minimal residual disease (MRD). Therefore, the recovery of ccfDNA in sufficient quantity and adequate purity is crucial to provide accurate and reproducible results. In this study, we investigate the efficiency of four different commercial kits to recover short fragments of DNA compatible with the ccfDNA. **Methods:** Ten mL of total peripheral blood from seven patients with Diffuse Large B-Cell Lymphoma (DLBCL) were collected in EDTA and were immediately processed. Plasma was obtained by centrifugation at 1.900 x g for 10 min at 4 °C, followed by other centrifugation at 20.000 x g for 10 min at 4 ° C to remove cellular debris. Aliquots of 1.0 mL of plasma were stored at -80 °C until ccfDNA extraction. The ccfDNA extraction was performed using the same samples by the following kits: QIAamp MinElute ccfDNA kit (CFDNA - Qiagen, Hilden, Germany), QIAamp DNA Mini Kit (QBLOOD - Qiagen, Hilden, Germany), Illustra Blood GenomicPrep Mini Spin Kit (GE - GE Healthcare Bio-Sciences Corp, UK) and ccfDNA isolation kit Quick-ccfDNA Serum & Plasma Kit (ZYMO - Zymo Research, Irvine, USA) according to the manufacturer's recommendations. The ccfDNA obtained was eluted in 30 μL of elution buffer for the subsequent experiments. The measurement of the ccfDNA obtained for each kit was performed in a Qubit 2.0 fluorometer using Qubit dsDNA HS assay kit (Thermo Fischer Scientific, Waltham, USA). The analysis of integrity, purity and the size of the fragments of DNA were performed in the Agilent 2100 Bioanalyzer (Agilent Technologies, Germany) equipment using the High Sensitivity DNA kit (Agilent Technologies, Germany). **Results:** The results obtained for each commercial kit in the Qubit-analysis were: *CFDNA* (0.27 ng/uL), *QBLOO* D (0.26 ng/uL), *ZYMO* (0.03 ng/uL) and *GE* (0.02 ng/uL). The Bionalyzer eletrophoresis showed that *CFDNA* kit was able to recover two different fragments sizes of ccfDNA one of 360–380 bp (0.114 ng/uL) and other of 160-180 bp (1.808 ng/uL). The DNA recovered with *QBLOOD* revealed sizes of 360-380 bp (0.031 ng/uL) and 160-180 bp (0.079 ng/uL). Both kits, *GE* and *ZYMO* were not able to isolate fragments of DNA smaller than 10.380 pb. **Conclusion:** We demonstrated that *QIAamp MinElute ccfDNA* kit (*CFDNA*) was capable to recovery high quantity of small fragments of DNA compatible with ccfDNA. This result supports the use of protocols based in magnetic beads in experiments working with ccfDNA. Additionally, we highlight the importance to verify the quality and not only the quantity of the DNA extracted to confirm the presence of ccfDNA.

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COEXISTENCE OF ACUTE MONOBLASTIC LEUKEMIA IN A CHRONIC LYMPHOCYTIC LEUCEMIA: CASE REPORT

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Background: Chronic lymphocytic leukemia (CLL) is associated with an increased risk of other malignancies, such as skin and. second hematological malignancies. In the majority of cases, this usually involves a disease transformation to an aggressive non-Hodgkin lymphoma, multiple myeloma or prolymphocytic lymphoma. CLL patients can rarely develop acute myeloid leukemia (AML). We have reported a case in which there was simultaneous presentation of AML and CLL. **Case report:** A 78-year-old man presented with tiredness and mucosal bleeding. Laboratory evaluation revealed a white blood count of 220,970 μ/l with 4% of blasts,69% lymphocytes and 20% monocytes, hemoglobin of 6.7 g/dl, platelet count of 29,000 μ/l. A bone marrow aspirate revealed markedly hypercellular bone marrow with 21% blasts and promonocytes and 47% mature lymphocytes. A peripheral blood flow cytometry demonstrated a mature CD5 and CD23 positive B-cell population expressing CD19, low-density CD 20, CD11c, CD25, CD81, CD39 and lambda light chains at 53.02% of lymphocytes, consistent with B-CLL.; In addition, a second population showed CD33, CD13 low-density, CD11b, CD64, CD14, CD36, HLA-DR, CD56, CD123, CD11c, CD38 and CD9 positives in 39.35% of immature monocytes, consistent with acute monoblastic leukemia. Cytogenetics on bone marrow aspirate showed 48,XY, +4,+8[18] / 46,XY [2]. **Conclusion:** The present report highlights the rare possibility of the development of a myeloid malignancy in a patient with lymphoid malignancy. Most of these cases more frequently present as a secondary event in patients receiving chemotherapeutic agents for CLL.Knowledge of this rare association is the key to timely and accurate diagnosis, particularly in patients with atypical presentation. Existing literature demonstrates that AML may also develop concurrently or even after the diagnosis of treatment-naïve CLL patients. Further studies of such patients have potential to provide insight into the pathogenesis of myeloid as well as lymphoid malignancies.

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COMPARAÇÃO ENTRE DUAS DIFERENTES CALCULADORAS ONLINE DE FARMACOCINÉTICA (WAPPS-HEMO VERSUS MYPKFIT)

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Introdução: A farmacocinética (PK) de fatores FVIII e FIX é essencial para a individualização da profilaxia em pacientes hemofílicos. O cálculo da meia-vida e do tempo superior à 1% são estimados por diferentes programas de farmacocinéticas, onde são introduzidos algoritmos matemáticos e podem ser utilizados para várias drogas. Nos últimos anos foram desenvolvidos softwares que realizam uma modelagem multicompartimental de uma derivação Bayesiana para estimativa de dados. **Objetivo:** Avaliar o desempenho e comparar curvas farmacocinéticas utilizando duas calculadoras diferentes, disponibilizadas na Web, para determinar a meia-vida e o tempo superior a 1% do Fator VIII recombinante (rFVIII)

administrado, seguindo o modelo Bayesiano de 2 amostras. **Métodos:** Foram comparados dados de meia-vida e o tempo estimado que o rFVIII demora para chegar à 1%, obtidos pelo Web Accessible Population Pharmacokinetic Service – Hemophilia (WAPPS-HEMO), versão 4.1 e myPKFit versão 3.1, de 53 pacientes com Hemofilia A grave, sem inibidor em profilaxia. Desse total, 75,5% tinham entre 12 a 55 anos e 24,5% entre 4 e 11 anos. Todos assinaram o Termo de Consentimento Livre Esclarecido (TCLE). Foram infundidos em média 25 UI/Kg de rFVIII e as amostras coletadas nos tempos de 3 e 24h. Os ensaios de FVIII e cálculos foram realizados em um Laboratório Central onde foi utilizado o ensaio coagulométrico de um estágio e os níveis de FVIII foram expressos como atividade coagulante do FVIII. **Resultados:** As médias e medianas das estimativas de meia-vida do rFVIII administrado obtidas pelo myPKFit foram Me=10,57 e Md=10,55, enquanto no cálculo equilibrado do WAPPS-HEMO foi Me=10,34 e Md=10,0. Em relação ao tempo estimado para o rFVIII administrado chegar à concentração de 1% no plasma, as médias e medianas das estimativas foram: myPKFit Me = 56,43h e Md = 55,5h, enquanto no cálculo conservador do WAPPS-HEMO a Me = 53,63h e Md = 52,5h. O teste de múltiplas comparações de Dunn's atribuiu o valor de p = 0,8961 (adultos) e 0,9703 (crianças) para a meia-vida e p = 0,0911 (adultos) e > 0,9999 (crianças), para o tempo superior a 1%, indicando não haver diferenças significantes entre as plataformas. Já a comparação para as demais estimativas do WAPPS-HEMO (otimista e equilibrado) foram p<0,0001, demonstrando uma diferença significante. **Discussão:** Esse estudo padronizou a dose administrada, horários de coleta em hemofilia A grave. Resultados divergentes interindividuais não foram comparados entre si e estão relacionados com parâmetros já bem estudados na literatura, como o volume de distribuição, taxas de clearance, concentração do fator de von Willebrand, idade e peso. Resultados relacionados à comparação cruzada entre essas duas ferramentas foram estudados anteriormente, entretanto, não foram diretamente relacionadas entre si, e apontaram uma diferença importante entre as calculadoras. Não foram observados diferença estatisticamente significante, quando comparados os dados de meia-vida pelo myPKFit vs WAPPS-HEMO estimativa equilibrada e no tempo superior a 1% pelo myPKFit vs WAPPS-HEMO estimativa conservadora. **Conclusão:** A escolha da ferramenta é importante no desenho da profilaxia e a critério clínico. As plataformas apresentam resultados semelhantes considerando a meia-vida equilibrada do WAPPS-HEMO e a estimativa conservadora para o tempo superior a 1%.

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COMPARISON BETWEEN MULTIPLEX LIGATION-DEPENDENT PROBE AMPLIFICATION (MLPA) AND CYTOGENETICS FOR VALIDATION OF CHROMOSOMAL ABERRATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Objective: Recently, a multiplex ligation-dependent probe amplification (MLPA®) has emerged as a method that is reported to be fast, sensitive, and cost-effective that can detect copy number variation. In the present study, MLPA analysis was compared with chromosome banding analysis (CBA) data to determine the efficiency of MLPA in patients with chronic lymphocytic leukemia (CLL). **Materials and methods:** CBA karyotyped 65 patients. Chromosomal analysis was performed on G-banded metaphase cells from cultured lymphocytes in a culture medium at 37°C for 72 hours. Numerical, as well as structural abnormalities were reported on at least 20 well-spread and well-banded metaphases. Genomic DNA was extracted from peripheral blood samples and subjected to MLPA through the probemix, kit SALSA®MLPA®P037 containing 54 MLPA probes, including 41 probes for 2p, 6q 8p/q, 9p21, 11q, 12p/q, 13q, and 17p chromosomal regions. The PCR products were separated by capillary electrophoresis using an ABI 3500 DNA analyzer. **Results:** Cytogenetics resulted in 41 normal karyotypes, and 25 karyotype aberrations (13 tris12; 3 del13; 2 del11; 4 del17; others 3). Genetic abnormalities observed by MLPA were 45 (14 tris12; 26 del13q; 2 del11q; 3 TP53 deletion), and 22 were normal by MLPA. Of the 41 normal cytogenetics, MLPA was concordant in 20 patients and discordant in 21 patients. Of those 21, MLPA identified 19 del13q, 1 delq11, and 1 tris12. Regarding abnormalities, cytogenetics and MLPA were concordant in 12 tris12, 2 del13, 3 del17, and 1 del11. Statistical difference was seen when comparing both methodologies in favor of MLPA (p= 0.003; chi-square test). **Discussion and conclusion:** In this study, we aimed to determine the usefulness of the MLPA probemix P037-B1 as a routine for detecting clinically relevant chromosome abnormalities in CLL in laboratories without cytogenetic routine. MLPA identified more abnormalities than cytogenetic (44%). We observed that MLPA was consistent with the cytogenetic results in 20 of 41 normal patients (48%). The main discordant results identified by MLPA were 13q deletion, the most common cytogenetic change in chronic lymphoblastic leukemia (CLL). In one patient, results of the MLPA did not show deletion in the TP53 gene, while isochromosome 17 was observed by cytogenetic. A low proportion of malignant cells with the relevant abnormality could be responsible for the discrepancies in the MLPA technique. Besides that pitfall, our findings highlight the advantage of MLPA compared with conventional cytogenetics. In