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PERIPHERAL BLOOD PICTURE IN PRIMARY HYPOCELLULAR REFRACTORY ANEMIA AND IDIOPATHIC ACQUIRED APLASTIC ANEMIA: AN ADDITIONAL TOOL FOR DIFFERENTIAL DIAGNOSIS

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ABSTRACT

Background and Objective. Hypoplastic myelodysplastic syndromes (MDS) are being reported with increasing frequency. Aplastic anemia (AA) needs to be differentiated from hypoplastic MDS particularly primary hypoplastic refractory anemia (PHRA) because of the impact on management and prognosis. This distinction may be morphologically difficult even with careful marrow examination which may provide insufficient material due to extreme hypocellularity. The value of peripheral blood (PB) parameters in making the distinction between AA and PHRA is not well studied. In this work, we attempt to examine peripheral blood findings as an additional tool for differentiating PHRA from acquired idiopathic AA.

Methods. PB findings in ten cases of PHRA, which are selected based on the following: less than 30% cellularity, multilineage dysplasia and/or clonal cytogenetic abnormality, are compared to ten cases of classic AA. The PB is examined for automated parameters, differential white cell count, morphologic changes in red cells, white cells, platelets, and the presence of circulating blasts, megakaryocytic fragments and micromegakaryocytes.

Results. AA patients tend to have lower platelet and monocytic counts and higher lymphocytic percentages. The following morphologic findings are seen only in PHRA but not in AA: hypochromic red cells, left shift, circulating blasts, hypersegmentation with long filaments, hypogranular, ring, and pelgeroid neutrophils, Dohle bodies, circulating micromegakaryocytes and megakaryocytic fragments.

Interpretation and Conclusions. We conclude that careful examination of peripheral blood may provide sufficient information to allow for the distinction between PHRA and AA early in the course of the disease. Similarly, patients with classic AA who subsequently develop unusual blood findings during routine follow up should be suspected of having a clonal evolution which needs to be confirmed by marrow examination and cytogenetic analysis.

Key words: myelodysplastic syndromes, aplastic anemia, peripheral blood, refractory anemia

The myelodysplastic syndromes (MDS) are a group of clonal stem cell disorders characterized by cytopenias, ineffective hematopoiesis and increased risk for developing acute leukemia.[1,2] The French-American-British (FAB) Cooperative Group proposed criteria for the diagnosis and classification of MDS based on the morphology of peripheral blood and bone marrow.[3] However, despite these criteria, some cases of MDS, particularly refractory anemia, can be difficult to diagnose partly due to subtle morphologic changes and partly due to overlapping morphologic features with other causes of anemia such as megaloblastic anemia, anemia of chronic disease and anemia of chronic renal failure.[4] Moreover, approximately 5% of MDS patients have no anemia at presentation but only neutropenia and/or thrombocytopenia.[5] An interesting feature in MDS is the variability in marrow cellularity. The majority of MDS cases have normo- or hypercellular marrow. A variable percentage of MDS patients particularly those with secondary MDS have hypocellular marrow.[1,5] The incidence of hypocellular MDS is reported to be between 8% and 29%.[6-12] However, a recent study of Brazilian patients reports a much higher incidence of hypocellular MDS of 46%.[13] The difference in the reported incidence between these studies may be related to case selection, marked topographic variation of hematopoietic cells in the marrow in MDS or the definition of hypocellularity. Most studies define hypocellularity as <30%.[8,9,11,12] Others used <20% for patients above 60 years of age[7,10] or age-matched normal cellularity.[11] Hypocellular MDS, in particular, can represent diagnostic difficulty since these cases can be confused with aplastic anemia (AA).

The differentiation between hypocellular MDS and AA is of great therapeutic and prognostic importance. With modern therapy such as bone marrow transplantation and immunosuppressive therapy such as antilymphocyte/antithymocyte

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globulins, patients with severe AA have long term survival of approximately 80% at 14 years.[14] Similarly, patients with moderate AA have median survival exceeding 174 months with androgen and supportive therapy.[8] On the other hand, several studies suggest that the median survival for patients with hypocellular MDS is not significantly different from hypercellular MDS and varies from 22 to 33 months.[7,9,10,11,13] Some cases of hypocellular MDS may show transient response to androgens and/or immunosuppressive therapy thus adding further diagnostic difficulty.[8,15,16] Marrow findings such as the presence of bi- or trilineage dysplasia, increased bone marrow blasts, micromegakaryocytes, high numbers of megakaryocytes, slight marrow fibrosis and irregular distribution of megakaryocytes in the marrow biopsy are features favoring hypocellular MDS rather than AA.[17] Similarly, the detection of a clonal cytogenetic abnormality early in the course of the disease is an indication of MDS. However, the problem is complicated by the fact that the majority of hypocellular MDS cases have no increase in marrow blasts and are, therefore, classified as refractory anemia.[9-13] Some cases of hypocellular MDS may have dysplastic features confined to erythroid cell line which can be observed in AA as well.[6,7,13] In addition, 50%-100% of patients with hypocellular MDS have normal karyotypes.[6,9,10,11] Acquired idiopathic AA and primary hypocellular refractory anemia (PHRA) may have no relevant past clinical history and both often present with symptoms related to cytopenia(s). Differentiating these two entities is rather difficult and challenging. Most earlier studies have focused on bone marrow criteria. In this study, we attempt to examine peripheral blood findings as an additional tool for differentiating PHRA from acquired idiopathic AA.

Materials and Methods

The files of the Department of Pathology between 1990-1995 were reviewed for confirmed cases of MDS. This time period was selected because of easy retrieval of peripheral blood (PB) smears. Sixty one cases of MDS were found. Sixteen cases (26%) of hypocellular MDS were identified (cellularity <30%). The diagnosis of PHRA was confirmed in ten cases (16%). These cases had morphologic and/or clonal cytogenetic abnormalities. The morphologic findings in the bone marrow aspirate and biopsy included some or all of the following: multilineage dysplasia, abnormal localization of immature precursor and increased marrow reticulin fibers.[12,17] These cases of PHRA were compared with ten cases of classic acquired idiopathic AA. The clinical charts of these patients were reviewed. The complete blood counts and peripheral blood (PB) smear at diagnosis and prior to therapy were obtained. Automated differential count was accepted after scanning the PB smear. When no five-part automated differential count was recorded, a manual 200 cell count by two observers was performed (400 cell total) as previously recommended.[18] The following parameters were compared: Red cell count, white cell count (WBC), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, red cell distribution width , platelet count, and mean platelet volume. The percentage and absolute counts of the following white cells were examined: neutrophils, eosinophils, basophils, lymphocytes, and monocytes. Peripheral blood smears were reviewed for the following parameters:

1) red cells: for the presence of oval macrocytes, elliptocytes, dacrocytes, hypochromic cells, basophilic stippling in 10 high power fields (1000×), and nucleated red blood cells;

2) white cells: for the presence of Dohle bodies, hypogranular, pelgeroid or ring neutrophils, left shift (>15% band neutrophils or any metamyelocyte or myelocyte), hypersegmentation, hypersegmentation with long filaments , similar to those described in myelokathexis,[19,20] and circulating blasts (<1%);

3) platelets: for the presence of giant platelets (at least the size of a lymphocyte nucleus), micromegakaryocytes and megakaryocytic fragments.

Statistical difference of numerical values of the two groups was analyzed using the Student's t-test.

Results

Patient population

The ten patients with PHRA included 7 males and 3 females with ages 23-81 years (median 66 years). The ten patients with AA included 7 males and 3 females with ages 17-80 years (median 60 years). Only one patient in the PHRA group had a chromosomal abnormality which involved deletion of the long arm of chromosome 13 (del (13)(q13q31)). All patients had normal or increased iron stores. Serum vitamin B_{12} and folate were assayed in 12 patients at diagnosis: all MDS patients and two AA patients; all had normal results.

Automated blood count parameters

The results are shown in Table 1. The total WBC count was significantly lower with AA (p=0.008). There was a trend for the platelet count to be lower in AA although it did not reach statistical significance (p=0.057). Similarly, a less significant trend was seen with the MCV which tended to be lower with AA (p=0.08).

Table 1. Comparison of automated blood parameters between primary hypoplastic refractory anemia (PHRA) and acquired idiopathic aplastic anemia (AA).

	PHRA (mean±SD)	*AA (mean±SD)*	*P value*
WBC (10^3/uL)	5.1±2.1	2.9±0.9	0.008
RBC (10^6/uL)	2.7±0.7	2.8±0.6	NS
Hgb (gm/dL)	9.2±2.1	8.9±1.1	NS
Hct (%)	27.5±6.3	26.2±4.9	NS
MCV (fl)	104.3±8.9	95.7±11.3	NS*
MCH (pg)	34.9±3.5	32.7±3.9	NS
MCHC (%)	33.4±1.3	33.2±2.7	NS
RDW	18.5±3.5	18.5±4.6	NS
Platelets (10^3/uL)	137±118	48±60	NS*
MPV	8.9±1.4	8.4±2	NS

*Abbreviations: WBC: white cell count, RBC: red cell count, Hgb: hemoglobin, Hct: hematocrit, MCV: mean corpuscuLar volume, MCH: mean corpuscuLar hemoglobin, MCHC: mean corpuscuLar hemoglobin concentration, RDW: red cell distribution width, MPV: mean platelt volume. NS: not significant; *See text for details.*

Differential white cell count

The results are shown in Table 2. Both the percentage and absolute counts of neutrophils were lower with AA (p=0.005 for both values). The percentage of lymphocytes was much higher in AA (p=0.008). However, the absolute lymphocytic count was not different between the two groups. Monocytopenia was detected in 3 patients with PHRA and 5 patients with AA. There was a trend for a lower absolute monocytic count in AA (p=0.053). No case with eosinophilia is seen except one case of PHRA with 19% eosinophils and an absolute eosinophilic count of 1.7×10^3/uL. All the other cases in both groups had eosinophils ranging from 0-4%. Basophils ranged from 0-2% with no case of basophilia identified.

Morphology of PB smears

The results are summarized in Table 3. White cell morphology seems to provide a reliable tool for discrimination. The presence of left shift in the absence of infection was seen only in PHRA. Hypersegmentation of neutrophils with long filaments was detected only in PHRA. Although seen in six cases, these neutrophils represented a small proportion of neutrophils in positive cases (<5%). Similarly, pelgeroid, hypogranular, and ring neutrophils, Dohle bodies and circulating blasts, though present in a small number of cases, seem to be indicative of PHRA. Hypochromic cells were only seen in 20% of PHRA while absent in all cases of AA. The presence of giant platelets was not specific. However, the identification of circulating micromegakaryocytes or megakaryocytic fragments proved to be of discriminatory significance, occuring only in PHRA.

Table 2. Comparison of percentage and absolute differential white cell count between primary hypoplastic refractory anemia (PHRA) and acquired idiopathic aplastic anemia (AA).

	PHRA (mean±SD)	*AA (mean±SD)*	*P value*
Neutrophils (%)	55±14	34±20	0.005
Neutrophils (10^3/uL)	2.8±1.5	1.1±0.8	0.005
Monocytes (%)	6±2	6±3	NS
Monocytes (10^3/uL)	0.3±0.17	0.17±0.1	NS*
Lymphocytes (%)	35±15	60±20	0.008
Lymphocytes (10^3/uL)	1.7±0.8	1.6±0.6	NS

*Abbreviations: NS: not significant. *see text for details*

Table 3. Peripheral blood morphologic findings which are helpful in differentiating primary hypoplastic refractory anemia (PHRA) from acquired idiopathic aplastic anemia (AA).

	PHRA (n=10)	*AA (n=10)*
Left shift	6*	0
Hypersegmentation with long filaments	6	0
Hypogranular neurophils	3	0
Pelgeroid neutrophils	2	0
Ring neutrophils	2	0
Circulating blasts	2	0
Hypochromic cells	2	0
Micromegakaryocytes/Megakaryocytic fragments	2	0
Dohle bodies	1	0

**number of patients positive for the examined parameter.*

Discussion

A fair percentage of MDS patients, as high as 46% in some studies, can present with low marrow cellularity.[6-13] Differentiating acquired AA from primary hypocellular refractory anemia (PHRA) at presentation can be problematic both clinically and pathologically. This differentiation is of considerable significance because of its therapeutic and prognostic impact. In this study, we found that careful PB examination may provide valuable information in this regard.

AA patients had lower total WBC count, percentage and absolute neutrophilic count and higher lymphocytic percentage. In addition, in AA there was a trend for lower absolute monocytic and platelet counts. Monocytopenia is known to occur in AA[21] but not well studied in MDS. In this study, monocytopenia was observed in 30% of PHRA and 50% of AA. Overall, in all our patients with MDS on file of subtypes other than chronic myelomonocytic leukemia, monocytopenia was present in 22 of 45 (45%). None of these patients with monocytopenia had refractory anemia with ring sideroblasts. In the majority of these patients monocytopenia was associated with granulocytopenia. However, the overall degree of monocytopenia was less severe than with AA.

Red cell morphology was not very helpful. The only finding indicative of PHRA was the presence of hypochromic red cells identified in 20% of cases. All AA and PHRA patients had adequate iron stores in their bone marrow. These hypochromic cells may be related to the abnormal iron metabolism and ring sideroblasts commonly associated with MDS.[22]

Left shift was not seen in any of our AA cases while was present in 60% of PHRA. Similarly, only PHRA had circulating blasts in 20% of cases. A previous study found no circulating blasts in patients with AA.[10] Qualitative abnormalities in granulocytes such as Dohle bodies, pelgeroid features, hypogranularity and ring neutrophils are seen only in PHRA although in a small proportion (10-30%). Hypersegmentation is seen in megaloblastic anemia, uremia and some cases of neutrophilia.[23] Hypersegmented neutrophils was a common finding in PHRA (60%) and to a lesser degree in AA (30%). All our patients with MDS had normal levels of vitamin B_{12} and folate. However, most patients with AA had no record of such measurements soon

after diagnosis. Therefore, in AA, early vitamin B_{12} and/or folate deficiency could not be excluded as an underlying mechanism for hypersegmentation. Hypersegmentation has been previously described as a manifestation of dysgranulopoiesis.[3,24] Hypersegmentation with long filaments as a predominant feature of myelodysplasia (myelokathexis) has been rarely reported.[19,20] Myelokathexis is an abnormality in neutrophilic maturation associated with granulocytopenia. It has been originally described in a small number of young patients with increased incidence of infection.[19] In our experience, neutrophils with similar morphology may be detected in patients with overt megaloblastic anemia. The present study shows that in the majority of MDS cases such cells can be seen in the peripheral blood if searched for, since they were present in a small proportion of neutrophils in positive cases. These cells were not found in AA. The presence of myelokathexis-like neutrophils may indicate an acquired defect in nuclear maturation. Morphological abnormalities are typically associated with functional alterations of neutrophils in MDS patients.[25]

Giant platelets have been reported with MDS particularly those presenting with isolated thrombocytopenia.[26] In this study, giant platelets were seen in 60% of PHRA. However, giant platelets were seen as well in 30% of our patients with AA. On the other hand, circulating micromegakaryocytes and megakaryocytic fragments were detected in 20% of our PHRA patients but not with AA. Circulating micromegakaryocytes were observed in 18% of patients with MDS presenting with isolated thrombocytopenia.[26]

We conclude that careful examination of peripheral blood may provide sufficient information to allow for the distinction between PHRA and AA early in the course of the disease. Similarly, patients with classic AA who subsequently develop unusual blood findings during routine follow-up should be suspected of having a clonal evolution which needs to be confirmed by marrow examination and cytogenetic analysis.[27]

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