. J.; ESTCOURT, L. FAB Classification of Leukemia. *Brenner's Encyclopedia of Genetics*, v. 3, p. 5-7, 2013.

BAHIA, D. M. M. et. al. Aberrant Phenotypes in Acute Myeloid Leukemia: A High Frequency and Clinical Significance. *Haematologica*, v. 86, n. 8, p. 801-806, 2001.

BALJEVIK, M. et. al. Telomere Length Recovery: A Strong Predictor of Overall Survival in Acute Promyelocytic Leukemia. *Acta Haematogica*, v. 136, p. 210–218, 2016.

BASSO, G. et. al. Diagnosis and Genetic Subtypes of Leukemia Combining Gene Expression and Flow Cytometry. *Blood Cells, Molecules, and Diseases*, v. 39, p. 164–168, 2007.

BÉNÉ, M. C. et. al. Immunophenotyping of Acute Leukemia and Lymphoproliferative Disorders: A Consensus Proposal of the European Leukemia Network Package. *Leukemia*, v. 25, p. 567-574, 2011.

BLACKBURN, E. H. et. al. Human Telomere Biology: A contributory and Interactive Factor in Aging, Disease Risks, and Protection. *Science*, v. 350, p. 1193-1198, 2015.

BOWER, H. et. al. Continued Improvement in Survival of Acute Myeloid Leukemia Patients: An Application of the Loss in Expectation of Life. *Blood Cancer Journal*, v. 6, p. 1-6, 2016.

BRUEDIGAM, C.; LANE, S. W. Telomerase in Hematologic Malignancies. *Current Opinion*, v. 23, n. 4, p. 346-353, 2016.

CALLICOTT, R. J.; WOMACK, J. E. Real-time PCR Assay for Measurement of Mouse Telomeres. *Comparative Medicine*, v. 56, n. 1, p. 17-22, 2006.

CAWTHON, R. M. Telomere Measurement by Quantitative PCR. *Nucleic Acids Research*, v. 30, n. 10, p. 1-6.

DESANTIS, C. E. et. al. Cancer Treatment and Survivalship Statistics, 2014. *CA: Cancer Journal for Clinicians*, v. 64, p. 252-271, 2014.

ELYAMANY, G. et. al. Abnormal Expression of CD79a, CD56 and CD7 in Acute Myeloid Leukemia. *Pathology Discovery*, p. 1-5, 2013.

FERRARA, F.; SCHIFFER, C. A. Acute Myeloid Leukaemia in Adults. *The Lancet*, v. 381, n. 9865, p. 484–495, 2013.

FINAK, G. et. al. Standardizing Flow Cytometry Immunophenotyping Analysis from the Human Immunophenotyping Consortium. *Scientific Reports*, v. 6, p. 1-11,2016.

GERBING, R. B., Shorter Remission Telomere Length Predicts Delayed Neutrophil Recovery After Acute Myeloid Leukemia Therapy: A Report from the Children's Oncology Group. *Journal of Clinical Oncology*, v. 34, p. 1-12, 2016.

AHMAD, I. N., et. al. Flow Cytometric Analysis: Four-Year Experience in a Tertiary Care Centre of Pakistan. *Cureus*, v. 8, n. 9, p. 1-10, 2016.

HARRISON, W. J. The Total Cellularity of the Bone Marrow in Man. *Journal of Clinical Pathology*, v. 15, n. 3, p. 254-259, 1962.

HASSAN, M.; Abedi-Valugerdi, M. Hematologic Malignancies in Elderly Patients. *Haematologica*, v. 99, n. 7, p. 1124-1127,2014.

IKOMA, M.R.V. et al. First Proposed Panels on Acute Leukemia for Four-Color Immunophenotyping by Flow Cytometry from the Brazilian Group of Flow Cytometry-GBCFLUX. *Cytometry Part B: Clinical Cytometry*, v. 88, n. 3, p. 194-203, 2015.

Ministério da Saúde, Estimativa 2016/Incidência de Câncer no Brasil. Disponível em: <http://www.inca.gov.br/estimativa/2016/estimativa-2016-v11.pdf > Acesso em: 18 de outubro de 2016.

JAHEDI, M. et. al. Aberrant Phenotype in Iranian Patients with Acute Myeloid Leukemia. *Advanced Pharmaceutical Bulletin*, v. 4, n. 1, p. 43-47, 2014.

JULIUSSON, G. et. al. Age and Acute Myeloid Leukemia: Real World Data on Decision to treat and Outcomes from the Swedish Acute Leukemia Registry. *Blood*, v. 113, n. 18, p. 4179-4187, 2009.

JULIUSSON, G. et. al. Acute Myeloid Leukemia in the Real World: Why Population-Based Registries Are Needed. *Blood*, v. 119, n. 17, p. 3890-3899, 2012.

JULIUSSON, G.; HOUGH, R. Tumors in Adolescents and Young Adults. *Progress in Tumor Research*, vol. 43, p. 87-100,2016.

KLATSKY, A. L. et al. Alcohol Consumption and Risk of Hematologic Malignancies. *Annals of Epidemiology*, v. 19, n. 10, p. 746–753, 2009.

JHA, R. et. al. Lymphoid Associated Antigen Expression in New Cases of Acute Myeloid Leukemia. *Journal of Pathology of Nepal*, v. 3, p. 487-490, 2013.

KHAN, Z.; PANDEY, M.; SAMARTHA, R. M. Role of Cytogenetic Biomarkers in Management of Chronic Kidney Disease Patients: A Review. *International Journal of Health Sciences*, v. 10, n. 4, p. 576, 2016.

LÉONARD, L. et. al. Recent Advances on Multi-Parameter Flow Cytometry to Characterize Antimicrobial Treatments. *Frontiers in Microbiology*, vol.7, p. 1-16, 2016.

LEY, T. J. et. al. Genomic and Epigenetic Landscapes of Adult *de novo* Acute Myeloid Leukemia. *The New England Journal of Medicine*, v. 368, n. 22 p. 2059-2074, 2013.

MAZHAR, N. et. al. Aberrant Expression of CD Markers in Acute Leukemia. *The Annals of Pakistan Institute of Medical Science*, v. 9, n. 2, p. 99-102, 2013.

NDIFON, W.; DUSHOFF, J. The Hayflick Limit May Determine the Effective Clonal Diversity of Naive T Cells. *The Journal of Immunology*, v. 196, p. 1-7, 2016.

NOVOA, V. et al. Inmunofenotipos Aberrantes en Leucemias Agudas en uma Población Hospitalaria de Buenos Aires. *Medicina*, v. 7, p. 9-16, 2013.

ORAN, B.; WEISDORF, D. J. Survival for Older Patients with Acute Myeloid Leukemia: A Population-Based Study. *Haematologica*, v. 97, n. 12, p. 1916-1924, 2012.

ORFAO, A. et. al. Clinically Useful Information Provided by the Flow Cytometric Immunophenotyping of Hematological Malignancies: Current Status and Future Directions. *Clinical Chemistry*, v. 45, n. 10, p. 1708-1717, 1999.

OSSENKOPPELE, G. J. et. al. Review of the Relevance of Aberrant Antigen Expression by Flow Cytometry in Myeloid Neoplasms. *British Journal of Haematology*, v. 153, p. 421–436, 2011.

PAIVA, R. M. A.; R. T. CALADO. Telomere Dysfunction and Hematologic Disorders. *Progress in Molecular Biology and Translational Science*, v. 125, p. 133-157, 2014.

ROSE-INMAN, H.; KUEHL, D. Acute Leukemia. *Emergency Medicine Clinics of North America*, v. 32, p. 579–596, 2014.

VISSER, O. et. al. Incidence, Survival and Prevalence of Myeloid Malignancies in Europe. *European Journal of Cancer*, v. 48, p. 3257–3266, 2012.

WALTER, R. B. at. al. Significance of FAB Sub-Classification of "Acute Myeloid Leukemia, NOS" in the 2008 WHO Classification: Analysis of 5,848 Newly Diagnosed Patients. *Blood*, v. 121, n. 13, p. 2424–2431, 2013.

WANG, L. et. al. The Role of Telomeres and Telomerase in Hematologic Malignancies and Hematopoietic Stem Cell Transplantation. *Journal of Hematology and Oncology*, v. 7, n. 61, 2014.

WASS, M. at. al. Value of Different Comorbidity Indices for Predicting Outcome in Patients with Acute Myeloid Leukemia. *Plos One*, p. 1-13, 2016.

WINTERS, N. at.al. Exposure to Ambient Air Pollution in Canada and the Risk of Adult Leukemia. *Science of the Total Environment*, v. 526, p. 153–176, 2015.

YAN, S. at. al. Telomerase Gene Mutation Screening and Telomere Overhang Detection in Chinese Patients with Acute Myeloid Leukemia, *Leukemia & Lymphoma*, v. 54, n. 7, p. 1437–1441, 2013.

YANG, D-H.at. al. Predictable Prognostic Factor of CD56 Expression in Patients with Acute Myeloid Leukemia with t(8:21) After High Dose Cytarabine or Allogeneic Hematopoietic Stem Cell Transplantation, *American Journal of Hematology*, v. 82, p. 1–5, 2007.

ZHANG, X. at. al. The DNA Methylation Inhibitor Induces Telomere Dysfunction and Apoptosis of Leukemia Cells that is Attenuated by Telomerase Over-expression, *Oncotarget,* v. 6, n.7 p. 4888-4900, 2014.

ZHENG, J. at. al. A Correlation Study of Immunophenotypic, Cytogenetic, and Clinical Features of 180 AML Patients in China, *Cytometry Part B (Clinical Cytometry)*, v. 74b, p. 25–29, 2008.

CAPÍTULO 1

ABERRANT PHENOTYPES IN ACUTE MYELOID LEUKEMIA AND ITS RELATIONSHIP WITH PROGNOSIS AND SURVIVAL: A SYSTEMATIC REVIEW

Lucio HS Pinheiro[1], Louise D Trindade[1], Amanda FO Costa[1], Dulce M Schimieguel[1]

[1]Department of Pharmacy, Laboratory of Hematology, Federal University of Sergipe, Aracaju, Sergipe, Brazil.

*Corresponding author:
pinheiro_lucio@hotmail.com (LP)
Phone: +55 (79) 998020508
Postal Address: Rua Projetad, 210 - Rosa Elze, São Cristóvão - SE, 49100-000

ABSTRACT

Background: Acute myeloid leukemia is a hematologic malignancy highly associated with poor prognosis and low survival rate. Aberrant phenotypes detected by immunophenotyping have been associated with a worse clinical course of the disease, specially, when related with prognosis and survival rate. The aim of this systematic review was to evaluate the influence of aberrant markers in prognosis and survival of acute myeloid leukemia.

Materials and methods: Following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, a systematic review of PubMed, Scopus, Science Direct, Web of Science and Cochrane Library was carried out through 1998 to 2016.

Results: We included 16 studies on this systematic review, which the aberrant phenotype expression of 30 markers were detected by flow cytometry using samples of 2,000 patients. From all those, the expression of nine aberrant markers were related to prognosis, and five had shown negative impact on prognosis in AML. This systematic review studied the following markers CD7, CD82, CD34, CD19, CD56, CD98, CD90, CD123, and CD15.

Conclusions: The prognosis implication of aberrant antigen remains controversy and new studies are essential for a better understanding of leukemia features. In order to lead to higher rates of complete remission, the development of a target therapy is becoming prominent.

Key words: Aberrant phenotypes, acute myeloid leukemia, prognosis, survival.

1. INTRODUCTION

Leukemia is a group of malignant and heterogeneous hematological disorders that affect the normal cellular maturation process[1]. Hematologic malignancies comprise 8% of all cancers types in developed countries, in which is estimated that about half of these cases are classified as acute leukemia [2].

Acute myeloid leukemia is characterized by a blockage of hematopoietic progenitor cells differentiation giving rise to an accumulation of blasts in the bone marrow[3]. In acute myeloid leukemia occurs the increase of neoplastic monocytic, erythrocytic, granulocytic and megakaryocytic lineages in the bone marrow while their levels are decreased in the peripheral blood, leading to the occurrence of clinical manifestations such as anemia, infections and hemorrhages[2].

Aberrant phenotypes include cross-lineage expression, maturational asynchronous expression and over expression of antigen, absence or reduction of their expression[4]. Besides, aberrant immunophenotypes have been reported as adverse prognostic factors, and some other antigens have been associated to survival[5]. Furthermore, age is a negative prognostic factor as demonstrated that survival decreases drastically when age increases concluding that older patients show low survival rate[6].

Nevertheless, there are few studies relating aberrant phenotypes with poor prognosis and patients' survival. Consequently, further studies are necessary, and through them will be possible to address patients to a better treatment. Therefore, the aim of this systematic review was to evaluate the influence of aberrant phenotype expression in prognosis and survival of patients with acute myeloid leukemia.

2. MATERIALS AND METHODS

A systematic review was performed based on a scientific research protocol describing the aims and methods used. This synthesis was performed according to *Preferred reporting items for Systematic Reviews and Meta-Analyses* (PRISMA).

This systematic review aimed to answer the following question: *Which are the aberrant phenotypes in acute myeloid leukemia and what their influence in prognosis and survival?*

Search strategy

The literature search was conducted using PubMed, Science Direct, Web of Science, Scopus and Cochrane Library databases looking for articles published from 1998 to 2016. The timeline was determined according to the beginning of the use of eight-color flow cytometry [7].

To this search were used the following terms: Aberrant phenotype (MeSH) or Aberrant immunophenotype (MeSH) or Aberrant expression (MeSH) or Aberrant marker (MeSH), and Prognosis (MeSH) or Survival rate (MeSH) or Survival analysis (MeSH) and Leukemia myeloid, acute (MeSH) or Acute leukemia (MeSH). Also, it was used their equivalents in Portuguese and Spanish.

Study selection

The articles found in this search were compared with the inclusion criteria previously defined to determine the study relevance: (1) articles published from 1998 to 2016; (2) articles published in English, Spanish and Portuguese; (3) articles that

used immunophenotyping in their methodologies; (4) articles about acute myeloid leukemia; (5) articles with available abstracts and full text.

Study cases, systematic and literature reviews, meta-analysis, editorials, conference proceedings and books were excluded from the study. Two reviewers independently evaluated the titles and abstracts from the articles applying the inclusion criteria. Articles that seem to be relevant were fully analyzed, and the articles that were included in this systematic review were based in agreement between the two reviewers. Disagreements between the two reviewers were inspected by a third reviewer that fully analyzed the articles, and made the final decision whether or not to use the article.

Rating quality of individual studies

The methodological quality of each individual study was evaluated using the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) scale, which consisted of 22 items. High scores meant that there was sufficient information and good design.

Data extraction and management

From the included studies, information regarding several parameters was obtained: (1) journal of publication; (2) The Journal Citation Reports impact factor; (3) location; (4) study design; (5) aim of the study; (6) number of samples analyzed; (7) Acute myeloid leukemia classification; (8) most incident subtype; (9) aberrant immunophenotypic marker; (10) prognostic value; (11) first induction treatment protocol; (12) follow up; (13) survival; (14) limitations; and (15) STROBE scores.

For inaccessible or incomplete full texts, authors were contacted for additional information.

3. RESULTS

The literature search

Findings in the databases (PubMed, Scopus, Science Direct, Web of Science and Cochrane Library) reached 20,660 articles. After primary readings of tittles and abstracts 31 potentially articles remained. Final analysis resulted in a total of 16 studies that were included into this systematic review as illustrated in the figure 1.

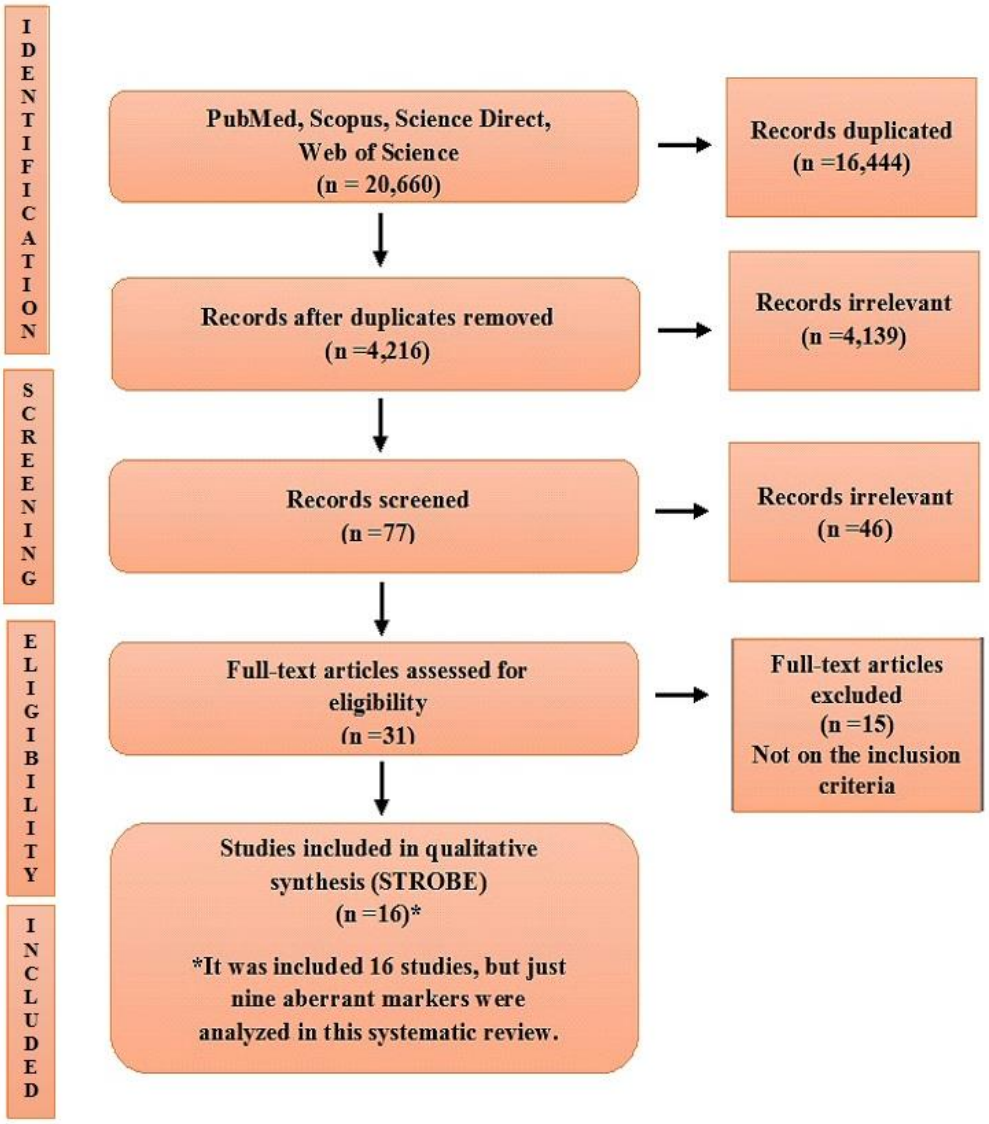

Figure 1. Flowchart of of the study selection process.

Study Characteristics

It was conducted an overview from the 16 studies[2,8-22] to extract relevant characteristic from them. A range of 12–706 patients with a total of 2,000 hematological samples were studied in all articles. Ten studies used the French-American-British (FAB) classification for AML, three studies used the World Health Organization (WHO) classification, and three of them does not classify AML into any subtype. In the articles, the most reported AML subtype was FAB M2 followed

by FAB M1.

The majority of the studies were conducted in developed countries where high technology is advanced, such as, United States of America, Germany, Japan, United Kingdom, and Saudi Arabia. From the articles analyzed, the Journal Citation Reports impact factor ranged from 11,847 to 0,138. In all articles were cited 30 different aberrant antigens and the way they are expressed in the cells; however, just 9 aberrant markers were correlated with prognosis, CD7, CD82, CD34, CD19, CD56, CD98, CD90, CD123, and CD15. From these nine aberrant markers, four of them analyzed patient's survival. The studies showed that the most common treatment protocol was anthracycline-based induction therapy, as a chemotherapy regimen, even although other treatment regimens were applied in some group of patients.

Related to the methodological quality of each study, it was evaluated by STROBE tool, and the articles ranged from 77,8% to 91%. The highest methodological quality is found in articles that scored >90%.

The following characteristics were found about CD7. It is a cell surface glycoprotein member of the immunoglobulin superfamily. This protein is found on thymocytes and mature NK and T-cells [23]. Five studies in this systematic review, conducted by El-Sissy et al.[8], Jahedi et al.[2], Bahia et al.[9], Rausei-Mills et al.[10]. and Baqai et al.[11], involved CD7 in their findings. It was showed that CD7 was the most common aberrant lymphoid antigen in AML cases, and it was found more frequent in M1, M2 and M7 AML subtypes. Besides this, they demonstrated low remission rate which confers a worse prognosis, and it is related to a more aggressive course of the disease. Furthermore, as showed in Rausei-Mills et al.[10] study, CD7

was associated with unfavorable cytogenetics, which can be classified as an adverse prognostic factor, and it has clinical significance. Association between CD7 antigen expression and FLT3/ITD mutation was found, and it has been associated with poor prognosis, as it was observed in Baqai et al.[11] findings.

CD82 is a member of the tetraspanin superfamily, identified as an accessory molecule in T-cell activation. Furthermore, CD82 may be associated to integrin molecules in $CD34^{+}/CD38^{-}$ AML cells what promotes adhesion to the bone marrow endosteal niche. A study was conducted by Nishioka et al.[12] about self-renewing leukemia stem cell (LSC) compartments ($CD34^{+}/CD38^{-}$ cells), showed that CD82 is highly expressed in $CD34^{+}/CD38^{-}$AML cells. These findings demonstrated that overexpression of CD82 is associated to LSC adhesion to the bone marrow (BM), and it appears to be direct related to the colony forming ability of the LSC regulating their proliferation. Therefore, CD82 looks to play a role in survival of $CD34^{+}/CD38^{-}$ AML cells, then it has relevant unfavorable influence in prognosis in AML.

CD56 as known as NCAM (neural cell adhesion molecule) expressed by NK and T cells[24]. In this systematic review, four studies conducted by Iriyama et al.[13], Oelschlägel et al.[14], Cui et al.[15] and Breccia et al.[16] have analyzed CD56 and its influence on prognosis. Iriyama et al.[13] found a frequent relationship between CD56 and AML t(8;21), as well as, unfavorable outcomes when white blood cells $CD56^{+}$ counts increased at diagnosis. CD56 was identified as an independent prognostic factor for relapse. Oelschlägel et al.[14] showed a decrease of the association of CD34FITC/CD56PE positive cells at treatment failure. Cui et al.[15] and Breccia et al.[16] studies showed an association of the high expression of CD56 to aggressive clinical behavior on patients with AML as well as $CD56^{+}$ higher frequency of

relapse (34%) than $CD56^-$ (20%) and its association to a high incidence of differentiation syndrome. Moreover, overall survivor for CD56 presence in acute promyelocytic leukemia was low (60%) when compared to $CD56^-$ (85%).

CD15 is a carbohydrate as known as Lewis X[25]. Three studies that analyzed CD15 aberrant expression were included in this systematic review, Oelschlägel et al.[14], Bahia et al.[9], and Breccia et al.[16]. The first two studies cited above showed that a decrease of $CD15^+$ leads to a bad prognosis, associated to more unfavorable chromosome abnormality when compared to patients at this positive group. $CD15^+$ cells decrease at relapse more than one half of the initial values and asynchronous expression of $CD15^+$ as important association between aberrant antigen and treatment response. Different of Breccia et al.[16] findings, that showed patients who expressed CD15, and they were correlated to a cumulative incidence of relapse of 45% in 5 years when compared to 11,3% of the negative group.

CD34 is a membrane glycoprotein that has been linked to increased resistance to apoptosis, and it has been associated to NPM1 mutation which is a poor prognosis factor. It was demonstrated in Zeijlemaker et al.[18] studies that $CD34^-$ cells showed high frequency of association to $NPM1^{mut}/FLT3^{mut}$. Besides this, $CD34^-$ patients were classified into intermediate and poor risk group, as a result of additional poor prognostic cytogenetic or molecular abnormalities, $NPM1^{mut}/FLT3^{mut}$. FAB-M5 was the most frequent leukemia subtype diagnosed in patients with $CD34^-$cells. Relapse incidence in CD34-negative patients was relatively low in agreement with the overall survival that showed a great clinical significance, when compared to CD34-positive patients. According to these findings, CD34-negative antigens showed as a favorable prognostic factor.

CD19, a major B-cell marker, was more frequent in AML-M2 cases by FAB classification. Five studies, conducted by Chen et al.[19], Iriyama et al.[13], El-Sissy et al.[8], Abdulateef et al.[20] and Walter et al.[21], had CD19 present in their findings. This antigen was highly associated to t(8;21) translocation, which is a cytogenetic alteration that confers a good prognosis to AML patients. Complete remission was found as a common factor in patients who showed CD19 positivity. Also, CD19 expression is significantly correlated with improved prognosis. Abdulateef et al.[20] study showed that the co-expression of CD19 and CD56 occurred just in cases that presented t(8;21). Walter et al.[21] showed that there is high association between CD19 expression in AML patients and t(8;21) cytogenetic abnormality. However, both findings are linked to a transcription factor, *PAX5*, which is related to an adverse prognosis involved in gene repression.

4. DISCUSSION

Based on antigen expression in different subtypes of hematologic cell lineages, multiparametric flow cytometry of high resolution had been used to identify the leukemic cells characteristics. Studies related to positive or negative and high or low antigen expression, as well as, translineage of some antigens on malignant cells, had obtained information about the immunophenotype of different leukemia subtypes. The wide application of immunophenotyping in acute myeloid leukemia has become essential to understand the relationship between aberrant immunophenotypes and cell morphology and its cytogenetic features[26].

During this systematic review, it was possible to notice that prognosis

implication of aberrant antigen remains controversy and new studies are essential for a better understanding of leukemia features. From the nine aberrant markers cited, five were associated with poor prognosis. CD7, CD83, CD56, CD123, and CD15 demonstrated to have influence on patient's survival. However, CD19, CD98, CD90, and $CD34^-$ are associated with a favorable prognosis.

As shown in Jahedi et al.[2] , El-Sissy et al.[8], and Bahia et al.[9], CD7 is associated to a more aggressive course of the disease. Other studies conducted by Nishioka also demonstrated the same result that was found in the article included in this systematic review. Nishioka et al.[27] demonstrated that CD82 is involved in survival of $CD34^+/CD38^-$ AML cells, and its downregulation decreases $CD34^+/CD38^-$ AML cells adhesion to bone marrow. Cui et al.[15] and Breccia et al.[16] studies showed that CD56 has a negative prognostic impact on AML patients, and their studies showed that patients with aberrant expression of CD56 had a higher frequency of relapse. CD19 was classified as a good prognostic factor due to patients who demonstrated its expression obtained complete remission as shown in Chen et al.[19] and Iriyama et al.[13] studies.

A review of the literature made by Mawad and Estey[28] showed that mutations on FLT3 gene can cause cell proliferation and inhibition of apoptosis. The most common mutation is the internal tandem duplication (ITD); therefore FLT3-ITD mutation confers a poorer prognosis, and it is related to high relapse rates. Rausei-Mills et al.[10] and Baqai et al.[11] demonstrated in their studies that CD7 is strongly associated with FLT3/ITD mutation. Peters et al.[29] oversaw a study that is in agreement with the articles used in this systematic review. His study showed that CD19 is strongly expressed in AML cases, and it has association with some

cytogenetic abnormalities, such as, t(8;21) translocation. Chen et al.[19] and Walter et al.[21] studies found that CD19 is highly associated with t(8;21), and CD19 is related to a favorable prognosis. Also, CD56 has strong association with t(8;21) as showed in El-Sissy et al.[8], Iriyama et al.[13], and Chen et al.[19] studies.

AML is heterogeneous hematologic disease, which is already classified by WHO. Different genetic subtypes confer different approaches and targeted therapies to the achievement of a correct treatment and cure. In this systematic review, Bahia et al.[9] recognize the importance of the evaluation of aberrant phenotypes for therapeutic decision and prognostic. In particular, $CD15^{+}$ and CD117+ as a good prognostic factor for AML.

Cui et al.[15] studies showed the most common leukemia-associated aberrant immunophenotype (LAIPs) at diagnosis and relapse, the most common ones were aberrant expression of CD7 and CD56, lack of lineage-specific antigen and asynchronous antigen expression. In addition to, minimal residual disease in the post induction phase is a negative factor, associated to decreased overall survivor. Similar findings exhibited by Baqai et al.[11] where the aberrant expression of CD7 in myeloid cells showed a positive correlation with FLT3(ITD) mutation, which is associated to bad prognosis.

El-Sissy et al.[8] article discoursed about the fact of the detection of aberrant markers of lymphoid antigens will turn the minimal residual disease easier to trace. These lymphoid antigens were detected in 47% of acute myeloid leukemia patients. CD123 overexpression found in many leukemia subtypes such as AML, B-ALL, and HCL, make its expression a helpful marker to establishment of diagnostic moreover CD123 could be useful as a clinical tool to identify the risk of treatment failure in

AML patients [30]. Chavez-Gonzaléz et al.[17] study showed that $CD123^+$ level decreased after relapse in pediatric AML patients.

New molecular and immunotherapeutic knowledges present benefits for AML patients, specially most needed patients as elderly and high risk patients, bringing better outcomes. Recent challenges are how to use these new approaches along with clinical data for better treatment choices [31].

Besides that, CD7+ CD15+ CD34+ HLA-DR+ immunophenotype were correlated as a significant predictor of overall survivor for patients with normal karyotype acute myeloid leukemia (NK-AML) and has latent role in risk stratification [32].

Limitations were found in our systematic review, as the large number of subject variation. Some articles, which had a small patient number could not to be representative as a data to immunophenotypic studies. Another observation was about the large difference of groups: pediatric patients, small ethnic populations for example, Indians, Arabians and Asians lead to specific characteristics that might cause miscomprehensions. The lack of new studies about acute myeloid leukemia, especially about the correlation between prognosis, clinical outcomes or therapy used for patients and aberrant immunophenotypic marker. Different methods to determine the positive or negative antigen expression varied among the studies included in this systematic review. Other limitation was related to insufficient information about the aberrant marker. Many of the aberrant marker cited in the articles do not have any correlation with their expression and prognosis or survival; therefore, some aberrant markers were not included in the results in this systematic review.

Nevertheless, limitations cited above, throughout this systematic review is

possible understand the importance of flow cytometry for antigen recognition. In eighteen years, new findings were discovered about the relationship between aberrant immunophenotype and clinical prognosis in acute myeloid leukemia. However the prognosis implication of aberrant antigen remains controversy and new studies are essential for a better understanding of leukemia features. Besides that, the development of a target therapy is becoming prominent, under these circumstances a more efficient and selectivity therapy has helped in a better clinical outcome and a role risk stratification. In order to lead to higher rates of complete remission and make possible the achievement of cure.

Conflicts of interest: none to declare

REFERENCES

[1] NOVOA V, NÚÑEZ NA, CARBALLO OG, LESSA CF. Inmunofenotipos aberrantes en leucemias agudas en uma población hospitalaria de buenos aires. Medicina (B Aires) 2013; 7:9-16.

[2] JAHEDI M, SHAMSASENJAN K, SANAAT Z, ALIPARASTI M, ALMASI S, MOHAMIDIAN M, et al. Aberrant phenotype in Iranian patients with acute myeloid leukemia, APB 2014; 4:43–47.

[3] FERRARA F, SCHIFFER, CA. Acute myeloid leukaemia in adults. Lancet (London) 2013; 381:484–495.

[4] SWERDLOW SH, CAMPO E, HARRIS NL, JAFFE ES, PILERI SA, STEIN H, THIELE J, VARDIMAN JW. WHO classification of tumors of haematopoietic and lymphoid tissues. 4th ed. IARC: Lyon; 2008.

[5] OSSENKOPPELE GJ, VAN DE LOOSDRECHT AA, SCHUURHUIS GJ. Review of the relevance of aberrant antigen expression by flowcytometry in myeloid neoplasms, Br J Haematol, v. 153, p. 421–436, 2011.

[6] DESANTIS CE, LIN CC, MARIOTTO AB, SIEGEL RL, STEIN KD, KRAMER JL, et al. Cancer treatment and survivalship statistics, 2014, CA Cancer J Clin, v. 64, p. 252-271, 2014.

[7] BANDALL SC, NOLAN GP, ROEDERER M, CHATTOPADHYAY PK. A deep profiler's guide to cytometry, Trends Immunol, v. 33(7), p. 323–332, 2012.

[8] EL-SISSY AH, EL-MASHARI MA, BASSUNI WY, EL-SWAAYED AF. Aberrant lymphoid antigen expression in acute myeloid leukemia in Saudi Arabia, J Egypt Natl Canc Inst 2006; 18:244–249.

[9] BAHIA DMM, YAMAMOTO M, CHAUFFAILLE MLLF, KIMURA EYS, BORDINI JO, FILGUEIRAS MAC, et al. Aberrant phenotypes in acute myeloid leukemia: a high frequency and clinical significance, Haematologica 2001; 86:801–806.

[10] RAUSEI-MILLS V, CHANG KL, GAAL KK, WEISS LM, HUANG Q. Aberrant expression of CD7 in myeloblasts is highly associated with *De Novo* acute myeloid leukemias with *FLT3/ITD* mutation, J Hematop 2008; 129:624–629.

[11] BAQAI J, CRISAN D. Correlation of *FLT3* mutations with expression of CD7 in acute myeloid leukemia, Appl Immunohistochem Mol Morphol 2015; 23:104–108.

[12] NISHIOKA C, IKEZOE T, FURIHATA M, YANG J, SERADA S, NAKA T, et al. $CD34^{+}/CD38^{-}$ acute myelogenous leukemia cells aberrantly express CD82 which regulates adhesion and survival of leukemia stem cells, Int J Cancer 2012; 132:2006–2019.

[13] IRIYAMA N, HATTA Y, TAKEUCHI J, OGAWA Y, OHTAKE S, SAKURA T, et al. CD56 expression is an independent prognostic factor for relapse in acute myeloid leukemia with t(8;21), Leuk Res 2013; 37:1021–1026.

[14] OELSCHLÄGEL U, NOWAK R, SCHAUB A, KOPPEL C, HERBST R, MOHR B, et al. Shift of aberrant antigen expression at relapse or at treatment failure in acute leukemia, Cytometry 2000; 42:247–253.

[15] CUI W, ZHANG D, CUNNINGHAM MT, TILZER L. Leukemia-associated aberrant immunophenotype in patients with acute myeloid leukemia: changes at refractory disease or first relapse and clinicopathological findings, Int J Lab

Hematol 2014; 36:636–649.

[16] BRECCIA M, PORPRIS MS, MINOTTI C, STEFANIZZI C, RAPONI S, COLAFIGLI G, et al. Aberrant phenotypic expression of CD15 and CD56 identifies poor prognostic acute promyelocytic leukemia patients, Leuk Res 2014; 38:194–197.

[17] CHAVÉZ-GONZÁLES A, DORANTES-ACOSTA E, MORENO-LORENZANA D, ALVARADO-MORENO A, ARRIAGA-PIZANO L, MAYANI H. Expression of CD90, CD96, CD117, and CD123 on different hematopoietic cell populations from pediatric patients with acute myeloid leukemia, Arch Med Res 2014; 45:343–350.

[18] ZEIJLEMAKER W, KELDER A, WOUTERS R, VALK PJM, WITTE BI, CLOOS J, et al. Absence of leukaemic $CD34^{+}$ cells in acute myeloid leukaemia is of high prognostic value: a longstanding controversy deciphered, Br J Haematol 2015; 171:227–238.

[19] CHEN SW, LI CF, CHUANG SS, TZENG CC, HSIEH YC, LEE PS, et al. Aberrant co-expression of CD19 and CD56 as surrogate markers of acute myeloid leukemias with t(8;21) in Taiwan, Int J Lab Hematol 2007; 30:133–138.

[20] ABDULATEEF NAB, ISMAIL MM, ALJEDANI H. Clinical significance of co-expression of aberrant antigens in acute leukemia: a retrospective cohort study in Makah Al Mukaramah, Saudi Arabia, Asia Pac J Cancer P 2014; 15:221–227.

[21] WALTER K, COCKERILL PN, BARLOW R, CLARKE D, HOOGENKAMP M, FOLLOWS GA, et al. Aberrant expression of *CD19* in AML with t(8;21) involves a poised chromatin structure and PAX5, Oncogene 2010; 29:2927–2937.

[22] NIKOLOVA M, GUENOVA M, TASKOV H, DIMITROVA E, STANEVA M. Levels of expression of CAF(CD98) have prognostic significance in adult acute leukemia, Leuk Res 1998; 22:39–47.

[23] ROGERS SL, ZHAO Y, JIANG X, EAVES CJ, MAGER DL, ROUHI A. Expression of the leukemic prognostic marker CD7 is linked to epigenetic modifications in chronic myeloid leukemia. Mol Cancer 2010; 9:1-14.

[24] HCDM Council. Available in <http://www.hcdm.org/index.php/component/molecule/?view=molecule&task=viewmolecule&moleculeid=246&search=> Access in January 10th, 2017.

[25] ENGEL P, BOUMSELL L, BALDERAS R, BENSUSSAN A, GATTEI V, HOREJSI V, et al. CD Nomenclature 2015: Human Leukocyte Differentiation Antigen Workshops as a Driving Force in Immunology. J Immunol 2015; 195:455-463

[26] ZHENG J, WANG X, HU Y, YANG J, LIU J, HE Y, et al. A Correlation Study of Immunophenotypic, Cytogenetic, and Clinical Features of 180 AML Patients in China, Cytometry B (Clin Cytom) 2008; 74:25–29.

[27] NISHIOKA C, IKEZOE T, YANG J, YOKOYAMA A. Tetraspanin family member, CD82, regulates expression of EZH2 via inactivation of p38 MAPK signaling in leukemia cells, Plos One 2015; 10:1–17.

[28] MAWAD R, ESTEY EH. Acute myeloid leukemia with normal cytogenetics, Curr Oncol Rep 2012; 14:359–368.

[29] PETERS JM, ANSARI Q. Multiparameter flow cytometry in the diagnosis and management of acute leukemia, Arch Pathol Lab Med 2011; 135:44–54.

[30] LIU K, ZHU M, HUANG Y, WEI S, XIE J, XIAO Y. CD123 and its potential clinical application in leukemias. Life Sci 2015; 122:59–64.

[31] KHALED S, AL MALKI M, MARCUCCI G. Acute myeloid leukemia: biologic, prognostic, and therapeutic insights. J Natl Compr Canc Netw 2016; 30:318–329.

[32] IRIYAMA N, ASOU N, MIYAZAKI Y, YAMAGUCHI S, SATO S, SAKURA T, et al. Normal karyotype acute myeloid leukemia with CD7+ CD15+ CD34+ HLA-DR+ immunophenotype is a clinically distinct entity with a favorable outcome, Ann Hematol, v.93, p. 957-963, 2014.

APPENDIX

Table 1. Individual studies analyzed on the systematic review.

MARKER	ARTICLE	JCR	LOCATION	DESIGN	AIM OF THE STUDY	STROBE
CD7 CD56 CD19	Chen et. al. (International Journal of Laboratory Hematology, 2007)	2.401	Taiwan	Cross-sectional observational	Retrospective study to characterize the frequency and significance of aberrant antigen expression of AML in Taiwan	18(81,8%)
CD7	Rausei-Mills et. al. (Hematopathology, 2008)	0.138	USA	Cross-sectional observational	Analysis of the clinical and pathologic features of 15 cases of de novo AML with normal cytogenetics and with the FLT3/ITD mutation.	17 (77.8%)
CD19	Walter et. al. (Oncogene 2010)	7.932	UK	Cross-sectional observational	Study of the characteristics of CD19 chromatin that is a direct target of PAX5 in cells with and without chromosomal translocation t (8; 21).	17 (77.8%)
CD9 CD7 CD56 CD79a	El-Sissy et. al. (Journal of the Egyptian Nat. Cancer Inst.,2006)	0.424	Saudi Arabia	Cross-sectional observational	Determination of aberrant lymphoid antigen expression in Saudi acute myeloid leukemia (AML), correlate them with FAB subtypes, evaluate early surface markers CD7 and CD56, and to investigate the role of cytoplasmic CD79a.	18(81,8%)
CD2 CD3 CD7 CD10 CD19 CD20 CD22	Jahedi et. al. (Advanced Pharmaceutical Bulletin, 2014)	2.01	Iran	Cross-sectional observational	Evaluate the incidence of aberrant phenotypes and possible prognostic value in peripheral and bone marrow blood mononuclear cells of Iranian patients with AML.	18(81,8%)

Table 1. Individual studies analyzed on the systematic review. (Continued)

MARKER	ARTICLE	JCR	LOCATION	DESIGN	AIM OF THE STUDY	STROBE
CD7 CD2 CD19 CD10 CD117+CD15+ CD34+CD56+ CD117+CD65+	Bahia et. al. (Haematologica, 2001)	6.671	Brazil	Cross sectional observational	Analyze 35 cases of AML, examining them for aberrant phenotypes by multiparametric flow cytometry.	19 (86,4%)
CD15 CD56	Breccia et. al. (Leukemia Research, 2013)	2.606	Italy	Cross-sectional observational	Assess the frequency of CD15 andCD56 expression, and their prognostic value in a large series of APLpatients, uniformly diagnosed and treated according to the AIDAschedule.	20 (91%)
CD34-	Zeijlemaker et. al. (British Journal of Haematology, 2015)	5.812	Netherland	Cohort	Review the importance of CD34 as an impact factor in AML in patients with intermediate risk and favorable risk	19 (86,4%)
CD82	Nishioka et. al. (International Journal of Cancer, 2012)	5.531	Japan	Cross sectional observational	Compare the protein expression profile of freshly isolated CD341/CD382 cells with that of CD341/CD381 counterparts from individuals with acute myelogenous leukemia	18(81,8%)
CD19 CD56 CD15 CD7 CD34	Iriyama et. al. (Leukemia Research, 2013)	2.606	Japan	Cross-sectional observational	Investigation of the clinical significance for the prognosis of surface antigen expression in patients with AML t (8; 21)	19 (86,4%)

Table 1. Individual studies analyzed on the systematic review. (Continued)

MARKER	ARTICLE	JCR	LOCATION	DESIGN	AIM OF THE STUDY	STROBE
CD56 CD7 CD19 CD2 CD33 CD13	Abdulateef et. al. (Asian Pacific Journal of cancer Prevention, 2014)	2,39	Saudi Arabia	Cohort	Determine the prevalence of aberrant antigen expression in acute leukemia, to assess clinical relevance, and to demonstrate immunophenotype-karyotypic correlations	18(81,8%)
CD 7	Baqai and Crisan(Applied Immunohistochemistry & Molecular Morphology, 2015)	1.553	USA	Cohort	Expand the evaluation of the evolution of the association of aberrant expression of CD7 in blasts into new AML with D835 mutation.	18(81,8%)
CD90 CD 96 CD117 CD123	Chávez-González et. al. (Archives of Medical Research, 2014)	2.219	Mexico	Cohort	Analyze the expression of four cell surface antigens relevant to human hematopoiesis—CD90, CD96, CD117, and CD123—in bone marrow from pediatric AML patients and normal control subjects.	20 (91%)
CD13 CD33 CD56 CD7 CD4 CD11b	Cui et. al. (International Journal of Laboratory Hematology, 2014)	2.401	USA	Cross-sectional observational	Changes in leukemia-associated aberrant immunophenotype (LAIP) in patient with refractory and relapsed acute myeloid leukemia (AML)	19 (86,4%)

Table 1. Individual studies analyzed on the systematic review. (Continued)

MARKER	ARTICLE	JCR	LOCATION	DESIGN	AIM OF THE STUDY	STROBE
CD98	Nikolova et. al.(Leukemia Research, 1998)	2.606	Bulgaria	Cross-sectional observational	Evaluation of CD98 expression levels in patients with leukemia	17 (77.8%)
CD15	Oelschla¨gel et. al. (Cytometry Part A, 2000)	3.181	Germany	Cross sectional observational	Investigation of the stability of aberrant antigen expression in the relapse or failure of initial chemotherapy treatment	17 (77.8%)

AML: Acute myeloid leukemia; JCR: journal citation reports; FLT3/ITD: fms related tyrosine kinase 3/internal tandem duplication; iTRAQ: isobaric tags for relative and absolute quantification; LAIP: leukemia-associated immunophenotypes.

Table 2. Main disease and treatment features of the individual studies included on the systematic review.

ARTICLE	MARKER	PATIENTS (N)	CLASSIFICATION	PROGNOSIS	FOLLOW-UP	SURVIVAL	TREATMENT	CUT-OFF	GENE MUTATION
Chen et. al. (International Journal of Laboratory Hematology, 2007)	CD7	111	M0: 10 M1: 15 M2: 36 M3: 15 M4: 21 M5: 8 M7: 6	POOR	NR	NR	ARA-C, ATRA or none treatment	NR	NR
	CD56								
	CD19								
Rausei-Mills et. al. (Hematopathology, 2008)	CD7	31	M0: 2 M1: 12 M2: 7 M4: 8 M6: 2	POOR	NR	NR	NR	NR	FLT3/ITD
Walter et. al. (Oncogene 2010)	CD19	NR	AML with t(8;21)	POOR.	NR	NR	NR	NR	PAX5
El-Sissy et. al. (Journal of the Egyptian Nat. Cancer Inst.,2006)	CD9	34	M1: 9 M2: 10 M3: 5 M4: 2 M5: 5 M6: 1 M7: 2	NR	NR	NR	NR	NR	NR
	CD7			POOR					
	CD56			POOR					
	CD79a			NR					

Table 2. Main disease and treatment features of the individual studies included on the systematic review. (Continued)

ARTICLE	MARKER	PATIENTS (N)	CLASSIFICATION	PROGNOSIS	FOLLOW-UP	SURVIVAL	TREATMENT	CUT-OFF	GENE MUTATION
Jahedi et. al. (Advanced Pharmaceutical Bulletin, 2014)	CD2	56	M0: 3 M1: 12 M2: 16 M3: 11 M4: 8 M5: 5 M7: 1	POOR	NR	NR	NR	>20%	NR
	CD3			POOR					
	CD7			POOR					
	CD10			POOR					
	CD19			POOR					
	CD20			POOR					
	CD22			POOR					
Bahia et. al. (Haematologica, 2001)	CD7	35	M0: 1 M1: 7 M2: 8 M3: 4 M4: 6 M5: 6 M6: 1 M7: 2	POOR	NR	NR	NR	> 20%	NR
	CD2			NR					
	CD19			GOOD					
	CD10			NR					
	CD117+CD15+			GOOD					
	CD34+CD56+			POOR					
	CD117+CD65+			GOOD					
Breccia et. al. (Leukemia Research, 2013)	CD56	114	M3	POOR	5 anos	58% vs 85%	ATRA + idarubicin: GIMEMA AIDA	>20%	PML/RARA
	CD15			POOR		60% vs 85%		>20%	

Table 2. Main disease and treatment features of the individual studies included on the systematic review. (Continued)

ARTICLE	MARKER	PATIENTS (N)	CLASSIFICATION	PROGNOSIS	FOLLOW-UP	SURVIVAL	TREATMENT	CUT-OFF	GENE MUTATION
Zeijlemaker et. al. (British Journal of Haematology, 2015)	CD34-	706	M0: 62 M1: 129 M2: 185 M4: 102 M5: 94 M6: 20 M7: 4 RAEB: 40 RAEB-t: 41 Not classified: 29	GOOD	4 anos	62% vs 39%	1st cycle: Idarubicin + Cytarabine 2nd cycle: Amsacrine + Cytarabine 3rd cycle: Mitoxantrone + Etoposide	5%, 10%, 20	NPM1/FLT
Nishioka et. al. (International Journal of Cancer, 2012)	CD82	18	NR	POOR	NR	NR	NR	NR	Inativation of MMP9
Iriyama et. al. (Leukemia Research, 2013)	CD19	144	M2: AML with t(8;21)	GOOD	NR	NR	NR	NR	NR
	CD56			POOR					
	CD15			POR					
	CD7			POOR					
	CD34			POOR					

Table 2. Main disease and treatment features of the individual studies included on the systematic review. (Continued)

ARTICLE	MARKER	PATIENTS (N)	CLASSIFICATION	PROGNOSIS	FOLLOW-UP	SURVIVAL	TREATMENT	CUT-OFF	GENE MUTATION
Abdulateef et. al. (Asian Pacific Journal of cancer Prevention, 2014)	CD56	73	M1: 10 M2:9 M3:3 M4: 12 M5: 4 M6:1 M7: 1 ALL: B 26 L: 7	POOR	NR	NR	NR	NR	NR
	CD7			POOR					
	CD19			GOOD					
	CD2			NR					
	CD33			NR					
	CD13			NR					
	CD56			NR					
Baqai and Crisan(Applied Immunohistochemistry & Molecular Morphology, 2015)	CD7	149	AML with FLT3	POOR	NR	NR	NR	NR	FLT3/ITD
Chávez-González et. al. (Archives of Medical Research, 2014)	CD90	12	M1: 2 M2:4 M4: 1 M5: 2 M7: 3	GOOD	NR	NR	First course: ATEDox Other: Cytarabine and Mitroxantone. >5% blasts at BM (Cytarabine, Etoposide)	M3 subtype: Promyelocitic leukemia	FLT3/NPM1
	CD96			NR					
	CD117			POOR					
	CD123			POR					

Table 2. Main disease and treatment features of the individual studies included on the systematic review. (Continued)

ARTICLE	MARKER	PATIENTS (N)	CLASSIFICATION	PROGNOSIS	FOLLOW-UP	SURVIVAL	TREATMENT	CUT-OFF	GENE MUTATION
Cui et. al. (International Journal of Laboratory Hematology, 2014)	CD 13 CD33 CD56 CD7 CD4 CD11b	47	AML	POOR POOR POOR POOR POOR POOR	NR	NR	NR	NR	NR
Nikolova et. al.(Leukemia Research, 1998)	CD98	62	ALL:B 17 T 7 ALL: M0:4 M1:8 M2:12 M3:1 M4: 7 M5: 5 M6:1	GOOD	13.5 months.	NR	ALL patients: induction with vincristine,novantrone/farmarubicine, prednisolone AML patients: induction with farmarubicine, cytosine arabinoside and 6thioguanine	CAF7 low group: 25th percentile (MFI < 20.4 channel) CAF7 intermediate group: 25th and 75th percentile (MFI between 20.4 and 47 channel) CAF7 high group up to 75th percentile (MFI > 47 channel)	NR
Oelschla¨gel et. al. (Cytometry Part A, 2000)	CD15	289	AML :59	POOR	NR	NR	NR	NR	NR

AML: Acute myeloid leukemia; FLT3/ITD: fms related tyrosine kinase 3/internal tandem duplication; MFI: Mean fluorescence intensity; ATEDox: cytarabine, 6-thioguanine, etoposide, doxorubicin; NPM1: nucleophosmin 1; PAX5: paired box 5; PML/RARA: Promyelocytic leukemia /retinoic acid receptor alpha; ARA-C: cytarabine; ATRA: vesanoid, treitinoin; MMP9: matrix metallopeptdase 9