

Adenosine Deaminase Activity in Chronic Lymphocytic Leukemia and Healthy Subjects

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Abstract

Background: B cell chronic lymphocytic leukemia is one of the most frequent hematologic malignancies in the world. Cellular surface CD markers and serum Beta-2-microglobulin may be used as a prognostic tool in CLL patients.

Objectives: In the present study we introduce serum adenosine deaminase as a diagnostic marker in CLL.

Materials and Methods: Blood samples were collected from B-CLL and healthy subjects. White blood cell, red blood cell and platelet count and blood Erythrocyte sedimentation rate was recorded and serum Beta-2-microglobulin, Lactate dehydrogenase and total ADA enzyme activity were determined.

Results: Serum ADA activity was significantly higher in patients group than that of controls. ADA had a significant and direct correlation with B2M, WBC, LDH and ESR. However, there was not any relation between ADA and the stages of disease. Diagnostic cut-off, sensitivity and specificity of the serum ADA test were 27.97 U/L, 91% and 94%, respectively.

Conclusions: A higher ADA activity in patients group and its correlation with CLL markers were seen in our study. High diagnostic value of serum ADA in our study suggests that it might be considered as a useful screening tool among the other markers in CLL.

Keywords: Adenosine Deaminase, Beta-2-Microglobulin, Chronic Lymphocytic Leukemia, Diagnostic Value

1. Background

Chronic lymphocytic leukemia (CLL) is characterized by the gradual accumulation of CD19-CD5-malignant B cells along with immune cell dysfunction (1). It has been reported that immune response and homeostatic control defects in the T cell and the leukemic B cell compartments (2). Abnormalities in these compartments may contribute to the inability of the immune system to recognize and destroy the leukemic cells (3-6).

CLL is the second most common type of leukemia in adults (7, 8). It often occurs during or after middle age, and is rare in children (9). Usually CLL does not cause any symptoms and clinical course of disease is variable (9). Some of these patients have a stable situation (even to the end) and do not require treatment, while others have progressed disease despite treatment (10). So, evaluation of new diagnostic and therapeutic strategies for B-CLL seems necessary and attractive. Prognosis of disease mainly depends on the clinical and laboratory outcomes of disease. However, many patients are asymptomatic at the time of diag-

nosis.

Adenosine deaminase (ADA) (EC 3.5.4.4) is a hydrolytic enzyme that involves in the deamination of adenosine and deoxyadenosine nucleosides, forming inosine and deoxyinosine, respectively (11). ADA is widely distributed in human tissues and is involved in immune system development (12, 13). In particular, ADA has an important role in proliferation and differentiation of lymphoid cells and plays a major role in various stages of lymphocyte maturation (14). ADA is also the main regulator of adenosine concentration in plasma which is involved in development of inflammatory response and cytokine production (15).

Alteration in serum ADA activity has been reported in a broad range of diseases such as tuberculosis, HIV, lung cancer, chronic heart failure and chronic obstructive pulmonary disease (16-20). ADA is known as a marker of T-lymphocyte activation (21). Furthermore, it has been shown in several studies that ADA activity may be useful both in the diagnosis and monitoring of some malignancies (21, 22).

Considerably, CLL is one of the most common lym-

phomas in Iran (23). In addition, the current tests for diagnosis and follow up of CLL patients are difficult, slow and expensive. ADA is measured calorimetrically and coefficient of variation (%CV) for this method is about 3%; it has a high reproducibility and very inexpensive materials used for this method (16).

2. Objectives

Because of simplicity, rapid use and low priced of ADA test, in present study, we measured total ADA, activity in serum of patients with B-CLL and healthy subjects to assess their diagnostic validity as a marker for diagnosis of CLL.

3. Materials and Methods

3.1. Patient Selection

All 87 B-CLL patients (68 males and 19 females with an average age of 66.27 ± 10.68 years) who admitted to the Sanandaj Tohid hospital (Kurdistan, Iran) during a period of January 2012 until April 2014 were enrolled in this case-control study. Diagnosis of disease was based on clinical and laboratory characteristics, including hematologic and immunologic data. The youngest patient was 44 years old and the oldest one was 91 years. Based on Rai staging system (24), staging of the patients were done. The control group consisted of 100 healthy individuals (50 women and 50 men) with a mean age of 53.84 ± 8.11 years, with non-malignancy (negative pathological tests). Written informed consent was obtained from all patients and the study was approved by the ethics committee of Kurdistan University of Medical Sciences. Criteria for inclusion of individuals and treatment conditions were determined previously (25, 26). Of all cases, 22 patients were treated with FC (fludarabine with cyclophosphamide) and CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone).

3.2. Measurement of ADA Activity

the fasting blood serum samples were collected and used to measure the enzyme activity. Adenosine was obtained from Sigma-Aldrich (Saint Louis, Missouri 63103, USA). Sodium di-hydrogen phosphate [$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$], disodium hydrogen phosphate [$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$], Ammonium sulfate [$(\text{NH}_4)_2\text{SO}_4$], Phenol [$\text{C}_6\text{H}_5\text{OH}$], Sodium nitroprusside [$\text{Na}_2(\text{Fe}(\text{CN})_5\text{NO})$], soda [NaOH] were obtained from (Merck Chemicals Ltd, Germany). Serum total ADA activities were determined by Giusti method (27). Briefly, duplicate 25 μL samples were incubated for 60 minutes at 37°C with 500 μL of 21 mM adenosine (one sample) in 50

mM phosphate buffer, and the released ammonia was determined by its reaction with 1.5 mL phenol nitroprusside in 30 minutes (106 mM phenol plus 0.17 mM sodium nitroprusside) in the presence of 1.5 mL of sodium hypochlorite (11 mM NaOCl plus 125 mM NaOH), using a spectrophotometer absorption read at 630 nm. To control the presence of ammonium before addition of exogenous adenosine, untreated samples were run in parallel. The results were expressed in U/L.

3.3. Statistical Analysis

Data analysis was carried out using the Kruskal-Wallis and two-way ANOVA tests. A P value < 0.05 was considered statistically significant. Spearman rank coefficients were used to express the correlation between variables. The significance of the correlation was denoted by a P value < 0.05 . All statistical analyses were done using software SPSS version 16. Receiver operating characteristic (ROC) curves were constructed to establish a sensitivity-specificity relationship. Cut-off values that provided the best combination of sensitivity and specificity were determined by ROC curve analysis. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR+), negative likelihood ratio (LR-) and accuracy were calculated (20).

4. Results

The means and standard deviations of ADA for B-CLL and healthy groups were 51.67 ± 20.99 U/L and 19.03 ± 6.17 U/L, respectively ($P < 0.0001$). Table 1 represents the ADA activity in patient group according to the stage of disease. As shown in this there were not any significant differences between groups in regard to stage.

Clinical and laboratory outcomes of study subjects are shown in Table 2. Our results show that the serum Beta-2-microglobulin (B2M) and Lactate dehydrogenase (LDH) were higher in the patient group than healthy subjects. In addition, the white blood cell (WBC) and red blood cell (RBC) count and Erythrocyte sedimentation rate (ESR) had similar patterns and were significantly higher in patients. Furthermore, platelet (Plt) count was significantly lower in patients compared to healthy group. However, we could not find any differences for B2M, WBC, RBC, LDH, ESR and Plt in various stages. In addition, treatment with chemotherapeutic agents significantly ($P < 0.05$) decreased the ADA activity in patient group (ADA activity before treatment was 56.55 ± 22.61 U/L while as ADA activity after treatment was 39.38 ± 18.87).

We also determined the possible correlation between ADA activity and laboratory markers in B-CLL group. Our

Table 1. Serum ADA Activity in Study Groups^a

	ADA Activity, U/L				
	Stage 0 (n = 6)	Stage I (n = 9)	Stage II (n = 7)	Stage III (n = 6)	Stage IV (n = 19)
Patients (n = 47)	32.59 ± 13.02	59.61 ± 20.56	54.4 ± 14.72	47.87 ± 23.53	54.1 ± 22.3
Controls (n = 100)	19.03 ± 6.17 ^b				
Total	51.67 ± 20.99 ^b				

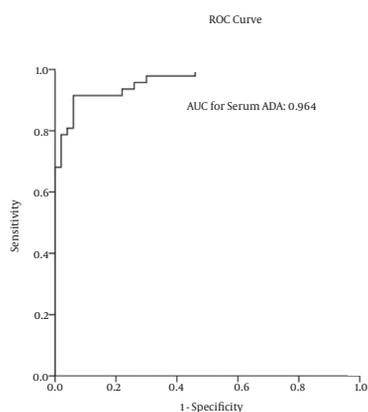
Abbreviations: ADA, adenosine deaminase; U/L, units per liter of enzyme activity.

^aValues are expressed as mean ± SD.

^bSignificant difference ($P < 0.05$).

finding demonstrated that there is a significant positive correlation between ADA activity and WBC, LDH, B2M and ESR (Table 3). On the other hand, RBC and Plt counts had no correlation with ADA activity (Data not shown).

ROC curve analysis revealed that cut-off level is 27.97 U/L for enzyme activity in serum sample. Using this cut-off level for serum ADA activity, sensitivity and specificity were 91% (95%CI = 0.8 - 0.98) and 94% (95%CI = 0.83 - 0.99), respectively. Besides, according to obtained sensitivity and specificity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio were evaluated (Table 4 and Figure 1).

Figure 1. ROC Curves for Serum ADA

ADA, Adenosine deaminase; AUC, area under the curve; ROC, receiver operating characteristic.

5. Discussion

Several investigations showed the role of adenosine deaminase in immune system development. It seems that ADA has an important role in maturation of lymphocytes and high ADA activity is seen in immature lymphocytes

(28, 29). In addition, there are several studies that represent high ADA activity in various malignancies. Patients with lung cancer, breast cancer, ovarian cancer, gastric carcinoma and colorectal cancer have increased level of serum ADA activity (21, 30-33). Our results also demonstrated higher enzyme activity in B-CLL patients compared to healthy subjects.

In addition, our results revealed that there is a direct correlation between B2M, WBC, LDH and ESR with serum ADA activity. These markers, especially B2M could be used to evaluate the severity and spread (stage) of multiple myeloma, to help determine the prognosis of cancers such as multiple myeloma and lymphoma, and may sometimes be ordered to evaluate disease activity and the effectiveness of treatment (34). However, in our study we could not find any correlation between these markers and the stage of disease. It may be because of the low number of patients dispersed in various stages. Furthermore, the direct significant correlation of ADA with these markers that could be seen in our study suggests the serum ADA activity as a good prognostic tool in CLL.

There are a few studies that determined ADA activity in lymphocytes (35-38). The results from these studies are completely contradicted to our results. They suggest that ADA activity in malignant lymphocytes is lower than normal ones. However, previous studies clearly showed that although the location of the enzyme is mainly cytosolic, ADA has been found in membrane fractions (39) and realization of ADA to blood stream may rise by increasing the cell numbers. To our knowledge, our study is the first investigation that evaluated the serum ADA activity with clinical outcomes in B-CLL patients. Our results revealed that serum ADA activity in B-CLL patients is significantly higher than controls. Furthermore, we showed that if we use a cut-off value equal to 27.97 U/L, the sensitivity and specificity of serum ADA test is 91% and 94%, respectively.

Besides, our previous studies showed that ADA activity tangibly depends on the wavelength used for measurement of ADA. In addition, it seems that it has a wide range

Table 2. Concentration of Different Variables in B-CLL Patients

	Stage 0	Stage I	Stage II	Stage III	Stage IV
B2M, mg/L	2.90 ± 1.43	4.29 ± 1.49	4.87 ± 2.55	4.27 ± 1.71	5.56 ± 2.03
Total			4.71 ± 2.04		
LDH, U/L	375.17 ± 101.49	360.67 ± 73.78	388.86 ± 11.52	315.67 ± 11.87	382.89 ± 141.97
Total			369.96 ± 116.03		
ESR, mm/h	12.07 ± 7.97	3.96 ± 1.83	11.86 ± 9.89	7.38 ± 5.96	9.02 ± 7.61
Total			8.65 ± 7.38		
Plt Count, × 10³/μL	1.84 ± 0.45	1.84 ± 0.56	1.96 ± 0.66	1.92 ± 0.56	1.27 ± 0.44
Total			1.64 ± 0.58		
WBC Count, × 10³ cell/μL	59.40 ± 42.65	85.54 ± 10.33	56.20 ± 44.26	61.00 ± 46.61	17.47 ± 23.01
Total			47.18 ± 58.84		
RBC Count, × 10⁶ cell/μL	12.85 ± 1.30	12.67 ± 2.46	13.64 ± 1.64	11.33 ± 0.78	12.41 ± 1.46
Total			12.56 ± 1.70		

Abbreviations: B2M, beta-2-microglobulin; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; Plt, platelet; RBC, red blood cell; WBC, white blood cell.

Table 3. Correlation of Serum Adenosine Deaminase With CLL Markers

	Pearson Correlation	P Value
WBC	0.49	< 0.05
LDH	0.446	< 0.05
B2M	0.621	< 0.01
ESR	0.373	< 0.01

Abbreviations: B2M, beta-2-microglobulin; ESR, erythrocyte sedimentation rate; LDH, lactate dehydrogenase; WBC, white blood cell.

Table 4. Diagnostic Values for Adenosine Deaminase Activity in Serum Samples

	Cut-Off, U/L	Sensitivity	Specificity	LR+	LR-	PPV	NPV	Accuracy, %
Values	27.97	0.91	0.94	15.17	0.1	0.91	0.92	93

Abbreviations: LR, likelihood ratio; NPV, negative predictive value; PPV, positive predictive value.

of results in different studies. It was suggested that this alterations could be due to sampling and/or patient selection and analysis methods. Furthermore, reference intervals for healthy subjects that have been reported in different ethnic groups are very diverse (16).

In summary, we showed that serum total ADA activity in B-CLL subjects is significantly higher than controls. In addition, our results also showed a direct correlation between WBC, LDH, B2M and ESR with ADA activity. Furthermore, we showed that serum ADA activity is a good test for differentiating B-CLL patients from healthy subjects and it may be used as an alternative or additional test along with the other tests for diagnosis and/or prognosis of B-CLL.

It can be concluded that determination of serum ADA

activity might be a simple, rapid, and inexpensive diagnostic tool for screening of B-CLL patients compared to current costly, laborious, and time consuming markers.

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Footnotes

Authors' Contribution: All authors contributed in the conception and design of the work, conducting the study,

analysis and interpretation of data, drafting and revising the draft, approval of the final version of the manuscript, and agreed for all aspects of the work.

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References

1. Everaus H, Luik E, Lehtmaa J. Active and indolent chronic lymphocytic leukaemia-immune and hormonal peculiarities. *Cancer Immunol Immunother.* 1997;**45**(2):109-14. [PubMed: [9390202](#)].
2. Bartik MM, Welker D, Kay NE. Impairments in immune cell function in B cell chronic lymphocytic leukemia. *Semin Oncol.* 1998;**25**(1):27-33. [PubMed: [9482524](#)].
3. Bonyhadi M, Frohlich M, Rasmussen A, Ferrand C, Grosmaire L, Robinet E, et al. In vitro engagement of CD3 and CD28 corrects T cell defects in chronic lymphocytic leukemia. *J Immunol.* 2005;**174**(4):2366-75. [PubMed: [15699173](#)].
4. Cantwell M, Hua T, Pappas J, Kipps TJ. Acquired CD40-ligand deficiency in chronic lymphocytic leukemia. *Nat Med.* 1997;**3**(9):984-9. [PubMed: [9288724](#)].
5. Kato K, Cantwell MJ, Sharma S, Kipps TJ. Gene transfer of CD40-ligand induces autologous immune recognition of chronic lymphocytic leukemia B cells. *J Clin Invest.* 1998;**101**(5):1133-41. doi: [10.1172/JCI1472](#). [PubMed: [9486984](#)].
6. Rossi E, Matutes E, Morilla R, Owusu-Ankomah K, Heffernan AM, Catovsky D. Zeta chain and CD28 are poorly expressed on T lymphocytes from chronic lymphocytic leukemia. *Leukemia.* 1996;**10**(3):494-7. [PubMed: [8642868](#)].
7. Shih LY, Liang DC. Non-Hodgkin's lymphomas in Asia. *Hematol Oncol Clin North Am.* 1991;**5**(5):983-1001. [PubMed: [1938764](#)].
8. Anderson JR, Armitage JO, Weisenburger DD. Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. *Ann Oncol.* 1998;**9**(7):717-20. [PubMed: [9739436](#)].
9. Kalpadakis C, Pangalis GA, Sachanas S, Vassilakopoulos TP, Kyriakaki S, Korkolopoulou P, et al. New insights into monoclonal B-cell lymphocytosis. *Biomed Res Int.* 2014;**2014**:258917. doi: [10.1155/2014/258917](#). [PubMed: [25295254](#)].
10. Nabhan C, Rosen ST. Chronic lymphocytic leukemia: a clinical review. *JAMA.* 2014;**312**(21):2265-76. doi: [10.1001/jama.2014.14553](#). [PubMed: [25461996](#)].
11. Phillis JW. Adenosine and adenine nucleotides as regulators of cellular function. CRC press; 1991.
12. Conway EJ, Cooke R. The deaminases of adenosine and adenylic acid in blood and tissues. *Biochem J.* 1939;**33**(4):479-92. [PubMed: [16746934](#)].
13. Van der Weyden MB, Kelley WN. Human adenosine deaminase. Distribution and properties. *J Biol Chem.* 1976;**251**(18):5448-56. [PubMed: [9388](#)].
14. Adams A, Harkness RA. Adenosine deaminase activity in thymus and other human tissues. *Clin Exp Immunol.* 1976;**26**(3):647-9. [PubMed: [1009688](#)].
15. Zidek Z. Adenosine - cyclic AMP pathways and cytokine expression. *Eur Cytokine Netw.* 1999;**10**(3):319-28. [PubMed: [10477388](#)].
16. Abdi M, Ahmadi A, Roshany D, Khodadadi I, Javid S, Shahmohammad-Nezhad S, et al. Diagnostic value of serum adenosine deaminase activity in HIV infected patients of Kurdish population. *Clin Lab.* 2013;**59**(7-8):757-62. [PubMed: [24133903](#)].
17. Goodarzi MT, Abdi M, Tavilani H, Nadi E, Rashidi M. Adenosine deaminase activity in COPD patients and healthy subjects. *Iran J Allergy Asthma Immunol.* 2010;**9**(1):7-12. [PubMed: [20548128](#)].
18. Khodadadi I, Abdi M, Ahmadi A, Wahedi MS, Menbari S, Lahoopour F, et al. Analysis of serum adenosine deaminase (ADA) and ADA1 and ADA2 isoenzyme activities in HIV positive and HIV-HBV co-infected patients. *Clin Biochem.* 2011;**44**(12):980-3. doi: [10.1016/j.clinbiochem.2011.05.020](#). [PubMed: [21640091](#)].
19. Khodadadi I, Wahedi MS, Abdi M, Daneshkhan N, Rahbari R, Menbari S, et al. Evaluation of adenosine deaminase (ADA) isoenzymes activity and tumor necrosis factor-alpha (TNFalpha) concentration in chronic heart failure. *EXCLI J.* 2014;**13**:58-66. [PubMed: [26417242](#)].
20. Nikkhoo B, Sigari N, Ghaderi B, Afkhamzadeh A, Azadi NA, Mohsenpour B, et al. Diagnostic Utility of Adenosine Deaminase in Serum and Bronchoalveolar Lavage Fluid for Screening Lung Cancer in Western Iran. *J Med Biochem.* 2013;**32**(2):109-15.
21. Urunsak IF, Gulec UK, Paydas S, Seydaoglu G, Guzel AB, Vardar MA. Adenosine deaminase activity in patients with ovarian neoplasms. *Arch Gynecol Obstet.* 2012;**286**(1):155-9. doi: [10.1007/s00404-012-2279-5](#). [PubMed: [22395862](#)].
22. Buyukberber M, Sevinc A, Cagliyan CE, Gulsen MT, Sari I, Camci C. Non-Hodgkin lymphoma with high adenosine deaminase levels mimicking peritoneal tuberculosis: an unusual presentation. *Leuk Lymphoma.* 2006;**47**(3):565-8. doi: [10.1080/10428190500395472](#). [PubMed: [16396783](#)].
23. Mozaheb Z, Aledavood A, Farzad F. Distributions of major sub-types of lymphoid malignancies among adults in Mashhad, Iran. *Cancer Epidemiol.* 2011;**35**(1):26-9. doi: [10.1016/j.canep.2010.09.009](#). [PubMed: [21036690](#)].
24. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Paster-nack BS. Clinical staging of chronic lymphocytic leukemia. *Blood.* 1975;**46**(2):219-34. [PubMed: [1139039](#)].
25. Binet JL, Caligaris-Cappio F, Catovsky D, Cheson B, Davis T, Dighiero G, et al. Perspectives on the use of new diagnostic tools in the treatment of chronic lymphocytic leukemia. *Blood.* 2006;**107**(3):859-61. doi: [10.1182/blood-2005-04-1677](#). [PubMed: [16223776](#)].
26. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood.* 2008;**111**(12):5446-56. doi: [10.1182/blood-2007-06-093906](#). [PubMed: [18216293](#)].
27. Giusti G, Galanti B. Adenosine deaminase. *Methods Enzym Analysis.* 1974;**2**:1092-9.
28. van Laarhoven JP, de Bruyn CH. Purine metabolism in relation to leukemia and lymphoid cell differentiation. *Leuk Res.* 1983;**7**(4):451-80. [PubMed: [6194380](#)].
29. van Laarhoven JPRM, Spierenburg GT, Collet H, Delespesse G, De Bruyn CHMM. Purine Interconversion Pathways in T, B, T γ and T-T γ Cells from Human Peripheral Blood. *Adv Exp Med Biol.* 1984;**165**:111-8. doi: [10.1007/978-1-4757-0390-0_23](#).
30. Aghaei M, Karami-Tehrani F, Salami S, Atri M. Diagnostic value of adenosine deaminase activity in benign and malignant breast tumors. *Arch Med Res.* 2010;**41**(1):14-8. doi: [10.1016/j.arcmed.2009.10.012](#). [PubMed: [20430249](#)].
31. Aghaei M, Karami-Tehrani F, Salami S, Atri M. Adenosine deaminase activity in the serum and malignant tumors of breast cancer: the assessment of isoenzyme ADA1 and ADA2 activities. *Clin Biochem.* 2005;**38**(10):887-91. doi: [10.1016/j.clinbiochem.2005.05.015](#). [PubMed: [16054616](#)].
32. Eroglu A, Canbolat O, Demirci S, Kocaoglu H, Eryavuz Y, Akgul H. Activities of adenosine deaminase and 5'-nucleotidase in cancerous and noncancerous human colorectal tissues. *Med Oncol.* 2000;**17**(4):319-24. doi: [10.1007/BF02782198](#). [PubMed: [11114712](#)].
33. Urschel JD, Blewett CJ, Bennett WF, Miller JD, Young JE. Handsewn or stapled esophago-gastric anastomoses after esophagectomy for cancer: meta-analysis of randomized controlled trials. *Dis Esophagus.* 2001;**14**(3-4):212-7. [PubMed: [11869322](#)].

34. Yoo C, Yoon DH, Suh C. Serum beta-2 microglobulin in malignant lymphomas: an old but powerful prognostic factor. *Blood Res.* 2014;**49**(3):148-53. doi: [10.5045/br.2014.49.3.148](https://doi.org/10.5045/br.2014.49.3.148). [PubMed: [25325033](https://pubmed.ncbi.nlm.nih.gov/25325033/)].
35. Martin M, Aran JM, Colomer D, Huguet J, Centelles JJ, Vives-Corrons JL, et al. Surface adenosine deaminase. A novel B-cell marker in chronic lymphocytic leukemia. *Hum Immunol.* 1995;**42**(3):265-73. [PubMed: [7759315](https://pubmed.ncbi.nlm.nih.gov/7759315/)].
36. Mesarosova A, Hrivnakova A, Babusikova O. A comparison of some leucocyte differentiation markers and the adenosine deaminase and purine nucleoside phosphorylase values in B and T cell leukemias and lymphomas. *Neoplasma.* 1995;**42**(6):307-12. [PubMed: [8592572](https://pubmed.ncbi.nlm.nih.gov/8592572/)].
37. Yasmineh WG, Brynes RK, Lum CT, Abbasnezhad M. Adenosine deaminase activity in lymphocytes of normal persons, leukemic patients, and kidney-transplant recipients. *Clin Chem.* 1977;**23**(11):2024-9. [PubMed: [334394](https://pubmed.ncbi.nlm.nih.gov/334394/)].
38. Simpkins H, Stanton A, Davis BH. Adenosine deaminase activity in lymphoid subpopulations and leukemias. *Cancer Res.* 1981;**41**(8):3107-10. [PubMed: [6972803](https://pubmed.ncbi.nlm.nih.gov/6972803/)].
39. Franco R, Canela EI, Bozal J. Heterogeneous localization of some purine enzymes in subcellular fractions of rat brain and cerebellum. *Neurochem Res.* 1986;**11**(3):423-35. [PubMed: [3010150](https://pubmed.ncbi.nlm.nih.gov/3010150/)].