

# AUTOIMMUNE COMPLICATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA: A SINGLE CENTER EXPERIENCE

AYESHA EHSAN,<sup>1</sup> AYAZ LONE<sup>2</sup> AND MUHAMMAD AAMIR KHAN<sup>1</sup>  
Department of Hematology, <sup>1</sup>Shaikh Zayed Federal Post Graduate Medical Institute  
and <sup>2</sup>Fatima Memorial Hospital College of Medicine and Dentistry, Lahore

## ABSTRACT

*Introduction:* Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of B cells with mature appearance and a distinctive immunophenotype in peripheral blood, bone marrow, lymph nodes and other lymphoid tissues. Autoimmune complications are common in CLL and by far the most common manifestation is autoimmune hemolytic anemia (AIHA), followed by immune thrombocytopenia (ITP) and pure red cell aplasia (PRCA). The study was carried out to see the spectrum of autoimmune complications in patients diagnosed with CLL.

*Materials and Methods:* This cross sectional study was carried out to describe the clinical presentation, haematological parameters and complications of CLL in patients coming to Shaikh Zayed Hospital (SZH), Lahore over a period of 6 years from June 2002 to July 2008. CLL cases were diagnosed according to the National Cancer Institute (NCI) criteria. The collected data was entered into SPSS version 13 for analysis.

*Results:* Thirty one patients were diagnosed with CLL and in 6 (19.4%) patients the disease was complicated by AIHA and in 1 (3.2%) patient by ITP at presentation.

*Conclusion:* Autoimmune cytopenias were observed in 22% of 31 patients. Autoimmune hemolytic anaemia was more common than ITP, no patients of PRCA were recognized.

*Key Words:* Chronic lymphocytic leukemia (CLL), Autoimmune Hemolytic anemia (AIHA), Immune Thrombocytopenia (ITP).

## INTRODUCTION

Chronic lymphocytic leukemia considered a homogenous disease of immature, immune – incompetent, minimally self – renewing B – cells, which accumulate because of a faulty apoptotic mechanism earlier is now viewed as two related entities, both originating from antigen – stimulated mature B lymphocytes, which either avoid death through the intercession of external signals or die by apoptosis only to be replenished by proliferating precursor cells.<sup>1</sup> It is characterized by the progressive accumulation of B cells with mature appearance but functionally incompetent and a distinctive immunophenotype (i.e, SmIg<sup>dim</sup>, CD<sub>5</sub><sup>+</sup>, CD<sub>19</sub><sup>+</sup>, CD<sub>20</sub><sup>dim</sup>, CD<sub>23</sub><sup>+</sup>) in peripheral blood, bone marrow, lymphnodes and other lymphoid tissues.<sup>2,3</sup>

Immune dysregulation, a hallmark of chronic lymphocytic leukemia manifests itself in 3 autoimmune diseases: warm autoimmune hemolytic, immune thrombocytopenia and pure red cell aplasia.<sup>4</sup> Autoimmunity is not confined to the formed elements of the blood as paraneoplastic pemphigus and acquired angioedema do occur in CLL but the non-hematologic autoimmunity is very rare. The pathogenesis of autoimmunity in CLL is unknown. The source of any putative autoantibody is not clear yet.

Recently, it has been hypothesized that leukemic B-cells may also act as professional antigen presenting cells.<sup>5,6</sup> The Binet clinical staging system is used to define disease extent and predict survival.<sup>7,8</sup>

Epidemiological data from Pakistan is minimal and only a few centres have published their information.<sup>3,8</sup> This study was conducted to see the clinical picture of CLL at presentation, frequency and spectrum of autoimmune complication seen in CLL patients at the time of initial diagnosis and to correlate autoimmune cytopenia with clinical and biological features.

## MATERIALS AND METHODS

This cross sectional study was carried out to describe the clinical presentation, haematological parameters and complications of CLL in patients coming to Shaikh Zayed Hospital, Lahore over a period of 6 years from June 2002 to July 2008. CLL cases were diagnosed according to the National Cancer Institute (NCI) criteria<sup>9,10</sup> and whenever possible the diagnosis was confirmed by flow cytometry. The diagnosis of AIHA was based on the presence of an otherwise unexplained hemoglobin level < 10 g/dL or hematocrit < 30% and a positive direct anti-globulin test (DAT) for either IgG or C3 and the presence of at

least one indirect marker of hemolysis: high reticulocyte count, low serum haptoglobin levels, increased serum LDH or bilirubin levels. For patients in whom DAT was negative, the diagnosis of AIHA was made if at least 2 of the indirect signs of hemolysis were present.<sup>11</sup> ITP was defined as a sudden and otherwise unexplained fall in platelet count to  $< 100.000 / \text{mm}^3$  with at least 2 of the following: evidence of normal bone marrow function (normal or increased megakaryocytes in bone marrow), no splenomegaly, no chemotherapy within the last month from study entry.<sup>12</sup> Patients were confirmed as having stage C “infiltrative” if they had either Hb less than 10 g/dL or platelet count less than  $100.000 / \text{mm}^3$  with no positive DAT and no indirect signs of hemolysis, and further confirmed whenever possible by a significant marrow infiltration as defined by either a diffuse bone marrow histological pattern or, when this was not available, by bone marrow aspirate (more than 80% lymphocytes) or reticulocytopenia (less than 1%).

Thirty one patients were diagnosed during the study time. They were included by non-probability purposive sampling. Informed consent was taken from the patient and sociodemographic data like name, age, sex, occupation was collected. A detailed history was taken about lymphadenopathy, fever, bleeding from any site, breathlessness on mild exertion and easy fatigability. General physical examination was carried out including pallor, fever, bleeding manifestations in the skin (e.g. bruises and purpura), accessible lymph adenopathy in the cervical, axillary and inguinal region. Hepatomegaly and splenomegaly were sought in abdominal examination and confirmed by abdominal ultrasound. The blood sample from the patients were tested for Complete Blood Counts (CBC) including Haemoglobin (Hb), Total leucocyte count (TLC), Platelet count, Mean cell volume (MCV). Blood film was stained by May – Grunwald – Giemsa stain and peripheral smear examination was carried out for differential leucocyte count (DLC), identification of spherocytes, schistocytes, thrombocytopenia and platelet anisocytosis. Bone marrow aspirates were done from right posterior iliac crest with Islam Bone Marrow Aspirate Biopsy Needle® in all cases. May – Grunwald – Giemsa staining was performed on the aspirate. The bone marrow trephine biopsies were done by Islam Bone Marrow Trephine Biopsy Needle® from the same site and stained with Haematoxylin and Eosin stains. Slides were evaluated for cellularity and infiltration pattern of lymphocytes. Megakaryopoiesis and erythropoiesis were also assessed.

The collected data was entered into SPSS version 13 for analysis. Nominal data of variables including, pallor, fever, bleeding, splenomegaly. Hepatomegaly, lymphadenopathy, bone marrow features including cellularity, pattern of infiltration were expressed as

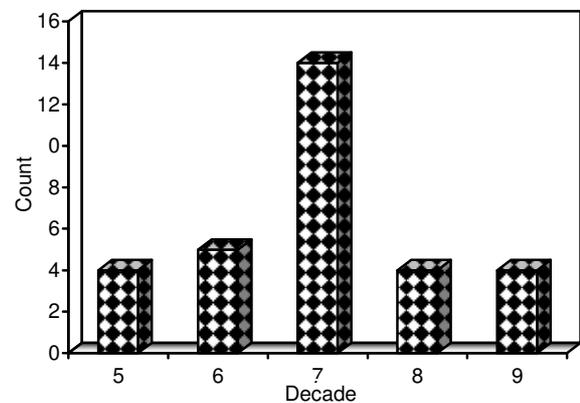
frequency percentage. The variables in CBC including Hb, TLC, Platelet count were recorded as Mean  $\pm$  SD

**RESULTS**

Thirty one patients of chronic lymphocytic leukemia were included in the study. Autoimmune cytopenia was seen in 7 (22.5%) cases. There were 6 cases (19.4%) who presented with autoimmune hemolytic anemia and 1 case (3.2%) of ITP and no case of PRCA.

There were 23 males (74%) and 8 females (16%) with a male to female ratio of 4.6:1.

The mean age of the entire cohort was 62.84 years. The maximum number of patients presented in the 7<sup>th</sup> decade (45.2%). Figure 1 describes the decade of presentation of the cohort.



**Fig. 1:** No. of patients presenting with CLL in different decades.

Seven cases presented in Binet A stage, 4 cases were in Binet B and the rest were in Binet C. The haematological parameters are detailed in Table 1. Binet A had 1 case of AIHA, all other cases of immune complications were in C stage. Most of the female patients presented in Binet stages B and C.

Seven patients presented with B symptoms of weight loss and fever (22.5%), 20 patients had lymphadenopathy (64.5%), 16 cases had an enlarged spleen (51.6%), 8 had hepatomegaly (25.8%). The patients with B symptoms did not have autoimmune complications.

Diffusely infiltrating lymphocytes were seen in bone marrow histology of 4 patients (12.9%), nodular infiltrates were present in 12 (38.7%) patients, interstitial in 3 (9.6%) patients and mixed histology was seen in the rest of the patients. The majority of patients with diffuse histology presented with B symptoms.

**DISCUSSION**

This study was carried out over a span of 6 years.

CLL constituted 31% of chronic leukemias presenting during this time period. Chronic myeloid leukemia was considerably more commonly seen in the patients presenting at SZH.

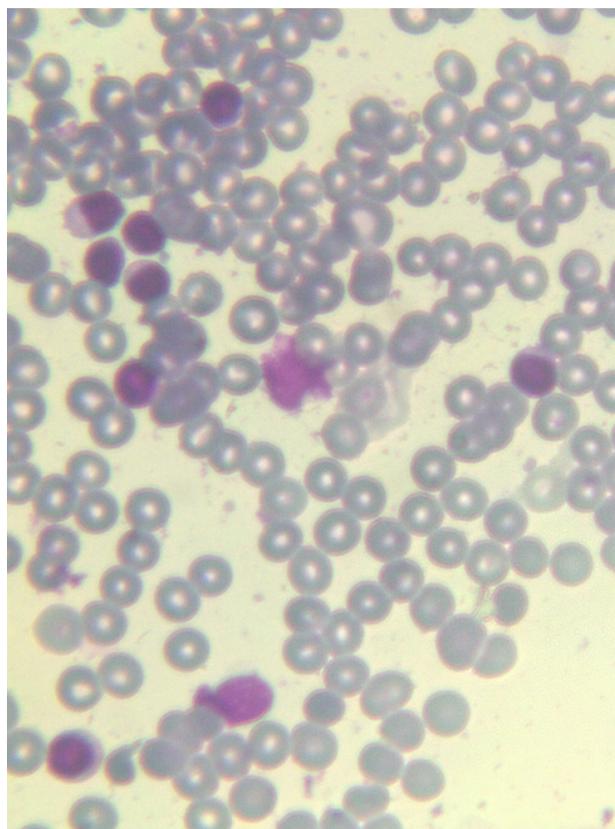
A similar study carried out at the same centre by Asif et al reported median age of presentation of CLL at 60 years, and a pronounced male predominance.<sup>13</sup> Anwar M reports from another city of Pakistan a median age of presentation of 65 years with 58% patients above 60 years.<sup>14</sup> Both studies gave a similar age range as the present study. Agrawal et al from India reported median age of 61 years.<sup>15</sup> Male to female ratio of 4.6:1 reported in the present study is very similar to 4:1 reported by Anwar M et al.<sup>14</sup>

Agarwal et al reported very similar frequency of clinical features: 25% had fever at presentation as compared to our result of 22%, lymphadenopathy

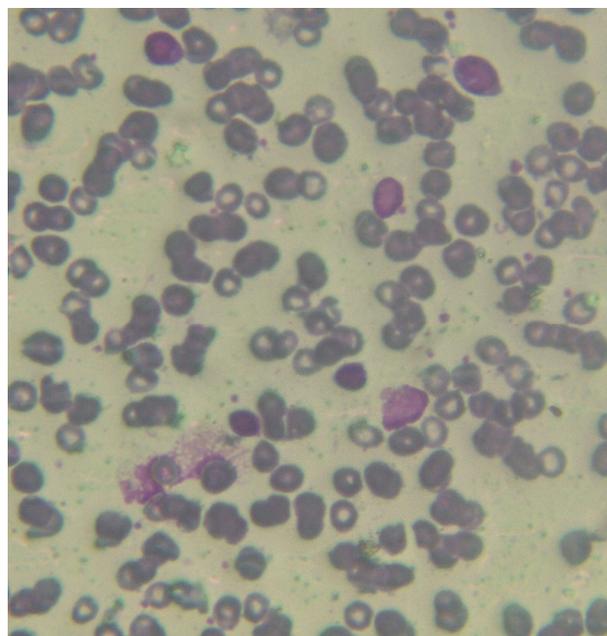
**Table 1:** Mean values for age, complete blood counts for the patients according to Binet stage.

Stage	A	B	C	Total
No of patients	7	4	20	31
Age	67.8 ± 12	56 ± 7.8	62.5 ± 11.7	67.8 ± 12
Haemoglobin (g/dl)	11.6 ± 0.7	11.4 ± 1.1	8.3 ± 2.1	9.38 ± 2.5
WBC (10 <sup>9</sup> /l)	68.6 ± 67	73.3 ± 44.2	115.3 ± 92.7	83.3 ± 68.8
Platelet (10 <sup>9</sup> /l)	207.5 ± 73	231.5 ± 79	114.5 ± 92.7	149 ± 97.8
Autoimmune complications	0	0	7	7

Key: WBC – White cell count



**Fig. 2:** Peripheral blood smear of CLL with ITP revealing lymphocytosis and thrombocytopenia.

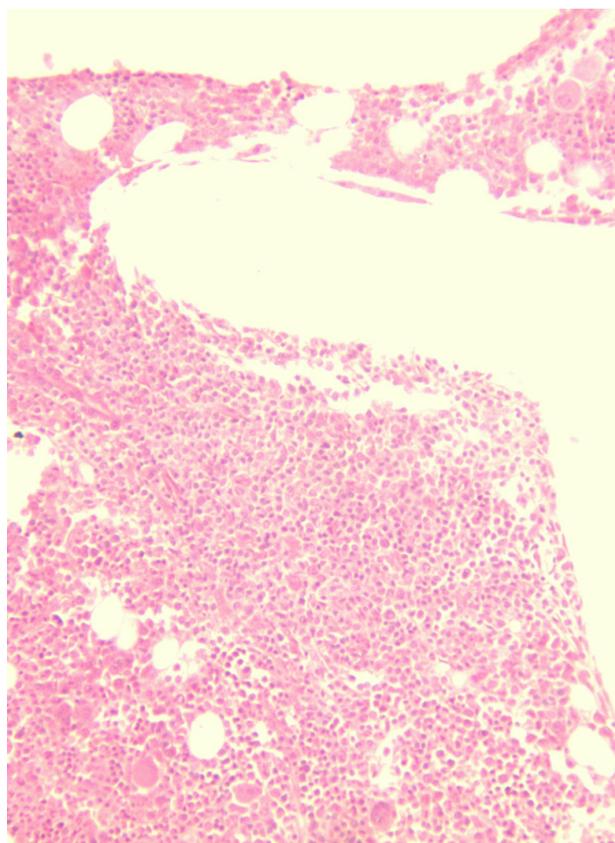


**Fig. 3:** Peripheral blood smear with CLL and AIHA revealing lymphocytosis and schistocytes.

was seen in 55% of their patients as compared to our 64.5%. Their reported median TLC was  $70.6 \times 10^9/l$  as compared to our  $83.3 \times 10^9/l$ .<sup>15</sup>

Diehl LF et al reported AIHA in 11% of CLL pts and was reported to be a late manifestation. They reported PRCA to be an under recognized entity in CLL which could be found in 6% cases in early stages of the disease. ITP occurred in 2 – 3% CLL pts in their study.<sup>4</sup> Moreno C et al found 7% autoimmune cytopenia in CLL pts, AIHA in 5.3% patients out of which 1.9% had this complication at the time of diagnosis and ITP in 2% patients.<sup>2</sup> Kyasa MJ et al reported AIHA in 4.5% and ITP in 3.8% patients and PRCA in 0.8% pts in a study of 132 patients from Little Rock USA.<sup>16</sup> Zent CS et al reported AIHA in 2.3%,

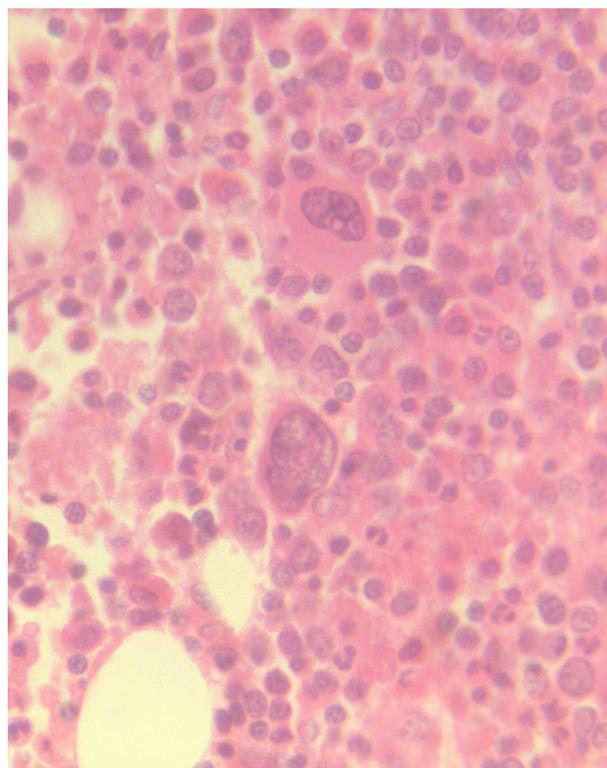
ITP in 2% and Pure red cell aplasia in 0.5% cases in their study at Mayo Clinic Rochester USA.<sup>17</sup> In another study carried out by Zent CS et al immune cytopenias occurred in 24.2% patients of lymphoproliferative disorders and was attributable to CLL in 17% cases with 75% of these having bone marrow failure and 25% having autoimmune disease.<sup>11</sup>



**Fig. 4:** Low power view of bone marrow biopsy revealing mixed infiltration by lymphocytes, active erythropoiesis and megakaryocytes.

Our cohort revealed a higher percentage of patients with autoimmune hemolytic anemia but a comparable percentage of ITP. The discrepancy may be due to the small number of patients in our cohort which may not be representative of a larger patient population. This study was limited by the lack of follow-up of these patients, so any autoimmune manifestation arising late in the disease process was not included.

Most of the studies reported a significantly high percentage of patients with stage C disease secondary to autoimmune complications. These patients showed an entirely different prognosis from the rest of the stage C patients if treatment was constituted appropriately. Diehl et al reported that 55% of their



**Fig. 5:** High power view of bone marrow trephine biopsy revealing active erythropoiesis, megakaryocytes and lymphocytic infiltration.

patients presented at Binet A whereas 33% presented with advanced stage C disease<sup>4</sup>. Advanced Binet C stage disease due to an autoimmune mechanism was associated with a significantly better survival than those with advanced stage related to a massive bone marrow infiltration (median survivals: 7.4 years vs. 3.7 years). These results emphasize the importance of determining the origin of cytopenia in patients with CLL for both treatment and prognostic purposes.<sup>2,15</sup>

From India Agarwal et al reported diffuse bone marrow involvement in 16.3% patients against our 12.3% patient, interstitial involvement in 10.2% pts against our 9.6% patients, nodular in 67.3% patients against our 38.7% patients and mixed in 6.1% pts as compared to our 38.7% cases. Most of our results matched those given by the Indian study. This goes on to suggest that the natural history of the disease is somewhat similar in the two neighboring countries.

It is **concluded** that CLL is a disease afflicting the elderly with a male preponderance. Autoimmune cytopenias were found in 22% of 31 patients studied. Autoimmune hemolytic anaemia was more common than ITP, no patients of PRCA were recognized in the cohort.

**REFERENCES**

1. Chiorazzi N, Rai. RK, Ferrarini M. Chronic lymphocytic leukemia. *N Engl J Med* 2005; 352: 804-815.
2. 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: 4771-4776.
3. Ikram N, Hassan K, Tufail S. Chronic lymphocytic leukemia (CLL) An overview. *Int J Pathol* 2003 Dec; 1: 48-59.
4. Diehl LF, Ketchum LH. Autoimmune disease and chronic lymphocytic leukemia: auto immune hemolytic anemia, pure red cell aplasia and autoimmune thrombocytopenia. *Semin Oncol.* 1998; 25 (1): 80-97.
5. Hamblin TJ. Autoimmune complications of chronic lymphocytic leukemia. *SeminOncol* 2006 Apr; 33 (2): 230-239.
6. Arena DG, CAscavilla N. Chronic lymphocytic leukemia – associated autoimmune hemolytic anemia. *Leukemia and Lymphoma* 2007; 48 (6): 1072-1080.
7. Saqatys Em, Zhang L. Clinical and laboratory prognostic indicators in chronic lymphocytic leukemia. *Cancer Control* 2012 Jan; 19 (1): 18-25.
8. Anwar M. Chronic lymphocytic leukemia: current concepts in management. *Pak Armed Forces Med J* 2005 Dec; 55 (4): 335-44.
9. Cheson BD, Bennett JM, Rai KR, Grever MR, Kay NE, Schiffer CA et al. Guidelines for clinical protocols for chronic lymphocytic leukemia: recommendations of the National Cancer Institute sponsored working group. *Am J Hematol.* 1988; 29 (3): 152-163.
10. Cheson BD, Bennett JM, Grever M, Kay N, Keating MJ, O’Brain S et al. National Cancer Institute – sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood.* 1996; 87 (12): 4990-4997.
11. Zent CS, Ding W, Schwager SM, Reinalda MS, Hoyer JD, Jelinek DF et al. The prognostic significance of cytopenia in chronic lymphocytic leukaemia / small lymphocytic lymphoma. *Br J Haematol.* 2008; 141 (5): 615-621.
12. Visco C, Ruggeri M, Laura Evangelista M, Stasi R, Zanotti R, Giaretta I et al. Impact of immune thrombocytopenia on the clinical course of chronic lymphocytic leukemia. *Blood.* 2008; 111 (3): 1110-1116.
13. Asif MJ, Chughtai NM, Iqbal Z, Ehsan A, Arif S. Pattern of chronic leukemia at Shaikh Zayed Hospital, Lahore. *Proceeding Shaikh Zayed Postgrad Med Inst* 2002; 16 (1): 24-9.
14. Anwar M, Chronic lymphocytic leukemia: current concepts in management. *Pak Armed Forces Med J* 2005; 55 (4): 335-44.
15. Agrawal N, Naithani R, Mahapatra m, Panigrahi I, Kumar R, Pati HP et al. Chronic lymphocytic leukemia in India – a clinico-haematological profile. *Hematology.* 2007; 12 (30): 229-33.
16. Kyasa MJ, Parrish RS, Schichman SA, Zent CS. Auto-immune cytopenia does not predict poor prognosis in chronic lymphocytic leukemia/small lymphocytic lymphoma. *Am J Hematol.* 2003; 74 (1): 1-8.
17. Zent CS, Ding W, Reinalda MS, Schwager SM, Hoyer JD, Bowen DA et al. Autoimmune cytopenia in chronic lymphocytic leukemia / small lymphocytic lymphoma: changes in clinical presentation and prognosis. *Leuk Lymphoma.* 2009; 50 (8): 1261-8.