patients (< 20 years of age). Acute Leukemias (AL) represent a heterogeneous group of diseases that originate from the interaction between genetic and epigenetic alterations in hematopoietic progenitors, leading to the dysregulation of multiple crucial signal transduction pathways, resulting in hematopoietic insufficiency due to a clonal proliferation of immature precursors. Clonal heterogeneity has been implicated as a driving mechanism of leukemogenesis since a high degree of genetic variability is associated with an increased risk of particular subclones having a proliferative advantage leading to clonal expansion. In this context, cytogenetics is still considered one of the most valuable tools for understanding the mechanisms which lead to AL transformation since chromosomal abnormalities are found in 80% of children affected by the disease. In this regard, due to the relative rarity and aggressive potential of childhood AL, the prognostic impact of these abnormalities remains to be elucidated. Thus, by combining cytogenetics and genomic high throughput sequencing (NGS), it may be possible to characterize the genomic profile of pediatric patients with AL, identifying putative genes/gene fusions and genomic variants to help determine the risk stratification and provide appropriate treatment to patients, including adaptations in therapeutic protocols. Herein we describe the cytogenetic and genomic profile of six children with acute leukemia. Firstly, using banding cytogenetics and FISH, patients were sorted for further genomic investigations by NGS. **Material and methods:** We performed G-banding and FISH experiments. NGS was performed on a targeted approach. **Results:** We characterized six (6) pediatric patients diagnosed with acute leukemias presenting with rare cytogenetic abnormalities. The cytogenetic abnormalities studied were associated with genomic pathogenic variants by NGS analysis. We observed variants in IDH1 (2 cases); multi-hit TP53, Li-Fraumei related (1); novel RUNX1 (1); KIT (1); WT1 (1), besides a CBFA2T3-GLIS2 fusion associated with a Philadelphia chromosome. **Discussion:** The prognostic evaluation of acute leukemias at diagnosis is a critical step for proper clinical management. According to the WHO classification, risk stratification is based on cytogenetic and molecular data, morphological characteristics, and mutational screening. In this regard, NGS can be a powerful tool, allowing the parallel sequencing of multiple targets and detecting variants with prognostic impact. NGS became relevant in discovering genes/variants and characterizing the clonal architecture. Thus, due to its capability to screen a high number of genes and detect different types of molecular aberrations, targeted NGS has been increasingly incorporated into the clinical routine for the management of AL patients. **Conclusion:** We highlight the importance of cytogenetic studies combined with NGS in identifying leukemic putative genes and selecting pediatric patients with AL as possible candidates for future targeted therapies. Cytogenetic, molecular, and genomic technologies combined have been shown to help understand the AL mutational landscape and impact clinical practice.

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RELIABLE FLOW CYTOMETRY METHOD FOR MONITORING OF MINIMAL RESIDUAL DISEASE IN PATIENTS WITH B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA AFTER BLINATUMOMAB THERAPY

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Introduction: The recent active use of immunotherapy based on CD19/CD3 bispecific T-cell engager (blinatumomab), brings significant problems on monitoring Minimal Residual Disease (MRD) based on Multiparameter Flow Cytometry (MFC), due to possible CD19 downregulation by tumor cells. This phenomenon breaks the well-established approach of MFC strategy analysis, based gating on CD19 positivity. Also, the selective pressure of the CD19-directed immunotherapy increases the incidence of the so-called lineage switch, which has become one of the possible mechanisms of BCP-ALL resistance to immunotherapy. At present, there is no consensus approach to MFC-MRD monitoring after blinatumomab therapy for BCP-ALL. There are several described changes in MRD methodology, including the addition of other early B-lineage antigens (e.g., CD22, CD24 and CD79a) or only the modification of the gating strategy using conventional sets of antibodies. **Aim:** Evaluated two different antibody panels to monitor MFC-MRD in patients Relapsed/Refractory (R/R) B-cell ALL after blinatumomab therapy. **Materials and methods:** All Bone Marrow (BM) samples the MRD detection was performed with panel EuroFlow BCPALL MRD protocol standardized and added two more tubes (8 colors) with early B-lineage antigens according to previously described in literature (e.g., Tube 1: CD66b/CD22/CD34/CD19/CD24/CD38/CD10/CD45; Tube 2: CD20/CD22/CD34/CD19/CD79a/CD38/CD10/CD45). All BM samples were studied following the EuroFlow SOPs with the acquisition at least $\geq 5 \times 10^6$ events per samples and in both panels. The cytometer FACSCanto 8-color and Infinicyt software was used for acquisition and analyzed data files. The study enrolled real-world children in public health care systems and was approved by the local ethics committees. **Results:** A total of 54 BM samples obtained from 11 children -48% M and 52% F with a median age of 10-years (0–19) with R/R B-cell ALL were studied at different time points after treatment with blinatumomab, including: pré-transplant (n = 24) and post-transplant (n = 30). As controls without use of blinatumomab were include 04 BM from children with B-cell ALL at D15 of treatment with standard chemotherapy protocol. MRD were detected in 35% (19/54) of BM studied and of these 2/11 relapsed. We found 100% agreement between the two panels used. Only one sample was discordant at first time, but upon review it was noted that this CD19 negative population were very early CD19- normal B cell progenitors. **Discussion:** The wide implementation of CD19-directed therapy in the treatment in protocol for R/R BCP-ALL significantly complicated the rather routine procedure of MRD monitoring. Despite the

subjective impressions by these investigators that other B-cell antigens such as CD22, CD24 and cyCD79a brings more confidence to analyze B-cell ALL MRD samples from patients treated with anti-CD19 therapy, for this studied cohort, we did not find difference on the results of MRD with the standard EuroFlow panel and the alternative panel. **Conclusion:** No difference was found on the MRD detection with the standard EUFOLOW panel and the alternative panel. Although introduction of CD22, and CD24 markers, with 8 colors strategy, it was not advantageous. Using 8 colors strategy, it may be recommended to investigate the comparative financial impact of the EuroFlow, and the alternative panel in the context of Blinatumomab therapy.

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LEUCEMIA AGUDA INDIFERENCIADA

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Introdução: A Leucemia Aguda Indiferenciada (LAI) é caracterizada pela proliferação clonal de células hematopoiéticas primitivas, por definição, não possui marcadores específicos de linhagem linfoide ou mieloide sendo necessário excluir leucemias de linhagens incomuns como as de células dendríticas plasmo citoides, células NK, basófilos ou mesmo tumores não hematopoiéticos. Portanto o diagnóstico de LAI pode ser complexo e requerer outras análises além da imuno fenotipagem convencional. Dada sua raridade, pouco se sabe sobre sua incidência, sobrevida e manejo ideal. Os pacientes apresentam leucocitose, anemia, contagem variável de plaquetas e uma variedade de sintomas inespecíficos relacionados à hematopoese ineficaz (fadiga, sangramento e hematomas, infecções recorrentes, dor óssea) e/ou envolvimento de locais extramedulares (linfadenopatia, esplenomegalia, hepatomegalia). **Relato de caso:** Paciente masculino, 7 anos, astenia, 4 dias com febrícula, perda ponderal de 2 Kg. Hemograma externo: Hb: 7,4g/dL; VCM: 77,8fL (2% eritroblastos). Leuco: 4.000 uL; Neutro: 1.080 uL; Plaquetas: 13.000 uL. Exame Físico: Presença de linfonodos palpáveis em cadeia cervical anterior, posterior e supraclaviculares, móveis, fibroelásticos, < 1 cm, baço a 4 cm da borda costal esquerda; fígado a 6 cm da borda costal direita. Exames na admissão: Hb: 10,0 g/dL; VCM 80 fL; Leuco: 4.320 uL; Linf.: 1.666 mm^3; Neutro: 648 uL; Blastos: 58,0%; Plt: 38.000 uL. Ferritina: 431 ng/mL; LDH: 269U/L; VHS: 50 mm. Medula óssea com celularidade diminuída, com presença de 55% de blastos de difícil diferenciação morfológica. Alguns blastos de tamanho pequeno e formato regular; núcleo ocupando quase todo o citoplasma, de cromatina frouxa e heterogênea sem nucléolo evidente; outros de tamanho mediano, formato regular; núcleo de formato irregular com cromatina frouxa e heterogênea por vezes com um nucléolo evidente; citoplasma escasso levemente basofílico e finamente granular. A Citometria de Fluxo mostrou no aspirado da MO 58,8% de blastos e na biópsia 67,0%. Positividade para: TdT-/+ nuclear (31,0%), CD15-/+ (58,0%), CD19+/++ heterogêneo, CD24+/++, (50,5%), CD33-/+ (26,6%), CD38++, CD45+/++, CD66c+/++, CD71-/+ (34,0%), CD123-/+ (40,0%), HLA-DR+/++ heterogêneo, NG2 (proteína 7,1)+/++ e negatividade para antígenos citoplasmáticos MPO, CD79, CD22, CD3, IgM e antígenos de membrana: CD3, CD7, CD10, CD11b, CD11c, CD13, CD16, CD22, CD34, CD35, CD36, CD41, CD42a, CD42b, CD56, CD61, CD64, CD105, CD117, IgM, IREM-2. Citogenética: 47, XY, +14/46, XY. Molecular: KMT2A-AFF1 por PCR Inconclusiva. ETV6-RUNX1: Negativo. FISH: KMT2A-AFF1 positivo, BCR:ABL negativo. As alterações cromossomo 10, 17,4 negativas. LCR sem infiltração. O paciente iniciou tratamento com protocolo GBTLI 2021, alto risco, e a Pesquisa de DRM D19 e D49 foram negativas. Técnica lise total Euroflow Positividade: CDD19, CD20, CD22, CD45, CD81, HLA-DR. Negatividade: CD10, CD15, CD34, CD38, CD66c/CD123, NG2 (proteína 7,1). LOD (*limit of detection*): 0,00086%. LLOQ (*lower limit of quantification*): 0,0021% e LOD: 0,0017% LLOQ: 0,0045% respectivamente. **Conclusão:** : Descrevemos um caso de leucemia aguda sem marcadores definidoras de linhagem. O prognóstico da AUL é geralmente ruim, mas tem melhorado ao longo do tempo. A imuno fenotipagem por Citometria de Fluxo Multiparamétrica (CFM) tornou-se uma ferramenta importante no manejo clínico das neoplasias hematológicas, tanto para fins de diagnóstico quanto de monitoramento (Doença Residual Mensurável – DRM).

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PÚRPURA TROMBOCITOPÊNICA TROMBÓTICA: DESAFIO DIAGNÓSTICO NA PEDIATRIA

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Introdução: A incidência mundial da Purpura Trombocitopênica Trombótica (PTT) é de 3 casos/1.000.000 adultos/ano, sendo ainda mais raro em crianças. A PTT é mais prevalente em mulheres, com média de idade de 40 anos e sua mortalidade é alta se não identificada e tratada precocemente. **Objetivos:** Relatar um caso de PTT em paciente jovem, com faixa etária atípica para esta doença, demonstrando a importância do diagnóstico precoce para a melhora no desfecho clínico. **Relato do caso:** Paciente de 13 anos, sexo feminino, previamente hígida, foi trazida ao pronto socorro devido à crise convulsiva tônico clônica. Na história pregressa, paciente com relato de febre, cefaleia holocraniana de forte intensidade, petéquias e equimoses em tronco e membros inferiores há 5 dias, com evolução para hemiparestesia a direta, seguida de hemiplegia e afasia. Inicialmente, pelo quadro neurológico agudo e febril, realizado suspeita de meningoencefalite e introduzida antibioticoterapia. Contudo, exames admissionais não foram sugestivos de infecção, evidenciando anemia e plaquetopenia de 11 mil. Solicitada então investigação com equipe de hematologia pediátrica. Paciente com Coombs