Original Article

Detection and Stratification of Antibodies in Autoimmune Haemolytic Anaemia in Chronic Lymphocytic Lukemia

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ABSTRACT

Objective: Chronic lymphocytic leukemia is characterized by proliferation, accumulation and sustained increase of morphologically mature but functionally incompetent lymphocytes. Autoimmune phenomena are well-known complications of lymphoproliferative diseases. Autoimmune Haemolytic Anaemia is the most frequent autoimmune disorder associated with chronic lymphocytic leukaemia.

Study Design: Detection of frequency of autoimmune haemolytic anaemia in chronic lymphocytic leukaemia.

Place and Duration of Study: This study was conducted at the Department of Pathology and Oncology, King Edward Medical University and Institute of Nuclear Medicine & Oncology Lahore from January 2013 to December 2016.

Materials and Methods: One hundred adult patients with chronic lymphocytic leukemia were enrolled. All patients age above 18 years to 85 years either gender and newly diagnosed cases and cold cases of chronic lymphocytic leukaemia included. Patients on treatment or having being treated with chronic lymphocytic leukaemia were excluded.

Results: 82% were males and 18patients were female. Mean age was 65.8±1.33 years with the greatest number of patients falling in the group of 71-80 years. Out of total patients 27% had Coombs positive. Most of the patients having Coombs positive were in stage 4. The maximum number of patients had haemoglobin of 8.1 to 14 g/dl and 70% patients had TLC up to 100,000 $\times 10^3$.The antibodies detected had the following percentages in terms of stratification. The antibody was of the IgG class in 85 of patients (85%) and C3d was present in 77 of them. IgM class in 15 patients (15%). Out of 15, only 8 showed presence of C3d.

Conclusion: The treatment modalities are different in different causes of anaemia and complications due to chronic lymphocytic leukaemia. Therefore, detection and stratification of antibody is significant and related with disease progression and overall survival in number of chronic lymphocytic leukaemia patients.

Key Words: Chronic lymphocytic leukaemia, Autoimmune haemolytic anaemia, Antibodies.

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INTRODUCTION

Autoimmune induced hemolytic anaemia is the product of auto antibodies to patients with red cell antigens typically present in the plasma of their individual.[1] Anaemia is the disorder that is inadequate in the amount of red blood cells (in turn the ability to carry oxygen) to satisfy body physiologic requirements.

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The haemolytic autoimmune anaemia can be either idiopathic or secondary. Lymphoproliferative and autoimmune diseases are mostly affected.[2] The most prevalent type of leukaemia in western countries is chronic lymphocytic leukaemia (CLL), but in Asia it is greatly reduced. In the USA, Europe and Australia the median age of diagnoses is approximately 70 years old, and in approximately a quarter of the patients under 65 years old.[3] The most common form of Western leukaemia with 4.2/100 000 per year is chronic lymphocytic leukaemia. At >80 years of age, this trend is rising to > 30/100 000 / year. The average age is 72 years for diagnosis. CLL patients estimated to be 10% younger than 55 years4. 30 percent of Caucasians with all leukemia display chronic lymphocytic leukaemia. The incidence of disease in Eastern Europe and the USA is high, while chronic leukaemia is rare in Asia and Africa.

The defined hematopoietic neoplasm of the World Health Organization defines the chronic lymphocytic leukaemia and the small lymphocytic lymphoma (SLL)

is only distinguished in terms of leukemia.[4] Chronic lymphocytic leukaemia is, by definition, often a neoplastic B-cell disease, while a person's lymphocytic entity does not.

Chronic lymphocytic leukaemia cells coexprime CD5, the surface antigen, along with the CD19, CD20 and CD23 B cell antigens, for at least 3 months.[5] The chronic diagnosis for lymphocytic cellular Leukemia needs 5 lymphocytes x 109/LB to be present in the peripheral blood. The expression of either K or L immunoglobulin light chains is limited to any leukaemia clone.[6] Autoimmune phenomena are a well-known complication of lymphoproliferative diseases. Three autoimmune hematologic conditions frequently associated with chronic lymphocytic leukaemia are autoimmune haemolytic anaemia, idiopathic thrombocytopenic purpura, and pure red cell aplasia.[7] Of these, autoimmune haemolytic anaemia is the most frequent autoimmune disorder and its pathogenesis complicating the course of chronic lymphocytic leukaemia remains a matter of considerable mystery.

Establishing a relationship between autoimmune haemolytic anaemias and chronic lymphocytic leukaemia will help the clinicians in modifying the treatment and to screen all chronic lymphocytic leukaemia patients for autoimmune haemolytic anaemia to alter timely management. Patients presenting with WAIHA present a perplexing problem for the blood bank because transfusion is commonly needed but it is to be avoided when possible because it can increase the haemolysis. Transfusion is reserved for situations that are life-threatening.

MATERIALS AND METHODS

This descriptive cross sectional study was conducted at Department of Pathology and Oncology, King Edward Medical University and Institute of Nuclear Medicine & Oncology Lahore from 1st January 2013 to 31st December 2016. A total of 100 adult patients with chronic lymphocytic leukemia were enrolled. All patients age above 18 years to 85 years, both males and females and newly diagnosed cases and cold cases of chronic lymphocytic leukaemia that have not taken any treatment were included. Patients on treatment or having being treated with chronic lymphocytic leukaemia, received transfusion within last three months, known cases of autoimmune disorders like systemic lupus erythematosus, lupus nephritis, pemphigus vulgaris and giant cell arthritis, taking drugs known to cause haemolytic anaemia like quinidine, penicillin, and methyldopa and steroid therapy were excluded. A thorough and methodical history and examination of all the diagnosed lymphoma patients was recorded. For every patient a fresh 3 ml blood sample was collected. A complete blood count was carried out using Automated Haematology Analyzer (*Sysmex KX-21)* and peripheral blood smears were prepared using Wright Giemsa stain to establish whether anaemia is present or not. Direct antiglobulin test using antihuman globulin (Coombs reagent) was done to determine the immune cause of anaemia. Bilirubin and Lactate dehydrogenase levels were measured using Beckman coulter. The antibody screening was done using3 cell screening pannel of DIA cell and antibody typing was done using 11 cell ID pannel of DIA cell. Data was entered and analysed on SPSS version 20.

RESULTS

There were 82 (82%) males and 18 (18) females with mean age of the patients was 65.8.1.5 years (Table 1). The mean haemoglobin of all chronic lymphocytic leukaemia patients was 9.8±2.62 g/dl and further stratification showed that most number of patients fell in the group of 11.1-14 g/dl. Among the total patients, 73.33% had Coombs test negative and 26.67% had a positive result. However, it was noted that those who had Coombs positive had a lower level of haemoglobin with a mean of 7.69±2.3 g/dl and with maximum number of patients were in range of 8.1-11 g/dl. The antibody was of the IgG class in 85 of patients (85%) and C3d was present in 77 of them, IgM class in 15 patients (15%).Out of 15 only 8 showed presence of C3d (Table 2).

Table 1: Demographic information of the patients

Variable	No.	%
Gender		
Male	82	82.0
Female	18	18.0
Age (years)		
40-50	19	19.0
51-60	17	17.0
61-70	31	31.0
71-80	30	33.0
81-90	3	3.0

Table 2: Means of the different variables

Variable	Mean±SD
Haemoglobin	9.8 g/dl±2.62
Coombs positive	7.69 g/dl±2.3

DISCUSSION

The results of this study showed a frequency of autoimmune haemolytic anemia in our patients of 22.66%. The mean age of the patients in our study was 65.8±1.5 years with most of patients falling in the group of 71-80 years. While in another local study by Ehsan et al[8] the mean age of cohort was 62.84years. The maximum number of patients presented in the 7th decade (45.2%). In another study by Diehl[9] reported the average age was 69.6 years with a peak incidence in the age bracket of 70–79 years.

The majority of the patients in our study were male i.e. 81.33% with a male to female ratio of 4.3:1. In one of the local study conducted at KPK by Hamayun[10] all cases reported were that of males. Similar to these results of 100% male predominance was found in another local study of leukaemic patients by Aziz at al.[11] The study by Ehsan[8] showed similar results of gender distribution with a male to female ratio of 4.6:1. Oppezo et al[12] conducted a study showing that 63% of the population under study was male. In another study conducted by Goldin et al[13] on a cohort of 660 showed that male population suffered from CLL twice as more.

Our study showed that patients with Coombs positive presented with advanced stage of 3 and 4. This is in concordance to the study by Kyasa et al[14] which establishes that DAT positive is a poor prognostic factor. In another study by Moreno et al[15] male sex and advanced disease have been classically associated with autoimmune cytopenia. Barcellini et al[16], also concludes in the study that Coombs positive is bad prognostic factor and patients present usually with advanced stages.

In this study patients were divided in two groups. Half of the patients were below 60 years and half of the patients more than 60 years. No significant relationship (p value = 0.136) was found between age and autoimmune anaemia in chronic lymphocytic leukaemia patients. The study by Flowers[17] also showed that age had no role in patients developing the complications of chronic lymphocytic leukaemia. However, a study Nicora[18] showed that with advancing age the complications of chronic lymphocytic leukaemia were also increased.

In our study 85 of the patients had IgG and 15 had IgM which in concordance to a study conducted by Wilson and Chabot.[19] In selecting blood for transfusion, precision can also be helpful. Some staff choose to transfuse RBCs that are autoantibody friendly. In few cases, any blood donor is typically incompatible with WAIHA patients. Any transfused blood being administered in the crosspatch is thus considered "least incompatible." Because of the low number of White Cells in this blood portion, leukocyte-reduced RBCs are the preferred blood product. Tiny volumes (100mL) of Leuco-filtered blood are steadily transfused and the patient is monitored for any adverse effects.

CONCLUSION

Frequency of autoimmune haemolytic anaemia is 22.66% among chronic lymphocytic leukaemia patients which reflects that autoimmune complications are significantly high with chronic lymphocytic leukaemia. Antibody detection and specificity may also be helpful in selecting blood for transfusion.

Author's Contribution:

Concept & Design of Study:	Arsala Rashid
Drafting:	Humera Rafiq, Mukarrama Rashid
Data Analysis:	Sobia Ashraf, Ambreen Hamid
Revisiting Critically:	Arsala Rashid, Humera Rafiq
Final Approval of version:	Arsala Rashid

Conflict of Interest: The study has no conflict of interest to declare by any author.

REFERENCES

1. Assessing the iron status of populations: report of a joint World Health Organization/ Centers for Disease Control and Prevention technical consultation on the assessment of iron status at the population level. 2nded. Geneva: World Health Organization; 2007.
2. Qazi H, Ikram N, Hassan K. Chronic Lymphocytic Leukaemia. Haematol Updates 2010; 74-8
3. Matutes E, Attygalle A. Diagnostic issues in chronic lymphocytic leukaemia. Clin Haematol 2010;23(1):3-20
4. Eichhorst B, Dreyling M, Robak T. Chronic lymphocytic leukemia ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Hematol Oncol 2011;22(6):50-4.
5. Shvidel L, Shtarlid M, Klepfish A, Sigler E, Berrebi A. Epidemiology and ethnic aspects of B cell chronic lymphocytic leukemia in Israel. Leukemia 1998; 12(10):1612-7.
6. Cheson B, Cheson BJ. National Cancer Institute Sponsored Working Group Guidelines for Chronic Lymphocytic Leukemia: Revised Guidelines for Diagnosis and Treatment. Oncologist 2012;87: 4990-97.
7. Mauro F, Foa R. Autoimmune hemolytic anemia in chronic lymphocytic leukemia: clinical, therapeutic, and prognostic features. Blood 2000; 95: 2786-92.
8. Ehsan A, Lone A, Khan MA. Autoimmune complications in chronic lymphocytic leukemia: a single center experience. Biomedica 2013;29: 37-41.
9. Diehl LF, Karnell LH, Menck HR. The National Cancer Data Base Report on age, gender, treatment, and outcomes of patients with chronic lymphocytic leukemia. Cancer 1999;86(12): 2684-92.
10. Hamayun M, Khan SA, Muhammad W. Investigation on the prevalence of leukemia in North West Frontier Province of Pakistan. Turkish J Cancer 2005;35(3):119-22.

11. Aziz F, Qureshi IZ. Clinical and cytogenetic analyses in Pakistani leukemia patients. Pak J Zool 2008;40(3):147.
12. Oppezzo P, Vasconcelos Y, Settegrana C, Jeannel D, Vuillier F, Legarff-Tavernier M, et al. The LPL/ADAM29 expression ratio is a novel prognosis indicator in chronic lymphocytic leukemia. Blood 2005;106(2): 650-7.
13. Goldin LR, Pfeiffer RM, Li X, Hemminki K. Familial risk of lymphoproliferative tumors in families of patients with chronic lymphocytic leukemia: results from the Swedish Family-Cancer Database. Blood 2004;104(6):1850-4.
14. Kyasa MJ, Parrish RS, Schichman SA, Zent CS. Autoimmune cytopenia does not predict poor prognosis in chronic lymphocytic leukemia/small lymphocytic lymphoma†. Am J Hematol 2003;74(1):1-8.
15. Moreno C, Hodgson K, Ferrer G, Elena M, Filella X, Pereira A, et al. Autoimmune cytopenia in chronic lymphocytic leukemia: prevalence, clinical associations, and prognostic significance. Blood 2010;116(23): 4771-6.
16. Barcellini W, Capalbo S, Agostinelli RM, et al. Relationship between autoimmune phenomena and disease stage and therapy in B-cell chronic lymphocytic leukemia. Haematologica 2006; 91: 1689-92.
17. Flowers C, Farber CM, Flinn I, Grinblatt DL, Kay NE, Kipps TJ, et al. Variation in health-related quality of life (HRQOL) by line of therapy, age, and gender among patients with chronic lymphocytic leukemia. J Clin Oncol 2013;31(15 Suppl):7086-9.
18. Nicoara AD, Niculescu Z, Ghinea MM, Gheorghe AD. Etiopathogenic aspects of autoimmune hemolytic anemias. Eur J Int Med 24:e173-e4.
19. Wilson JA, Chabot S. Antibody Identification in a Patient with Chronic Lymphocytic Leukemia: A Case Study. American Society for Clin Laborat Sci 2002;15(3):136-9.