# Hyperleukocytosis and Chronic Lymphocytic Leukemia: comparing clinical, immunophenotypic, and cytogenetic data


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## Introduction

Chronic lymphocytic leukemia (CLL) is one of the most common leukemias with 14,570 new diagnoses and 4,380 deaths per year in the US. Hyperleukocytosis (absolute leukocyte count exceeding 100,000 cells/mm$^3$) in other lymphoproliferative diseases is associated with coagulopathy, pulmonary symptoms, and also symptoms of hyperviscosity. The clinical picture of hyperleukocytosis in CLL has been similarly described in the literature but immunophenotypic and cytogenetic characteristics have not been explored.

## Aim

The aim of this project was to determine and analyze the clinical, immunophenotypic, and cytogenic differences between typical CLL and hyperleukocytic CLL. We were particularly interested in prognostic factors such as lymphoid involvement, anemia, thrombocytopenia, CD38 and ZAP-70 expression.

## Methods

**Data Collection:**
Tissue samples (peripheral blood, lymphoid tissue, bone marrow) from CLL patients were collected and submitted to Ameripath Central Florida in Orlando, FL for pathology work-up including diagnosis, prognosis, and progression. Data was collected from the hematopathology reports of the submitted tissue samples. Patient data was placed into one of two groups based on absolute leukocyte count: hyperleukocytic CLL (greater than or equal to 100 x 10$^6$ cells/mL) and typical CLL (5.0-99.9 x 10$^6$ cells/mL). Data from 82 typical CLL patients and 10 hyperleukocytic CLL patients was included in the study.
Patients diagnosed with small lymphocytic lymphoma or prolymphocytic leukemia were excluded from the study.

**Data points:**
Clinical: age, peripheral blood cellularity, erythrocyte count, hemoglobin, platelet count, white blood cell count, lymphocyte percentage, percentage of CLL cells, lymphoid involvement (lymphadenopathy, organomegaly).

Immunophenotype: CD5, CD10, CD13, CD19, CD20, CD23, 45, CD52, HLA-DR, kappa, lambda, and the prognostic markers CD38 and ZAP-70.

Cytogenetics: Cytogenetic data, when available was recorded and the counts or proportion of each genetic lesion were compared between the two groups.

## Results

**Clinical Findings:**
Anemia was present in both groups and there was no significant difference between them. Platelet count was within the normal range in the typical CLL group but in the thrombocytopenic range in the hyperleukocytic group (p= 0.016).There was little difference in lymphoid enlargement between the typical CLL (14 yes, 68 no) and hyperleukocytic (3 yes, 7 no) groups (Fisher exact, p=0.5415).

Table 1: Comparison of clinical findings.

| | Hyperleukocytosis-Mean (SD) | Typical CLL-Mean (SD) | Difference | p-value** |
|---|---|---|---|---|
| Gender | 7M / 3F | 54M / 29F | -- | -- |
| Age (yrs) | 65.20 (12.91) | 70.96 (9.72) | -5.76 | 0.20 |
| Cellularity (4.0-10.0 x10$^3$ cells/uL, blood) | 127.21 (98.45) | 25.43 (26.55) | +101.78 | **0.035** |
| RBC (4.5-6.3 x 10$^6$ cells/mL) | 3.29 (1.11) | 4.22 (0.70) | -0.94 | 0.092 |
| Hgb (14.0-18.0 g/dL) | 10.60 (2.64) | 12.52 (1.88) | -1.92 | 0.11 |
| Platelets (144-440 x 10$^6$ cells/mL) | 128.29 (60.24) | 204.30 (105.60) | -76.02 | **0.016** |
| WBC (4.2-10.0 x 10$^6$ cells/mL) | 210.2 (107.98) | 24.00 (21.85) | +186.20 | **0.00087** |
| % Lymphocytes (15.0-40.0%) | 79.2 (12.25) | 57.13 (24.54) | +22.07 | **0.0039** |
| Lymphocyte count (1.2-3.4 x 10$^6$ cells/mL) | 94.47 (84.73) | 16.73 (19.04) | +77.74 | 0.25 |
| % Abnormal (CLL) cells (NNV) | 90.2 (7.61) | 55.18 (26.8) | +35.68 | **4.19 x 10$^{-12}$** |

**Comparison of the mean values was analyzed using a two-tailed t-test, assuming unequal variance.

**Immunophenotypic Findings:**
There were no apparent differences in immunophenotypic expression between the two groups with regard to diagnostic or prognostic marker expression. Both groups showed CD5+, CD10-, CD19+, CD20+, CD23+, CD45+, HLA-DR+, FMC7-, CD52+ (p=0.13-1.00). Prognostic markers: there was no difference in the proportion of CD38+ or ZAP-70+ expression between the two groups (p=1.00, 0.73, respectively).

Table 2: Comparison of Flow Cytometry Findings.

| Marker | Expression | Hyperleukocytosis | Typical CLL | p-value** |
|---|---|---|---|---|
| CD5 | Positive | 9 | 77 | 0.90 |
| | Negative | 1 | 4 | |
| CD10 | Positive | 0 | 0 | 1.00 |
| | Negative | 10 | 81 | |
| CD13 | Positive | 0 | 4 | 0.13 |
| | Negative | 2 | 0 | |
| CD19 | Positive | 10 | 81 | 1.00 |
| | Negative | 0 | 0 | |
| CD20 | Positive | 8 | 65 | 1.00 |
| | Negative | 2 | 15 | |
| CD23 | Positive | 10 | 78 | 1.00 |
| | Negative | 0 | 1 | |
| CD45 | Positive | 10 | 81 | 1.00 |
| | Negative | 0 | 0 | |
| HLA-DR | Positive | 9 | 61 | 1.00 |
| | Negative | 1 | 7 | |
| FMC7 | Positive | 3 | 20 | 1.00 |
| | Negative | 7 | 57 | |
| CD52 | Positive | 10 | 30 | 1.00 |
| | Negative | 0 | 0 | |
| CD38 | Positive | 6 | 44 | 1.00 |
| | Negative | 4 | 36 | |
| ZAP-70 | Positive | 3 | 39 | 0.73 |
| | Negative | 4 | 29 | |

**Analysis of counts of expression was done using Fisher exact test.

**Cytogenetic Findings:**
There were no significant differences in cytogenetic abnormalities. However, statistical analysis was hampered by low sample size and incomplete data in both groups.

Table 3: Cytogenetic Aberration Incidence and Percentage

| | Percent Observed-% (count) | | | Percent in Literature-% | |
|---|---|---|---|---|---|
| Karyotype | Hyperleukocytosis | Typical CLL | p-value[A] | Reported | X$^2$ value[B] |
| Normal Karyotype | 0 (0) | 10 (2) | 0.616 | 18 | 0.86 |
| 13q deletion | 71 (5) | 20 (4) | | 55 | |
| Trisomy 12 | 43 (3) | 20 (4) | | 16 | |
| 17p deletion | 29 (2) | 15 (3) | | 7 | |
| 11q deletion | 29 (2) | 5 (1) | | 58 | |
| Various abnormalities | 85 (6) | 70 (14) | | 82 | |

[A] Incidence or counts of each genetic aberration were analyzed between the two groups using a Fisher exact test in a 2 x 6 contingency table. [B] Percentage has been reported in the literature and is compared to our findings using Chi-square analysis.

## Conclusions

**Clinical:**
Thrombocytopenia in the hyperleukocytic group suggests a direct impact of the increased leukemic cells on platelet formation in bone marrow.

**Immunophenotype:**
These findings suggest that hyperleukocytic CLL B-cells are immunophenotypically identical to typical CLL cells. The lack of any significant difference in CD38 or ZAP70 suggests that progression to hyperleukocytosis is an event independent from IgVH mutation status and may have independent prognostic significance.

**Cytogenetic:**
There is no apparent difference in cytogenetic lesion between the two groups. Statistic analysis was limited due to sample size and methodology. We cannot consider the timing of mutation appearance or exclude any novel, undiscovered mutations.

## References


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