nas amostras analisadas no perfil linfocitário, sendo também possível a aplicação destas em uma maior variedade de casos. Estes métodos computacionais elucidam a identificação de padrões relevantes nas amostras analisadas, o que é especialmente importante em estudos com grandes quantidades de dados, onde torna-se possível a aplicação de estudos de *big-data* e *machine learning*, além do comparativo com a literatura relacionada, a exemplo do entendimento e definição da ontogenia das populações B, T e NK, frente à diferentes quadros clínicos. Por fim, a visualização dos dados celulares pode ser retratada de forma mais completa, com recursos mais variados que podem reforçar o rigor e acompanhamento analítico. **Conclusão:** A integração de bibliotecas R especializadas, como *FlowSOM*, *OpenCyto*, e outras, proporcionou uma análise mais robusta e eficiente dos dados de citometria de fluxo. As abordagens avançadas de clustering permitiram identificar as populações B, T e NK, além de caracterizar subpopulações com maior precisão e eficiência, favorecendo o entendimento da ontogenia das mesmas. Essas técnicas têm o potencial de impulsionar significativamente a pesquisa e clínica em citometria de fluxo, trazendo novos parâmetros de análise e abrindo novas possibilidades para o controle interno e entrega dos resultados, por exemplo, na investigação e monitoramento de processos biológicos complexos, além do auxílio no desenvolvimento e acompanhamento de terapias personalizadas.

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ACUTE MYELOID LEUKEMIA WITH A RARE IMMUNOPHENOTYPE AND COMPLEX GENETIC PROFILE

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Acute Myeloid Leukemia (AML) presents a wide immunophenotypic variability and defining molecular alterations, used for the revised WHO 2022 classification. The genetic profile is also used for risk stratification according to the European LeukemiaNet (ENL) 2021 and treatment definition. Here we present a case with an unusual immunophenotype and complex genetic alterations. Case report: 42-year-old woman, with increased menstrual flow in the last 10 days and no other alterations. On physical examination, she was in good condition, with slight paleness, without hemorrhagic suffusions, absence of lymph node enlargement and hepatosplenomegaly. Complementary tests showed Hb 9.4 g/dL WBC 2850/mm^3 (2% blast cells) Platelets 48,000/mm^3 Fibrinogen 56 mg/dL (normal: 200 – 400), normal biochemistry and coagulogram. The myelogram showed massive infiltration by hyper granular blasts suggesting basophilic leukemia. Immunophenotyping revealed 3 blast populations, all expressing cyMPObright, CD13, CD33bright, CD34, CD38dim/negative, CD44, CD45intermediate, CD45RA, CD96, CD97, CD99, CD123, CD244, CLL1, TIM3. The differences between the expressions of the blast populations were: 1st population (41%) expressed partial CD7, CD117bright and homogeneous, heterogeneous HLA-DR (positive to negative), not expressing CD11b and CD203c; 2nd population (40%) expressed CD11b, CD203c bright, without expressions of CD7 and HLA-DR (basophilic component); 3rd population (19%) did not express CD7, CD11b, CD203c and HLA-DR (promyelocytic component). The 3 populations did not express cyCD3, SmCD3, CD4, CD10, CD14, CD15, CD16, CD19, CD36, CD42a+CD61, CD54, CD56, CD64, CD71, cyCD79a, CD105, CD300e. This profile was consistent with AML with basophilic and acute promyelocytic (APL) components. The karyotype was complex: 46,XX, del(3)(p21), del(5)(q15), t(15;17)(q22;q21)[6]/46,XX[14]. The NGS panel revealed the presence of the *PML::RARA* gene fusion, negative for *NPM1*, *FLT3 ITD* and *TKD* mutations and others. The patient was treated with Cytarabine plus Daunorubicin (7+3) and ATRA. Assessment of measurable residual disease (MRD) by flow cytometry (FC) on D28 of induction showed 0.2% non-APL myeloblasts and 0.29% basophilic blasts. Despite preventive therapies, the patient experienced life-threatening bleeding and sepsis, but had a favorable outcome. She received 2 cycles of consolidation and maintenance therapy. FC MRD and *PML::RARA* RT-PCR was undetectable after the 2 consolidation cycles and during maintenance therapy. *PML::RARA* was also undetectable at the end of therapy and 3 months later. The probability of severe bleeding in APL with the concomitant presence of basophilic blasts has already been described, as was seen in this case. The patient had a good therapeutic response. Monitoring MRD in AML is essential to guide treatment, and the methods must be complementary, especially in cases with multiple leukemic clones. The identification of different leukemia clones by immunophenotype reflected the genetic complexity of this case. Therefore, it is important to integrate diagnostic methods for better patient management.

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FLOW CYTOMETRY IMMUNOPHENOTYPING OF HEALTHY PLATELETS AND HOSPITALIZED PATIENTS WITH SUSPECTED PLATELET DYSFUNCTION: CHALLENGES FOR ESTABLISHING A CUTOFF VALUE

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Introduction/Objective: Platelets play an essential role in hemostasis, for their characterization, several platelet surface glycoproteins (GP) were identified as GPIIb, evaluated by the expression of CD41, and non-covalently binds to GPIIIa, recognized by the CD61 marker, making up the GPIIb-GPIIIa complex (CD41/CD61). GPIX is identified by CD42a and GPIb by CD42b that interacts together with GPV (CD42d), forming the

other complex, GPIb-IX-V. Flow Cytometry (FC) is one of the techniques which allows the identification and characterization of platelets. The detection of absent or reduced expression of the glycoproteins is the main objective of this technique. Abnormalities of glycoproteins lead to hemorrhagic syndromes. Among the main diseases, Bernard Soulier (BS) and Glanzmann's Thrombasthenia (GT) stand out. BS is an disease caused by several molecular changes that produce altered proteins that would have a fundamental role in the stability of the expression of the von Willebrand receptor GPIb/IX/V complex (CD42a/CD42b/CD42d). GT is an thrombocytopathy caused by deficiency or decrease in the fibrinogen receptor GPIIb-GPIIIa (CD41/CD61). We aimed to show a FC based platelet assessment test for diagnostic use, which measures the expression of markers in normal patients, and evaluate these markers in patients with platelet disorders. **Materials and methods:** We examined a control group of 41 healthy adults to establish reference values and assess the variability of the relative expression of platelet markers and compare to 30 patients with suspected platelet dysfunctions. Were determined the MFI of the expressed parameters by FC using CD41, CD42a, CD42b and CD61, and SSC/FSC platelet gated cells. **Results:** We determined our baseline panel of markers and compared them with suspected platelet dysfunctions. Patients with suspected BS presented increased levels of MFI for GPIIIa (CD61) and GPIIb (CD41). They showed significantly reduced levels of GPIb (CD42b) and GPIX (CD42a). Patients with suspected GT showed normal expression of GPIX (CD42a), increased expression of GPIb (CD42b) and reduced levels of GPIIIa (CD61), showing expression patterns like those described in the literature, which characterizes GT as deficiency or decrease of one of the markers of the GPIIb/GPIIIa complex (CD41/CD61). **Discussion:** We describe the FC assay to support the diagnosis of different platelet disorders. We standardized and implemented the technique in which the expression profile of platelet-associated immunophenotypic markers was determined in a population of hematological normal individuals. It was possible to incorporate the test into laboratory research, to assist in the study of various pathologies associated with platelet dysfunction through the evaluation of the expression of these markers. With the analyzes in our laboratory, it was possible to evaluate 30 patients with suspected platelet dysfunction and contribute to diagnostic elucidation. **Conclusions:** Our study made it possible to implement a technique that brought benefits to care. These analyzes can be implemented in clinical practice to improve diagnosis for patients with suspected platelet disorders.

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CLONAL EVOLUTION IN ACUTE MYELOID LEUKEMIA DETECTED BY FLOW CYTOMETRY

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Acute Myeloid Leukemia (AML) is, in fact, a set of diseases with variable clinical and immunophenotypic presentation, determined by genetic alterations. During treatment, new leukemic clones may arise, or even the dominant clone may undergo genetic and immunophenotypic modifications in the process of clonal evolution. This process is responsible for leukemia relapse and failure to detect measurable residual disease (MRD). Pre-leukemic stem cells detected or not at the time of diagnosis may also undergo clonal evolution and determine the relapse of leukemia. This case report exemplifies these situations. A 61-year-old woman was referred to our hospital after 2 months of treatment for a monocytic AML (30% monoblasts, 18% promomocytes) in a fragile clinical status. She had been treated with 2 cycles of Cytarabine and Daunorubicin (5 +2). The immunophenotypic diagnosis was made at our institution: the monoblasts expressed CD4, CD36, CD56, CD71, CD97, CD99, dim/partial myeloperoxidase, partial CD7, heterogeneous CD13, bright and homogeneous CD15, CD18, CD33, CD38, CD44, CD45, CD64, LLC1 and HLA-DR. Monoblasts did not express CD2, cyCD3, SmCD3, CD10, CD11b, CD14, CD16, CD19, CD22, CD34, CD35, CD45RA, CD54, cyCD79a, CD96, CD105, CD117, CD123, CD244, CD300e, NuTdT, TIM3, NG2. She did not have access to molecular tests. After the 1st and 2nd cycle of chemotherapy, the MRD results were 0.12% and 0.06% respectively, but the blast cell immunophenotype was different from the diagnosis, not expressing CD7, CD15, CD18, CD36, CD56 and CD64, with asynchronous expression of CD13, CD33, CD117 and HLA-DR in CD34 positive cells, with 40% of CD34 positive blast cells without expression of CD38 and with aberrant expression of CD54, characterizing leukemic stem cells (LSC). She underwent 2 consolidation cycles with Cytarabine (1 g 12/12 hours for 3 days) adapted to her clinical status and had febrile neutropenia after the 1st cycle. Immediately before the 2nd consolidation cycle, the myelogram showed morphological remission and undetectable MRD with a limit of detection and quantification of 10^{-5}. One month after the 2nd consolidation cycle, she was admitted with sepsis and overt disease progression assessed by myelogram. Patient not eligible for intensive chemotherapy due to her clinical and nutritional conditions, she received palliative treatment and died. Unfortunately, immunophenotyping was not performed after relapse, but in the MRD monitoring we could observe immunophenotypic changes from differentiated monocytic cells to myeloid precursor cells at a less mature stage of differentiation including LSC, which support the hypothesis of clonal evolution. In conclusion, to detect MRD in AML patients, who potentially may undergo clonal evolution, it is essential to use a comprehensive panel to detect not only the leukemia-associated immunophenotypes (LAIPs) at diagnosis, but also the "non-normal" immunophenotypes acquired during the treatment in addition to the LSC.

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