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VISÃO PANORÂMICA DAS APLICAÇÕES CLÍNICAS DO TESTE DE GERAÇÃO DE TROMBINA PELO MÉTODO CAT

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Objetivo: Realizar uma revisão narrativa sobre o uso da técnica de geração de trombina (TGT) pelo método CAT (*Calibrated Automated Thrombogram*) para dar um panorama geral da sua utilização pelo mundo. **Materiais e métodos:** A busca foi realizada na base de dados PubMed utilizando os seguintes termos: *calibrated automated thrombogram; calibrated automated thrombogram assay; calibrated automated thrombography, calibrated automated thrombin generation*. Derivou-se então 407 artigos, os quais foram transferidos à plataforma Rayyan, onde passaram por uma seleção em que foram excluídos, com base na leitura do título e resumo: estudos com experimentos em animais, estudos de padronização e validação do TGT e revisões narrativas e sistemáticas. **Resultados e discussão:** Os temas mais recorrentes e descritos neste trabalho foram o uso no TGT na avaliação da trombose associada ao câncer, na pediatria (neonatos e crianças), nas hemofilias, nas doenças tromboembólicas e uso de medicamentos. A maioria dos estudos abordados neste trabalho correlacionam positivamente os parâmetros gerados pelo método CAT e o diagnóstico ou prognóstico dos pacientes em questão. Os principais parâmetros que mostraram associação com a clínica foram *lagtime* (corresponde ao período entre a adição dos reagentes disparadores e o início da produção de trombina), *time-to-peak* (tempo necessário para chegar ao máximo de produção de trombina), *peak* (concentração máxima de trombina produzida na fase de amplificação/propagação) e ETP (potencial de trombina endógena, que corresponde a quantidade total de trombina produzida). Em condições hipocoagulantes foram encontrados menores valores de ETP e *peak* e maiores valores de *lagtime* e *time-to-peak*. Já em condições hipercoagulantes foram encontrados maiores valores de ETP e *peak* e menores valores de *lagtime* e *time-to-peak*. Testes para avaliação da hemostasia têm grande importância no diagnóstico e prognóstico de diversas doenças, sejam elas hematológicas ou não. Nesse sentido, o TGT pelo método CAT apresentou-se como um teste capaz de analisar a GT de forma global. A capacidade deste método de sofrer variações de acordo com as condições de reação é um grande diferencial já que determinam quais vias pró e anticoagulantes irão contribuir para o resultado do ensaio. **Conclusão:** Pode-se observar que o TGT pelo método CAT tem aplicabilidade clínica no manejo de pacientes com desequilíbrio hemostático (hipo e hipercoagulabilidade), tanto no diagnóstico quanto no prognóstico. Entretanto, apesar de permitir uma avaliação mais ampla da hemostasia, esse método tem como principal desvantagem sua variabilidade, o que implica em resultados inconsistentes e algumas vezes incomparáveis. Essa inconsistência é consequência das possibilidades de manejo deste método, ou seja, as concentrações de fator tecidual, fosfolipídios, bem como o uso de inibidores da via intrínseca, condições ambientais e de coleta como por exemplo, a temperatura, transporte e tempo de punção, também causam interferência. Dessa forma, esse método ainda está muito concentrado em centros de pesquisas em países como Suécia, Áustria, Países Baixos, França, Alemanha, Irlanda, Reino Unido, Bélgica, Itália, Estados Unidos, Canadá, México, Malta, Noruega, Austrália, Turquia, Coreia do Sul, Finlândia, Brasil, Arábia Saudita, Grécia. Contudo, com os constantes avanços na padronização deste teste, a presença desta ferramenta na rotina de mais laboratórios torna-se iminente.

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CITOMETRIA DE FLUXO

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CD26+ LEUKEMIC STEM CELLS IDENTIFICATION AS A TOOL FOR CHRONIC MYELOID LEUKEMIA DIAGNOSIS

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Background: Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by proliferation of immature myeloid cells maintaining their capacity to differentiate. The increase of myeloid precursors is due to an acquired genetic alteration of the hematopoetic stem cells that behave as leukemic stem cells (LSC). It is characterized by the chromosomal translocation t(9;22)(q34.1;q11.2), which results in the formation of the Philadelphia chromosome, containing the BCR-ABL1 fusion gene. The diagnosis must be confirmed by cytogenetic analysis and RT-qPCR. In 2019, Raspadori et al. described a flow cytometry protocol for CML diagnosis and identified the expression of CD26 as a marker for CML LSC in peripheral blood and bone marrow. **Aim:** To compare a flow cytometry protocol for CML investigation with the gold standard diagnostic method BCR-ABL PCR assay. **Methods:** Peripheral blood and bone marrow samples received at Sabin Medicina Diagnostica lab between April 2019 and January 2020 for CML investigation with medical order for flow cytometry and PCR for BCR-ABL assays were retrospectively analyzed. For flow cytometry, samples were processed according to protocol previously described by Raspadori et al. and stained with the following anti-human monoclonal antibodies: HLA-DR-FITC (G46-6), CD123-PE (9F5), CD34-PerCP-Cy5.5 (8G12), CD117PE-Cy7 (104D2), CD38-APC-H7(HB7), CD33 BV421 (WM53), CD45-V500 (2D1) from BD Biosciences and CD26-APC (BA5b) from Exbio. Acquisition was performed on a 3-lasers, 8-colors FACSCanto^{TM}II flow cytometer (BD Bioscence). Analysis and quantification of CML LSC (CD34+/CD26+/CD38-) was performed using Infinicyt (Cytognos). BCR-ABL1 was identified by an in house routine one-step RT-qPCR using $\Delta\Delta$Cq method. The buffy coats were removed from EDTA-whole blood (8 mL)

or bone marrow samples (4mL), nucleic acids were stabilized and then extracted using Magna 96 (Roche). A one-step RT-qPCR QuantiNova Probe master mix (Qiagen) with primers and probes described by Gabert et al. 2003 (EAC) and by Pane et al. 1996 were used. BCR-ABL1 and ABL RNAs were co-amplified at Roche LightCycler 480II for e14a2/e13a2 (p210), e1a2 (p190), and e19a2 (p230) fusions. The degree of agreement between the test methods (flow cytometry) and the comparative methods (PCR for BCR-ABL) was quantified using Kappa statistics with three categories. **Results:** In this period, 21 samples from different patients were received at Sabin Medicina Diagnostica lab for CML investigation and medical order for flow cytometry and BCR-ABL assays. 10 samples were from peripheral blood and 11 from bone marrow. In 10 samples (3 bone marrows and 7 peripheral blood), flow cytometry assay did not show a CD26+ CML LSC population, and BCR/ABL PCR assay resulted negative. In 11 samples (8 bone marrows and 3 peripheral blood), a CD26+ CML LSC population was identified by flow cytometry and BCR-ABL PCR assay resulted positive. There were no discordant results. The degree of agreement between the test methods (flow cytometry) and the comparative methods (PCR for BCR-ABL) was a perfect agreement (kappa=1). **Conclusions:** In conclusion, our data are in accordance with the results previously described by Raspadori et al. Although we still believe that further studies are necessary, the identification of a CD26+ CML LSC population by flow cytometry may be a diagnostic tool for CML when a BCR-ABL PCR assay is not available.

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CYTOLOGICAL AND MULTIPARAMETRIC FLOW CYTOMETRY ANALYSIS FOR DIAGNOSTIC OF BREAST IMPLANT-ASSOCIATED ANAPLASTIC LARGE CELL LYMPHOMA

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Background: Breast implant-associated anaplastic large cell lymphoma (BIA-ALCL) is a provisional entity with morphological and immunophenotypic characteristics indistinguishable from anaplastic large cell lymphoma (ALCL), as hallmark morphology and CD30 positivity. However, unlike ALCL, BIA-ALCL often presents as unilateral effusion associated to silicone breast implants. Diagnostic confirmation of BIA-ALCL can be difficult. In this setting, multiparametric flow cytometry (MFC) looking for CD30, HLA-DR and CD25 positivity may be a good option for help in diagnostic assistance. **Objective:** To describe cytological and flow cytometric founds of patients with suspected periprosthetic fluid and compare confirmed BIA-ALCL to negative patients. Methods: From mar/2018 and Jul/2020, all periprosthetic fluid (PF) collection sent to our lab to cytology and MFC analysis to quantification and characterization of pathologic, T and B cells were included. All specimens were collected in dry tubes and sent immediately to the lab. A cytospin was prepared and cored with Wright-Giemsa staining for morphological evaluation. A total of 100 uL of the concentrated cells were stained with CD4-V450, CD45-V500, HLA-DR-FITC, CD30-PE, 7AAD, CD19-PE-Cy7, CD14+CD3-APC, CD8-APCH7 and Lymphocyte Screening Tube (Euroflow®). For each sample, 100,000 cells were acquired using FACSCanto-II cytometer and data were analyzed with Infinicyt(tm) software. Positive cases were submitted to a confirmation tube with HLA-DR-V450, CD45-V500, CD45RO-FITC, CD25-PE, CD5-PerCPcy5.5, CD2-PE-Cy7, CD14-APC, CD43-APCH7. Cases with less than 1000 cellular events available in flow cytometry acquisition were considered unavailable. **Results:** 83 PF collection from 77 patients were analysed in 28 months. Five patients had bilateral breast collection and one patient repeated the evaluation 2 weeks after first analysis. Median age was 50 years (31–57 years). We found seven positive cases (9.1% of patients); in one of them, the first sample was considered unavailable. Thus, the MFC sensitivity was 85.7% and specificity 100% in our cohort. From 76 negative samples, 9 (11.8%) were considered unavailable cause of lack of viable cells, 7 (9.2%) were blood contaminated, 11 had neutrophilic exudate (14.5%) and 49 (64.5%) had transudates with a predominance of mature lymphocytes. Cytological examination of all seven positive cases revealed numerous large, anaplastic cells with pleomorphic nuclei, prominent nucleoli, and moderate basophilic cytoplasm with frequent vacuoles. MFC immunophenotyping showed large tumor cells (increased FSC/SSC scatter) with bright expression of CD30, CD25 and HLA-DR, $CD45^{dim}$ and absence of monocytic, B and NK cell antigens (CD14, CD19, CD20, CD38, CD56 and light chain expression). All had absence of CD3, five cases had CD4 heterogeneous expression, one had weak CD8, and one had CD5 dim. In negative cases available, scant or rare CD30 positive lymphocytes with normal morphology was considered reactive and corresponded to activated T cells. Furthermore, when we compared BIA-ALCL and normal cases, we detected a significant MFI difference, with overexpression of CD30, HLA-DR and CD25 and dim expression for T cell markers in tumor cells compared with normal samples. **Conclusion:** Here we describe seven patients with BIA-ALCL and could highlight the utility of cytologic evaluation and multiparametric flow cytometry immunophenotyping in diagnostic workup.

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DESENVOLVENDO UM ALGORITMO SIMPLES BASEADO EM CITOMETRIA DE FLUXO MULTIPARAMÉTRICA PARA RASTREAMENTO RÁPIDO DE LEUCEMIA PROMIELOCÍTICA AGUDA

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