



# Hematogones: The Supreme Mimicker and a Cytomorphological Confounder in Acute Lymphoblastic Leukemia

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## Abstract

**Objective** B-lymphocyte progenitors, namely the hematogones (HGs), may pose problems in morphological assessment of bone marrow, not only during the diagnostic workup but also while evaluating bone marrow for remission status following chemotherapy. Here, we describe a series of 12 cases of acute lymphoblastic leukemia (ALL) that included both B-ALL and T-ALL cases, which were evaluated for remission status and revealed blast-like mononuclear cells in bone marrow in the range of 6 to 26%, which on immunophenotypic analysis turned out to be HGs.

**Materials and Methods** This is a case series of 12 ALL cases who were undergoing treatment at the Army Hospital (Referral and Research), New Delhi. All these cases were under workup for post-induction status (day 28) and to check for suspected ALL relapse. Bone marrow aspirate (BMA), biopsy, and immunophenotyping were performed. Multicolored flow cytometry was performed using CD10, CD20, CD22, CD34, CD19, and CD38 antibodies panel.

**Results** BMA assessment of 12 cases revealed a maximum of 26% blastoid cells and a minimum of up to 6%, raising the suspicion of hematological relapse. However, on clinical assessment, these patients were well preserved, with preserved peripheral counts. Hence, marrow aspirates were subjected to flow cytometry using the CD markers panel, as discussed above, which revealed HGs. These cases were followed by minimal residual disease (MRD) analysis that revealed MRD-negative status, further confirming our findings.

**Conclusion** This case series highlights the importance of morphology and bone marrow immunophenotyping in unveiling the diagnostic dilemma in post-induction ALL patients.

## Keywords

- ▶ bone marrow
- ▶ flow cytometry
- ▶ hematogones
- ▶ immunophenotyping

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## Introduction

Hematogones (HG) are the normal B-lymphocyte precursors present in bone marrow and in Latin HGs means “blood-maker.” These were first described in 1937 as “lymphoid-appearing cells” by Peter Vogel in bone marrow aspirates from children. HGs are non-neoplastic precursors of B-lymphocytes originally acknowledged by their morphologic features in bone marrow smears. These are generally found in small numbers in most marrow specimens analyzed by flow cytometry. However, HGs are reported to occur in large numbers in some healthy infants and young children in a variety of diseases in both children and adults. Though the HGs are identified as regenerative cells of bone marrow, these may be particularly significant after chemotherapy or bone marrow transplantation and in some diseases such as autoimmune, congenital cytopenias, neoplasms, and acquired immunodeficiency syndrome.<sup>1-4</sup>

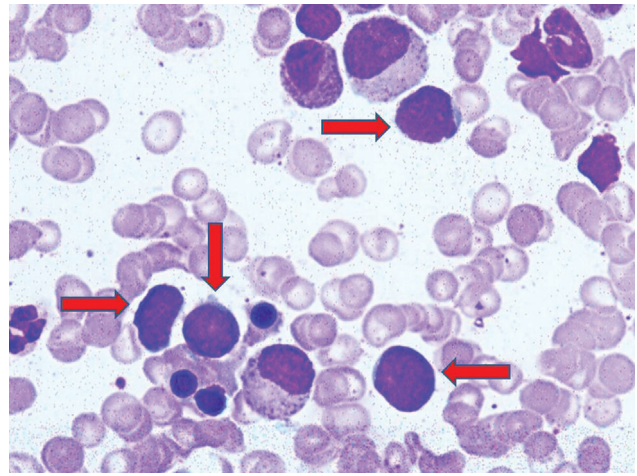
Recently, several studies have demonstrated a role of HGs level in predicting outcomes after conventional chemotherapy or allogenic hematopoietic stem cell transplantation.<sup>5-8</sup>

Increased numbers of HGs may cause problems in diagnosis of acute lymphoblastic leukemia (ALL) because of the morphologic features they commonly share with the neoplastic lymphoblasts. Their immune phenotype also has features in common with neoplastic B cell precursor lymphoblasts. Also, raised number of HGs (more than 5%) are seen to occur in cases following chemotherapy when marrow is recovering, further contributing to the confusion among the hematopathologists and clinical hematologists regarding the remission status and thus posing greater challenge in morphological assessment of the patient.<sup>4,9-12</sup> Good clinicopathological correlation and multiparametric flow cytometry play vital role to distinguish between these cell populations in nearly all instances.<sup>4,9-12</sup>

In light of above findings, we present a case series of 12 known ALL cases in which we were able to surpass the diagnostic dilemma of HGs vis-à-vis blasts while evaluating the patients for their post-induction remission status (day 28) by collaboration of good clinical input, cytomorphology, and bone marrow aspirate multiparametric flow cytometry.

## Materials and Methods

This study was conducted at tertiary care center in North India and 12 patients, suffering from ALL, were studied during the time span of 16 months in year 2020 to 2021. All the patients were of ALL diagnosed at this center and bone marrow samples were obtained on day 28 for look for remission status in post-induction phase. Both bone marrow biopsy and bone marrow aspirate (BMA) were obtained from all patients to carry out cytomorphological and histopathological evaluation. First BMA slides were stained with Leishman–Giemsa stain using standard protocol and then cytomorphological evaluation was performed under the microscope. The BMAs that showed increased blast-like cells/HGs as evident by size more than small-intermediate sized lymphocyte, high N:C ratio, compact to open chromatin and inconspicuous nucleoli were further

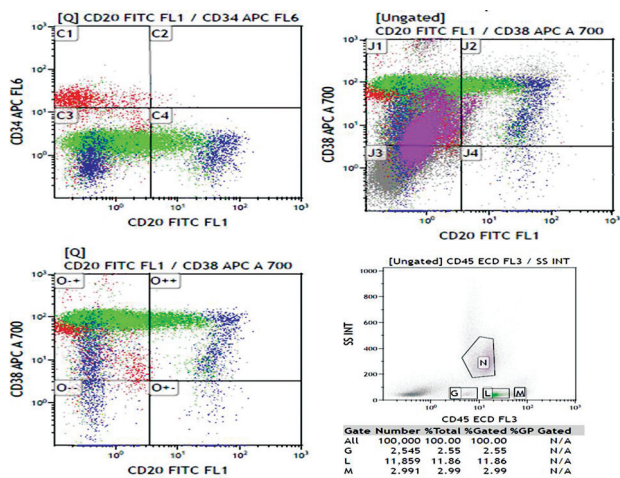


**Fig. 1** Bone marrow aspirate showing hematogones (HGs) as depicted by red arrows. These HGs have size more than small-intermediate sized lymphocyte and show high N:C ratio, compact to open chromatin and inconspicuous nucleoli.

subjected to flowcytometry analysis (FCA) to solve the diagnostic dilemma raised blasts (→Fig. 1). Background milieu in bone marrow aspirates of almost all the patients showed trilineage hematopoiesis with maturation of all cell lineages that further strengthened our suspicion of these blastoid cells as possible HGs.

For flow cytometry, 02 mL of bone marrow aspirate specimen in ethylenediaminetetraacetic acid vacutainer was taken and first washed with phosphate buffer saline (PBS) and resuspended in PBS. The samples were then stained with monoclonal antibodies directed against cell surface markers using a stain-lyse-wash method. Cell suspensions will be incubated for 15 minutes with various cocktails of fluorochrome-conjugated antibodies that will include CD19, CD10, CD34, CD79a, CD38, CD20, and TdT. All antibodies were obtained from Beckmann coulter and analysis was performed in Beckmann FC500 flow cytometer equipment. Red blood cell lysis was done by ammonium chloride solution followed by rinsing with PBS and resuspension in PBS containing 1% formaldehyde. Gating on lymphoid cells was done based on CD45 versus side scatter and CD 19 versus side scatter. The antigenic expression was rated as positive when the percentage of positive blast cells was more than or equal to 20% either in aberrant or conventional case. Similarly, aberrant phenotypes were defined when at least 20% of the blast cells expressed that particular phenotype. FCA using primary panel of monoclonal antibodies was performed. The primary panel of antibodies comprised of CD45 (immunologic marker for gating purpose for identifying blasts in all the tubes), CD19, CD10, CD34, CD38, CD20, and TdT.

On CD45/side scatter gating, HGs and blasts have low expression of CD45 with scattering of HGs in two to three dim CD45 populations as compared with single dim CD45 population of blasts that gives good diagnostic clue on FCA. Sequence and stages of evolution and maturation pattern of HGs are as follows; the earliest B-lineage precursors express CD34, CD38, CD19, and strong CD10 while they lack CD20

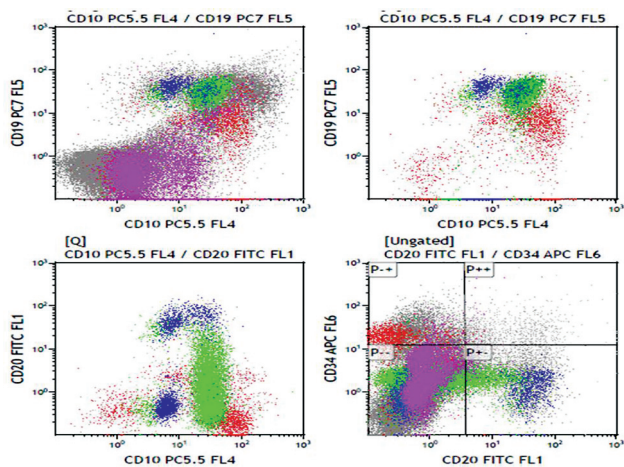


**Fig. 2** Flow cytometry on bone marrow showing two distinct populations of hematogones (green and red) that are CD45 dim as compared with CD45 bright mature lymphoid population (blue). These are approximately 14% and show distinct immunophenotyping features when tagged with CD20, CD38, and CD34.

expression (stage 1). In next stage, there is loss of expression of CD34 completely and CD10 partially and upregulation of CD20 (stage 2). Stage 3 HGs show a progressive expression of CD20 and surface immunoglobulin light chains. Lastly CD10 is downregulated completely, CD38 partially, and CD20 upgraded to high intensity in mature B-lymphocytes. CD45 expression progressively increases during maturation from stage 1 to stage 3 HGs (►Figs. 2–3) Ethical approval for the study protocol was obtained from institutional ethical committee and written informed consent was taken from all patients.

**Results**

The general characteristics, clinicohematological parameters, and flow cytometry findings are summarized in ►Table 1. In



**Fig. 3** Flow cytometry on bone marrow showing hematogones with distinct immunophenotyping features when tagged with CD19, CD10, CD20, and CD34. Color coding is as follows: Mature lymphocytes: blue, hematogones: green and red.

this series of 12 cases, flow cytometry was used to quantify HGs as a percentage of total events in bone marrow aspirate samples of post induction ALL patients. The percent HGs was compared for density gradient and lysis cell processing methods. The immunophenotype of HGs was determined using one of two 4-color combinations of antibodies and compared with that of neoplastic lymphoblasts. Among 12 cases of ALL, 8 were B-ALL and 4 were T-ALL. The age range of these patients was 10 ± 8 years in B-ALL and 9 ± 5 years in T-ALL cases, with male to female ratio 3:1 in B-ALL and 1:1 in T-ALL. All the cases were day 28 post-induction to access the patient for remission status. Percentage of HGs was ranging from 6 to 26%. Average HGs were 11% in B-ALL and 7% among T-ALL cases and approximately 9% among total 12 ALL cases (►Table 2). On detailed clinical assessment, these patients were well preserved, with preserved peripheral counts. Hence, the marrow aspirates were subjected to flow cytometry using the CD markers panel as discussed above, which revealed HGs. These cases were followed by minimal residual disease (MRD) analysis by flow cytometry that revealed MRD-negative status, further confirming our findings. However, next-generation panel (NGS) panel was not done in these patients because of two reasons: first cost-effective and standardized flow cytometry MRD analysis correlated with hematological findings along with our flow cytometry findings and second nonavailability of the expensive NGS setup in this laboratory.

**Discussion**

As per the best of our knowledge, there is sparse research content available regarding HGs from North India after through search in literature and this is first study to discuss HGs, diagnostic confusion imposed by them, and the relevance of multicolor flow cytometry in assessment of post-induction ALL cases from North India. Study by Sędek et al in year 2014 from population in Poland showed increased bone marrow HGs leading to diagnostic confusion because of their similarities to neoplastic lymphoblasts especially while evaluating the patient following treatment for ALL. These findings are in line with this study because HGs are often expanded in regenerating marrow and can potentially be mistaken for residual disease.<sup>12</sup> As in our case series, all 12 cases of ALL including both B-ALL and T-ALL showed HGs ranging from 6 to 26% with average number of HGs being 11% in B-ALL (mean standard deviation of ± 6.32%) and 7% in T-ALL cases (mean standard deviation of ± 4.2%).

Our study is in concurrence with a study by Lúcio et al in the year 2001 in European population, which showed that the average number of HGs among 130 patients with less than 1 year of age was 9%, which drops to 3.9% by 2 to 5 years, and in patients more than 50 years of age it is less than 1%.<sup>1</sup> Furthermore, the result of our study is comparable to study by Campana in American population in which percentage of HGs exceeds 10% in the bone marrow among the adult and pediatric patients with age ranging from 2 to 16 years.<sup>11</sup> The morphological distinction from neoplastic lymphoblasts is very difficult especially when the percentage of HGs exceeds 5 to 10% in the bone marrow.<sup>9–11</sup>

**Table 1** Hematological profile and hematogones count on flow cytometry in 12 ALL patients

Sl. no.	Age (y)	Gender (M/F)	Hb (gm/dL)	WBC ( $\mu\text{L}$ )	Platelet ( $\mu\text{L}$ )	Blast count bone marrow (%) at time of diagnosis	Hematogones bone marrow (%) flow cytometry (day 28)
1.	20	M	10.0	53,717	15,000	94	7
2.	10	F	10.6	56,781	17,000	60	12
3.	6	M	8.8	48,596	69,000	94	10
4.	11	M	11.0	64,408	80,000	45	6
5.	3	M	4.8	60,778	19,000	60	16
6.	21	F	10.7	94,154	240,000	58	26
7.	7	M	6.5	42,576	7,000	90	7
8.	4	M	4.2	57,445	31,000	94	14
9.	10	M	10.7	47,369	5,000	70	11
10.	5	M	7.9	71,624	80,000	80	6
11.	4	F	8.0	47,624	7,000	80	6
12.	16	F	6.5	77,302	40,000	90	9

Abbreviations: ALL, acute lymphoblastic leukemia; Hb, hemoglobin; WBC, white blood cell.

**Table 2** Distribution of ALL patients into B- and T-ALL immunophenotype and their clinicohematological profile

Clinical profile	B-ALL	T-ALL
Age (years)	10 + 8	9 + 5
Sex (M:F)	3:1	1:1
Number of cases	08/12	04/12
%HG's	11% mean SD (+6.32)	07% mean SD (+4.2)

Abbreviations: ALL, acute lymphoblastic leukemia; HG's, hematogones; SD, standard deviation.

Neoplastic lymphoblasts may exhibit one or more aberrancies relative to normal B-lymphocyte precursors such as uniform expression of TdT and CD34; negative or under-expression of CD45, CD20, human leukocyte antigen-DR isotype (HLA-DR), and CD38; overexpression of CD10; an abnormal spectrum of CD22; and co-expression of CD34 and CD20.<sup>11-15</sup>

FCA with combination of CD19/CD10/CD20/CD34/CD38 was efficiently used to differentiate the two depending upon the difference in the fluorescence intensity between blasts and HG's.<sup>9-11,16</sup> Among all these studies, a study by McKenna et al in year 2001 analyzed immunophenotypic characteristics of HG's in 662 consecutive bone marrow samples and stressed upon importance of FCA in delineating the uniqueness of HG's. These results are in line with our study as we were also able to delineate the HG's using FCA.<sup>16</sup>

The increased number of HG's have been reported in the bone marrow of children recovering from chemotherapy, regenerating marrow of treated ALL, after bone marrow transplant in acute myeloid leukemia, aplastic conditions, other forms of bone marrow injury, infections like cytomegalovirus, HIV, and immune thrombocytopenia disorders.<sup>1-3,16</sup> They pose diagnostic dilemma and potentially

can be mistaken for misdiagnosis of ALL or residual disease in known cases of ALL because of their morphological similarities to neoplastic lymphoblasts. On morphology, HG's vary in shape from 10 to 20  $\mu$  in diameter with round or oval nucleus with condensed but homogeneous chromatin. Nucleoli are absent or small and indistinct. Cytoplasm is generally scant but when present is moderately to deeply basophilic and devoid of inclusions, granules, or vacuoles (**Fig. 1**).

In recent studies, it was found that HG's (> 5%) have now also been seen to be associated with good prognosis and increased survival rates in case of post-chemotherapy or post-engraftment leukemia's.<sup>4,7-9</sup> So, more long-term follow-up studies are required to access the therapy response. In this study, we did not analyze the correlation of HG's with clinical outcome due to relatively lesser number of patients with HG's; however, we intend to study the same in near future for prognostication of the ALL patients.

## Conclusion

Distinction of benign HG's from neoplastic lymphoblasts in cases of ALL is difficult on morphology and very essential for disease management in cases of post-chemotherapy or post-marrow transplant and flow cytometry can reliably distinguish HG's from residual lymphoblasts in almost all cases when optimal antibody combinations are used.

### Conflict of Interest

None declared.

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