Hematogones in immune thrombocytopenic purpura: diagnostic implication

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Hematogones (HGs) are benign immature B cells in bone marrow with a variety of benign and malignant conditions, including idiopathic thrombocytopenic purpura, leukemia, lymphoma, red blood cell aplasia, iron deficiency anemia, amegakaryocytosis, regenerative bone marrow following viral injury, chemotherapy or bone marrow transplantation, copper deficiency, autoimmune cytopenias, neuroblastoma, and acquired immunodeficiency syndrome (AIDS). HGs may cause diagnostic problems because of their morphologic and immunophenotypic similarities to neoplastic lymphoblasts. Herein, two patients with thrombocytopenia and three lineage dysplasias in the bone marrow suggesting myelodysplastic syndrome (MDS) with excess blasts are presented. Light microscopic evaluation of marrow from both patients revealed periodic acid-Schiff (PAS)-negative blasts. However, flow cytometric analysis revealed excessive HGs in both patients, implying that the cells that were considered as blasts were actually large HGs. Thus, the patients were diagnosed as immune thrombocytopenic purpura due to the isolated thrombocytopenia, large platelets on blood and bone marrow smears and increased megakaryocytes in the bone marrow.

These cases emphasize the importance of distinction of hematogone-rich conditions from leukemia and MDS for accurate diagnosis and treatment, and the reliability of multiparameter flow cytometry for the differential diagnosis.

Key words: hematogones, immune thrombocytopenic purpura, myelodysplastic syndrome.

Hematogones (HGs) are benign immature (early and mid-stage) B cells that increase in the bone marrow of children and adults with a variety of conditions, including idiopathic thrombocytopenic purpura (ITP), leukemia, lymphoma, red blood cell aplasia, iron deficiency anemia, amegakaryocytosis, regenerative bone marrow following viral injury, chemotherapy or bone marrow transplantation, copper deficiency, autoimmune cytopenias, neuroblastomas, and acquired immunodeficiency syndrome (AIDS)[1-3]. The biological significance of this condition is unclear, but it is considered as a phenomenon of marrow regeneration[1]. HGs can be detected during the regenerative phase of bone marrow after viral injury[1,3]. The percentage of marrow HGs may fluctuate with disease status or persistent elevations may occur. Persistent elevations have been observed following cessation of chemotherapy for acute lymphoblastic leukemia (ALL) for more than a year following marrow transplantation[4].

Hematogones (HGs) may cause diagnostic problems because of their morphologic and immunophenotypic similarities to neoplastic lymphoblasts[1-3], and accurate distinction of HG-rich conditions from leukemia and myelodysplastic syndrome (MDS) are critical for patient care.

Herein, we report two children in whom further investigations had to be carried out to be able to differentiate between blasts and HGs and therefore to differentiate between MDS and ITP, before the final diagnosis of ITP.

Case Reports

Case 1

A 16-month-old girl was admitted to our hospital with generalized rash all over her body for two days. It was learned that she did not have any infection history for the last two weeks. She was the only child of the family, and there was no consanguinity between her parents. She had a cousin with diagnosis of ALL (Phi+).

Her physical examination revealed petechial rash all over her body and ecchymoses on her forehead and right costal arch. Her hemoglobin (Hb) was 122 g/L, hematocrit (Htc) 34.9%, platelet (plt) 58 x 10^9/L, and white blood cell count (WBC) 10.6 X 10^9/L with a differential count of 40% lymphocytes, 34% neutrophils, 6% monocytes, 20% blast-like cells, and paucity of thrombocytes. Her biochemical profile was normal.

Bone marrow aspiration smear revealed a cellular bone marrow with a mean 11% blasts (Fig. 1), 3% myelocytes, 1% eosinophilic myelocytes, 1% basophilic myelocytes, 3% metamyelocytes, 23% neutrophils, 5% eosinophils, 1% basophils, 9% band forms, 23% lymphocytes (some immature), 1% monocytes, 19% orthochromatic erythroblasts, and a myeloid/erythroid ratio of 2.4. Immature megakaryocytes were increased. There were dysmorphic findings in the granulocytic, erythroid and megakaryocytic series. The granulocytic cell line displayed pseudo-Pelger-Huet abnormality (3%), macrocytosis (22%), chromatin clumping (40%), hypo- and agranulation (12%), and nuclear-cytoplasmic asynchrony (2%); the erythropoietic cell line displayed nuclear bridge (3%), nuclear lobulation (0%), multinuclearity (1%), cytoplasmic granules (3%), and megaloblastic changes (9%); the megakaryocytic cell line displayed separated nuclei (0%), micromegakaryocytes (10%), small binucleated megakaryocytes (4%), and round, nonlobulated megakaryocytes (22%) according to the standard MDS criteria of dysmorphism[5]. Additionally, naked megakaryocyte nuclei made up 28% of all evaluated megakaryocytes, some of which displayed emperipolesis. Periodic acid-Schiff (PAS) was negative, and myeloperoxidase staining was not available.

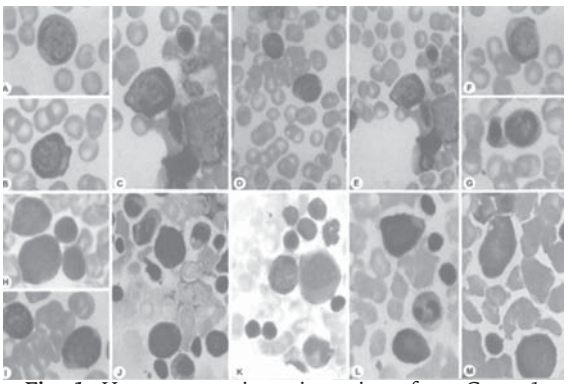

Fig. 1. Hematogones in various sizes, from Cases 1 and 2, under light microscope (x100).

Bone marrow flow cytometry revealed 36% HGs. There were three different HG populations with different sizes, as CD45 bright, dim and moderate (Fig. 2A). The analysis of these cells revealed positivity of CD34 (dim, 10%), CD10 (moderate, 81%), CD10+CD19 (73%), CD38 (bright, 98%), CD19 (moderate, 79%), CD22 (dim, 91%), CD20 (dim, 40%), sIgM (38%), cIgM (49%), and HLA-DR (89%), compatible with HGs. In the CD45 population, CD41 and additionally glycophorin A were negative. Aberrant or asynchronous antigen expression was not established (Fig. 2B-F). Bone marrow cytogenetic examination revealed 46 XX.

Case 2

A 4.5-month-old boy who was vaccinated (DTP, polio, Hib) 15 days before was admitted with generalized rash of two days' duration. His physical examination revealed an otherwise healthy baby with petechiae covering his body and a few ecchymoses on the extremities. Hb was 97 g/L, Htc 26%, plt 5 x 10^9/L, and WBC 8.8 X 10^9/L, with a differential count of 49% lymphocytes, 40% neutrophils, 9% monocytes, 3% eosinophils, and no thrombocytes. His biochemical profile was normal. Bone marrow aspiration smear revealed a cellular bone marrow with megakaryocytic hyperplasia. The differential count was 8% blasts (range: 5-15%) (Fig. 1), 5% myelocytes, 1% metamyelocytes, 7% neutrophils, 2% eosinophils, 7% band

forms, 60% lymphocytes (some immature), 3% monocytes, 1% proerythroblasts, 6% orthochromatic erythroblasts out of 500 cells, and a myeloid/erythroid ratio of 3.3. Immature megakaryocytes were increased. There were dysmorphic findings in the granulocytic, erythroid and megakaryocytic series. The granulocytic cell line displayed pseudo-Pelger-Huet abnormality (3%), macrocytosis (1%), chromatin clumping (12%), hypo- and agranulation (2%), and nuclear-cytoplasmic asynchrony (0%); the erythropoietic cell line displayed nuclear bridge (1%), nuclear lobulation (0%), multinuclearity (1%), cytoplasmic granules (1%), and megaloblastic changes (2%); the megakaryocytic cell line displayed separated nuclei (0%), micromegakaryocyte (4%), small binucleated megakaryocyte (4%), and round, nonlobulated megakaryocyte (12%) according to the standard MDS criteria of dysmorphism[5]. Additionally, naked megakaryocyte nuclei made up 62% of all evaluated megakaryocytes, some of which displayed emperipolesis. PAS was negative, and myeloperoxidase staining was not available.

Bone marrow flow cytometry revealed 43% HGs. There were three different HG populations (Fig. 3A). The analysis of these cells revealed positivity of CD10 (77%), CD19 (63%), CD10+CD19 (62%), CD20 (60%, dim), CD22 (21%,dim), CD34 (50%), CD38 (80%), CD45 (85%), and HLA-DR (71%), and negativity of kappa (4%), lambda (negative), sIgM (5%), Tdt, and additionally negativity of CD41 and glycophorin A in the CD45 population. CD34, CD10, CD19, CD22 (dim), CD38, and CD20 (dim) positivity was thought to be compatible with HGs. Aberrant or asynchronous antigen expression was not established (Fig. 3B-G).

Vitamin B_{12}, folic acid, ferritin, and immunoglobulin levels and abdominal ultrasonography were normal, and antinuclear antibodies (ANA), antiDNA, anticardiolipin antibodies, Coombs tests, and viral serologies were negative in both patients. Diagnosed as acute ITP, they were administered mega-dose methylprednisolone protocol[6]. For the first patient, the platelet count rose to 245 x 10^9/L on the seventh day of the therapy and was sustained over 150 x 10^9/L during her 1.5-year follow-up. For the second patient, the platelet count rose to 352 x 10^9/L on the seventh day of therapy and was sustained over 200 x 10^9/L during his 1.5-year follow-up.

Discussion

Hematogones (HGs) are found in highest numbers in marrow from infants and young children and decline significantly with increasing age. Patients younger than 16 years were reported to have increased HGs compared to patients 16 and older, although many adults with advanced age were also reported to have increased HGs[7]. Thus, while there are no "normal values" for HGs, 5% or more is generally considered as "increased".

Hematogones (HGs) show prominent variability in size, ranging from cells only slightly larger than normal lymphocytes to cells that approximate the size of a myeloblast[8]. Most HGs are small (10-12 μm) and characterized by scant cytoplasm and dense nuclei with smooth homogeneous chromatin and round or notched contours and with generally absent nucleoli, which sometimes are small and indistinct and can be distinguished from lymphocytes easily[9]. While intermediate forms carry the features of both HGs and mature lymphocytes without nucleoli, larger HGs (17-20 μm) have a slightly more reticulated chromatin pattern with small, inconspicuous nucleoli, and a thin rim of homogeneous blue cytoplasm without granules, inclusions or vacuoles[9], and large HGs resemble some leukemic lymphoblasts, which show high nuclear-cytoplasmic (N/C) ratios, fine stippled chromatin, uniform size, and small nucleoli and/or more condensed chromatin without nucleoli[3]. In our patients, the cells that could not be distinguished from blasts were large HGs. The blast types to be distinguished in the clinical and other laboratory settings of both patients were, first of all, type 1 blasts of MDS according to the old nomenclature, which lack cytoplasmic granules and have basophilic cytoplasm, uncondensed fine nuclear chromatin, often with one or two nucleoli[10], and lymphoblasts in a preleukemic condition. Therefore, differentiation between blasts and HGs is of utmost importance to reach an accurate diagnosis. In these two children, we distinguished blasts from HGs based on their flow cytometric and cytochemical characteristics.

The maturation of B-lineage precursors can be

conveniently divided into three stages based upon the predictable immunophenotype that also characterizes their evolution into mature B cells. Stage 1 of maturation is defined by CD34 positivity, and Stage 3 of maturation is defined by bright CD20 expression. Stage 2 characterizes all HGs that are CD34 (-) and CD 20 dim/(+)[7]. The majority of HGs observed are in the intermediate stage (stage 2), while the rest are usually nearly equally divided between stages 1 and 3. CD19, CD22, CD38, and CD10 are expressed in all three stages[7].

Asynchronous expression of the earliest and latest antigens, e.g. concurrent CD34 and CD20, and aberrant over- or under-expression of antigens like negative or under-expression of CD45, CD20, HLA-DR and CD38, overexpression of CD10, and an abnormal spectrum of expression of CD22 are not observed in HG populations[7]. ALL samples consistently express a more immature, but homogeneous immunophenotype, with the majority of cases expressing TdT, CD34, or both[3]. Asynchronous and aberrant expression is not observed in HG populations. Appropriately applied 3- and 4-color multiparametric flow cytometry has distinguished HGs from leukemic lymphoblasts that have asynchronous and/or multiple aberrant expressions of antigens[7,9]. Thus, in our patients, the HG populations exhibited a typical complex spectrum of antigens and lack of asynchronous and aberrant expressions that define the normal evolution of B-lineage precursors, from stage 1 to stage 3 HGs (Figs. 2, 3).

Another characteristic of HGs in flow cytometry is their position on the dot plot template. Especially in pre B ALL, the aberrant immunophenotype is often found at a separate place on the dot plot template, which is a so-called 'empty space' where no healthy cells are normally found. HGs are found in the "small lymphocyte region", and have lower average forward light scatter than mature B cells[11].

Another characteristic of HGs is that they lack bone marrow clustering compared to ALL cells. Expression of adhesion antigens like CD44 and CD54 heterogeneously on HGs and homogeneously on lymphoblasts may explain the propensity of blasts to form clusters[3] (Fig. 1). In our patients, no definite clustering of HGs on the bone marrow smears was noted.

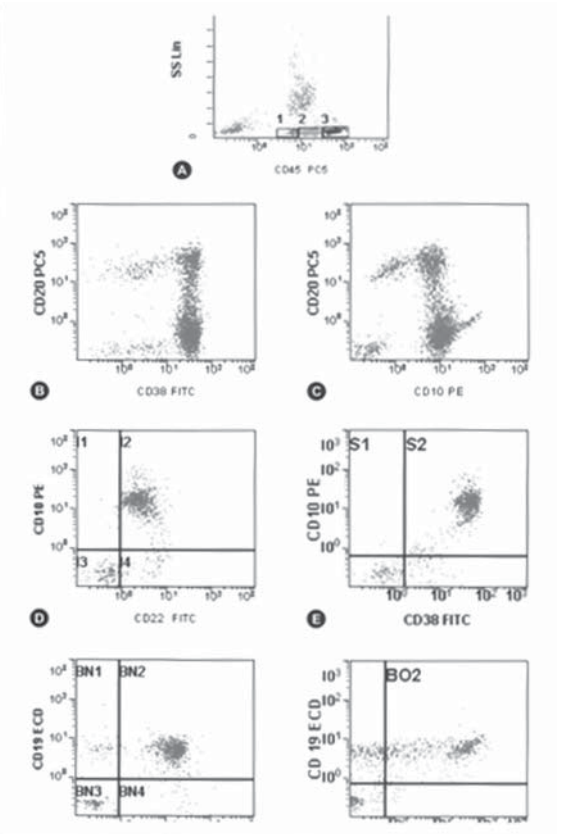

Fig. 2. Bone marrow flow cytometric diagrams of Case 1. A. Hematogones with very low side scatter of CD45 and three different populations of hematogones, which progressively increase in intensity and size.
B-F. The histograms illustrating the normal pattern of maturation of B-lymphocyte precursors (hematogones) with the 5-color antibody combination of CD34, CD10, CD20, CD19, and CD38. Hematogones in all maturation stages from stage 1 to 3 are best seen in diagrams b and c, yielding an inverse C.

PAS negativity might be also considered to support our hypothesis that the blast-like cells were actually HGs, although there can be PAS-negative lymphoblasts[8].

Our first patient had unilineage cytopenia, dysmorphic findings in more than 10% of each of three cell lineages and blast percentage between 5-19% (11%). The second patient had bilineage cytopenia, dysmorphic findings in more than 10% of each of three cell lineages and blast percentage between 5-19% (8%). Presence of cytopenias, increased dysplastic changes[12], megakaryocyte hyperplasia, megakaryocyte emperipolesis, micromegakaryocytes and naked megakaryocyte nuclei, and lack of cytogenetic abnormality (in 60% of MDS) were some of the similarities shared by both ITP and MDS[13],

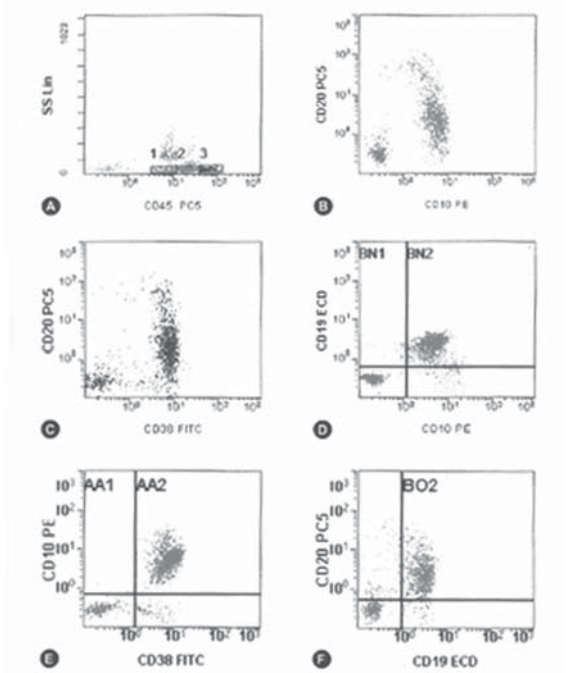

Fig. 3. Bone marrow flow cytometric diagrams of Case 2.

A. Hematogones with very low side scatter of CD45 and three different populations of hematogones, which progressively increase in intensity and size.

B-G. The histograms illustrating the normal pattern of maturation of B-lymphocyte precursors (hematogones) with the 5-color antibody combination of CD34, CD10, CD20, CD19, and CD38. Hematogones in all maturation stages from stage 1 to 3 are best seen in diagrams b and c, yielding an inverse C.

although the specific criteria of World Health Organization (WHO)-MDS classification, which defines the presence of dysplasia in more than 10% of two or more cell lines, has not been utilized for childhood MDS classification[5].

Thus, both of the patients at first insight could easily be diagnosed as 'MDS with excess blasts' or a preleukemic era of leukemia, instead of ITP, since increased blast cell count is not expected in ITP. After determining that the cells that were considered as blasts were actually large HGs, and that they were not micromegakaryocytes[13], we diagnosed the patients as ITP with excess HGs, although the excessive number of naked megakaryocyte nuclei in Case 2 may also have been attributed to MDS itself[12]. A silent viral infection for both patients and/or the DTP, polio and Hib vaccinations in the second patient could have triggered the thrombocytopenia and HG increment.

In summary, it should be kept in mind that HGs might increase in several conditions in childhood (e.g. ITP, MDS, lymphoma, red blood cell aplasia, iron deficiency anemia, and regenerative bone marrow following viral injury, chemotherapy or bone marrow transplantation), and they should be distinguished from blasts before making the diagnosis of MDS or leukemia.

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