Application of Flow Cytometry in the Diagnosis of Primary Immunodeficiency Disease (PID): King Hussein Medical Center Experience

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ABSTRACT

Objective: To highlight some of the significant applications of flow cytometric immunophenotyping in the diagnosis of Primary Immunodeficiency Disease.

Methods: We reviewed the medical records of 135 consecutive patients who were referred to the Immunology Clinic at King Hussein Medical Center with a flow cytometry based diagnosis of Primary Immunodeficiency Disease between January 2000 to August 2009.

Results: The medical records of 135 patients with history of recurrent or persistent infections were reviewed. Seventy seven (57%) patients were males and 58(43%) were females. They aged between 2 and 120 months with a mean age of 13 months. Flow cytomerty-based diagnosis was identified in 68 (50.3%) patients. Predominant antibody deficiency was diagnosed in 14 (10.3%) patients. There were 35(26%) patients with T and B cell immunodeficiency. There were 6 patients' satisfied diagnostic criteria of possible HyperIgM Immunodeficiency syndrome. Diagnosis of severe combined immunodeficiency was retrieved in 22(16.2%) patients. Primary phagocytic disorder was the diagnosis in 34 (25%) patients. Dihydrorhodamine flow cytomerty-based burst test was confirmatory for Chronic Granulomatous Diseases in one patient while in the other 14 patients diagnosis was based on nitroblue tetrazoleoum test and genetic mutation study. There were 8 (6%) patients with other well defined immunodeficiency syndromes; one patient with Wiskott Aldrich Syndrome, 5 patients with Ataxia Telangectasia, one with Bloom syndrome, and one with DiGeorge anomaly. Eight (6%) patients were found to have an immunedysregulation syndrome. There were 8(6%) patients with an undefined primary immunodeficiency. Post Bone marrow transplantation Immunereconstitution of T-, B-cells and Leukocyte adhesion molecules were identified in 14 patients with appropriate Flow cytomerty immunophenotyping assay.

Conclusion: Flow cytometric immunophenotyping of leucocytes appears to be an efficient and rapid tool in the diagnosis and follow-up of immunodeficient patients, supporting early recognition, which is reflected on reduced morbidity and improved survival

Key words: Flow cytometry, Immunodeficiency, King Hussein Medical Center

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Introduction

Primary immunodeficiency diseases (PID) are a heterogeneous group of disorders defined by defects in genes involved in host defense.[1] More than 150 different PID currently recognized by the World Health Organization.[2] Accurate diagnosis and

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classification of PID are necessary to decide on appropriate clinical management, to enable informed genetic counseling and to permit the systematic collection of data on PID through registries.[1] It was since the last 3 decades when flow cytometry has emerged as an invaluable technology contributing significantly to the understanding and evaluation of the immune system.[3] Clinicians can more clearly define defects and better understand the cellular responses in immunodeficiency diseases and determine the effects of therapy on these patients.[4] The range of PIDs in which flow cytometry has proven to be useful from a clinical and diagnostic purpose has significantly expanded. This now includes not only patients presenting with clinical histories consistent with classical antibody deficiencies and severe combined immune deficiency, but also patients with more limited infectious histories.[5]

This study was conducted to highlight some of the significant applications of flow cytometric immunophenotyping in the diagnosis of primary immunodeficiency in Jordanian population, which otherwise may be difficult to diagnose by using other standard diagnostic approaches.

Methods

We retrospectively reviewed the medical records of 135 consecutive patients who were referred to immunology clinic at King Hussein medical center with a history of recurrent or persistent infections, who underwent extensive diagnostic work up for primary immunodeficiencies, including flow cytometric assessment of peripheral blood cells between January 2000 to August 2009. The files were reviewed for the use of flow cytometric immunophenotyping of leucocytes in the diagnosis and follow-up of immunodeficient children according to the main clinical categories. Our laboratory utilizes a panel of monoclonal antibodies that allows the measurement (both as a percentage of total lymphocytes and as absolute counts per mm 3 of whole blood) of the major lymphocyte subsets including B-cells (CD19), total T-cells (CD3), T-helper (CD3 and CD4), T-suppressor/cytotoxic (CD3 and CD8), and natural killer (NK) cells (CD3 and CD16 and/or CD56).[1] This is in addition to class of adhesion molecules which includes three receptors each with a different alpha chain iC3b (CD11b), C3dg (also called p150, 95, CD11c), and LFA-1 (CD11a) and sharing a common beta chain, CD18.[1] The DHR respiratory burst assay by flow cytometry was available for one patient with chronic Granulomatous Diseases (CGD). Peripheral blood samples were anticoagulated with K_3EDTA and stained for 10 minutes with various combinations of monoclonal antibodies conjugated by fluorescein isothiocyanate (FITC) and phycoerythrin (PE). A special panel of markers were used for immune deficiency diseases which are constituted of CD45/CD14, CD3/CD4, CD3/CD8, CD3/CD19, CD3/CD16+56 in addition to CD11b/CD18 and CD11c/CD18 for granulocytes (CALTAG™ Laboratories). After that ,cells were lysed for 10 minutes, using FACS lysing solution from Becton Dickenson (BD), centrifuged and the pellets were washed two times with FACS wash (BD), then the centrifuged pellets were resuspended in FACS flow diluent (BD).[6] Two- color flow cytometry was performed on FACS Calibur, (BD). Cells were analysed using the cell-Quest software from BD by collecting 10,000 ungated list mode events, lymphocytes were identified using light scatter gating procedure and differential staining with monoclonal antibodies;CD45/CD14 and analyzing cells with the lymphocyte gate, for granulocytes appropriate gate selected and cells were analysed for CD11b/CD18 and CD11c/CD18.[6]

Results

Files of 135 patients with history of recurrent or persistent infections were reviewed. Seventy seven (57%) patients were males and 58(43%) were females. They aged between 2 and 120 months with a mean age of 13 months. Flow cytomerty-based diagnosis was identified in 68(50.3%) patients while in 59(43.7%) patients the diagnosis was made according to appropriate clinical and laboratory diagnostic criteria. There were still 8 (6%) patients in whom diagnosis couldn't be identified.

Predominant antibody deficiency was diagnosed in 14(10.3%) patients, 6 patients with X-linked agammaglobulinemia, 2 with autosomal recessive hypogammaglobulinemia, 3 with common variable immunodeficiency and 3 with selective IgA deficiency with IG subclass deficiency. There were 35(26%) patients with T and B cell immunodeficiency. Twenty two patients with SCID (16.2%), two patients with Omenn syndrome, 6 patients with HyperIgM syndrome, one patient with MHC class 2 deficiency and 4 patients with undefined combined immunodeficiency.

In the SCID group, there were 8 patients with T(CD3) negative, B(CD19) positive and NK(CD16-56) negative SCID, 3 patients with T(CD3) negative, B(CD19) positive and NK(CD16-56) positive, 5 patients with T(CD3) negative, B(CD19) negative and NK(CD16-56) negative SCID, 6 patients with T(CD3) negative, B(CD19) positive and NK(CD16-56) negative SCID.

There were 8(6%) patients with other well defined immunodeficiency syndromes; one patient with WAS, 5 patients with AT, one with Bloom syndrome, and one with DiGeorge anomaly. Eight (6%) patients were found to have an immunedysregulation syndrome; Chediac Higashi syndrome was diagnosed in 2 patients, Griscelli syndrome in one patient, familial hemophagocytic lymphohistiocytosis in 2 patients, X-linked lymphoproliferative syndrome in 2 patients, and autoimmune lymphoproliferative syndrome in one patient.

Primary phagocytic disorder diagnosis was made in 34(25%) patients; one patient with Kostmann syndrome, 8 with cyclic neutropenia, 8 with leukocyte adhesion deficiency, 2 with possible gamma interferon receptor deficiency and 15 with chronic granulamatous disease. DHR-Flowcytomerty-based respiratory burst assay was confirmatory for Chronic Granulomatous Diseases (CGD) in one patient while in the other 14 patients diagnosis was based on NBT and genetic mutation study.[8,9]

Fifty nine (43.7%) patients with inconclusive Flow cytomerty assay were diagnosed according to ESID clinical diagnostic criteria,[10] as shown in Table I.

Post Bone marrow transplantation Immunereconstitution of T-, B- cells and Leukocyte adhesion molecules were identified in 14 patients with appropriate Flow cytomerty immunophenotyping assay.

Discussion

Primary immunodeficiency is not uncommon Its true incidence and prevalence will not be known unless there is national newborn screening and an established registry.[12] Flow cytometry immunophenotyping should precisely determine the relative frequencies of leukocyte subpopulations by detection of surface and intracellular markers using fluorochrome labeled monoclonal antibodies.[13]

Reliability of this helpful diagnostic tool is largely dependent on proper blood sample handling, cell separation methodology and labeling techniques.[13]

We reviewed the files of 135 patients with recurrent or persistent infections to study the frequency of flow cytometry-based diagnosis of PID. Diagnosis of PID was made by FCM in 61(50.3%) subjects. There were still 8(6%) patients who were grouped with undefined PID. It is due to limitation of flow cytometry diagnostic panel at our facility.

The positive yield of flow cytometry in our study was exclusively limited to patients with B cell (CD19) deficiency either autosomal recessive or X-linked hypogammaglobulinemia, patients with severe combined immunodeficiency (SCID), patients with leukocyte adhesion deficiency and only one patient with chronic granulamatous disease (CGD), while common variable immunodeficiency (CVID), HyperIgM immunodeficiency syndromes, combined immunodeficiency (CID), familial HLH, and the other 14 patients with Chronic Granulomatous Diseases (CGD) and others were diagnosed by appropriate clinical, assay of total immunoglobulin, functional antibodies and Nitroblue tetrazolium test (NBT). This insufficiency of immunophenotypic evaluation of PIDs will make it difficult to provide diagnostic clues as well as information useful to classify patients and predict clinical outcome in some of our patients at high risk to have PIDs.

In 8 patients with clinical and laboratory findings in keeping with Leukocyte Adhesion deficiency Syndrome (LADI), the diagnosis was made by appropriate flow cytometry immunophenotyping. This was found very useful to confirm this diagnosis in many patients evaluated with similar clinical presentations. Three of them were transplanted successfully before irreversible end organ damage happened. This is also applied to 4 patients with SCID one of them was transplanted at age of 3 weeks when SCID diagnosis was made by flow cytometry immunophenotyping assay at birth due to positive family history of PIDs.

Seventy eight percent of our SCID patients were diagnosed late with severe respiratory damage which made bone marrow transplantation unfeasible and risky. The suboptimal awareness of PIDs and inaccessible Flow cytomerty immunophenotyping are behind this delay in diagnosis.

Bone marrow transplantation was done in only 6 patients out of 22 who were diagnosed with SCID. The range of PIDs in which flow cytometry has proven to be useful from a clinical and diagnostic purposes has significantly expanded.[5,14] This, now,

Table I. Number of patients and methods of diagnosis of different types of PID

Primary immunodeficiency	No. of patients	%	Method of diagnosis
Antibody deficiency	14	10.3	
X-linked agammaglobulinemia	6		CD19 below 1% in male
Autosomal recessive Hypogamma-globulinmemia	2		CD19 below 1 % in female
Common variable immunodeficiency	3		ESID diagnostic criteria with no flow cytometry assay
Selective IgA deficiency with IG subclass deficiency	3		ESID diagnostic criteria with no flow cytometry assay
Combined Immunodeficiency	35	26	T positive, B negative and characteristic clinical features
Omenn syndrome	2		
Hyper IgM immunodeficiency	6	4.4	ESID diagnostic criteria
MHCII deficiency	1		
Un defined combined immunodeficiency	4		
Wiskott's Aldrich Syndrome	1	0.74	ESID diagnostic criteria
Ataxia Telengectasia	5	3.7	ESID diagnostic criteria
Bloom syndrome	1	0.74	Characteristic clinical features
DiGeorge syndrome	1	0.74	ESID diagnostic criteria
Chediak Higashi Syndrome	2	1.5	Characteristic clinical features and characteristic giant granules on neutrophils
Griscelli syndrome	1	0.74	Characteristic clinical features
Familial Hemophagocytic Lymphocytosis Histocytosis(HLH)	2	1.5	Diagnostic guidelines for HLH-2004 (11)
XL-lymphoproliferative syndrome	2	1.5	ESID diagnostic criteria
AI-lymphoproliferative syndrome	1	0.74	ESID diagnostic criteria
Primary Phagocytic disorders	34	25	ESID diagnostic criteria
Kostmann syndrome	1		
Cyclic Neutropenia	8		
Leukocyte adhesion deficiency I	8		
Gamma receptor interferon deficiency	2		
Chronic Granulomatous deficiency	15		
Severe Combined immunodeficiency	22	16.20	ESID diagnostic criteria
T-, B+, NK-	8		
T-,B+,NK+	3		
T-,B-,NK-	5		
T-, B+, NK-	6		

includes not only patients presenting with clinical histories consistent with classical antibody deficiencies and severe combined immune deficiency, but also patients with more limited infectious histories. Included among these are patients with genetic defects associated with Mendelian susceptibility to mycobacterial disease focusing the evaluation on specific surface protein expression and cell function analysis.[15] We failed to retrieve a definite diagnosis in one patient presented with disseminated BCG infection and in another one with recurrent salmonella osteomyelitis. We were unable to study the CD40 ligand assay in 6 patients presented with clinical and serum immunoglobulin assay suggestive of possible HyperIgM immunodeficiency syndrome. Even though we did successful bone marrow transplantation in 2 of them but still the Immunereconstitution of the CD40 ligand was not proven and bone marrow transplantation was not performed in 2 patients who showed typical clinical presentation and immunoglobulin assay. We were also unable to make definite diagnosis of this syndrome at birth within sibling of same families.

The 3 patients with common variable immunodeficiency in our series, were not diagnosed by flow cytometry because of lack switched memory B cells (CD27+IgM-IgD-) detection in our laboratory.

Immunereconstitution was evaluated in 15 patients underwent BMT. An initial indication of T-cell reconstitution was found by looking at absolute CD3 numbers in peripheral blood at 6 week after transplant in all patients.[16] B cell reconstitution was

done by B cell enumeration. It was demonstrated as early as 3 months in all patients except one with mismatched related unconditioned BMT done for SCID, which is expected in such a case as B cell engraftment might be delayed and may not occur.[17]

Conclusion

Flow cytometric immunophenotyping of leucocytes appears to be an efficient and rapid tool in the diagnosis and follow-up of immunodeficient patients, supporting early recognition, which is reflected on reduced morbidity and improved survival.

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