Journal of
Biomedical and Pharmaceutical Sciences

Research Article Open Access

CD147 Expression as a Prognostic Marker in Patients with Chronic B-cell Lymphoproliferative Disorders

Eman Hassanin Bakr[1], Alaa El-Din Saad Abd El-Hamid[1], Fadia Mostafa Atia[1], Ahmed Mostafa Elzaway[1] and Basma Badreldin Hasan[2]*

[1]*Clinical Pathology Department, Suez Canal University Teaching Hospital, Egypt*

[2]*Clinical Pathology Department, Port Said University, Egypt*

Abstract

Background: CD147 is expressed at different levels on numerous cell lineages and contains a single highly conserved transmembrane domain containing a glutamic acid that mediates interactions with other transmembrane proteins. CD147 is overexpressed in a broad range of human malignant tumours. CD147 promotes invasive properties, proliferation, and survival of tumour cells. Thus, the overexpression of BSG in tumours is generally regarded as an unfavourable prognostic marker.

Aim: We investigated the expression and prognostic relevance of CD147 in 20 CLL patients vs. 10 DLBCL patients.

Patients & Methods: We examined the 50 individuals (30 NHLs patients, 20 healthy controls) for lymphadenopathy and splenomegaly then, we withdraws peripheral blood samples from the study groups which were analyzed for CBC with blood film, LDH, UA, and immunophenotyping for CD147 on peripheral blood B lymphocytes by FC. Then we reassessed the patient's at 6, 12 month.

Results: At the initial assessment, CD147 positive expression on $CD19^{+}$ gated population was (26.8 ± 25.7%) compared to the control group with P-value (<0.05) , these patients also showed associated high LDH, UA, B symptoms, leucocytosis & absolute lymphocytosis. In the reassessment the patients with high CD147 expression the following were observed; fall in the Hb level, PLT count, development of organomegaly, and B symptoms.

Conclusion: High expression of CD147 by immunophenotyping correlates with the patient poor outcome according to international prognostic index (IPI) for lymphoma.

Keywords: Chronic Lymphocytic Leukemia (CLL); Diffuse large B-cell Lymphoma (DLBCL)

Introduction

CD147 is expressed at different levels on numerous cell lineages and contains a single highly conserved transmembrane domain containing a glutamic acid that mediates interactions with other transmembrane proteins. That should clarify why CD147 has been implicated in a broad scale of physiologic and pathologic functions. It has been reported to relate with the monocarboxylate transporters MCT1, MCT4, integrins, and caveolin-127 and acts as a receptor for extracellular cyclophilins. Still, it was originally recognized and has been habitually studied as an inducer of MMPs in adjacent fibroblasts or in tumor cells [1].

MMPs considered one of the most important groups that mediate communication between tumors and their microenvironment; they are enzymes that are produced mainly by various stromal cells rather than tumor cells. MMPs help cancer cells spread by breaking down the extracellular matrix (ECM) and other barriers. In many tumors, MMP expression is regulated mainly by tumor-stroma interactions via extracellular MMP inducer (EMMPRIN) also called CD147, HAb18G/CD147, or basigin [2,3].

Peripherally, CD147 is expressed on all leucocytes, red blood cells, platelets and endothelial cells. It is highly expressed on cells with high metabolic activity such as lymphoblasts, and tumor cells. Presently, four basigin transcript variants encoding different isoforms (basigin isoforms 1,2,3 and 4) are reported, of these four variants, the basigin-2 transcript (accession number NM_198589) is the most predominant splice variant and mostly expressed on B-lymphocytes [4,5].

Upregulated CD147 expression in tumor tissues, but not elevated serum levels of CD147, was associated with poor prognosis of HCC patients, and revealed that tissue CD147 expression levels are an independent prognostic indicator in HCC.

CD147 expression was associated with aggressive clinicopathologic features in HNSCC. CD147 promoted tumor progression in HNSCC and might be a potential prognostic and treatment biomarker for HNSCC.

BSG-2 was reported to be upregulated in NHL tumor cells. CD147 expression intensity was linked to clinical myeloinfiltration, tumor size, LDH value, and clinical staging, rather than age, gender, or immune typing. Currently the interaction between tumor cells & extracellular matrix in tumor metastasis becomes an era of great interest and debate. And the further explore of the tumor cell expression of CD147, signal transduction mechanism of inducing MMPs, and the studies about the inhibition on functions and activity of CD147, are supposed to provide new therapeutic targets for tumor metastasis control. Also CD147 is becoming an important outlook for cancer treatment [6].

So in the current study we aim to investigate the role of CD147 expression in NHL patients (CLL and DLBCL) and its relation to the

***Corresponding author:** Basma Badreldin Hasan, Lecturer of Clinical Pathology, Clinical Pathology Department, Faculty of Medicine, Port Said University, Egypt, Tel: +1001593877; E-mail: basmabdr@yahoo.com

Received: January 28, 2019; **Accepted:** February 14, 2019; **Published:** February 24, 2019

Citation: Bakr EH, El-Hamid AEDSA, Atia FM, Elzaway AM, Hasan BB (2019) CD147 Expression as a Prognostic Marker in Patients with Chronic B-cell Lymphoproliferative Disorders. J Biomed Pharm Sci 2: 119

disease outcome to identify its importance as a prognostic factor. So we can introduce CD147 expression assessment to the algorism of CLL and /or DLBCL follow up and assessment of response to therapy. Also if CD147 was found to be highly expressed in CLL and/or DLBCL, it can be used as a method of treatment through being targeted.

Patients and Methods

Our study population is composed of thirty adult NHL patients composed of B-CLL (twenty patients); DLBCL (ten patients), these patients were hospitalized in the oncology inpatient unit and were diagnosed according to the international diagnostic criteria. Twenty healthy individuals as a control group with matched age and sex attending Suez Canal University Hospital in Ismailia City. These patients were followed up (through clinical examination & performing periodically complete blood count & serum LDH) at 6, 12 month intervals to detect the relation between the level of expression at the initial assessment and the clinical and laboratory outcome according to lymphoma IPI.

Children (less than 18 years old), patients that diagnosed to have other malignancies, patients exposed to certain risk factors that may affect gene expression such as alcohol, and patients refused to join the study were excluded from the study.

Data were collected from the included individuals using the following methods:

Interview questionnaire

(Gender, age, residence, familial history, exposure to infections such as; Epstein Barr virus (EBV), Human Immunodeficiency virus (HIV), Hepatitis C & B viruses, exposure to chemical agents such as benzene, alcohol intake, smoking, "B" symptoms such as: low grade fever, weight loss, night sweats, generalized body aches, past history of previously treated malignant diseases, receiving medical treatment after being diagnosed with B-cell NHL).

Clinical examination

(General examination; General appearance, vital signs, Lymph node examination: detection of lymphadenopathy, and abdominal examination; assessment of liver and spleen)

Laboratory investigations

Assessment of complete blood count on (Sysmex XT 1800 i), examination of peripheral blood smear by the hematologist, assessment of serum LDH, uric acid and serological screening of HCV, and immunophenotyping on (FacsCalibur, Bicton Dickston, Germany).

Interpretation of the lab results were made upon the normal reference values for age and sex for these patients , as for immunophenotyping results; Gating was made using SSC against CD19 and CD19+ population was selected, CD147 expression was assessed on $CD19^{+}$ population. CD147 was considered positive, if the expression was more than 20% based on the cut-off point for CD38 positivity in the CLL as a prognostic marker [7].

Results

Regarding the age difference and PLT count between the control group and NHLs patients, there were no significant differences. But significant differences were detected when we compared; **Hb** in the control group (12.5 ± 1 g/dl) to the NHLs group (10.7 ± 2.4 g/dl) with P-value (<0.05), **TLC** in the control group (6.7 ± 2.476 × 103 / cmm) to the NHLs group (25.359 ± 38.296/cmm) with P-value (<0.05), **lymphocytes** in the control group (2.570 ± 0.825 /cmm) to the NHLs group (18.774 ± 32.625/cmm) with P-value (<0.05), **LDH** in the control group (269.9 ± 20.8 IU) to the NHLs group (445.1 ± 315.8 IU) with P-value (<0.05), **UA** in the control group (3.48 ± 0.89 mg/dl) to the NHLs group (4.76 ± 1.37 mg/dl) with P-value (<0.05), and finally for **immunophenotyping** (**CD147 expression %**) in the control group (14.6 ± 2.5 %) to the NHLs group (26.8 ± 25.7 %) with P-value (<0.05) as shown in Table 1.

As for B Symptoms, lymphadenopathy, Splenomegaly, and HCV; when we compared the 2 groups, there were no significant differences between the 2 groups in B Symptoms, Splenomegaly or HCV. On the other hand there were significant differences between the 2 groups regarding Lymphadenopathy with P-value (<0.05) as shown in Table 2.

When we monitored these patients through a year (at 6 month & 12 month intervals), then compared the results of initial examination & investigations to them in the follow up reassessment, we found that there were significant differences between the NHLs cases at the initial assessment then at 6 month, and at 12 month intervals in the following; Hb initially was (10.7 ± 2.4 g/dl) compared to it in the follow up reassessment (10.2 ± 2.5 g/dl) with P-value (<0.05), **PLT count** initially was (181.433 ± 101.321 /cmm) compared to it in the follow up reassessment (193.300 ± 119.868 /cmm) with P-value (<0.05), and **Lymphadenopathy** initially, 20 cases were positive for lymphadenopathy (66.7 %) then it increased to 22 cases in the follow

Title	Control (n=20)		Cases (n=30)		P-value#
	Mean	SD	Mean	SD	
Age/year	52.2	12.2	58.0	15.1	0.69
Hb g/dl	12.5	1.0	10.7	2.4	0.02*
TLC x10^3 /cmm	6.7	2.476	25.359	38.296	0.00*@
PLT count /cmm	226.800	67.590	181.433	101.321	0.27@
Absolute Lymphocytosis / cmm	2.570	0.825	18.774	32.625	0.00*@
Immunophenotyping for CD147%	14.6	2.5	26.8	25.7	0.00*
LDH /IU	269.9	20.8	445.1	315.8	0.01*@
UA mg/dl	3.48	0.89	4.76	1.37	0.00*
Uric acid (UA). *The test was statistically significant at 95% level of confidence. # Student t-test was used @Mann-whitney test was used					

Table 1: Comparison between NHLs cases and healthy controls regarding age, CBC, LDH, UA, and immunophenotyping for CD147 expression.

Title		Control (n=20)		Cases (n=30)		P-value#
		No.	%	No.	%	
Gender	Female	12	70.0	14	46.7	0.28
	Male	8	30.0	16	53.3	
B Symptoms	Present	0	0.0	9	30.0	0.08
	Absent	20	100	21	70.0	
Lymphadenopathy	Yes	0	0.0	20	66.7	0.00*
	No	20	100	10	33.3	
Splenomegaly	Yes	0	0.0	9	30.0	0.08
	No	20	100	21	70.0	
HCV Ab	Negative	20	100	27	90.0	0.56
	Positive	0	0.0	3	10.0	
Antibody (Ab). *The test was statistically significant at 95% level of confidence. #Fisher's Exact Test @ Chi-Square Test						

Table 2: Comparison between NHLs cases and controls regarding gender, B symptoms, HCV, lymphadenopathy, and splenomegaly.

up reassessment (73.3 %) with P-value (<0.05). While there are no significant differences between the 2 groups in TLC, LDH, B symptoms, splenomegaly and lymphocytosis as shown in Tables 3 and 4.

When we compared the 2 subgroups of NHLs with each other, we found that there were significant differences between the 2 subgroups in; TLC, lymphocytosis, and immunophenotyping with P-value (<0.05). While for the Hb, PLT count, LDH, and UA, there were no significant differences. But By applying the post hoc test between the 2 subgroups in the following variables; TLC, immunophenotyping, and lymphocytosis we concluded that there was statistically significant difference between CLL as a subgroup and DLBCL, which means that marked elevation in the TLC, absolute lymphocytosis, and high expression of CD147 by flow cytometry were noticed most in CLL subgroup as shown in Tables 5 and 6.

Title	Case n=30		Cases after Follow up		P-value#
	Mean	SD	Mean	SD	
Hb g\dl	10.7	2.4	10.2	2.5	0.00*@
TLC x 10^3\cmm	25.359	38.296	13.705	23.676	0.84
PLT count \cmm	181.433	101.321	193.300	119.868	0.00*
Lymphocytosis \ cmm	18.774	32.625	7.673	20.317	0.83
LDH\ IU	445.1	315.9	390.0	122.2	0.41
Hemoglobin (Hb),Total lecuocytic count (TLC), Platelet (PLT), Lactate dehydrogenase (LDH) @Paired Samples Test, #Kruskal wallis test was used.*the test was statistically significant at 955 level of confidence.					

Table 3: Comparison between NHLs cases in the initial assessment and in the follow up regarding CBC and LDH levels.

Title		Cases n=30		Cases after Follow up		P-value#
		No.	%	No.	%	
B Symptoms	Present	9	30.0	16	53.3	0.08
	Absent	21	70.0	14	46.7	
Splenomegaly	Present	9	30.0	17	56.7	0.07
	Absent	21	70.0	13	43.3	
Lymphadenopathy	Present	20	66.7	22	73.3	0.01*
	Absent	10	33.3	8	26.7	
#Fisher's Exact Test						

Table 4: Comparison between cases in the initial assessment and after follow up regarding symptoms, splenomegaly, and lymphadenopathy.

Title		Cases (n=30)		P-value#
		Mean	SD	
Hb g\dl	CLL	11.0	2.4	0.40
	DLBCL	9.8	1.6	
TLC x 10^3\ cmm	CLL	42.826	46.183	0.02*
	DLBCL	5.852	2.579	
PLT count \cmm	CLL	175.188	80.432	0.63
	DLBCL	207.111	124.024	
Lymphocytosis \cmm	CLL	33.909	39.166	0.02*
	DLBCL	1.421	0.571	
Immunophenotyping for CD147%	CLL	41.1	27.4	0.00*
	DLBCL	9.6	6.4	
LDH IU	CLL	479.9	385.4	0.82
	DLBCL	397.7	229.4	
UA mg\dl	CLL	5.1	1.4	0.42@
	DLBCL	4.3	0.8	
@ANOVA, # Kruskal wallis test *the test was statistically significant at 95% level of confidence.				

Table 5: comparison between subtypes of NHLs (CLL and DLBCL) regarding CBC, UA, LDH and immunophenotyping.

Title		CLL N=20		DLBCL N=10		P - value
		No.	%	No.	%	
CD147 expression	<20%	7	35	7	70	0.02*
	>20%	13	65	3	30	
#Chi-square						

Table 6: Comparison between the 2 subgroups of NHL regarding CD147 expression (expressed as high expression (more than 20%) and normal expression(less than 20%).

Discussion

The strongest and most robust predictors of outcome in NHL are age, stage, number of lymph node or extranodal sites, performance status, and certain biochemical measurements such as serum lactate dehydrogenase, and hemoglobin level. These factors have been collected into various clinical prognostic indices in addition to multiple tumor biomarkers that have also been widely evaluated as predictors of NHL prognosis [8].

Among these tumor biomarkers is Basigin, which also known as CD147 or extracellular matrix metalloproteinase inducer (EMMPRIN), BSG plays a controlling role in the regulation of MMP expression via tumor-stroma interactions. In addition to several physiological and pathological functions that have been described [2].

Regarding the Hb level at the initial CBC assessment; most of the patients showed anemia ranged from mild to moderate (10.7 ± 2.4 g/dl) compared to the control group (12.5 ± 1 g/dl). Cases that showed severe anemia were associated with the highest CD147 expression immnuophenotypically, this altogether may referee to BM infiltration and replacement with the malignant lymphocytes.

Leucocytosis ranged from moderate to marked and was observed mainly in CLL cases and was associated with; absolute lymphocytosis, "B" symptoms, and increased CD147 expression which indicates BM replacement by the malignant lymphocytes. Leucopenia was detected in five cases among the study group as a part of pancytopenia.

Clinically, nine cases were manifested with "B" symptoms, eight cases of them showed increased CD147 expression on their peripheral blood B lymphocytes.

Regarding the flow cytometric analysis of CD147, there was a significant difference between the two groups; being (14.6 ± 2.5%) in the control group compared to (26.8 ± 25.7%) in the NHLs group with P-value (<0.05); which mean that 50% NHLs cases were positive for CD147 expression on their peripheral blood B lymphocytes (with a cut off value 20% for CD147 positivity); thirteen CLL cases, two DLBCL cases. $CD147^+$ cases were mostly associated with anemia, leucocytosis, and absolute lymphocytosis in addition to its occurrence in older age (>50 years in females and >60 years in males), which agree with [6] that CD147 was detected to be positive in 73% of the B- NHL cases and were associated with high LDH, anemia, and "B" symptoms.

For DLBCL cases (n=10), three cases of these patients showed high CD147 expression that may denote BM involvement had occurred which in turn correlate with poor outcome for these patients. This also confirms the metastatic role of CD147 as mentioned [5].

As for serum LDH level, there was a significant difference between the two groups; being (269.9 ± 20.8 IU) in the control group compared to (445.1 ± 315.8 IU) in the NHLs group with P-value (<0.05). LDH was elevated in nine patients; six CLL cases and was associated with high leucocytosis with absolute lymphocytosis, positive expression of CD147 on B cells, "B" symptoms, anemia, lymphadenopathy; three

DLBCL cases and it was associated with anemia or pancytopenia, and organomegaly. So cases that showed elevated LDH level were found to have high CD147 expression FC. From these data we conclude that elevated LDH correlate with high disease burden and poor outcome which agrees with [6] that CD147 expression intensity was linked to clinical BM infiltration, tumor size, LDH value.

For UA, there was a significant difference between the two groups; in the control group it was (3.48 ± 0.89 mg/dl) to the NHLs group (4.76 ± 1.37 mg/dl) with P-value (<0.05). It was elevated in 4 CLL cases and was associated with increased expression of CD147 on their B-cells and "B" symptoms which correlate with high tumor burden and poor outcome.

So higher expression of CD147 was associated with presence of "B" symptoms, anemia, leucocytosis, absolute lymphocytosis, high LDH, organomegaly, and these mostly occurred in elder patients which reflected the patient associated poor clinical condition at the time of the initial assessment.

When we monitored these patients for a year, we found that there was a significant difference between NHLs cases in the initial assessment and at the follow up reassessment at 6 month, and at 12 month intervals in the following; **the Hb level** in the initial assessment was (10.7 ± 2.4 g/dl) compared to it in the follow up reassessment (10.2 ± 2.5 g/dl) with P-value (<0.05), **PLT count** in the initial assessment was (181.433 ± 101.321 /cmm) compared to it in the follow up reassessment (193.300 ± 119.868 /cmm) with P-value (<0.05), and **Lymphadenopathy** in the initial assessment was (66.7%) then it became (73.3%) in the follow up reassessment with P-value (<0.05). This agrees with [9] who stated that increased CD147 expression is associated with lymph node metastasis and poor outcome.

Finally we conclude that most of the NHLs patients that showed high CD147 expression by flow cytometry were found to be elder in age (>50 years in females, and >60 years in males), developed more severe anemia, thrombocytopenia, organomegaly (lymphadenopathy & splenomegaly), "B" symptoms, and elevated serum LDH level, which carries poor prognosis according to the IPI for lymphoma. While the NHLs patients that showed low CD147 expression on peripheral blood B lymphocytes showed no changes from the initial assessment [10].

From the above analytical discussion of the current study we concluded that increased CD147 expression on peripheral B lymphocytes correlates with the patient poor general condition and it can predict inferior outcome for NHLs patients especially CLL patients [11].

Recommendations

a. CD147 can be used as a predictor for CLL outcome and it is more favorable to be routinely used as a part of the CLL panel of diagnosis and prognosis. It can be used as a baseline for follow up [12].

b. CD147 can be used in the staging of DLBCL patients, because it can denote BM involvement, but is it possible that it can replace BM biopsy?! .

c. High expression of CD147 in CLL and DLBCL can be used in the treatment of these patients as a target therapy "Anticancer therapy" [13].

References

1. Solstad T, Bains SJ, Landskron J, Aandahl EM, Thiede B, et al. (2011) CD147 (Basigin/Emmprin) identifies FoxP3_CD45RO_CTLA4_-activated human regulatory T cells. Blood 118: 5141-5151.
2. Liao CG, Kong LM, Song F, Xing JL, Wang LX, et al. (2011) Characterization of Basigin Isoforms and the Inhibitory Function of Basigin-3 in Human Hepatocellular Carcinoma Proliferation and Invasion. Mol Cell Biol 31: 2591-2604.
3. Halestrap AP (2012) The Monocarboxylate Transporter Family-Structure and Functional Characterization. IUBMB Life 64: 1-9.
4. Robert JJB, Chen L, Mesquita FS, Nowak RA (2008) Basigin-2 is a cell surface receptor for soluble basigin ligand. J Biol Chem 283: 178050-17814.
5. de Vries JF, Te Marvelde JG, Wind HK, van Dongen JJ, van der Velden VH (2010) The potential use of basigin (CD147) as a prognostic marker in B-cell precursor acute lymphoblastic leukaemia. Br J Haematol 150: 618-641.
6. Aiguo Liu, Qun Hu, Shuangyou Liu, Ying Hu (2009) The expressions of CD147 and MMP-9 in non-Hodgkin's lymphoma cells and the relation to prognosis. Chinese-German J Clin Oncol 8: 705-P708.
7. Pepper C, Majid A, Lin TT, Hewamana S, Pratt G, et al. (2011) Defining the prognosis of early stage chronic lymphocytic leukaemia patients. Br J Haematol 156: 499-507.
8. Geyer SM, Morton LM, Habermann TM, Allmer C, Davis S, et al. (2010) Smoking, Alcohol Use, Obesity, and Overall Survival from Non-Hodgkin Lymphoma. Cancer 116: 2993-3000.
9. Zhao SH, Wang Y, Wen L, Zhai ZB, Ai ZH, et al. (2013) Basigin-2 is the predominant basigin isoform that promotes tumor cell migration and invasion and correlates with poor prognosis in epithelial ovarian cancer. J Transl Med 11: 92.
10. Grass GD, Dai L, Qin Z, Parsons C, Toole BP (2014) CD147: regulator of hyaluronan signaling in invasiveness and chemoresistance. Adv Cancer Res 123: 351-373.
11. Takashi M (2016) Basigin (CD147), a multifunctional transmembrane glycoprotein with various binding partners. J Biochem 159: 481-490.
12. Xiao W, Zhao S, Shen F, Liang J, Chen J (2017) Overexpression of CD147 is associated with poor prognosis, tumor cell migration and ERK signaling pathway activation in hepatocellular carcinoma. Exp Ther Med 14: 2637-2642.
13. Yu B, Zhang Y, Wu K, Wang L, Jiang Y (2018) CD147 promotes progression of head and neck squamous cell carcinoma via NF-kappa B signaling. J Cell Mol Med 23: 954-966.