Short Communication

Hematogones Detection in Hematological Malignancies and Bone Marrow Transplantation

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Abstract

Hematogones (HGs, normal B-lymphocyte precursors) were first described in 1937 by Peter Vogel as "lymphoid-appearing cells" in bone marrow aspirates from children. HGs in Latin mean "blood-maker". The function and significance of these cells remained unclear till the 1970s when flow cytometry was used to identify them. HGs may be particularly prominent in the regeneration phase following chemo-therapy or bone marrow transplantation. Moreover, Increased HGs may cause misinterpreted because they share morphologic features with neoplastic lymphoblasts. In this review, we tried to focus on current morphologic and immunophenotypical characterization of HGs and their clinical significance as diagnostic and prognostic tool in predicting patient outcomes. We have examined several studies to evaluate risk ratio of HGs level at certain cut off limit which has been proposed in majority of studies. Out of 21 studies which included 1150 patients with different hematological malignances and bone marrow failure syndromes and 590 post transplantation patients. We report how HGs detection would help in diagnosis and predict outcome in bone marrow transplantation patients.

ABBREVIATIONS

HGs: Hematogones; AIDS: Acquired Immunodeficiency Syndrome; aHSCT: Allogeneic Hematopoietic Stem Cell Transplantation; ALL: Acute Lymphoblastic Leukemia ; CLL: Chronic Lymphocytic Leukemia; MDS: Myelodysplastic Syndrome ; AA: Aplastic Anemia; CML: Chronic Myeloid Leukemia; MRD: Minimal Residual Disease; GVHD: Graft Versus Host Disease; AML: Acute Myeloid Leukemia; NHL: Non-Hodgkinlymphoma; MM: Multiple Myeloma; SAA: Severe Aplastic Anemia; ATCL: Adult T-Cell Leukemia/Lymphoma; BMT: Bone Marrow Transplantation; CBT: Cord Blood Transplantation; LFS: Leukemia Free Survival; ITP: Immune Thrombocytopenic Purpura ; LPD: Lymphoproliferative Disorder; FL: Follicular Lymphoma

INTRODUCTION

Hematogenes (HGs), B-lymphocyte precursors, primarily recognized by their morphologic features in bone marrow [1,2]. Total HGs may be classified into early, intermediate or late developmental stages. Reports showed HGs may increase in healthy infants and children as well as in several diseases of children and adults, known as reactive HGs [3,4]. While HGs, identified as regenerative, may be particularly noticeable after chemotherapy or bone marrow transplantation and in some diseases such as autoimmune, congenital cytopenias, neoplasms, and acquired immunodeficiency syndrome (AIDS) [3,5-7]. Recently, several studies have demonstrated a role of HGs level in predicting outcomes after conventional chemotherapy [8-10] or allogeneic hematopoietic stem cell transplantation (HSCT) [11-14]. In this review, we discussed HGs potential role in certain diseases as a biomarker for either diagnostic and prognostics purposes, especially blood disorders and other malignancies. Our strategy in searching current literature using these specific keywords such as normal B precursor, HGs in disease diagnosis, post transplantation prognosis, HGs levels in relation to post chemotherapy prognosis, and HGs used to be misinterpreted and lead to miss diagnosis. We have applied few filters such as reports human studies reported in English, Chinese studies were translated into English through Google translate. We aimed to examine HGs detection throughout literature to-date and discussed pros and cons with spotlight on certain precautions for HGs assessment. Moreover, we have tried to find HGs predictive

values in clinical lab and its clinical significance for cancer patients.

Characterization

Morphologic: The most immature HGs have common cytological features with lymphoblasts and even in some cases they may be indistinguishable from neoplastic lymphoblastsin acute lymphoblastic leukemia (ALL) (Figure 1). Early HGs have round nuclei with indentations, scant cytoplasm, and inconspicuous homogeneous chromatin with indistinct or at times variably prominent nucleoli. Presence of one or more nucleoli reflects HGs immaturity, cytoplasm is moderately to deeply basophilic and lacks inclusions, granules or vacuoles [15]. The most mature HGs resemble mature B-lymphocytes with condensed chromatin (Figure 1). Late HGs typically exhibit highly condensed, uniform nuclear chromatin and scant cytoplasm. The nucleoli are absent or indistinct (Figure 1). HGs are usually not present in a peripheral blood smear, except for samples from neonates [16] or umbilical cord blood [2,17].

Immunophenotypic: The earliest recognizable B-lineage precursors expressed the progenitor cell marker CD34 in combination with CD38, CD19, high levels (bright) of CD10, and low levels of CD22 while lacking CD20. These progressed to the next stages by down-regulating CD34 completely and CD10 partially, prior to progressive up-regulation of CD20. CD22 levels also increased slightly as CD20 was up-regulated. Finally, CD10 was down-regulated completely, CD38 partially, and CD22 upgraded to high intensity. Late stage, in which CD10 is completely down-regulated, is considered a mature stage of B-cell development. TdT expression parallels CD34 in the B-cell maturation sequence. Surface Ig is variable among individual cells in each case, occurring shortly before to after acquisition of a high level of CD20 expression. Asynchronous expression of the earliest and latest antigens, e.g., concurrent CD34 and CD20,

and aberrant over- or under-expression of antigens was not observed in HGs populations [18]. However, after chemotherapy or stem cell transplant for hematological disorders, early HGs may coexpress CD20 (dim) and CD34 because of alteration of the maturation pathways of benign B-cell precursors. All stages of HGs express CD19, CD22, CD38 and CD10 [4,19-21]. Studies on cytoplasmic IgM expression in HGs have shown that cytoplasmic IgM expression occurred during HG maturation along with increasing CD19 expression, decreasing CD10 expression and loss of TdT/CD34. Cytoplasmic IgM is acquired early during the Stage 1 to Stage 2 transition in HG development (Figure 1) [20,22-32]. HGs are mainly detected by 4-color flowcytometry assay; where single cell suspensions from tissue, marrow aspirate or whole blood stained with fluorochrome-conjugated monoclonal antibodies against CD10, CD34, CD45 and IgM, HGs may be sub classified into (a): immature – CD34^{high+}, IgM^{Neg}, CD10^{high+} and CD45^{dim+}; (b): intermediate – CD34^{Neg}, IgM^{Neg}, CD10^{high+} and CD45^{high+}; (c): mature CD3^{Neg}, IgM^{high+}, CD10$^{Neg/dim+}$ and CD45^{high+}). Extended panel may be required to discriminate abnormal clone by staining for CD3, CD5, CD10, CD19, CD20, CD34, CD38, CD45, IgM, TdT, kappa and lambda immunoglobulin light chain [33,34]. Another flowcytometer report used 2 different 4-color antibody combinations of CD10, CD20, CD19, and CD38. The second 4-color antibody combination of CD10, CD20, CD22, and CD34. Both combinations categorize between early-stage; intermediate and late-stage HGs; and mature B cells [35,36].

Genomics: Gene expression of HGs, (Figure 2), showed differences of these maturation genes along differentiation stages. CD19 and CD22 are statistically significant over expressed in mature B cell than in HSCs, while CD 34 is statistically significant over expressed in HSCs than mature B cells. CD38 was not statically significant different in expression in both HSCs and Mature B Cells (Figure 2).
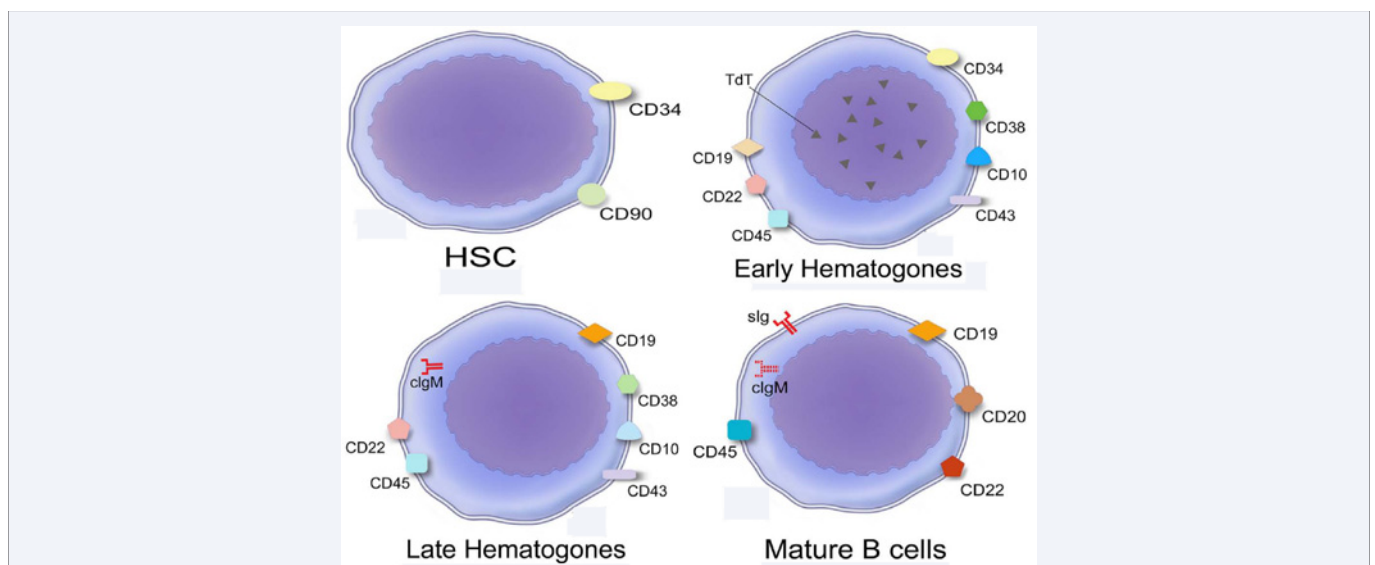

Figure 1 Representative figure for few stages of B cell maturation and differentiation along with their important biomarker expression, which may be used for characterization of HG with comparison to HSC, hematopoietic stem cellearly HGs, late HGs and mature B lymphocyte. Markers were marked to each stages based upon data collected from previous published reports. Each marker is labeled with unique color code, intensity of color used to represent its relative expression, i.e. light color means dim or low expression of this marker at this stage of normal differentiation while dark color means string expression [15, 50-55].
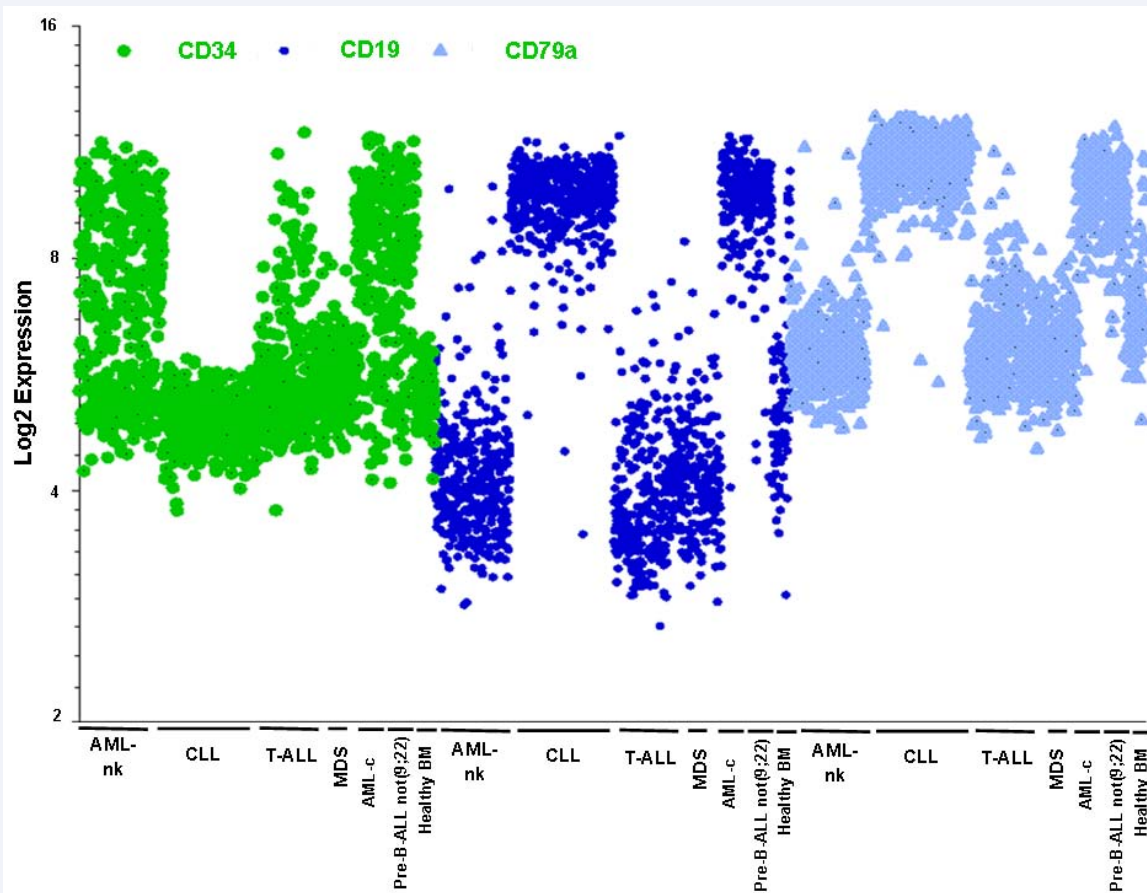
Figure 2 Scatterplot for expression of important early (CD34) and late (CD19, CD79a) genes involved in Maturation and differentiation stages of HG which may be used for detecting HGs by flowcytometer-based assayData was extracted from public availableGSE13159 [56].
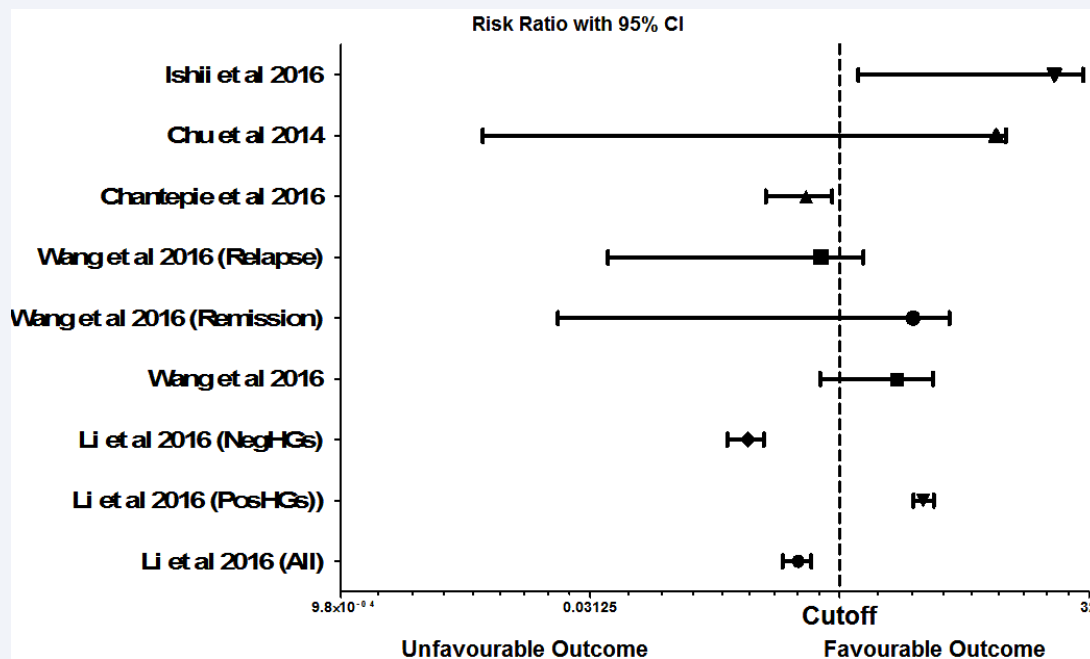

Figure 3 Risk ratio with 95% CI plotted for HGs level detected by flowcytometer 4-color assays as reported by five studies based and their value in predicting patient's outcome. Favorable and unfavorable outcome refer to remission or relapse after bone marrow transplantation.

PAX-5, a transcription factor expressed throughout B-cell maturation, has been evaluated in non-neoplastic bone marrow sections, which showed scattered positive nuclear staining in small B-lymphocytes/HGs [37-39]. Expression of the receptor tyrosine kinase-like orphan receptor 1 (ROR-1) was studied in a different subset of the lymphoid population. ROR1 was not expressed on the earliest B-cell precursors (CD34/CD38/CD19/CD10 positive). However, ROR1 was positive on the B-cell precursors at an intermediate stage of maturation (CD34−/CD38+/CD19+/CD10$^{intermediate\ to\ high}$).B-cell precursors (CD34−/CD38+/CD19+/CD10^{low}) and mature B cells (CD34−/CD38+/CD19+/CD10−) were negative for ROR [4,7,20,26,29,40-47]. Recently ZAP-70, Zeta-chain-associated protein kinase 70, a member of protein-tyrosine kinase family, expression was reported to be low in HGs [37,38]. Reports showed low BCL2, B-cell lymphoma, a human proto-oncogene located on chromosome 18, expression in mature and immature HGs [38,39,41,48,49]. The pathological CD5 expression in patients with increased HGs. CD5 was expressed in normal subset of polytypic B cells on a continuum, predominantly at the later stages of maturation, specifically on mature HGs and mature B cells. Thus, the difference in CD5 and surface Ig light chains expression allows for the distinction between normal CD5+ B-cells and CD5+ lymphoma cells chronic lymphocytic leukemia (CLL) or mantle cell lymphoma [18,38].

Factors affecting HGs levels

Presence and demography group: HGs could be detected morphologically in peripheral blood of neonates [6,46-57] and in umbilical cord blood [17,18,47]. They are better identified nowadays using a 4-color flow cytometry approach [2,8,58]. HGs can also be identified using a sensitive flow cytometer in peripheral blood in 65% of adults with certain clinical conditions [47,54]. HGs were reported to be present in high numbers in marrow from infants and young children, and then they showed a statistically significant decline with aging. There was no significant difference between males and females [15,36,59].

Conditions that affect HGs detection: Specimens processed by density gradient separation had a significantly higher percentage of HGs than those processed by ammonium chloride lysis mostly related to the removal of neutrophils, which would relatively increase HGs number [18]. There was a decline in mean percent HGs with increasing marrow involvement by neoplastic cells. The reason may be related to encroachment on HGs compartment by the neoplastic infiltrate, although other factors that inhibit Blymphocytogenesis may play a role [18]. HGs are above 5% in these clinical conditions ; lymphomas, various non-neoplastic blood cytopenias, post-chemotherapy, post–bone marrow transplantation, and AIDS were the most common [18].

HGs may be low (<0.1%) or even absent in hematological disorders, such as myelodysplastic syndrome (MDS) and aplastic anemia (AA) [31,60,61], possibly due to a T-cell mediated inhibition of hematopoiesis. Down-regulation of genes involved in B lymphopoiesis has been described in MDS (Figure 2) [62,63]. In chronic myeloid leukemia (CML), a significant but unexplained decrease in stage 1 and total HGs compared to age-matched controls has also been reported. The percentage of total HGs, as defined by the population of CD10/CD19 expressing event, decreased in the BM aspirates of patients with CML when compared to age-matched controls (0.26% vs. 0.87%, $p<0.001$). These differences were maintained in the BM of untreated patients with CML at diagnosis (0.29% vs. 0.87%, $p=0.001$), as well as the follow-up aspirates post-treatment (0.17% vs. 0.87%, $p<0.001$) [64].

Clinical significance of HGs

Minimal Residual Disease (MRD): The most important application of HG's phenotype knowledge is to distinguish HGs from minimal residual disease in patients treated for B-ALL, non-invasive molecular and flow cytometric MRD analysis are promising tools for future prospective trial using risk stratification with the level of MRD [65,66].

B Cell neoplasia: There are morphologic and phenotypic similarities between HGs and leukemic lymphoblasts in B-ALL [29]. B-lymphoblasts showed concordant expression of CD123 and CD34 in 91% of B-ALL cases (80% CD123+/CD34+ and 11% CD123−/CD34−cases), whereas the HGs had discordant expression(CD34+/CD123− in immature HGs and CD34−/CD123+ in mature HGs [67]. HGs can be distinguished from neoplastic immature lymphoid cells in bone marrow trephine biopsies by their specific morphological features, unique CD34 <TdT<CD20< PAX5 immunophenotypic pattern.CD20 positive cells in (78%) of cases in comparison to TdT positive cells in (76%) [52]. HGs should be identified from B-CLL cells during the flow cytometric analysis to reduce ZAP-70 or CD38 false negatives in samples with a high percentage of HGs [68-80].

Prognostic role for hematological malignancies: The exact role of HGs in myeloid malignancies is intriguing. Patients with >0.01% HGs had a significantly better leukemia free and overall survival rates [8]. Moreover, patients who had a negative residual disease after induction and detectable HGs in the bone marrow by flow cytometry had a better (relapse free survival) RFS and OS (overall survival). HGs could be a useful tool to identify prognostic subgroups in MRD-negative patients [80].

Prognostic factor after stem cell transplantation: After stem cell transplantation HGs were quantified at day +30 after (HSCT) and were inversely correlated with the donor's age. Patients with >5% HGs had a significantly longer overall survival and a lower rate of acute graft-versus host disease [18,81]. Also the percentage of CD10+/CD19+ bone marrow cells at day +100 was associated with improved event-free survival in the multivariate analysis after controlling for the disease stage, cytogenetic group, remission status and chronic graft-versus-host disease (GVHD) [81]. Following cord blood transplantation, a high percentage of HGs at day +21 was found to be associated with a lower rate of grade 3/4 acute, [13]. Disease relapse or complete remission in patients following bone marrow transplantation was examined in several recent published reports to examine risk ratio with 95% confidence interval for each study and/ or patient groups extracted from these studies (Figure 3). Systematic review did not calculate overall risk ratio as more valid studies with homogenous patients are required for reliable calculation.

COMMENTS

We have examined 21 published reports and prepared a diagnostic HGs table that contains 10 studies reported how HGs

Table 1: studies used HGs as a diagnostic tool in various blood disorders.

Study	Disease	Outcome
A-Diagnosis		
Zeidan et al 2016 [63].	B-ALL (MRD)	80%of B-ALL showed concordant expression pattern of the 2 antigens: 63% cases expressed both antigens, whereas 17% expressed neither. The distinct patterns of CD34/CD123 expression on HGs(discordant) and B-ALL blasts (concordant) are useful in differentiating small populations of residual blasts fromHGsafter induction therapy to detect MRD.
Carulli et al 2015 [69].	NHL	-55non-Hodgkin lymphoma (NHL)patients treated with rituximab-containing regimens.Maturation arrest at the level of stage 2 HGs, along with complete depletion of naïve, mature B-lymphocytes (short-term effects, 2 month). Long term 12 months follow up while treated with rituximab maintenance therapy, showed complete normalization of B-lymphocyte ontogeny. Hypo-gammaglobulinemia was still observed in nine of the 21 patients.
Carulli et al 2014 [34].	NHL	-171 cases of B-cell NHL, either at diagnosis or during follow-up.The combination of K/λ/CD20/CD19/CD10/CD45/CD5immunophenotyping,was specific in detection of HGs in 97.6% of samples and distinguish normal B-lymphocytes, neoplastic lymphocytes and HGsin a single step. HGspercentage showed a significant inverse correlation with the degree of neoplastic infiltration.
Juranovic et al 2014 [70]	Cyclic Neutropenai	-The heterozygous mutation of ELANE gene generated severe cyclic neutropenia, granulocytic maturation arrest, an increased number of HGs(26% of marrow cells) in the bone marrow, an absence of neutrophils, and the presence of stage 3 HGsin peripheral blood. The percentage of HGswas inversely proportional to the absolute number of neutrophils.
Tang et al 2012 [61]	MDS	-73 MDS patients and 53 non-MDS patients increased total CD34(+) myeloblasts; decreased stage-IHGs; altered CD45/side scatter; altered expression of CD13, CD33, CD34, CD38, CD117, and CD123; aberrant expression of lymphoid or mature myelomonocytic antigens on CD34(+) myeloblasts; and several marked alterations in maturing myelomonocytic cells.
Anton-Harisi et al 2012 [71].	Acute megakaryoblastic leukemia	-2 acute myeloid leukemia (AML) M7 patientswith 38% and 20% of HGs. Both had thrombocytopenia.BM smear in 1st patient shows 60% cells with morphologic features consistent with acute megakaryoblastic leukemia and the2nd shows dry tap but on the following aspirate 10% cells with lymphoblastic morphology. Repeated work up gives diagnosis of M7.
Chow et al Feb 2011 [64].	CML	-CML patientschecked for the presence of HGsand compared with an age-matched controls. -The percentage of total and stage I HGswere decreased in CML at diagnosis and at follow-up post therapy when compared to age-matched controls (diagnosis, total: 0.29% vs. 0.87%, p = 0.001; diagnosis, stage I: 0.06% vs. 0.20%, p = 0.008; follow-up, total: 0.17% vs. 0.87%, p < 0.001; follow-up, stage I: 0.04 vs. 0.20, p = 0.003).
Akyay et al 2011 [72].	ITP	-2 patients with thrombocytopenia and three lineage dysplasia in the bone marrow suggesting MDSwith excess blasts. Light microscopic evaluation of marrow from both patients revealed periodic acid-Schiff (PAS)-negative blasts, flow cytometric analysis revealed excessive HGs in both patients, implying that the cells that were considered as blasts were actually large HGs.
Sevilla et al 2009 [31].	AA & MDS	-The percentage of total and early (stage I) HGswere significantly decreased in AA compared to controls, and they returned to normal numbers after hematopoietic stem-cell transplant. This demonstrates early B-cell lineage involvement in AA, similar to recent findings in MDS.
Babusíková et al 2008[73].	AML	-Multiple stages of HGswere observed twice as frequently in B-ALL (73.8%) and T-ALL (69.2%) samples as in AML aspirates (34.1%). Stage 3 HGswere found usually in children and were thus frequent in B-ALL. The HGshad an extremely high phenotypic stability unaffected by disease or therapy or by their coincidence with leukemia cells. After each leukemia therapy phase, characteristics of normal regenerating B-cells may be mistaken for a relapse.
B-Prognosis		
Li et al 2016[74].	AML	-Phenotypic analysis revealed a significantly lower proportion of abnormal chromosome karyotype and CD34 expression in HG-positive patients.Survival duration (both leukemia-free and overall) was significantly greater in the HG positive group than in the HG negative group and a lower relapse rate.
Wang et al 2016[75]	ALL	-High-risk group had a significantly lower number of HGs than the intermediate-risk and low-risk groups (p<0.05). HGs in the complete remission group was significantly higher than in relapse group (p<0.05). Children with HGs ≤1.0% had a significantly lower event free survival (EFS).
Chantepie et al2016[76]	AML	- Patients with HGs in bone marrow samples had a significantly better RFS and OS than patients without HGs (p = 0.0021, and p = 0.0016). Detectable HGs(51%) independently predicted RFS (HR = 0.61, 95%CI: 0.42 - 0.89, p = 0.012) and OS (HR = 0.59, 95%CI: 0.38 - 0.92, 0.019) controlling for age, ELN classification, the number of chemotherapy cycles to achieve complete remission (CR), performance status, secondary AML and flow cytometric MRD.
Chu et al2014[9]	AML	- Patients who had detectable MRD≥0.2 % exhibited significantly lowerleukemia free survival (LFS) than those who did not (13.5 vs. 48.0 months; p = 0.042). - B-progenitor-related cluster size ≥5 % predicted improved outcome, with longer LFS (not reached vs. 13.5 months; p = 0.023) and better overall survival (not reached vs. 24.0 months; p = 0.027).
C-Misdiagnosis		

Table 1: studies used HGs as a diagnostic tool in various blood disorders.

Study	Disease	Outcome
Guillory et al2016 [77].	LPD	Flow cytometry analysis of his bone marrow revealed a small distinct population of cells expressing dim CD10, CD19, CD22, CD38, dim CD58, HLA-DR, and dim CD45, which are characteristic ofHGs however, demonstrated dim surface immunoglobulin lambda light-chain restriction. This case raises awareness of the potential pitfalls of working up bone marrow for involvement by B cell LPDs.
Matsuda and Hirota2015[78]	FL	A case where bone marrow infiltration of follicular lymphoma histopathologically mimickedHGs or B-ALL/LBL when CD20 expression was downregulated in follicular lymphoma after R-CHOP therapy
Chaudhariet al 2014[79].	AML	A case in a 16 months old male who was unsuccessfully treated for a pre-B cell ALL on the basis of flow cytometry of the bone marrow which showed CD19 and CD10 expressing 'blast' cells. A diagnosis of AML M7 was made on a subsequent bone marrow biopsy in which the blast cells expressed CD61 and Factor VIII, while they were negative for CD10 and CD20. Also present were a few CD10 and CD20 expressing small lymphoid cells, which were interpreted as HGs. The problem of mistaking HGs for 'blast' cells on flow cytometry, especially in the marrow of very young children where HGs are often prominent.

Table 2: Bone marrow transplantation studies used HGs detection as a prognostic biomarker in patient with various blood disorders.

Study	Pt #	Age (Y)	Gender %	Diagnosis (%)	Type of transplant	Detection Time	Detection Method	Outcome
Hiroto et al 2016[82]	134	Median (range) 42 (16–64)	M:F 59:41	AML (52%) ALL (25%) MDS (12%) CML (5%) NHL (5%) MM (1%) SAA (1%)	CBT	Day 90	-quantitative analysis (morphology)-Flow cytometry of bone marrow samples	- In 25 of 134, the HGswere significantly (r=0.85,p<0.001) related to?? -The rates of OS, relapse, and TRM did not differ significantly between patients with<1% andwith>1%HGsof CD34+ve cells. -Standard-risk patients, cumulative incidence of TRM was significantly lower in patients with>1% than with<1% HGs. No significant difference in the high-risk patients.
Noriko et al 2015[83]	260	Median (range) 47 (16–71)	M:F 60:40	CML (n=7) AML (n=89) ALL (n=45) MDS (n=33) SAA (n=7) NHL (n=10) ATCL (n=1)	BMT (n=192) PBSCT (n=43) Unrelated CBT (n=25)	Day 30	-Flow cytometry analysis - HGs are defined by very low side scatter, intermediate expression of CD45, and bright expression of CD19, CD10, and CD38.	-HGspositive group≥ 0.25 % HGs in BM MNCs. -HGs detected in(40.6 %) of patients at day 30. - HGs at day 30 was an independent prognostic factor influencing OS in BMT. -No difference in relapse rate between HG(+)and HG (−) patients. HG (−) patients had a high NRM and developed infections and frequent grade II to IV acute GVHD compared to HG(+) patients.
Shima et al2013[14]	108	BMT 49.2 (mean) (20-66) CBT 47.3 (mean) (19-67)	M:F 56:44	AML MDS ALL NHL	BMT (n=59) CBT (n=49)	Engraftment (day when circulating granulocytes reached > 0.5 X 109/L for 3 days) -Day 90 Peripheral blood -Day 25 in BMT and day 32 in CBT.	-BM MNC prepared by the gradient centrifugation method Cells were stained with anti-CD34 ,anti-CD38 , anti-CD10, anti-CD20, anti-CD19 and conjugated lineage (Lin) mixture (anti-CD3, -CD4, -CD8), CD11b, CD14, and -CD56. -PB cells stained with anti-CD10, anti-CD20, anti-CD19. -Analyze clonality of IGHgene rearrangements status of HGs, DNA was obtained from 10 000 double-sorted cells from all recipients presenting ->0.1% MNCs of HGs on FACS. Then PCR amplification of DJH and VDJH gene rearrangement.	-B-cell precursors within the BM MNCs at the time of complete donor-type engraftment were higher in CBT recipients than in BMT recipients (6.37% vs 1.75%; P<.001). - In 106 of 108 patients, B-cell precursors were polyclonal based on the rearrangement analysis of the IGH genes. -The frequency of BM B-cell precursors significantly correlated with the number of blood B cells at the time of engraftment (R = 0.47, P <.001) - Inversed correlation between donor age and percentage of BM HGs in patients treated with BMT (R = 0.32,P < .02) and CBT (R= 0.42, P < .001) - Patients developed HGs>5% of MNCs had significant better 3-year OS (100%) and RFS (93.3%). -HGs>5% of MNCs was more in patients without viral infection; BMT: P< .03; CBT: P< .01) and no severe acute GVHD of grade II-IV (BMT: P <01; CBT: P< .01).

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Study	Pt #	Age (Y)	Gender %	Diagnosis (%)	Type of transplant	Detection Time	Detection Method	Outcome
Honebrink et al 2012[13]	88	N/A	N/A	AML	Cord blood bonemarrow transplant.	Day 21 & day100	Morphological: 94.8% of cases the HGspercentages reported by the two observers were within 3.6%. At day the percentage of marrow HGs range from 0% to 10.8% Flow cytometry: Performed on BM aspirates that were collected in sodium heparin	-Day 21;Patients with HGs = 0% develop grade III to IV aGVHD (38%, 95%, P=.05) - Patients who had a high BM HGs percentage at day 21 tended to have a high percentage at day 100 (85%) -At day100, HGsrange from0%to 29.6%. - Patients with high. HGs percentages ≥0.9% had a 3-year OS of 76% Vs 49% for patients with HGs percentages<0.9%; P = .02) -This improvement in OS was due to decrease in TRM, patients with HGs (≥0.9%) at day 100 showed a 1-year TRMrate of 2% Vs 31%; P<.01)

Table 2: Bone marrow transplantation studies used HGs detection as a prognostic biomarker in patient with various blood disorders.

Abbreviations: LAML: Acute Myeloid Leukemia; ALL: Acute Lymphoblastic Leukemia; MDS: Myelodysplastic Syndrome; CML: Chronic Myeloid Leukemia; NHL: Non-Hodgkin Lymphoma; MM: Multiple Myeloma; SAA: Severe Aplastic Anemia

could help in disease diagnosis, additional 4 studies showing how HGs could give clues on acute leukemia prognosis and 3 studies pointing how HGs might lead to misdiagnosis. A second table with four studies dedicated HGs in patients before and after BM transplantation and show how HGs levels affects outcome. HGs are precursors of normal B lymphocytes, which are detected morphologically and more accurately by flow cytometry nowadays. A 4-color assay is currently the best method to report HGs percentage in bone marrow samples from patient suffering from several blood diseases or healthy individual [15,18,34,36], these biomarkers may be selected from a list of surface and cytoplasmic biomarkers reported and represented in Figure (1) along with morphologic distinction. However, current HGs measurement by flowcytometry may be affected by changes in gene expression of normal B precursors in various disease status as shown in Figure (2), presence of complex cytogenetic may alter such biomarker expression pattern as shown in AML with normal or complex cytogenetic. Certain blood cancer may lack HGs detection such as MDS which may be related to low biomarker gene expression or other genes such as PAX5, ROR1 and ZAP-70 as earlier [81-84]. Detection of HGs may require additional precautions regarding specimen preparation as Ficol isolation or RBCs lysis may alter HGs level [85]. Sampling time may be critical, prior treatment basal sample may be a great asset for future comparison. Patient age has a strong impact on HGs levels variation, while no gender difference has been reported [21,86].

HGs Level may play a predictive role in transplantation patients as well as diagnostic role in MRD of B-ALL and LPDs. HGs may be proposed as a diagnostic assay to a spectrum of hematological diseases. In acute leukemia ; ALL has concordant expression of CD34/CD123 which help differentiate ALL blast and reactionary HGs [63] while in AML multiple stages of HGs observed in 34% of cases which is much less than percent in ALL around 70% [86] . In CML HGs are less exiting than in age matched controls [64] . In NHL percent of HGs is inversely proportion to degree of neoplastic infiltration [87]. In bone marrow failure syndromes decrease HGs percent in patients with AA compares to controls help in distinguish of AA from MDS [88]. In cyclic neutropenia HGs were inversely proportion to neutrophil count [70].

In prognosis HGs mainly help in acute leukemia with a cut of limit around 1%. Patient with HGs level>1% have better survival [9,74,75,89]. Post BMT; patients with HGs >1% in most studies had less TRM and GVHD which leads to better survival in bonemarrow, peripheral blood and cord blood BMT in wide spectrum of hematological disorders as leukemia , LPD and bonemarrow failure [83].

HGs detection may also play a critical role in non-B cell malignancies such as AML, MDS which help in predicting disease free or relapse free survival outcome. Post-chemotherapy or following transplantation, HGs regenerate and their level may be detrimental to patient prognosis and prognostic outcome. Comparison to basal level of HG may be recommended to avoid false prediction of increase level or depleted one. False increase/ decrease in HGs may be a requirement to the lab before using it as a reliable biomarker for cancer patients and following BM transplantation. Moreover, considering a basal level may be a better approach for correcting for changes in gene expression of CDs used in HGs immunophenotyping assay.

CONCLUSION

Current literature confirmed that HGs detection may play a critical prognostic role in BMT patients as well as a diagnostic tool for B-Cell neoplasia. However, reliable HGs assay cutoff requires valid large studies to estimate positive and negative predictive values. Basal detection may be recommended for patient follow up considering measurement limitations.

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