CORRELATION BETWEEN CD200 EXPRESSION ON LEUKEMIC STEM CELLS AND RESPONSE TO TREATMENT IN DE-NOVO ADULT ACUTE MYELOID LEUKEMIA PATIENTS

Nouran A Hassan[1], Nermeen A Nabih[1] , Tamer M Ibrahim[1] and Rana G Abdelfatah[2]

[1]Internal medicine & hematology Department, Faculty of medicine Ain shams university and
[2] Internal medicine Department, Heliopolis Hospital, Cairo, Egypt.

Corresponding author:
Rana G Abdelfatah
Mobile: +2 01067869081
e.mail:
Raanagamal@gmail.com

Received: 22/2/2023
Accepted: 8/3/2023

Online ISSN: 2735-3540

ABSTRACT

Background: Acute myeloid leukaemia is considered one of the heterogeneous hematologic cancers that have a range of therapeutic modalities, genetic abnormalities, and prognoses. AML treatment that is effective is still difficult. Increasing anti-tumor response by inhibiting immunological checkpoints is an appealing approach for leukaemia treatments. An essential immunological checkpoint known as CD200 is the ligand for CD200 receptor (CD200R), which is present on myeloid and lymphoid cells. CD200R limits anti-tumor immune responses.

Aim of work: To research the relationship between CD200 and the response outcome to induction therapy in adult AML Egyptian patients.

Methods: Ain Shams University's clinical pathology department, internal medicine department, clinical hematology and bone marrow transplantation center, and flow cytometry laboratory all participated in this prospective cross-sectional study on 68 adult patients who were recently diagnosed with acute myeloid leukaemia.

Results: Median of CD200 expression was 7.8 (1.3–45) for the responder group compared to 87.7 (77–88.6) for the non-responder group, Compared to the responder group, the CD200 % in the non-responder group had a statistically significant greater value (p 0.001). According to the CD200 level, there was a statistically significant difference between the responder group and the non-responder group with a p-value of (p0.001). The higher positive CD200 was found in non-responder group 31 patients (100%) compared to responder group 18 patients (48.6%).

Conclusion: AML development may be influenced by CD200 expression in myeloid blasts from patients with the disease. In the future, this marker's analysis may be used as a prognostic indicator and to direct treatment for AML patients.

Key words: Myeloid, CD200, Leukemia, De-novo.

INTRODUCTION:

Scientists consider acute myeloid leukemia as a life-threatening haemato-poietic cancer that blocks myeloid differentiation in addition it causes aberrant myeloid progenitors to multiply uncontrollably, that accumulate in blood and bone marrow [(1)]. when haematopoietic stem or progenitor cells undergo genetic changes AML develops, these abnormalities include many genitic oncogeneses and chromosomal rearrangements[(2)]. Similar to normal hemato-poiesis, the hematopoietic arrangement in

acute myeloid leukemia is hierarchically structured. The cells that probably start and sustain AML within such a hierarchy known as self-renewing leukemic stem cells (LSCs)[3].

By researching the cell surface structures -specially glycoproteins- we found an important transmembrane glycoprotein that is expressed in many tissues for example the cns, T and B lymphocytes, and testis; this glycoprotein is known as CD200 and its function is to safeguard immune-privileged areas and encourage peripheral tolerance.

There is no proved signaling pattern inside the cells for CD200, When CD200 interacts with CD200R -a receptor homolog found in the cell-surface- found on leukocytes of the myeloid lineage such as basophils, mast cells, dendritic cells, certain T-cell populations and macrophages, immunosuppression is induced[4].

AIM OF WORK:

To research the relationship between CD200 and the response outcome to induction therapy in adult AML Egyptian patients.

PATIENTS AND METHODS:

We chose 68 adult patients that were recently diagnosed with AML to be observed for our prospective cross-sectional study in internal medicine department, clinical hematology & bone marrow transplantation unit flow cytometry laboratory, and clinical pathology department, Ain Shams university during one year.

All participants provided their written informed permission, and the research was authorized by the research ethics committee of the faculty of medicine at Ain Shams University. The work was done in conformity with the Declaration of Helsinki, which is the international medical association's code of ethics for human subjects studies.

Patients with de-novo (newly diagnosed) AML, found to be 18 to 65 years old who were deemed fit (eligible) for intensive chemotherapy of curative intent (free from cardiac, renal or liver impairment) were only included in our study while patients aged less than 18 years or more than 65 years, relapsed with AML, with AML with chemotherapy given before enrolment in the study, with secondary AML with preceding hematologic disorder, with APL (acute promyelocytic leukemia), with other types of acute leukemia other than actue myloid leukemia (acute lymphocytic leukemia, biophenotypic acute leukemia, etc..) were excluded. Patients with past history of other co-morbidities, autoimmunity and other immune disorders, other solid tumors or any end organ failure or who were considered ineligible to receive intensified treatment (patients with cardiac, renal or liver impairment) were also excluded. Complete history was taken from all patients including personal data, clinical data and any co-morbid or chronic medical illnesses. We fully examined all the patients by clinical evaluations, laboratory and radiological tests in order to establish the diagnosis, determine patient eligibility to chemotherapy and to assess tumor burden and risk stratification. These investigations involved complete blood picture (CBC) with differential count, blood chemistry including renal, hepatic profiles & lactate dehydro-genase, bone marrow aspirate & immune-phenotyping, cytogenic analysis, echo-cardiography, pelviabdominal ultrasound and magnetic resonance imaging for brain & spine if available. Regarding sample collection, heparinized peripheral venous blood (a full 2.5 ml was needed if an ESR was also performed) was collected from each participant (68 patients) at time of diagnosis, prior to starting medications and

induction chemotherapy therapy, and after receiving informed consent from AML patients being treated in the Hematology department, Ain Shams university hospital. The CD200 protein expression on AML patient samples was examined using fluorescence flow cytometry. It was performed in the flow cytometry laboratory, clinical pathology, Ain Shams university hospital.

Statistics/data explanation:

The data collected were examined with the statistical package for social sciences, version 20.0. (SPSS Inc., Chicago, Illinois, USA). The mean and standard deviation of quantitative data were used. Frequency and percentage were used to convey qualitative data. When comparing two means, the independent-samples t-test of significance and Mann Whitney tests were employed. For two-group comparisons in non-parametric data, U test was utilized. Only when the predicted count in any cell was less than 5 was the Fisher's exact test instead of the Chi-square test used to compare groups with qualitative data. Analysis of multivariate logistic regression To analyse the overall correlation between each conceivable risk factor and the occurrence of poor prognostic variables, 'odds ratios (OR) with 95% confidence intervals' were computed. The receiver operating characteristic (ROC curve) analysis was done to determine the overall predictability of the parameter and the optimal cut-off value with detection of sensitivity and specificity at this cut-off value. The confidence interval was set at 95%, while the acceptable margin of error was set to 5%. As a result, the p-value was defined as follows: P-value 0.05 was regarded significant, P-value 0.001 was considered highly significant, and P-value >0.05 was considered insignificant.

Ethics approval and consent to participate:

Written informed consent was obtained from all participants and the study was approved by the research ethical committee of Faculty of Medicine, Ain Shams University. The work has been carried out in accordance with the code of ethics of the world medical association (Declaration of Helsinki) for studies involving humans.

RESULTS:

Table (1) describes CD200 expression distribution in the entire study candidates. CD200 expression was between 1.3 and 92.8 with mean± SD of 49.20±34.61. There were 19 patients (27.9%) were “negative CD200” and 49 patients (72.1%) were positive CD200. There were 37 patients (54.4%) were complete remission, 31 patients (45.6%) were relapse/resistance as shown in Figure (1).

Table (2) shows that median of CD200 expression was 7.8 (1.3–45) for the responder group compared to 87.7 (77–88.6) for the non-responder group, A statistically significant difference existed between the responder and non-responder groups according to CD200 level with p-value ($p<0.001$). The higher positive CD200 was found in non-responder group 31 patients (100%) compared to responder group 18 patients (48.6%). Table (3) shows that t (15;17) cytogenetics was positive in 6 (8.8) patients & negative in 62 (91.2) patients.31 patients (100%) of the non- responder were t (15;17) negative.

Table (3) shows that t (15;17) was positive only in 6 patients of the 49 patients which were CD200 positive (12.2%), these 6 patients were complete remission. while t (15;17) was negative in 43 patients which were CD200 positive (87.7%), only 12 patients of them were complete remission & 31 patients of them were relapse. There was statistically significance of high CD200

expression in the t (15;17) negative AML group.

(Table 4) shows that age (years) was a significant predictor of poor prognostic variables, according to multivariate analysis, TLC, as for the CD200 were the best independent predictors of bad prognostic factors with [OR (C.I.95%), p-value] [3.519 (2.062-6.000), P=0.032; 8.340 (4.409-23.422), P=0.012; 7.356 (3.351-19.968), P=0.024; 2.352 (1.823-3.032),

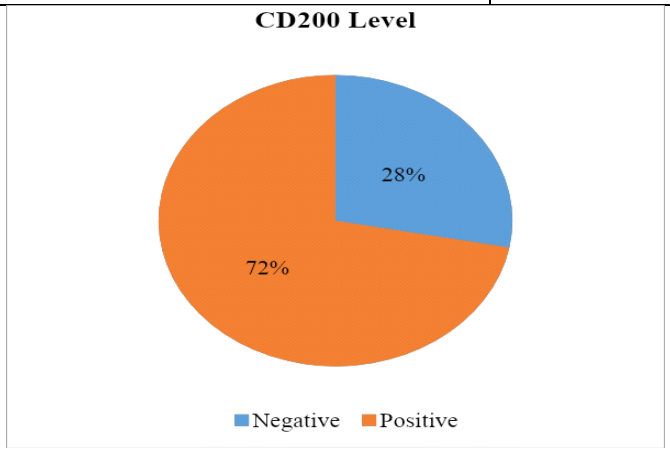
P=0.042; 2.168 (2.007-2.341), P=0.044; 2.160 (1.935-2.411), P=0.046; 2.752 (2.133-3.547),

P=0.037; 2.695 (2.015-3.605), P=0.040; 6.287 (2.864-17.066), P=0.028; 2.118 (2.017-2.224),

P=0.048; 8.961 (3.333-17.299), P=<0.001] respectively. Indicators of CD200 % were used using Receiver Operator Characteristics (ROC) curves as indicators of poor prognosis in patients who were included. The optimum cutoff value for CD200 was 45, with sensitivity of 96.8%, specificity of 83.8%, positive predictive value of 83.3%, negative predictive value of 96.9%, diagnostic area under the curve of 0.963 with p-value 0.001 (Figure 2). CD200 expression indices were significant predictors as indicated by the substantially high area under the curves (AUCs).

Table (1): CD200 expression descriptive among AML group (n=68).

CD200 expression	Total (n=68)
Negative	19 (27.9%)
Positive	49 (72.1%)



. (1): Pie chart CD200 expression descriptive among AML group.

Table (2): relationship of CD200 expression inresponder group and non-responder group in the form of comparison table.

CD200 expression	Responder (n=37)	Non reponder (n=31)	Test Value	P-value
Negative	19 (51.4%)	0 (0%)	*FE:*	<0.001*
Positive	18 (48.6%)	31 (100%)	22.092	*

Using: FE: Fisher's Exact and U=Mann-Whitney test
p-value more than 0.05 NS, *p-value less than 0.05 S and **p-value less than 0.001 HS

Table (3) correlation between CD200 & t(15;17) expression on AML group &their response to treatment

	CD 200+ve	CD200-ve
T(15;17)+ve	6 (100%)	0 (0%)
T(15;17)-ve	43 (69.35%)	19 (30.64%)

CD200 expression& cytogenetics	Resonders(CR) (n=37)	Non-responders(R/R) (n=31)
CD200 +ve&t(15;17)+ve	6(100%)	0(0%)
CD200+ve&t(15;17)-ve	12(27.9%)	31(72%)
CD200-ve&t(15;17)+ve	0(0%)	0(0%)
CD200-ve&t(15;17)-ve	19(100%)	0(0%)

Table (4): Multivariate regression analysis to risk factors as predictors of bad prognostic factors.

Items	β	SE	Wald	OR	95% C.I. for OR		p-value
					Lower	Upper	
Patient age in years	1.580	0.269	5.852	3.519	2.062	6.000	0.032*
TLC	3.303	0.562	12.233	7.356	3.351	19.968	0.024*
CD200	4.024	0.684	14.903	8.961	3.333	17.299	<0.001*

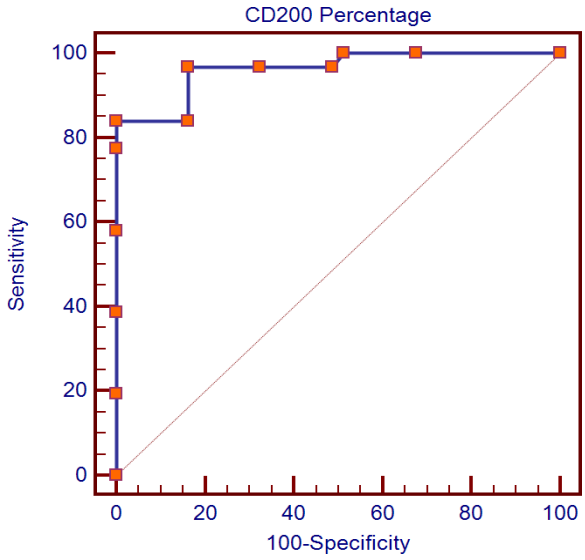


Figure (2): Receiver-operating characteristic (ROC) curve for CD200 expression level-based prediction of poor prognosis.

Cut-off	Sen.	Spe.	PPV	NPV	AUC [95% C.I.]	p-value
45	96.8%	83.8%	83.3%	96.9%	0.963 [0.887-0.994]	<0.001

DISCUSSION:

Scientists described that neoplastic proliferation of improperly differentiated blood stem cells in the bone marrow and monoclonal cells are major criteria of Acute Myeloid Leukemia (AML) –which is a form of blood Acute Leukemia- these neoplastic cells infiltrates many organs in addition to

bone marrow. patients who have never previously had myelodysplastic syndrome (MDS), myeloproliferative condition, or exposure to substances or treatments that might cause leukemia are described by the term De novo AML[5]. The interaction between CD200R and CD200 (which is a type-1 membrane glycoprotein with two immunoglobulin domains that is found in a variety of cells) can cause an immunosuppressive signal, promoting tumour development. Regarding malignancies of the blood the first instance of CD200 expression was in chronic lymphocytic leukemia, where it aids in differentiating it from mantle cell lymphoma. More aggressive multiple myeloma has been linked to plasma cells lacking CD200 expression. CD200 aberrant expression has recently been suggested as a poor prognostic factor in AML[6].

Our aim during this paper was evaluating the expression of CD200 in leukemic stem cells and how it affects how adult patients with acute myeloid leukemia respond to induction treatment. We collected blood samples from each patient at the time of diagnosis and during the evaluation of the response to induction treatment for 68 individuals; with the same inclusion and exclusion criteria. Our research included participants ranging in age from 21 to 75 (mean age, 44.43 - 13.57 years). Males predominated, with a male: female ratio around 1.72:1. Our findings showed that there were 37 patients (54.4%) were complete remission, 6 patients (8.8%) were primary induction failure, and 25 patients (36.8%) were relapse; as for the response it was 31 patients (45.6%) were non responder and 37 patients (54.4%) were responder.

Tiribelli and colleagues conducted a similar study in which one hundred thirty-nine patients with AML, their mean age was 60 years (range from 22 to 81), started their treatment at their institutions during a period of eight years. Complete remission (CR) was attained in 98 patients (70%) following induction therapy: 56/71 (79%) CD200- and 42/67 (63%) CD200+ individuals. Patients with high CD200 intensity had a lower CR rate (9/18, 50%) [7]. Chen and colleagues analyzed the results of acute myeloid leukemia induction treatment. 245 patients in all obtained complete remission, incomplete platelet recovery, or incomplete blood count recovery, together with 165 newly diagnosed patients with AML, 27 and 80 individuals had relapsed or were resistant to induction treatment (greater than or equal to 5% blasts by morphology unrelated to blood count recovery); of these 80 patients, 16 had relapsed AML[8].

Our analysis of data revealed that the following characteristics were significant predictors of poor prognostic factors: Age,TLC, as for the CD200 were the best independent predictors of bad prognostic factors. Indicators of CD200 % were used via Receiver Operator Characteristics (ROC) curves as indicators of bad prognosis in patients who were included. There was utilized to identify the optimal cut off value of CD200, which was 45, with sensitivity of 96.8% specificity of 83.8% positive predictive value of 83.3%, negative predictive value of 96.9%, diagnostic area under the curve of 0.963 with p-value 0.001. CD200 percentage indices were significant predictors as indicated by the considerably large area under the curves (AUCs).

Recently, Miao and colleagues in a series of patients emphasized the significance of CD200 expression intensity. High CD200 intensity was shown to have a deleterious effect in particular in the group of individuals with favorable clinical characteristics[9]. Coles and colleagues hypothesized that CD200 works in conjunction with other local factors to protect leukemia stem cells in a favorable milieu by raising the amount of Tregs in bone marrow, downregulating Th1-mediated cytokines necessary for an effective

cytotoxic T-cell activation, inhibiting macrophage activity, and expanding myeloid derived suppressor cells[10].

Conclusion:

In conclusion, the presence of CD200 in myeloblast in AML patients might contribute to the development of acute myloid leukemia. In the future, this marker's analysis may be used as a prognostic indicator and to help determine how to treat AML patients. It is acknowledged that CD200 plays a poor prognostic function in AML. Notably, CD200 overexpression is linked to a poorer prognosis in individuals with De-novo adult acute myeloid leukemia in addition to having a cumulatively detrimental effect. Further longitudinal studies are warranted to explore the correlation between CD200 expression on leukemic stem cells and response to treatment in adult patients diagnosed with De-novo AML.

Acknowledgement:

The authors are grateful for the patients without whom this study would not have been done.

Funding:

None.

Consent for publication :

Not applicable, The manuscript does not contain data from any individual person.

Author's contribution:

1. N H: collected the data, analyzed the patients data

2. T I: supervising the data and the statistics

3. NA: : supervising the data and the statistics

4. R A: revised the data , edited the manuscript, major contributer in writing the mauscript

All authors read and approved the final manuscript

Conflict of interest:

The authors affirm that they have no conflicts of interest.

The material is original research, has not been previously published and has not. been submitted for publication elsewhere while under consideration

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الارتباط بين cd 200 على الخلايا الجزعية اللوكيمياء والاستجابة للعلاج فى مرضا اللوكيمياء النخاعى الحاد عند البالغين

نوران على حسن[١] ، نرمين عادل نابيه[١] ، تامر احمد محمد ابراهيم[١] ، رنا جمال عبدالفتاح[٢]

[١]قسم الباطنة العامة وامراض الدم بكية الطب – جامعة عين شمس

[٢] قسم الأمراض الباطنه العامه – مستشفي هليوبلس – القاهرة

المقدمة: لوكيميا الدم النخاعي الحاد (AML) هو مرض مكون للدم يهدد الحياة ويتميز بإعاقة التمايز النخاعي والتكاثر غير الطبيعي للأسلاف النخاعية غير الطبيعية التي تتراكم في نخاع العظام والدم. يتميز بالنمو السريع للخلايا غير الطبيعية التي تتراكم في نخاع العظام والدم وتتداخل مع إنتاج خلايا الدم الطبيعية. ينتج ابيضاض الدم النقوي الحاد (AML)عن تحول الخلايا الجذعية المكونة للدم أو الخلايا السلفية من خلال اكتساب عيوب وراثية تتضمن إعادة ترتيب الكروموسومات وطفرات جينية متعددة. يتم تنظيم نظام المكونة للدم في AML بطريقة هرمية تمامًا كما هو الحال في تكوين الدم الطبيعي. ضمن هذا التسلسل الهرمي، يبدو أن الخلايا الجذعية اللوكيميا ذاتية التجديد (LSCs) هي الخلايا التي تبدأ وتحافظ على.AML

CD200عبارة عن بروتين سكري على سطح الخلية عبر الغشاء من النوع ١ aيتم التعبير عنه عادةً في الأنسجة الحساسة مثل الجهاز العصبي المركزي والخصية، بالإضافة إلى بعض الكريات البيض، بما في ذلك الخلايا الليمفاوية T وB ، حيث يتمثل دوره في تعزيز التسامح المحيطي وحماية المناعة. المواقع المميزة. لا يحتوي CD200 على فكرة إشارات داخل الخلايا معروفة، ولكنه يحث على تثبيط المناعة من خلال الارتباط بـ CD200R ، وهو متماثل لمستقبل سطح الخلية، والذي يتم التعبير عنه في الكريات البيض من سلالة النخاع، بما في ذلك الخلايا البدينة، والبلاعم، والخلايا القاعدية، والخلايا المتفرعة وكذلك مجموعات معينة من الخلايا التائية.

الهدف من الدراسة: كان هدفنا خلال دراستنا هو تقييم التعبير عن CD200 في الخلايا الجذعية لسرطان الدم وتأثيره على الاستجابة لعلاقة العلاج التعريفي لدى المرضى البالغين المصابين بسرطان الدم النخاعي الحاد. ١٠ كانت المرضي والطريقة:الدراسة عبارة عن دراسة مقطعية مستقبلية أجريت في قسم الطب الباطني وأمراض الدم السريرية ونخاع العظام وحدة الزرع بجامعة عين شمس. تم أخذ التاريخ الكامل من المرضى. تم إجراء الفحص السريري الكامل والفحوصات المخبرية والإشعاعية. جمعت عينات الدم من ٦٨ مريضا على النحو التالي ، تم جمع عينات الدم من كل مريض: في وقت التشخيص وأثناء تقييم الاستجابة للعلاج التعريفي ؛ بنفس معايير التضمين والاستبعاد. تم إجراء قياس التدفق الخلوي الفلوري لتحليل تعبير بروتين CD200 على عينات من مرضى .AML تم الحصول على الموافقة على إجراء الدراسة من مجلس المراجعة المؤسسية بجامعة عين شمس.(IRB)

الاحصاء: تم تحليل البيانات المسجلة باستخدام الحزمة الإحصائية للعلوم الاجتماعية ، الإصدار ٢٠.٠ SPSS) .Inc، شيكاغو ، إلينوي ، الولايات المتحدة الأمريكية). تم التعبير عن البيانات الكمية على أنها تعني ± الانحراف المعياري .(SD) تم التعبير عن البيانات النوعية بالتكرار والنسبة المئوية. تم تحليل النسبة المئوية للانفجار وتوزيع النسبة المئوية CD200 لإجمالي مجتمع الدراسة خلال دراستنا. تراوحت نسبة الانفجار من ٢٠ إلى ٨٥ بمتوسط SD ± من ٤٤.٠٩ ± ٢٠.٦٦ وتراوحت النسبة المئوية CD200 من ١.٣ إلى ٩٢.٨ بمتوسط SD ± يبلغ ٤٩.٢٠ ± ٣٤.٦١. كان هناك ١٩ مريضا (٢٧.٩٪ CD200" (سلبية" و ٤٩ مريضا (٧٢.١٪) كانت موجبة .CD200 كان متوسط النسبة

المئوية للانفجار ٥٥ (٢٠-٧٣) لمجموعة المستجيبين مقارنة بـ ٤٠ (٤٠-٤٥) لمجموعة غير المستجيبين ، ولا يوجد فرق معتد به إحصائيًا بين مجموعة غير المستجيبين مقارنة بمجموعة المستجيبين ذات القيمة.(p (p> 0.05 NS

النتائج: كشفت نتائجنا أن متوسط النسبة المئوية لـ CD200 كان ٧.٨ (١.٣-٤٥) لمجموعة المستجيبين مقارنة بـ ٨٧.٧ (٧٧-٨٨.٦) لمجموعة غير المستجيبين ، وكانت هناك دلالة إحصائية ١١ أعلى قيمة لنسبة CD200 في مجموعة غير المستجيبين مقارنة بمجموعة المستجيبين ذات القيمة .(p <0.001) p وفي الوقت نفسه ، كان هناك فرق معتد به إحصائيًا بين مجموعة المستجيبين وغير المستجيبين مجموعة المستجيبين وفقًا لمستوى CD200 مع قيمة p) p .(0.001>تم العثور على CD200 أعلى إيجابي في مجموعة غير المستجيبين ٣١ مريضًا (١٠٠٪) مقارنة بمجموعة المستجيبين ١٨ مريضًا (٤٨.٦٪). كشف تحليلنا متعدد المتغيرات عن تنبؤات مهمة للتنبؤ السيئ كانت العمر (بالسنوات) ، والجنس ، و TLC ، و Lymph ، و Net ، و BUN ، و Creat ، و eGFR ، و PTT ، و INR ، أما بالنسبة إلى CD200 ، فكانت أفضل تنبؤات مستقلة لعوامل الإنذار السيئة. كانت منحنيات خصائص مشغل المستقبل (ROC) مؤشرات لنسبة CD200 كمؤشرات للتنبؤ السيئ في المرضى المشمولين. كانت مؤشرات النسبة المئوية لـ CD200 تنبئًا مهمًا على النحو المشار إليه من خلال المساحة الكبيرة بشكل ملحوظ تحت المنحنيات (AUCs) ، وقد تم استخدامها لتحديد أفضل قيمة قطع لـ CD200 والتي كانت ٤٥ ، مع حساسية ٩٦.٨٪ خصوصية بنسبة ٨٣.٨٪ قيمة تنبؤية إيجابية لـ ٨٣.٣٪ ، قيمة تنبؤية سلبية ٩٦.٩٪ ، منطقة تشخيصية تحت منحنى ٠.٩٦٣ بقيمة احتمالية <٠.٠٠٠١.

المناقشة والختام: في الختام ، يمكن أن يلعب تعبير CD200 في الانفجار النخاعي في مرضى AML دورًا في تطوير .AML يمكن أن يكون تحليل هذه العلامة بمثابة علامة تنبؤية وقد يوجه العلاج في مرضى AML في المستقبل. ويلاحظ الدور النذير السلبي لـ CD200 في .AML من الجدير بالملاحظة ، إلى جانب التأثير السلبي الإضافي في المرضى الذين يعانون من ابيضاض الدم النخاعي الحاد لدى البالغين De-novo ، يرتبط الإفراط في التعبير عن CD200بتشخيص أسوأ.

هناك ما يبرر المزيد من الدراسات الطولية لاستكشاف العلاقة بين تعبير CD200 على الخلايا الجذعية لسرطان الدم والاستجابة للعلاج في مرضى سرطان الدم النخاعي الحاد لدى البالغين.De-novo