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803.EMERGING TOOLS, TECHNIQUES AND ARTIFICIAL INTELLIGENCE IN HEMATOLOGY

One High Efficient Detection Panel of Multiparamer Flow Cytometry for Non-Hematopoietic Tumors

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Background: In the past half century, flow cytometry has been successfully applied to detection of hematopoietic tumors. In fact, the incidence rate of non-hematopoietic tumors is higher than hematologic malignancies. However, the application of flow cytometry has been limited for a long time due to samples, lack of specific markers and other reasons. With the development of immunological technique, the detection of solid tumors by flow cytometry has attracted renewed attention. We designed a highly sensitive and specific assay panel for multiparameter flow cytometry(MFC) screening, diagnosis and follow-up of non-hematopoietic tumors.
Aims:find a highly efficient panel of MFC for screening, diagnosis and follow-up of non-hematopoietic tumors.
methods:
From Aug. 1st, 2008 to Mar. 31st, 2022, 858 samples from patients with non-hematopoietic tumor were detected in the flow cytometry lab of Hebei Yanda Lu Daopei Hospital, of which 617 samples were BM, 3 PB, 196 cerebrospinal fluid(CSF), 9 Pleural effusion or ascites, 3 lymph node, and 30 fine needle aspiration(FNA) from various tissue. In these 850 person, 476 were males, 374 females, the median age was 4 years old (0-80 years), and the median percentage of tumor cells in the sample was 0.16% (0.01%-96.23%).After rounds of optimization, CD9 FITC/GD2 PE/CD3 percp/CD4 pecy7/CD56 APC/CD36 Apccy7/CD81 BV421/CD45 V500, cytokeratin(CK) or CD326 FITC/HLA-ABC PE/CD38 percp/CD19 pecy7/CD56 APC/CD36 Apccy7/CD7 BV421/CD45V500 were selected as the final detection panel. The analysis was performed using 3 laser 8-10 color canto flow cytometers, and diva/flowjo software. The results were compared with pathological and clinical diagnosis to evaluate the sensitivity and specificity.
Results:
Firstly, Sensitivity and specificity analysis. Of 858 samples, 567 were positive, of which 442 were BM, 2 PB, 84 CSF, 9 fluid, 2 lymph nodes, and 28 FNA. The coincidence rate was more than 90% with pathology, other laboratory examinations and clinical diagnosis. It covers most of tumors nominated by WHO diagnostic criteria. The coverage and specificity were more than 99%, and the sensitivity was 0.01%. Both false positive rate and false negative rate were less than 1%. Secondly, the selection and verification of immunological markers. The positive rate and specificity of each immunological markers were analyzed in 567 positive samples, as shown in Figure 1. The specificity of CK combined with GD2 in the diagnosis of non-hematopoietic tumors was almost 100%, and the positive rate of CD56 was significantly different between epithelial and non-epithelial tumors. CD3/CD19/CD4/CD38/HLA-ABC/CD36/CD7 is almost not expressed in non-hematopoietic tumors but expressed at least one by almost all hematopoietic cells, which can exclude hematological malignancies. CD9/CD81, as a sensitive but non-specific marker, can assist in judgment, and combined with CD45-/CD36-, exclude non-specific staining to prevent missed diagnosis. Thirdly, tumor sources can be roughly distinguished. PCA (Principal component analysis) can accurately classify epithelial and non-epithelial tumors by analyzing 38 cases of neuroblastoma and 5 cases of epithelial-derived tumors. Flowsom cluster analysis of some common solid tumors in children showed that the expression intensity of different tumor markers was different. As shown in Figure 2.
Conclusion:
Although the high heterogeneity of non-hematopoietic tumors and the lack of specific immune markers, we found one high sensitive and specific MFC panel for detection of solid tumors after rounds of trials. The panel includes some non-lineage specific markers but commonly expressed in hematologic neoplasms and rarely in non-hematopoietic tumors. Different from the precise classification of euroflow protocol, our panel focuses on the identification of tumor cells, and exclusion of hematological malignancies and non-specific staining. It is an important detection and analysis panel with high coverage, strong practicability, high sensitivity and specificity, which may reduce the rate of missed diagnosis.

Disclosures No relevant conflicts of interest to declare.

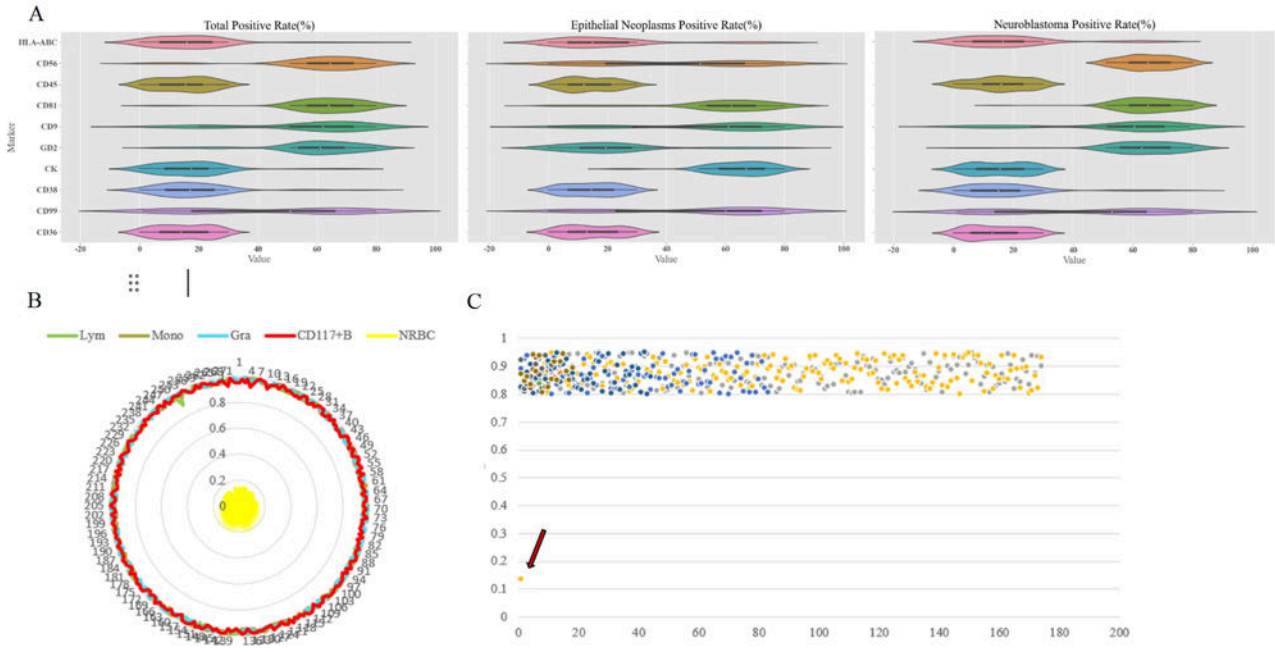

Figure 1: Analysis of the expression rate and differential efficiency of each immunological marker in 567 positive samples. A 、 The expression rate of each marker in the total positive sample, epithelium-derived tumors and neuroblastoma. B 、 In order to analyze the differential efficiency of HLA-ABC between hematopoietic cells and non-hematopoietic cells, 272 non-blood disease samples were selected to analyze the expression of HLA-ABC in normal hematopoietic cells. Except for nuclear red cells, all normal hematopoietic cells expressed HLA-ABC. C 、 The expression of HLA-ABC was analyzed in 869 cases (including ALL - B/ALL - T/AML/MPAL/MPN/MDS/MPN/MM/NHL - B/NHL - T/NHL - NK) of hematopoietic tumors, but only one case of AML tumor cells lost expression of HLA-ABC.

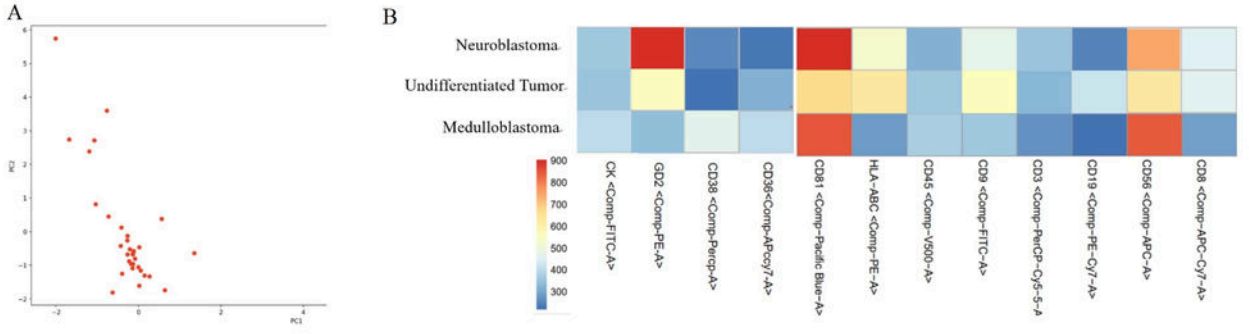

Figure 2: The panel could make a general distinction between epithelial and non-epithelial tumors. A. First principal component (PC1) versus second principal component (PC2) bivariate plots of the non-hematolympoid tube show discrimination between epithelium-derived tumors and neuroblastoma. B、Heat map summarizing the intensity and pattern of expression of different markers in distinct diagnostic subtypes of pediatric solid tumors based on mean fluorescent intensity per/cell level.

Figure 1

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