

DEDICATION

- ✦ *To my family for their good-natured forbearance with the process and for their pride in this accomplishment and for being a source of encouragement and inspiration to me throughout my life, this thesis is dedicated to them who taught me that the best kind of knowledge to have is that which is learned for its own sake.*
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Abstract

Flow cytometer (FC) became one of the most pivotal and definitive techniques in the diagnosis and classification of mature B cell neoplasm (MBCN). Since flow cytometer exploits the laser and photomultiplier technology for reliable high quality result with extremely high sensitivity and specificity, we used these important specifications in this cross sectional descriptive hospital based study to study the properties of B cells in the adult Sudanese patients whose have initial diagnosis as mature B cell neoplasm in the period of October 2010 and March 2013.

For the studying of these properties, we depended up on the evaluation of immunophenotypic antibodies using in the diagnosis and classification of MBCN against (CD45, HLA, CD34, CD3, CD5, CD23, CD22, CD79b, FMC7, Kappa, Lambda, CD10, CD11c, CD25 and CD103) with calculation of the mean of their flow cytometric parameters for each markers (Percentage, Fluorescence Intensity and Positive Peak Width), and focusing on the substantial feature which important in differentiation between CLL and other NHL. Also we evaluated the best type of sample between venous blood, bone marrow aspiration and lymph node aspiration in the highlighting of accurate result of markers.

One hundred and forty-six samples were conducted. (17.7 %) was lymph nodes (LN) samples, (48.8 %) was bone marrow (BM) samples and (33.5%) was venous blood samples. We prepared the lymph node samples which collected as Fine Needle Aspirations in a 3.0 ml PBS (pH=7.2), Mononuclear cells for flow cytometry preparation were separated from LN and BM samples using HISTOPAQUE-1077. Mononuclear cells from LN suspensions, BM suspensions and PB samples were conjugated with fluorescence labelled antibodies in the dark place. Tubes of kappa and lambda were going through washing procedure for 3 times by PBS before adding of Abs. Then all tubes were analyzed by flow cytometer and all flow cytometric parameters were recorded for each marker.

Data acquisition and analysis were performed with an EPICS XL Beckman Coulter flow cytometer and SYSTEM II software. Both 3 and 4 color protocols were performed using CD45 and light scatter gating system to identify cell populations and exclude the cells debris.

The majority (70%) of MBCN were males and the rest were females (30%). The mean age was (60.7) years. (66.5%) of patients had initial diagnosis as CLL, (28.7%) diagnosed as NHL and (4.8%) were normal samples. The sub-classification of NHL was done

and there were (6) cases diagnosed as DLBCL, (13) cases as PLL, (2) cases as MCL, (2) cases as LPL, (1) case as SLVL, (2) cases as FL, (1) case as Hairy cell leukaemia and (17) cases had inconclusive diagnosis. The mean Hb% value for MBCN patients was (84.7 %), (82×10^3) for TWBC mean, (164.4×10^3) for platelets mean and (78.6%) for lymphocytes mean.

CD45 showed an important role in the identification of MBCN (p.value = 0.0021). CD45 Min had significant difference between CLL and NHL (p.value = 0.004). CD34 was insignificant for diagnosis of MBCN or differentiation between CLL and NHL (p.value = 0.598). While HLA-DR could differentiate between them (p.value = 0.001). When we used CD20 and CD19 together, they showed very high significant values to differentiate between CLL and NHL. NHL cases showed high CD20 Min than CLL cases (p.value = 0.000), high CD20Min:CD19Min ratio (p.value = 0.000) and low CD20 Pw (p.value = 0.000).

The immunophenotyping features of CLL was (s+ve CD45, -ve CD34, +ve HLA, +ve CD19, w+ve/-ve CD20, +ve CD5, +ve CD23, -ve/w+ CD22, -ve/w+ CD79b, -ve/w+ Ig), while the immunophenotyping of NHL was (s+ve CD45, -ve CD34, +ve HLA, +ve VD19, s+ve CD20, -ve or +ve CD5, -ve/rare +ve CD23, s+ve CD22, s+ve CD79b, s+ve Ig). The characteristic markers for diagnosis of hairy group were CD11c, CD25 and CD103 and for follicular lymphoma was CD10. Venous blood and LN samples showed the best results to differentiate between CLL and other NHL.

As a conclusion of this study, Flow cytometer have a very distinctive role in the diagnosis of MBCN and also ability to differentiate between CLL and NHL. Diversity of FC parameters can help in the minimization of markers following into the minimization of panel cost without affecting in the result accuracy like using of CD20 & CD19 with their flow cytometric parameters and without the other markers showed a significant role in the differentiation between the two diseases. MBCN immunophenotyping feature of Sudanese patients was not much differing from other immunophenotyping feature in the other world especially when using the scoring system of Matutes as a guide.

ملخص الدراسة

عداد الخلوياى التدفقى (FC) اصبح واحدا من الأساليب الأكثر محورية وحسم استخداما فى تشخيص وتصنيف اورام الخلوياى البائية الناضجة (MBCN) منذ استغلال عداد الخلوياى التدفقى لتكنولوجيا الليزر ومضخم الفوتونات للوصول الى نتيجة ذات جودة عالية مع حساسية وخصوصية مرتفعة للغاية ، استخدمنا هذه المواصفات الهامة فى هذه الدراسة الوصفية لدراسة خصائص الخلوياى بي فى المرضى السودانين البالغين المشخصين مبدئيا باورام خلوياى بي الناضجة فى الفترة من أكتوبر 2010 الى مارس 2013.

لدراسة هذه الخصائص، اعتمدنا على تقييم النمط المناعى الظاهري للاضداد المستخدمة فى تشخيص وتصنيف اورام خلوياى بي الناضجة مقابل (CD45، HLA، CD34، CD3، CD5، CD23، CD22، CD79b، FMC7، Kappa، Lambda، CD10، CD11c، CD25 و CD103) مع حساب متوسط مؤشرات عداد الخلوياى التدفقى لكل واسم (النسبة المئوية، كثافة ضور الفلورسنت وعرض المنحنى الايجابى)، والتركيز على الميزة الجوهريه والهامة فى التفريق بين ابيضاض الدم اللمفى المزمن و لمفوم لا هودجكن الاخرى. أيضا قمنا بتقييم أفضل نوع من العينات بين الدم الوريدي ورشف نقي العظم والعقدة الليمفاوية فى تسليط الضوء على نتائج دقيقة للواسات.

تم الكشف عن مائة وستة وأربعين عينة. (17.7%) كانت من العقد الليمفاوية (LN) ، (48.8%) كانت من نقي العظم (BM) العينات و(33.5%) كانت عينات الدم الوريدي. قمنا بتحضير عينات العقد الليمفاوية التي كانت على شكل عينات رشف الابر الناعمة فى 3.0 مل من PBS (الرقم الهيدروجيني = 7.2)، الخلوياى وحييدات النوى المعدة لعداد الخلوياى التدفقى فصلت من نقي العظم والغدد الليمفاوية باستخدام محلول HISTOPAGUE-1077. كانت الخلوياى وحييدات النوى لى LN، ومعلقات BM وعينات PB مرتبطة مع الأجسام المضادة الموسمة بالفلورسنت فى مكان مظلم. أناييب Kappa و Lambda كانوا فى طريقتهم من خلال إجراء الغسيل لمدة 3 مرات بواسطة PBS قبل اضافة الاجسام المضادة. ثم كانت جميع الأناييب تحلل بواسطة عداد الخلوياى التدفقى وسجلت جميع مؤشرات عداد الخلوياى التدفقية لكل واسم.

تم اجراء تحليل البيانات باستخدام عداد الخلوياى التدفقى EPICS XL بيكمان كولتر وبرنامج النظام الثانى. أجريت على حد سواء بروتوكولات اللون الثلاثى والرابعى باستخدام نظام العزل CD45 والضوء المبعثر للتعرف على فئات الخلوياى واستبعاد حطام الخلوياى.

كان هناك (70 بالمائة) من مرضى اورام خلوياى بي الناضجة من الذكور و (30 بالمائة) من الإناث. وكان متوسط عمرهم (60.7) سنة. (66.5 بالمائة) من المرضى كان التشخيص الأولي لهم ابيضاض دم لمفى مزمن، (28.7 بالمائة) مشخصون بلمفوم لا هودجكن و (4.8 بالمائة) كانت عينات طبيعية. وقد تم عمل التصنيف الفرعى للمفوم لا هودجكن حيث كانت هناك (6) حالات تم تشخيصها بالمفوم المنتشر بالخلوياى البائية الكبيرة، (13) حالة لمفوم يلمفات

المفاوية، (حالتين) MCL، (حالتين) LPL، (حالة واحدة) SLVL، (حالتين) FL، و (حالة واحدة) لايبضاض الدم المزمن للخلايا الشعرية و(17) حالة غير محسومة التشخيص. وكان متوسط نسبة الهيموغلوبين المثوية لمرضى اورام الخلايا البائية الناضجة (84.7 بالمائة) ، ومتوسط مجموع خلايا الدم الابيض (82×10^3) ، ومتوسط الصفائح الدموية ($10^3 \times 164.4$) ومتوسط الخلايا الليمفاوية (78.6 بالمائة).

وأظهرت الدراسة ان لـ CD45 دورا هاما في اكتشاف اورام الخلايا البائية الناضجة (قيمة p تساوي 0.002). اوجد CD45 Min دلالة فرقية بين ايبضاض دم اللمفي مزمن ولمفوم لا هودجكن (قيمة p تساوي 0.004). ولم يكن لـ CD34 اي دلالة في التشخيص اورام الخلايا البائية الناضجة أو التمييز بين ايبضاض دم لمفي مزمن ولمفوم لا هودجكن (قيمة p تساوي 0.598). بينما امكن HLA التفريق بينها (قيمة p تساوي 0.001). وعند استخدام CD19 و CD20 ومعا فإنها أظهرت قيم ذات دلالات عالية جدا في التفريق بين ايبضاض دم لمفي مزمن ولمفوم لا هودجكن. وأظهرت حالات لمفوم لا هودجكن ارتفاعا في CD20 Min أكثر منها في حالات ايبضاض دم اللمفي مزمن (قيمة p تساوي 0.000). ، وارتفاع معدل CD20Min الى CD19Min (قيمة p تساوي 0.000) وانخفاض CD20 Pw (قيمة p تساوي 0.000).

وكانت السمات المناعية الظاهرية لايبضاض دم اللمفي مزمن (مرتفعة الايجابية لـ CD45، سلبية لـ CD34، ايجابية لـ HLA، ايجابية لـ CD19، منخفضة الايجابية او سلبية لـ CD20، ايجابية لـ CD5، ايجابية لـ CD23، سلبية او منخفضة الايجابية لـ CD22، سلبية او منخفضة الايجابية لـ CD79b، سلبية او منخفضة الايجابية لـ Ig)، بينما كانت السمات المناعية الظاهرية للمفوم لا هودجكن (مرتفعة الايجابية لـ CD45، سلبية لـ CD34، ايجابية لـ HLA، ايجابية لـ CD19، مرتفعة الايجابية لـ CD20، سلبية او ايجابية لـ CD5، سلبية او نادرا ما تكون ايجابية لـ CD23، مرتفعة الايجابية لـ CD22، مرتفعة الايجابية لـ CD79b، مرتفعة الايجابية لـ Ig).

الواسات المميزة لتشخيص مجموعة ايبضاض الدم المزمن للخلايا الشعرية كانت CD11c و CD25 و CD103، وللمفوما الجرابية كانت CD10. عينات الدم الوريدية وعينات العقد المفاوية أظهرت افضل النتائج للتفريق بين ايبضاض دم اللمفي مزمن و لمفوم لا هودجكن.

ملخص هذه الدراسة يبين ان عداد الخلايا التدفقي له دور مميز جدا في تشخيص اورام خلايا بي الناضجة والتفريق بين ايبضاض دم اللمفي مزمن لمفوم لا هودجكن. التنوع في مؤشرات عداد الخلايا الجرابي ساعد في تقليص الواسات والذي ادى الى تقليل تكلفة مجموعة الواسات من دون احداث تأثير على دقة النتيجة مثل استخدام CD20 و CD19 مع مؤشراتهم وبدون استخدام الواسات الاخرى أظهرها دورا هاما في التفريق بين المرضين. الملامح المناعية الظاهرية لاورام خلايا بي الناضجة في المرضى السودانيين لم يختلف كثيرا عن الملامح المناعية الظاهرية للدول الاخرى خاصة عند استخدام نظام النقاط لماتيتوس كوجه.

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LIST OF ABBREVIATIONS

ADCC	Antibody Dependent Cell Mediated Cytotoxicity	MCL	Mantle Cell Lymphoma
ALL	Acute Lymphoblastic Leukemia	MDS	Myelodysplastic syndrome
BCR	B Cell Receptor	MFI	Mean Fluorescence Intensity
BL	Burkitt Lymphoma	MHC	Majorhistocompatibility
BM	Bone Marrow	MRD	Minimal Residual Disease
CD	Cluster Differentiation	MYC	Myelocytomatosis
CLL	Chronic Lymphocytic Leukemia	MZL	Marginal Zone Lymphoma
CNS	Central Nervous System	NHL	Non-Hodgkin Lymphoma
DF	Degree of Freedom	NK	Natural Killer
DLBCL	Diffuse Large B Cell Lymphoma	NPV	Negative Predictive Value
DNA	Deoxyribonucleic acid	PALS	Periarteriolar Lymphoid Sheath
EDTA	Ethylenediaminetetraacetic acid	PB	Peripheral Blood
FISH	Fluorescence in situ hybridization	PBS	Phosphate Buffer Saline
FITC	Fluorescein Isothiocyanate	PE	Phycoerythrin
FL	Follicular Lymphoma	PLL	Prolymphocytic Leukemia
FNA	Fine Needle Aspiration	PPV	Positive Predictive Value
FSC	Forward Scatter Light	Pw	Peak width
Hb	Haemoglobin	RICK	Radiation and Isotopes Centre-Khartoum
HCL	Hairy Cell Leukemia	RNA	Ribonucleic acid
HIV	Human immunodeficiency virus	SCF	Stem Cell Factor
HL	Hodgkin Lymphoma	SLL	Small Lymphocytic Lymphoma
HLA	Human Leukocyte Antigen	SLVL	Splenic Lymphoma with Villous Lymphocytes
Ig	Immunoglobuline	SMZL	Splenic Marginal Zone Lymphoma
IL	Interleukin	SSC	Side Scatter Light
IPBSS	Isotonic Phosphate Buffered Saline Solution	TCR	T Cell Receptor
IPSID	Immuno proliferation small index disease	TdT	Terminal Deoxyribonucleotidyl tranferase
LN	Lymph Node	TRAP	Tartrate-resistant acid phosphatise
LPD	Lymphoproliferative Disorders	TWBC	Total White B cells
LPL	Lymphoplasmacytic Lymphoma	WHO	World Health Organization
MALT	Mucosal-Associated Lymphoid Tissue	ZAP-70	Zeta-chain-associated protein kinase 70
MBCN	Mature B Cell Neoplasm		

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