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Disorders Mimicking Myelodysplastic Syndrome and Difficulties in its Diagnosis

Lale Olcay and Sevgi Yetgin

Additional information is available at the end of the chapter

Abstract

Myelodysplastic morphology of blood cells can be encountered not only in myelodysplastic syndrome (MDS) but also in nonclonal disorders like viral, bacterial, parasitic infections, juvenile rheumatoid arthritis, polyarteritis nodosa, immune thrombocytopenic purpura (ITP), iron deficiency anemia, megaloblastic anemia, dysgranulopoietic neutropenia, congenital neutropenia, cases with microdeletion 22q11.2, malignant lymphoma, after administration of granulocyte colony stimulating factor, chemotherapy, steroids, smoking, alcohol, posttransplantation, copper deficiency also, together with or without cytopenia. Absence of cytogenetic abnormality in 50–70% of cases with MDS, some overlapping morphological and/or pathophysiological features make it challenging to differentiate between MDS and other diseases/disorders like aplastic anemia, refractory ITP, copper deficiency. Transient genetic abnormalities including monosomy 7 in megaloblastic anemia; increased immature myeloid cells in bone marrow of cases with copper, vitamin B12, or folic acid deficiency in the setting of cytopenia and dysmorphism may also lead to the misdiagnosis of MDS. On the other hand, there are also cases of transient MDS. In this chapter, a literature is be presented to draw attention of the readers on the disorders that mimic MDS. Additionally, our personal experiences are also be shared. Awareness of disorders mimicking MDS may prevent over- or underdiagnosis of MDS.

Keywords: secondary myelodysplasia, cell death, cell cycle, transient MDS, apoptosis, rapid cell senescence

1. Introduction

Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by ineffective hematopoiesis in bone marrow and cytopenias in peripheral blood. It is heterogeneous reflected by a number of subgroups with different characteristics both in adulthood and childhood [1–11].

A single diagnostic parameter specific to MDS has not been discovered yet, and a considerable number of patients with MDS lack chromosomal abnormality [1, 4]. Currently, the diagnosis of MDS is mainly dependent on quantitative and qualitative dysplastic abnormalities [5]. Establishment of special characteristics of dysplasia like the number of dysplastic cell lines, the percentage of the dysplastic cells, and characteristic megakaryocytes as in del(5q) syndrome are critical in order to be able to assess which subgroup of MDS the patient fits according to the World Health Organization (WHO) classification. Because uni- or multilineage dysplasia may be the only criterion that differentiates the subgroups refractory cytopenia with unilineage dysplasia (RCUD) and refractory cytopenia with multilineage dysplasia (RCMD) [1, 5, 6, 10, 11]. Additionally, for the lineage to be considered as dysplastic, at least 10% of the lineage should display displastic findings [1, 2, 5, 6, 8, 10, 11] both in adulthood and childhood MDS.

	Dyserythropoiesis	Dysgranulopoiesis	Dysmegakaryopoiesis
Peripheric blood	-Anisocytosis	-Nuclear hypolobulation	-Platelet anisocytosis
	-Poikilocytosis	(pseudo-Pelger-Huet cells)	-Giant platelets
	-Basophilic stippling	-Cytoplasmic hypo/agranulation	
		-Blasts	
Bone marrow	-Nuclear budding	-Anisocytosis	-Micromegakaryocytes
	Irregular nuclear edges	-Nuclear hypolobulation	-Nuclear hypolobulation
	Internuclear bridging	(pseudo-Pelger-Huet cells)	-Large monolobular forms
	-Karyorrhexis	-Nuclear hypersegmentation	-Small binucleated elements
	-Multinuclearity	-Irregular hypersegmentation	-Dispersed nuclei
	-Nuclear hyperlobulation	-Bizarre nuclear shapes	-Degranulation
	-Binuclearity	-Decreased granules	
	-Megaloblastic changes	-Agranularity	
	-Ring sideroblasts	-Pseudo Chediak Higashi granules	
	-Vacuolization	-Auer rods	
	-Periodic acid-Schiff positivity		
	-Cytoplasmic inclusions		
	Incomplete		
	-Hemoglobinization		
	-Fringed cytoplasm		
	-Cytoplasmic bridging		

Table 1. Recent definitions of morphological features of myelodysplasia (adulthood) [2, 5].

Nearly half of which is constituted by refractory cytopenia of childhood (RCC) [4]. Minimal diagnostic criteria for childhood MDS require fulfillment of at least two of the following criteria: sustained, unexplained cytopenia (neutropenia, thrombocytopenia, or anemia); at least bilineage morphologic myelodysplasia; acquired clonal cytogenetic abnormality in hematopietic cells; increased blast count (>5%) [3].

	Dyserythropoiesis	Dysgranulopoiesis	Dysmegakaryopoiesis
Abnormality	-Megaloblastic changes[1] -Lobulated nuclei in erythroblasts (kidney-shaped, bilobulated, multilobulated, bizarre irregular nuclear profile) -Multinuclearity (two or more distinctly separated nuclei of the same or of different sizes) -Cytoplasmic granules or inclusions[2]	-Bizarre nuclear shape[3] -A- or hypogranularity[4] -Nuclear/cytoplasmic (N/C) asyncrony[5] -Pseudo-Pelger anomaly[6]	-Micromegakaryocytes[7] -Small binucleated megakaryocyte[8] -Megakaryocyte with small round separated nuclei[9] -Megakaryocytes with nonlobated round nucleus[10]

[1]Megaloblastic changes: At least 1.5 times the size of a normal poly- or orthochromatic erythroblast with coarse condensation of chromatin and an increased nuclear-to-cytoplasmic ratio or orthochromatic erythroblasts with decreased nuclear-to-cytoplasmic ratio and at least double the size of a normal erythrocyte of the same maturational state.

[2]Cytoplasmic granules or inclusions: Presence of granules or nuclear fragments that can be definitely differentiated from ribosomal RNA.

[3]Bizarre nuclear shape: Abnormal nuclear shape, including irregularly lobulated nuclei of segmented granocytes with chromatin clumping or large twisted bands, large bands or metamyelocytes, multinuclearity (two distinctly separated neutrophilic bands or segmented nuclei).

[4]A- or hypogranularity: Neutrophilic or azurophilic granules should be markedly or completely absent and the cytoplasm of mature neutrophilic granulocytes has to stain pale blue/gray or translucent in the Romanowsky-Giemza stain. All maturation stages except blast cells should be affected.

[5]Nuclear/cytoplasmic (N/C) asyncrony: Mature neutrophilic granulocytes and metamyelocytes with basophilic cytoplasm and myelocytes with neutrophilic cytoplasm.

[6]Pseudo-Pelger anomaly: Mature granulocytes with either a centrally located round to ovoid nucleus (monolobated type) or two round nuclei of similar size connected by a slender chromatin bridge (bilobated type).

[7]Micromegakaryocytes: Mononucleated megakaryocyte with a size comparable to that of a promyelocyte or less, lacking features of a blast cell.

[8]Small binucleated megakaryocyte: Small megakaryocyte with the size of a micromegakaryocyte or slightly larger, with two round well-separated nuclei.

[9]Megakaryocyte with small round separated nuclei: Megakaryocytes of any size with multiple, at least three, round separated nuclei.

[10]Megakaryocytes with nonlobated round nucleus: Megakaryocytes of normal or reduced size with a nonlobated round nucleus and a mature granular cytoplasm.

Table 2. Morphological features of myelodysplasia (childhood-EWOG-MDS Group, 2005) [13].

The unilineage dysplasia in RCUD of adult MDS should have lasted for at least 6 months if no clonal cytogenetic abnormality is found and/or ring sideroblasts are less than 15% [12], so for these patients a repeated bone marrow examination is recommended after a 6 months' observation [5].

The dysplastic changes have been standardized in childhood [13, 14] and adulthood MDS [2, 5, 15, 16] (**Tables 1–4**) being restricted to three lineages as erythroid, granulocytic, and megakaryocytic lineages. Although monocytes are rarely affected in MDS, their presence rather is associated with CMML and AML [5].

	Dyserythropoiesis	Dysgranulopoiesis	Dysmegakaryopoiesis
Abnormality	-Abnormal nuclear lobulation -Multinuclear cells -Nuclear bridges	-Pelger-Huet cells -Hypo- or agranularity -Giant bands (in severe neutropenia, this criteria may not be complied)	-Megakaryocytes with variable size and separated nuclei or round nuclei (absence of megakaryocytes does not rule out RCC)

Table 3. Morphological features of myelodysplasia in refractory cytopenia of childhood (RCC) (WHO, 2008) [14].

Dyserythropoiesis	Dysgranulopoiesis	Dysmegakaryopoiesis
-Polychromasia	Neutrophilic lineage	-Megakaryocyte fragments
-Cleaves in nuclear membrane	-Macropolycyte (PMNL >14 µm)	-Giant granules in thrombocyte
-Pyknosis	-Ring nucleus	-Bothryoid nucleus in megakaryocytes
-Gigantism	-Increase in nuclear chromatin clumping	-Hypogranulation in megakaryocytes
-Punctate basophilia	-Nuclear sticks	
	-Vacuolated cytoplasm	
	-Increased granules/giant granules	
	-Hypergranular promyelocytes	
	-Increased apoptotic forms	
	Eosinophilic lineage	
	-Ring nuclei	
	-Charcot-Leyden crystals in nucleus	
	-Basophilic granules	

Table 4. Other parameters for myelodysplasia that were suggested previously but had not been included in the recent guidelines [15, 16].

On the other hand, myelodysplastic findings in blood cells arise due to any challenge during the course of normal differentiation and therefore these changes can be encountered not only in clonal disorders like MDS (primary myelodysplasia), but also in various nonclonal disorders affecting bone marrow like viral, bacterial, parasitic infections [17–27], autoimmune disorders (juvenile rheumatoid arthritis, polyarteritis nodosa, systemic lupus erythematosis, immune thrombocytopenic purpura) [17, 28–32], hemophagocytic histiocytosis (HLH), nutritional problems (malnutrition, iron deficiency anemia, megaloblastic anemia, copper deficiency, vitamin D deficiency, hyper vitaminosis A) [27, 33–43], neutropenia (congenital dysgranulo-poietic, congenital severe, idiopathic) [32, 44, 45], inherited disorders [27, 46, 47], malign lymphoma [48], due to effects of drugs and toxins [17, 27, 49–54], during posttransplantation period [27, 55], and other reasons [27] also and called "secondary myelodysplasia" [27]. These

findings are neutrophils with nuclear shape, hypoagranulation, abnormal nuclei, cytoplasm and granulation, anisocytosis, poikilocytosis, microspherocytes, giant thrombocytes, lymphocytes with cytoplasmic protrusions and vacuoles, monocytes with dysmorphic nuclei, cytoplasmic vacuoles and cytoplasmic protrusions, chromatin clumping, nucleocytoplasmic asynchrony, interchromatin bridges between erythroid precursors, oligonuclear megakaryocytes, naked megakaryocyte nuclei and cytoplasm, most of which have been included in the dysplasia criteria in MDS of childhood [13, 14], and adulthood [2, 15, 16].

While MDS is potentially preleukemic, disorders with secondary myelodysplasia are not neoplastic or preleukemic and are reversible when the underlying factor is removed [27].

Such cases with additional cytopenia in one or more cell lines, due to transient suppression of hematopoiesis may erroneously lead the physician to the diagnosis of MDS, especially when no cytogenetic abnormality can be attained. Additionally, assessment of morphological abnormalities in MDS is still not completely objective [56], in spite of that a number of dysmorphic findings were simplified, categorized [2, 13–16] and cut-off values were established [5]. This situation is valid especially for low-risk MDS cases without excess blasts and any detectable cytogenetic abnormalities. Additionally, necessity to wait without definite diagnosis and therefore therapy for at least 6 months in cytopenia cases with unilineage dysplasia [5, 12] is distressing for the patient and the family.

On the other hand, it is also challenging to differentiate cases which present as ordinary aplastic anemia, refractory immune thrombocytopenic purpura (ITP) [31], chronic neutropenia [44, 45] from MDS [4, 9, 14, 57–60]. Transient MDS or MDS-like disorders with or without [61–70] chromosomal abnormalities, acute myeloblastic leukemia (AML) cases with low blast cell count [71] should also be considered for accurate diagnosis. Additionally, it should not be forgotten that autoimmune disorders may also be a component of MDS itself [72–74] and there may be cases complying the criteria of other MDS subtypes like idiopathic cytopenia of undetermined significance (ICUS), idiopathic dysplasia of undetermined significance (IDUS) [5, 7, 58].

This chapter reviews on these diagnostic problems, in the following order:

• Nonclonal disorders which present as dysplasia and cytopenia

• Cases with hypoplastic bone marrow mimicking hypocellular MDS

• Transient chromosome abnormalities in the setting of cytopenia/spontaneous remission in MDS

• Mutations in the elderly and other cases

• Acute myeloblastic leukemia

• ICUS-IDUS

• Autoimmune disorders

• Common features in pathogenesis

• Differential diagnosis

• Conclusion and future recommendations

2. Nonclonal disorders which present as dysplasia and cytopenia

2.1. Viral and bacterial infections

In a pilot and unpublished study that we carried on in our clinic, we compared the dysmorphic parameters in the neutrophils of patients with viral (n:6; infections: rubella, rubeola, viral eruption of unknown origin, Ebstain Barr virus infection), bacterial (n:7; infections: preseptal cellulitis, urinary infection, tonsillitis, maxillary sinusitis, lymphadenitis, otitis media) infections and those of healthy controls. We found that the neutrophil diameter of those with bacterial infections; the percentage of pseudo Pelger-Huet cells and irregular distribution of granules in both viral and bacterial infections; the percentage of chromatin clumping in viral diseases were higher than the control. These findings showed that nonspecific infections can also give rise to dysmorphic findings in neutrophils.

In another study, we reported that those with bacterial diseases additionally displayed comparable diameter, macropolycyte (neutrophils with diameter >14 μm) percentage, bizarre nucleus, irregular distribution of granules with those of pretreatment ITP who also displayed myelodysplasia [17].

Striking dyserytropoiesis was reported in tuberculosis [27]. Several viral infections which closely mimick MDS will be delineated below.

2.1.1. Parvovirus infection

In the literature, there are cases of parvovirus infection, with [19–21] or without [22] immunodeficiency or chronic hemolytic anemia which transiently or chronically mimicked MDS or dyserythropoietic anemia [22]. Among them the two [19, 20] are of note.

The reported case of Hasle et al. [19] was an 8-year-old, previously healthy boy who admitted to the hospital with severe anemia, moderate thrombocytopenia, and granulocytopenia and a 2 weeks' history of intermittent fever. Physical examination was normal except for pallor. Bone marrow was hypercellular with marked erythroblastopenia and maturation arrest of the erythropoietic cell line. No giant pronormoblast and hemophagocytosis was noted. Dysplasia in myeloid and megakaryocytic lineage was evident. He had increased immunoglobulin (Ig) M, low IgG, slightly decreased natural killer (NK) cells which reduced during follow-up; impaired in vitro proliferation of blood mononuclear cells on stimulation.

The patient was assumed as MDS and was administered prednisolon, androgenic steroid, cyclophosphamid and cyclosporine, IgG infusion and frequent blood transfusions and developed hemochromatosis and hepatosplenomegaly. Thrombocytopenia deepened; hemoglobin transiently normalized. Parvovirus antibody studies revealed negative but when polymerase chain reaction technique became available, serum samples of the previous two years of the disease course and bone marrow smears were found positive for parvovirus infection.

The reported case of Baurmann et al. [20] was a 36-year-old, previously healthy woman who admitted to the hospital with fever, pancytopenia, and atypical lymphoid cells with dysplastic hematopoietic changes. She had frank splenomegaly, slightly increased bilirubin, lactate dehydrogenase (LDH), negative Coombs test. Since the bone marrow was hypocellular with multiple abnormal megakaryocytes, absence of erythropoiesis and 15% blasts carrying monocytic and histiocytic characteristics, she was diagnosed as MDS-refractory anemia with excess blasts (RAEB). A second bone marrow aspiration performed 6 days after admission revealed hypercellularity, no excess of blasts, erythropoietic hyperplasia with giant proery-throblasts, megakaryocytes which were in normal number but still dysplastic.

Parvovirus antibodies and DNA were positive while the serologic tests for other viruses were negative. Reticulocytosis, spherocytes, increased osmotic fragility test, and persistent subclin-ical hemolysis indicated at transient aplastic crisis mimicking MDS-RAEB due to parvovirus infection in the setting of hereditary spherocytosis.

2.1.2. Cytomegalovirus (CMV) infection

Miyahara et al. [23] reported a 41-year-old, previously healthy man who developed severe thrombocytopenia with myelodysplastic changes of bone marrow and multiple autoimmune abnormalities, low CD4/CD8 ratio following CMV infection. The bone marrow aspiration was hypocellular with decreased megakaryocytes, atypical lymphocytes, and trilineage dysplasia. After a short-course prednisolone therapy, he improved.

It was suggested that direct CD34+ multipotent stem cells were infected with CMV giving rise to injury to the bone marrow cells. The inhibitory effect of cytokines [tumor necrosis factor alpha (TNF-α), interferon gamma (IF-)] produced by CMV-infected leukocytes and stromal cells on hematopoiesis and autoimmunity might have been responsible for myelodysplastic changes and thrombocytopenia.

2.1.3. Human immunodeficiency virus (HIV) infection

At the time of primary infection, transient pancytopenia, lymphocytosis, increased hemato-gones, increase in CD8+ lymphocytes, isolated autoimmune thrombocytopenia, anemia, reticulocytopenia, neutropenia, trilineage myelodysplasia both in the peripheral blood and bone marrow were reported. Megakaryocytes which were in normal or increased numbers showed apparent naked nuclei and were occasionally dysplastic [18]. Dysplastic findings were found increased and erythropoiesis became megaloblastic during antiretroviral therapy.

2.1.4. Hepatitis C virus (HCV) infection

HCV-infected patients frequently had varying degrees of bone marrow dysplasia and patients with pancytopenia were those who had the most frequent bone marrow abnormalities. In the cohort of HCV-infected patients, those with hematopoietic malignancy also existed [24]. However, bone marrow was a site where HCV replicated extrahepatically which contributed to the etiology of HCV-associated neutropenia and thrombocytopenia. Peripheral clearance or

consumption of platelets might have increased in HCV infection also [75, 76] like in other abnormalities in infections [77].

2.1.5. Virus infections in MDS

It should not be forgotten that more than 50% of patients with myelodysplasia and chronic myeloproliferative diseases showed elevated antibody titers against viruses like EBV and HHV-6 [78].

2.2. Parasitic infections

2.2.1. Visceral leishmaniasis

Yaralı et al. [25] reported seven cases with leishmaniasis all of whom had pancytopenia, dysplasia in erythroid myeloid, and megakaryocytic lineages. The all qualitative and quantitative findings disappeared after 2 months' therapy.

The authors postulated that increased TNF-α which was shown to be associated with increased macrophages, increased oxidized pyrimidine nucleotides, decreased glutathione concentration and presumably reduced clearance of free oxygen radicals might be responsible for myelodysplasia in visceral leishmaniasis, and other hematological findings.

Dhingra et al. [26] also reported 18 cases with leishmaniasis who had various combinations of cytopenia with increased bone marrow cellularity. Trilineage myelodysplasia (22%), bone marrow fibrosis (16.6%), hemophagocytosis (11.1%), and increased iron stores (33.3%) were evident.

It was thought that infected bone marrow stromal macrophages with leishmania, selectively enhanced myelopoiesis by granulocyte macrophage colony stimulating factor (GM-CSF) and TNF-α overproduction, giving rise to hypercellularity and trilineage myelodysplasia. Increased iron stores were attributed to cytokine overproduction which also led to anemia.

2.2.2. Others

Secondary myelodysplasia due to plasmodium falciparum and *P. vivax* infection was also reported [27].

2.3. Autoimmune disorders

2.3.1. Juvenile rheumatoid arthritis (JRA)

Yetgin et al. [28] reported myelodysplasia in 17 patients with JRA, none of whom had received iron, corticosteroids, immunosuppressive drugs, or any transfusions, and none had acute infection or gross bleeding. Bone marrow of all cases revealed normal along with abnormal maturation at different levels, like left shift, along with trilineage dysplasia, the most prominent dysplasia being in myeloid lineage. Increased bone marrow cellularity, fatty changes,

erythroid hypoplasia, myeloid and mild-moderate megakaryocytic hyperplasia were detected. They all had anemia, mostly being microcytic; the most had leukocytosis and thrombocytosis.
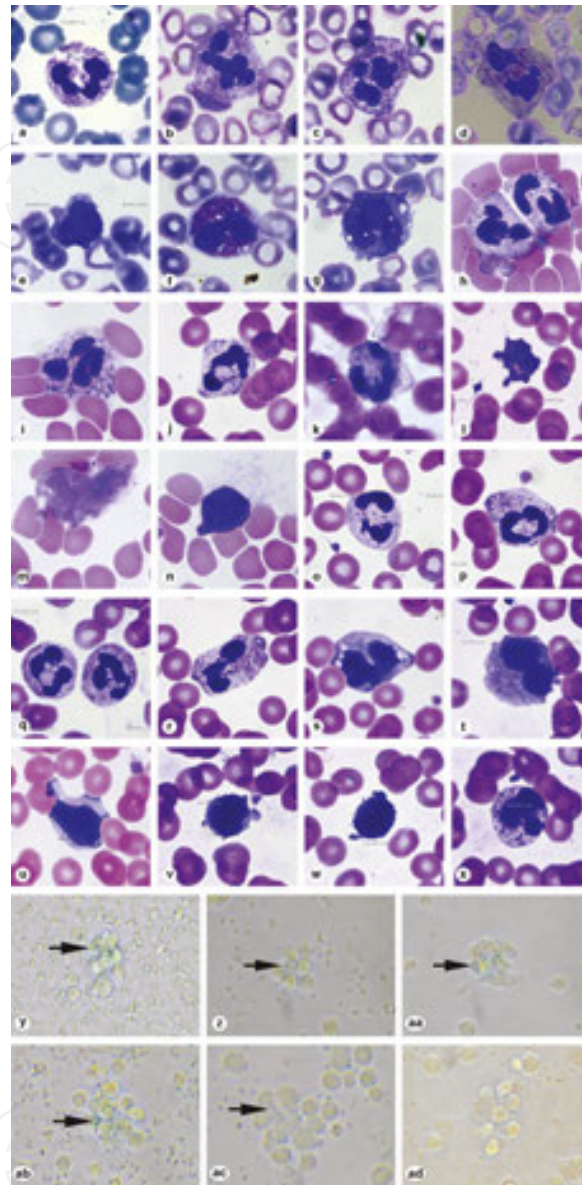
Figure 1. Dysmorphic hematological features of the peripheric blood smears of the patient ASİ with chronic ITP (a–g), patient MEY with JRA (h–n), patient TÇ with JRA (o–x). Courtesy of Turk J Med Sci [79]. **Neutrophils**: Macropolycytes (neutrophil > 15 μm) (b, c, h, i), hypersegmentation (c), cytoplasmic vacuoles (a), hypogranulation (d, o), cytoplasmic protrusions with or without granules (h, k), irregular distribution of granules (a, c, j), abnormal nuclei with nucleic protrusions (q, r), neutrophils with long chromatin between the nuclei (j, o), pseudo Pelger-Huet cells (o, p) **Lymphocytes**: Cytoplasmic protrusions (e, n, u, v, w). Basophils: Centralization of granules (f), abnormal nuclei and hypogranulation (x). **Monocytes**: Abnormal nuclei (g, s, t), cytoplasmic vacuoles (g, s), cytoplasmic protrusion (g). **Platelets**: Big or giant platelets (l, m) **β-gal staining photographs of the patients**. Patient TÇ JRA (y), patient MBY with JRA (z), patient KÇ with SLE (aa), patient HA with acute ITP (ab), patient ASİ with chronic ITP (ac), control (ad) (×100).

The score of myelodysplastic peripheral blood findings but not those of bone marrow correlated significantly with CRP and ferritin.

It was postulated that abnormally regulated cytokines and other local intracellular messengers by cellular immune system lead to alterations of the microenvironment of bone marrow giving rise to the myelodysplastic features (**Figure 1**).

On the other hand, clinicians should keep cautious since rheumatoid arthritis and other rheumatoid disorders may present as a part of immune abnormalities in MDS also [72–74].

2.3.2. Polyarteritis nodosa (PAN)

Yetgin et al. [29] reported a child with hematopoietic dysplastic characteristics in an 11-year-old girl with PAN. While her blood smear revealed occasional trilineage dysplasia, her bone marrow displayed moderate cellularity, fatty changes, and trilineage dysplasia in addition to blast and blast-like mononuclear cells. She received therapy of methyl prednisolone and cyclophosphamide and all of the hematologic abnormalities were found to have resolved after 6 months. It was suggested that these dysplastic findings were associated with the primary inflammatory process and increased cytokines.

2.3.3. Systemic lupus erythematosis (SLE)

Voulgarelis et al. [30] reported bone marrow biopsy and aspiration findings of 40 SLE cases in comparison with 10 MDS-refractory anemia (RA) cases. The patients had mono-bi- or trilineage cytopenia. The bone marrows were hyponormocellular with increased erythroid and megakaryocytic lineages in the majority of cases. All patients had dyserythropoiesis and dysmegakaryopoiesis. Dysmyelopoiesis was less striking with a left shifting. While the rate of dyserythropoiesis and dysmegakaryopoiesis were similar in patients with SLE and MDS-RA (100% vs 100%), the features of bone marrow biopsy specimens differed in that normo-hypercellularity and abnormal localization of immature progenitors (ALIP) aggregates were less but bone marrow necrosis was higher in SLE. Dilated sinuses (20%) were seen in SLE while no dilated sinus was noted in MDS-RA (0%). Increased reticulin, striking stromal edema, lack of inflammatory vascular damage and lack of microvascular obstruction by thrombus plugs, aggregates of T and B lymphocytes with polyclonal immunoglobulin expression were other striking features of SLE [30]. Specific lupus erythematosus (LE) cells (neutrophils containing a round, amorphous mass of purple, degraded nuclear material) were reported to be rarely seen when the bone marrow aspirate is anticoagulated and spreading of films were delayed [27]. These findings showed that bone marrow was a main target in SLE.

In SLE, it was shown that bone marrow fibroblasts could not produce enough hematopoietic growth factors and stromal cells of SLE patients failed to support allogenic progenitor cell growth in culture leading to defective hematopoietic microenvironment and altered cytokine expression. Additionally, autoreactive lymphocytes in the bone marrow of SLE patients might have directly caused immune destruction of both stromal cells and hematopoietic cells and indirectly affected them via releasing pro-inflammatory cytokines like TNF-α [35]. Secondary dysplastic changes in autoimmune diseases, in particular SLE, were demonstrated to closely mimick those in HIV [27] (**Figure 1**).

	MDS (RCC, RCUD, RCMD with unilineage cytopenia as thrombocytopenia)	Chronic ITP
Increased platelet destruction	-Present (peripheral) [81]	-Present (intrasplenic) [82]
Thrombocyte life span	-Short [81, 91]	-Short [82]
Thrombocyte production rate	-Decreased [81, 91]	-Decreased/normal [82]
Micromegakaryocytes	-Present [83]	-Present [84]
Naked megakaryocyte nuclei and megakaryocyte emperipolesis	-Present (more prominent) [31]	-Present (less prominent) [31]
Other dysplastic changes	-Present [14, 31]	-Present [17, 79]
Megakaryocyte apoptosis	-Only in micromegakaryocytes [31, 83] -Necrosis-like programmed cell death (mature + immature MKs) [81]	-Stage 3 megakaryocytes (apoptosis/ paraapoptosis) [82] -No apoptosis [85]
Apoptosis in other cell lines	-Apoptosis in all cell lines [86, 87]	-Lymphocytes: Resistant to apoptosis [88] -Granulocytes: No increased apoptosis [89]
Thrombocyte microparticles (TMPs)	-Present (TMPs/thrombocyte > normal) [90]	-Present (TMPs/thrombocyte > normal both in acute and chronic ITP) [90]
Response to splenectomy	-Response in several patients with different rates* [91, 92]	-Sustained response (70–80%) [93]

*Complete thrombocyte response in 50% (at 3 months) and 33% (at 12–54 months) of patients with short thrombocyte lifespan (<3.5 days), no transfusion requirement, but sustained neutropenia [91, 92].

Table 5. Characteristics of chronic ITP and MDS with unilineage cytopenia as thrombocytopenia (RCC subgroup of childhood MDS and RCUD, RCMD in adulthood MDS).

2.3.4. Immune thrombocytopenic purpura

In a previous study, we established neutrophil and eosinophil dysmorphism and increased macropolycytes in patients with acute and chronic ITP before treatment, in comparison with normal children. Several dysplastic features increased at the end of mega dose steroid (methyl prednisolone 30 mg/kg/day × 3 days followed by 20 mg/kg/day for the consecutive 4 days) therapy but decreased within 1–4 weeks after therapy was stopped. Hyperdiploidy in neutrophils which developed during steroid therapy normalized 7 days after therapy was stopped [17]. Dysplastic features were noted in other cell lines too [79].

These findings suggested that not only an intrinsic megakaryocyte proliferative defect giving rise to deficient platelet production were present in refractory chronic ITP patients but a defect before or at the level of colony forming unit-granulocyte-erythroid-monocyte-megakaryocyte (CFU-GEMM) also. The antiplatelet antibodies and the increased cytokines in ITP [80] might have been effective at this level (**Figure 1**).

*2.3.4.1. Differentiation between MDS (refractory thrombocytopenia) and chronic ITP*

Myelodysplastic syndrome with isolated thrombocytopenia (RCC subgroup of childhood MDS and RCUD, RCMD, MDS-U subgroups of adulthood MDS) can be masqueraded as refractory chronic ITP and the accurate diagnosis may be challenging due to close similarities between the two entities like decreased life span, decreased production rate in thrombocytes, increased thrombocyte destruction, presence of dysplastic findings in myeloid and megakaryocytic cell lines including micromegakaryocytes, naked megakaryocyte nuclei and megakaryocyte emperipolesis; and additionally megakaryocyte apoptosis, platelet microparticles and good response to splenectomy in several patients [17, 31, 81–93] (**Table 5**).
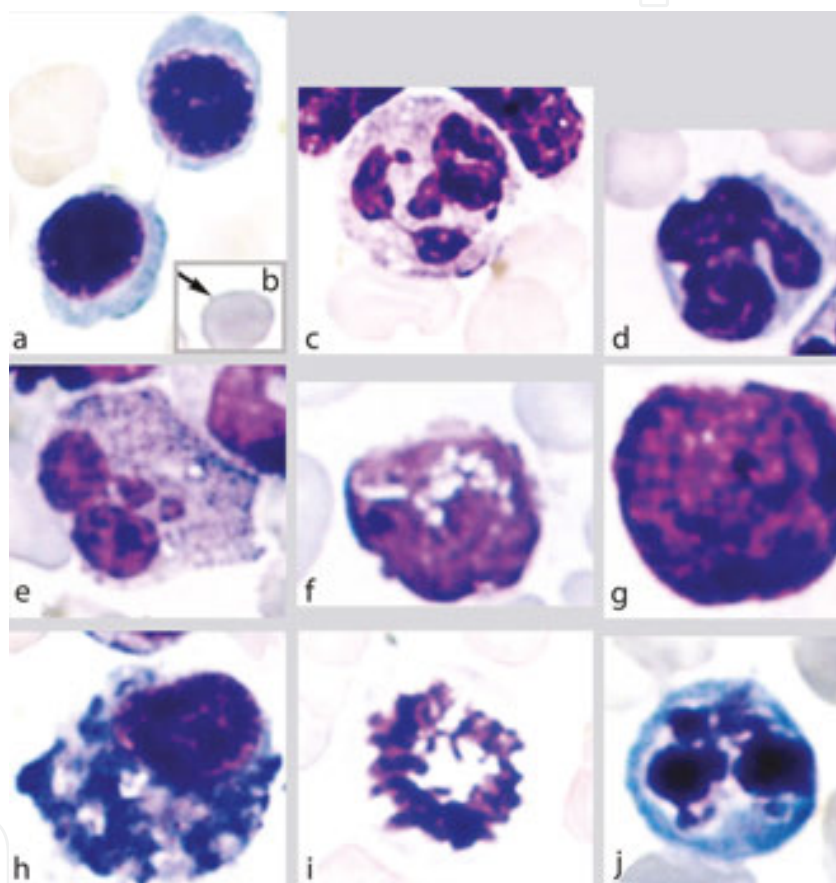
Figure 2. Myelodysplastic findings in a patient with MDS (refractory thrombocytopenia). Courtesy of Ped Hemat Oncol [31]. **Erythroid serie**: Interchromatin bridge between erythroblasts (a), spherocytes (b). **Neutrophilic serie**: Bizarre nucleus, nuclei with striking chromatin clumping, abnormal projections, cytoplasm with irregular distribution of granules and vacuoles (c), nucleocytoplasmic asyncrony (c, d). **Eosinophilic serie** with micronuclei, cytoplasmic vacuolation, cytoplasm with both eosinophilic and basophilic granules (e). **Monocytic serie** with cytoplasmic vacuoles (f). **Blast-like cells** (g). **Histiocytic serie**: Sea-blue histiocyte (h). **Mitotic cells**: Mitosis in an unknown cell (i). **Apoptotic cells** with condensed and fragmented nuclei and condensed cytoplasm (j).
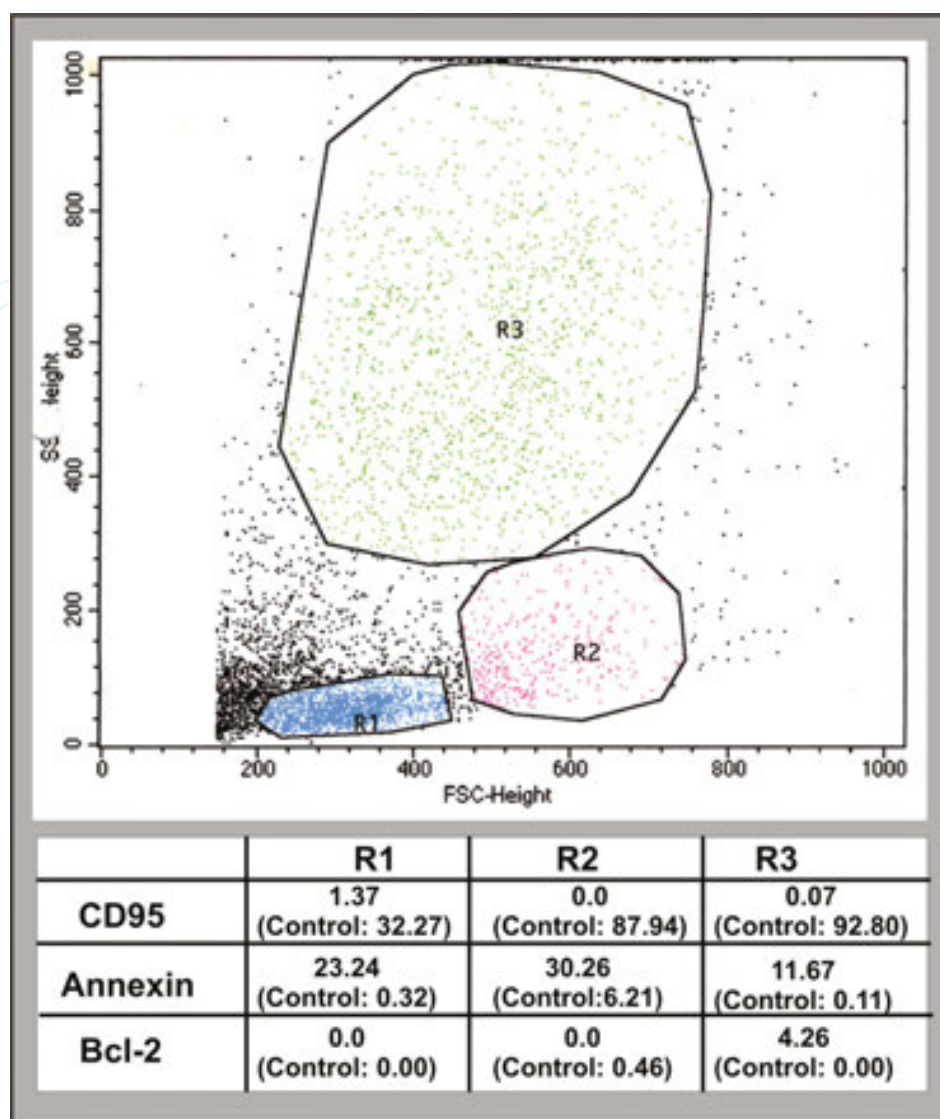
Thrombocyte microparticles per thrombocyte were reported more than normal in both disorders [90] and the both can benefit from splenectomy although with different success rates [91–93]. Two of these characteristics can be used to differentiate between the two entities. The first is that, while megakaryocyte apoptosis in ITP starts at stage 3 (mature) megakaryocyte

level [82] (or apoptosis does not take place in megakaryocytes of ITP patients [85], megakaryocyte apoptosis in MDS is detected in micromegakaryocyte level [31, 83]. The second is that while apoptosis takes place in myeloid, lymphoid, and monocytic cell lines in MDS [86, 87], no increase in apoptosis (in granulocytes and lymphocytes) on the contrary resistance to apoptosis (in lymphocytes) [88, 89] were reported in ITP (**Table 5**). We followed a patient with RCC that mimicked therapy resistant chronic ITP, who developed intracranial hemorrhage twice but underwent a successful bone marrow transplantation [31] (**Figures 2–4**).
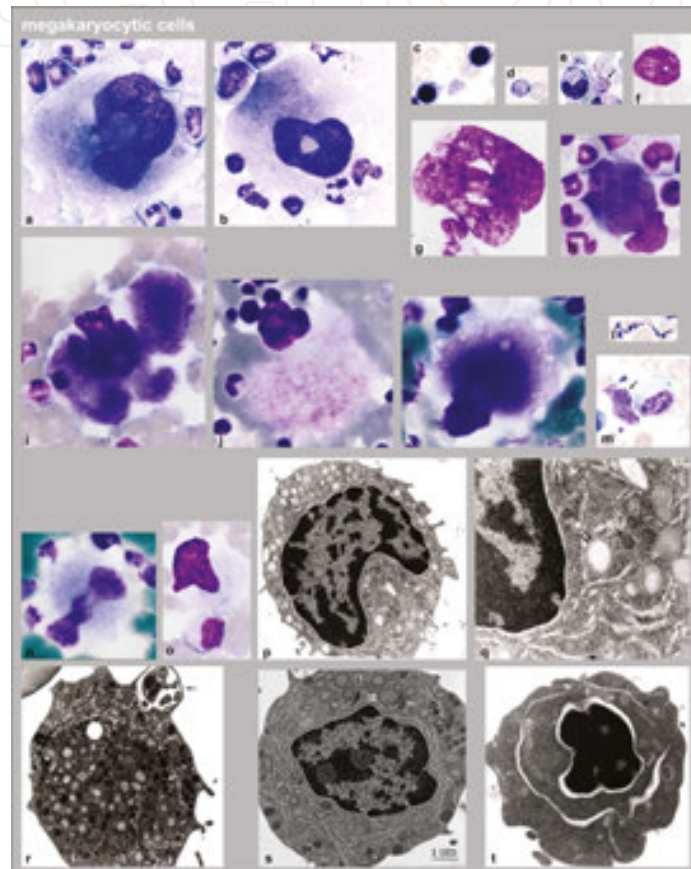
Figure 3. Dysmorphism in megakaryocytic serie, in a patient with MDS (refractory thrombocytopenia). Courtesy of Ped Hemat Oncol [31]. **Evaluation by light microscopy**: Mononuclear megakaryocytes (a, b), megakaryocyte with ring-shaped nucleus (b), megakaryocytes with nuclei that are being extruded out of the cell (i–k, o), the cytoplasm which is lobulated (i), basophilic and condensed (i, k), naked megakaryocyte nuclei (f–h) with abnormal nuclear shape (g–i), macroplatelets (c–e, m), dysmorphic platelets (e, l, m) are seen (×100). **Evaluation by transmission electron microscopy**: Stage I megakaryocyte with large, oval, and intended nucleus and a cytoplasm containing abundant ribosomes and granules. Demarcation system of membranes and granules are abundant in cytoplasm, all of which indicates nucleocytoplasmic asyncrony. The granules were identified as azurophilic granules (single arrow) and unidentifiable, large, oval, and electron lucent, abnormal granules (double arrow) (×10,000) (p) and lots of free ribosomes, azurophilic (single arrow) and abnormal unidentifiable, large, oval, electron lucent granules (double arrow) with demarcation membranes in the cytoplasm of the same cell (×27,800) (q). A megakaryocyte that shows abundant demarcation membranes, ribosomes, and granules. The granules are heterogenous as to both size and electron density. A phagosome (emperipolesis) is also seen (×12,930) (r). A stage I megakaryocyte with double nucleoli and abundant demarcation membranes, abundant azurophilic (single arrow) and unidentifiable, large, oval, electron lucent abnormal granules (double arrow) (s). Apoptotic stage I megakaryocyte that shows a condensed nuclear fragment and condensed cytoplasm, but mitochondria, mitochondrial crystae, and demarcation membranes are still intact (×16,700) (t).

	R1	R2	R3
CD95	1.37 (Control: 32.27)	0.0 (Control: 87.94)	0.07 (Control: 92.80)
Annexin	23.24 (Control: 0.32)	30.26 (Control:6.21)	11.67 (Control: 0.11)
Bcl-2	0.0 (Control: 0.00)	0.0 (Control: 0.46)	4.26 (Control: 0.00)

Figure 4. Fas, annexin and bcl-2 values in lymphocytes (R1), monocytes (R2), granulocytes (R3) from the bone marrow of the child with MDS (refractory thrombocytopenia), established by flow cytometry (Coulter Elite). Courtesy of Ped Hemat Oncol [31].

2.3.5. Autoimmune neutropenia

Although chronic idiopathic and autoimmune neutropenia are considered as benign disorders, it was reported that the bone marrow of these patients displayed dyserythropoiesis by 55% and they transformed to clonal hematological diseases including NK expansion, hairy cell leukemia, myelomonocytic leukemia, and MDS (RCUD, RCMD) within 30 months, with a rate up to 6.5%. Therefore, these patients should be closely followed up [32].

2.4. Hemophagocytic lymphohistiosis (HLH, hemophagocytic syndrome)

All patients whom we followed in our clinic due to primary or secondary HLH had myelo-dysplastic features in addition to cytopenia involving at least two cell lines. The common

findings in the bone marrow were erythroid hyperplasia, chromatin bridges between erythroid precursors, multiple nuclei in erythroblasts, mild megaloblastic changes, vacuoles in erythroblasts, myeloid precursors and monocytes, sometimes in thrombocytes; hypogranulation in neutrophil myelocytes, micromyelocytes, irregular distribution of cytoplasmic granules, large and sometimes hybrid granules in eosinophils; anisocytosis in thrombocytes including giant thrombocytes, naked megakaryocyte cytoplasm, megakaryocyte emperipolesis, oligomononuclear megakaryocytes. These changes were probably due to the cytokine storm that played the major role in the pathogenesis of HLH (**Figure 5**).
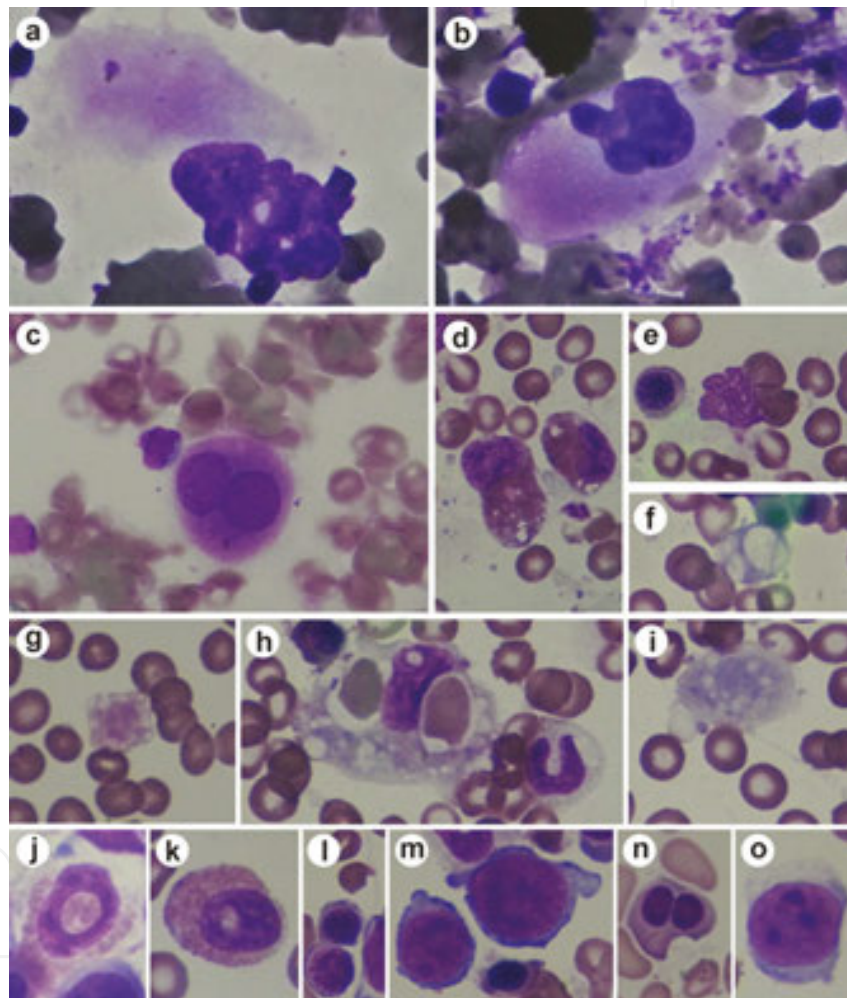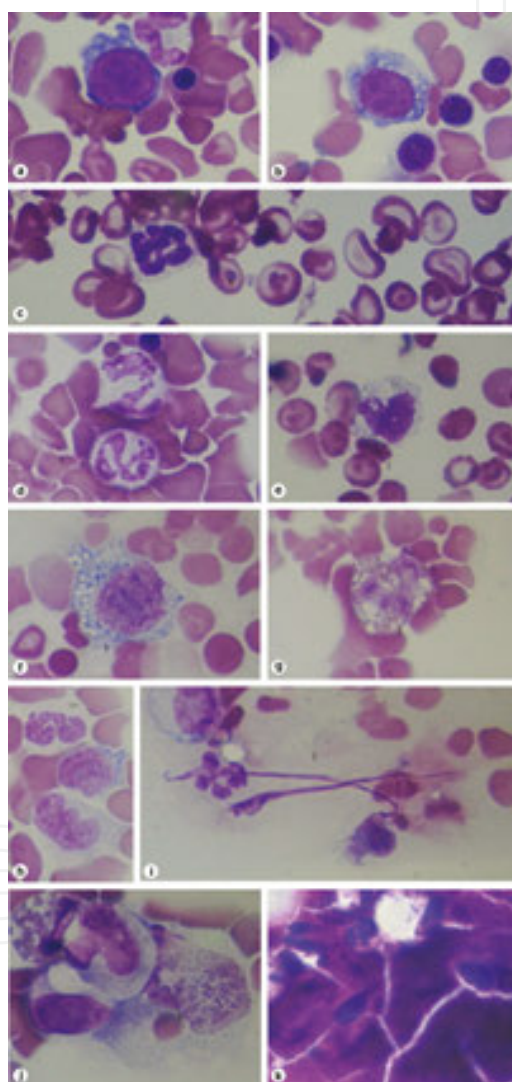

Figure 5. Myelodysplastic bone marrow findings of two patients with secondary (a, b) and primary (c–o) hemophagocytic histiocytosis (HLH) (personal archives). Megakaryocytes with mononuclear nuclei that have irregular edges and condensed cytoplasm are seen just after (a) and during (b) the process of extruding the nuclei out of the cell, developing naked megakaryocyte cytoplasm and nucleus (a). Bilobed erythroid precursor (c), eosinophil myelocytes with cytoplasmic vacuolation and rare basophilic granules, cytoplasmic protrusion (d), a naked nuclei of an unknown cell (e), cells or formations with heterogenous morphology consisting of numerous vacuoles with various sizes (f, g, i), indistinguishable from abnormal trombocytes and detached cytoplasm of hemophagocyting histiocyte (h), hypogranulated and vacuolated band (h), ringed nuclei in eosinophilic myelocytes (j, k), internuclear chromatin bridge (slightly dim) (l), cytoplasmic protrusions in basophilic erythroblasts (m), bilobed normoblast (n), cytoplasmic vacuolation in a proerythroblast (o) (vitamin B12, folic acid, Cu, Zn levels were normal).

2.5. Nutritional deficiencies

2.5.1. Malnutrition

Mono-, bi-, pancytopenia, and hematopoietic dysplasia were reported in patients with malnutrition, who were generally deficient in iron, vitamin B12, folate, and trace elements also. Bone marrow cellularity was reduced with normoblastic dyserythropoiesis. Giant metamyelocytes, vacuolation in erythroid and granulocytic precursors, abnormal sideroblasts including ring sideroblasts [27], hemophagocytosis, necrotic cells, and rarely dysmegakaryocytosis were reported.

Figure 6. Myelodysplastic bone marrow findings of a child with malnutrition and gelatinous transformation (personal archives). Basophilic erythroblasts with cytoplasmic protrusions (a) and vacuoles (b), agranular neutrophil (c, i) with numerous cytoplasmic vacuoles and hypersegmented nucleus (c), hypoagranular neutrophils with hypolobulated nuclei with abnormal protrusions, mild chromatin clumping (d, h), eosinophil with abnormal nucleus, irregular distribution of granules (g), eosinophilic band with few basophilic granules distributed irregularly (j), vacolated monocytes (e, j) and histiocytes (f), degenerating histiocytes (j), hypha and amorphous material representing the gelatinous material (i, k).

In anorexia nervosa, severe diseases with cachexia and starvation, acantocytosis in the peripheral blood, hypocellularity with/without "gelatinous transformation" were noted. In gelatinous transformation, the fat cells in the bone marrow are lost, and hematopoietic cells are replaced by extracellular matrix material composed of acid mucopolysaccharide rich in hyaluronic acid is detected [18, 33]. All the reported cases had anemia, leukopenia ± thrombocytopenia [33, 34], rarely only anemia [35] (**Figure 6**).

Trilineage dysplasia in peripheric blood of iron deficient patients were reported, being higher than the control group. In addition, microspherocytes which were observed in 20% of iron-deficient patients were not noted in the control group [36]. Leukopenia and thrombocytopenia may accompanied these changes as iron deficiency deepened, making the differential diagnosis between iron deficiency anemia and MDS more difficult. Alcantara et al. [94] showed that iron supported 11 genes in phorbol myristate acetate-induced HL-60 cell lines which were involved in critical cell decision points to pursue a differentiation or cell death pathway.

Additionally, increased erythropoietin in iron deficiency anemia might also have activated hematopoietic lineages. Presence of microspherocytes was attributed to a putative involvement of Rac1 and Rac2 GTPase, also known as Ras-related C3 botulinum toxin substrates 1 and 2 GTPase deficiency which reportedly altered actin assembly in red cells in mice. Further factors like cellular metabolic enzyme changes, cell growth and differentiation, and gene expression regulation might have been involved in the pathogenesis [36].

On the other hand, leukemia cases who were under chemotherapy and had myelodysplastic features were shown to have hypochromic macrocytes and increased serum iron and ferritin levels and increased soluble transferrin receptor implying at a disturbance in utilizing iron (functional iron deficiency) [49].

2.5.2. Megaloblastic anemia

See Section 4.2.

2.5.3. Copper deficiency

Copper is a cofactor of a number of enzymes (cuproenzymes) including those important for hematologic system like hephaestin, seruloplasmin (ferroxidases) and others like cytochrome *c* oxidase, superoxide dismutase 1, extracellular superoxide dismutase, and zyklopen, a new member of the vertebrate multicopper ferroxidase family

Pregnant and lactating women, premature infants, those with malabsorption, inflammatory bowel diseases, celiac disease, long lasting diarrhea, short bowel syndrome, those under total parenteral nutrition, or nutrition through jejunal tube, those who underwent gastric resection, bariatric surgery; Wilsons' disease patients who consumed copper-depleting drugs, conditions with excess zinc are under risk of copper deficiency [37–41].

The most important problem in copper deficiency lays in its diagnosis. It may be misdiagnosed as MDS or underdiagnosed. The time which lapses until appropriate diagnosis is made was

reported as approximately 1 year. Early diagnosis is important since therapy after neurological symptoms have developed is difficult [40].

On the other hand, it is of note that 11 out of 32 MDS patients were found to have copper deficiency [95].

The most striking hematologic findings in copper deficiency are mono-, bi-, and pancytopenia [39–41]. Anemia which is the most common hematologic finding (97.5%) in copper deficiency is generally normochromic or macrocytic but rarely microcytic, being dependent on the severity of the deficiency. When the activity of copper-dependent enzymes decrease, iron absorption is expected to be impaired, iron transport across intestinal cells be decreased, conversion of ferrous iron to the ferric form which is necessary for transport of iron by transferrin be impaired, conversion of ferric iron to ferrous iron which is necessary for incorporation of iron into the protoporphyrin molecule during hemoglobin synthesis be inadequate. The latter defect gives rise to both formation of ring sideroblasts and possibly erythrocyte membrane defect due to low levels of antioxidant zinc/copper dismutase activity necessary to convert superoxide-free radicals to hydrogen peroxide [37, 39]. However, the mechanism of anemia is not fully understood [37].

	Copper deficiency	MDS
Vacuolization	-In erythroid + myeloid lineages [39]	-In erythroid lineage [39]
Dysmegakaryopoiesis (nuclear lobulation and abnormal sizes)	-Not present [39] -Present [37]	-Generally present [2, 5, 14, 39]
Dysplasia	-No [39] -Bilineage [38] -Three lineage dysplasia [37]	-Unilineage: RCUD; RARS (erythroid) -Two or more lineages (RCC, RCMD; MDS-U) [2, 5, 14, 39]
Increased hematogones	-Present [37]	-Absent [39]
Other	-Left shift in myelopoiesis -Reduced terminally differentiated myeloid cells, myeloid arrest [39] -Erythroid arrest at proerythro blast stage [37]	-Erythroid hyperplasia (generally) [15] -Myeloid arrest or left shift (generally in RAEB) [96]
Ring sideroblast	-Present [37–39]	-Present only in RARS subgroup [2, 5]

Table 6. Bone marrow findings in copper deficiency in comparison with MDS.

Leukopenia in copper deficiency is together with neutropenia which was reported to be the most frequent and earliest manifestation of copper deficiency [41]. The neutrophils in peripheric blood smear are dysplastic. Impaired and delayed maturation, differentiation and regeneration of hematopoietic precursor cells, increased destruction of myeloid precursors in the bone marrow, defective neutrophil egress from the bone marrow, shortened life-span of

neutrophils, and presence of antibodies to neutrophils are the possible etiologic factors for neutropenia [39, 41].

The bone marrow is generally hypercellular [40] with increased myeloid and/or erythroid precursors mimicking myeloid and erythroid arrest, vacuolization in erythroid and myeloid precursors, increased iron stores, prominent ring sideroblasts, plasma cells in which hemosiderin is incorporated, increased hematogones [37] with [37, 38], without [39] myelodysplasia. In MDS, generally erythroid hyperplasia [15] is encountered. Myeloid arrest or left shift in granulopoiesis is seen generally in RAEB subgroup of MDS [96]. Characteristics of MDS and copper deficiency are summarized in **Table 6**.

2.5.4. Vitamin D deficiency

Vitamin D has both proliferating and differentiating effect on hematopoiesis [42]. The bone marrow taken from infants with vitamin D deficiency rickets and anemia showed early signs of myelofibrosis with increase of reticulin which was reversed by vitamin D treatment [97]. Anemia, thrombocytopenia, hepatosplenomegaly, hypocellularity and increased osteoblast count in bone marrow, and hematopoietic precursors in spleen aspirates were striking. Hypochromia, macrocytosis, tear drop cells, young myeloid elements along with nucleated red blood cells were evident [98]. Dysdifferentiation due to its deficiency might have been aggravated by coexistent malnutrition in many of several patients.

2.5.5. Hypervitaminosis A

An infant with hypervitaminosis A reportedly had eversible severe anemia, thrombocytopenia, and dyserythropoiesis. It was shown that in overdoses, vitamin A strongly inhibited the proliferation of multipotent hematopoietic cell line and bone marrow mesenchymal stem cells, through upregulating $p21^{Cip1}$ and $p27^{Kip1}$, cyclin-dependent kinase inhibitors [43].

2.6. Severe congenital neutropenia (SCN)

We, previously detected hematopoietic dysmorphism in congenital neutropenia, their non-neutropenic parents and one sibling, irrespective to the neutropenia mutation that the patients had [45, 99]. All the tested patients were negative for molecular genetics of MDS and were normal in conventional cytogenetics. Apoptosis of lymphocytes, granulocytes [45, 99], and monocytes [45], of both patients and parents and rapid cell senescence (RCS) in leukocytes of a few patients and their mothers were established [45, 99]. A substantial portion of cases had clinical or laboratory evidence of hemorrhagic diathesis and low NK and CD4+ cells.

These findings showed that pluripotent stem cells were involved in SCN irrespective to the genetic defect and non-neutropenic family members were also affected ([45, 99], study in submission) and congenital neutropenia and MDS shared the same death types and involved pluripotent stem cells.On the other hand, it should not be forgotten that MDS can present as isolated neutropenia (RCC, as refractory neutropenia). In our clinic, we followed a 4-year-old girl who was admitted to our hospital for chronic neutropenia, but the genetic evaluation

revealed trisomy 8 and a complex karyotype; while the molecular genetic studies for congenital neutropenia (*HAX1, ELANE, and G6PC3*) were negative (unpublished data).
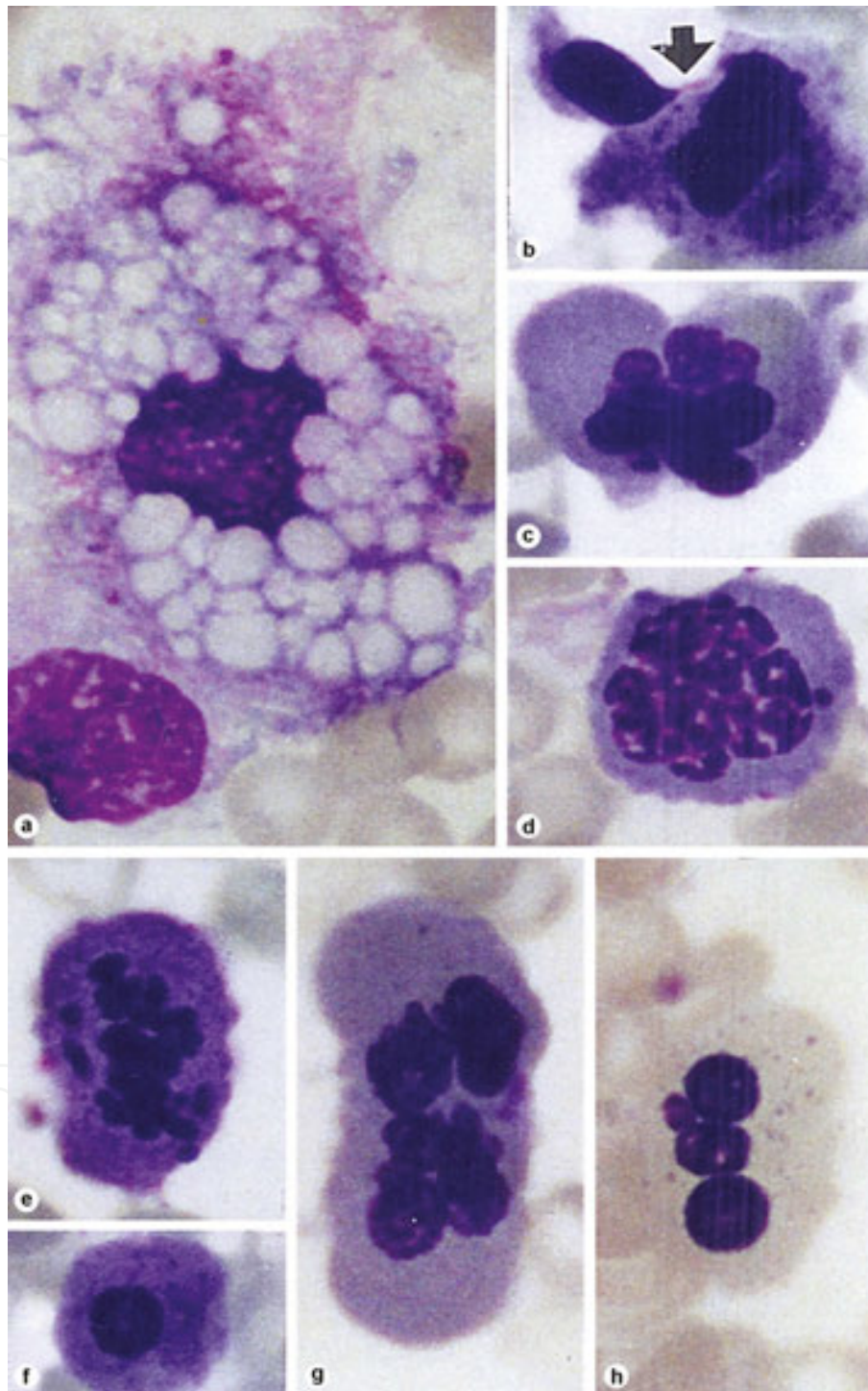
Figure 7. Myelodysplastic bone marrow findings of a 5-year-old patient with ALL who was on chemotherapy and had coexistent autoimmune hemolytic anemia [100] (personal archives). A Gaucher-like histiocyte that is hemophagocytosing a cell (a), internuclear chromatin bridge between two erythroblasts (b) (arrow), multinucleated erythroblasts with various sizes (c, e, g, h), striking megaloblastic changes (d), basophilic stippling (b, e–h).

2.7. Inherited conditions

Özbek et al. [46] reported myelodysplastic features in myeloid and erythroid cell lines, in 20 patients with microdeletion 22q11.2 (del22q11.2) with slight cytopenia. Their smears showed dysmorphism in erythroid and myeloid cell lineages, in addition to a few vacuolated plasmatoid lymphocytes; monocytic cells mimicking hypogranular myelocytes with cytoplasmic vacuoles and protrusions and blast-like cells.

Myelodysplasia scores in the myeloid cells and eosinophils and macropolycyte percentages were higher than those with conotruncal heart defects, viral and bacterial infections, and healthy children. Genes in the deleted region, like human cell division cycle-related (hCDCrel) gene was proposed to be responsible for these changes.

Other inherited conditions which present as dyserythropiesis and anemia are congenital dyserythropoietic anemia, thalassemia, congenital dyserythropoietic porphyria, mitochondrial myopathies, hereditary sideroblastic anemia, homozygous hemoglobin C, heterozygous unstable hemoglobins, some cases with thiamine-responsive anemia with diabetes and deafness, homozygote pyruvate kinase deficiency, stress erythropoiesis like severe hemolytic anemia [100] (**Figure 7**).

Those who present as dysgranulopoiesis with/without neutropenia are mitochondrial cytopaties, myelokathexis, and congenital neutropenia [45]. Those with dysmegakaryopoiesis and thrombocytopenia are inherited thrombocytopenias. Patients with GATA1 mutations have anemia and neutropenia together with trilineage dysplasia [27]. Patients with mevalonc aciduria due to mevalonate kinase deficiency have anemia, thrombocytopenia with/without fluctuation, dysplasia in erythroid and myeloid lineages [47].

2.8. Malignant lymphoma

In non-Hodgkin lymphoma (NHL) and Hodgkin lymphoma (HL) patients, myelodysplasia in granulocytic and erythroid lineages were noted without any marked myelodysplasia in megakaryocytic lineage. The myelodyspastic features were found comparable in patients with and without bone marrow infiltration of lymphoma cells.

The bone marrow was normal or hypercellular, with normal, reduced number of erythroid and increased number of myeloid cells, normal, or increased megakaryocytes; ALIP was not encountered. Reticulin fibrosis was rare (6.1%).

The myelodysplasia in lymphoma was thought to be a reaction to the lymphoma or to result from an impaired bone marrow stem cell [48].

## 2.9. Effect of drugs and toxins

2.9.1. Chemotherapy

Most of chemotherapeutic and immunosuppressive agents give damage to the bone marrow, inducing megaloblastic dyserythropoiesis in low doses, and hypoplasia in high doses. Drugs

that cause megaloblastosis are methotrexate, cyclophosphamide, daunorubicin, doxorubicin, cytarabine, hydroxyurea, azathioprine, and zidovudine [27]. Mycophenolate mofetil is known to cause Pelger-Huet anomaly, abnormal chromatin clumping, detached nuclear fragments in granulocyte lineage. Alemtuzumab was also reported to be associated with increased dysplastic features and virus-related hemophagocytic syndrome [27]. In our experience, the most consistent finding of dysplasia in patients who receive chemotherapy was hypoagranulation of myeloid cells (**Figures 7** and **8**).

We previously showed that leukemia patients displayed hypochromic macrocytes in their peripheric blood due to failure to utilize iron ([49], study in submission). Additionally, serum reticulocyte counts in the beginning of chemotherapy blocks declined significantly in the end of the blocks when erythropoiesis was markedly depressed and were found to have increased significantly at the beginning of the next chemotherapy block when the bone marrow regenerated and erythropoiesis increased [49].

Increased apoptosis, increased hemophagocyting macrophages, erythroid and megakaryocytic regeneration generally preceding granulocytic regeneration, megakaryocytic clustering and ALIP together with myelodysplasia were described after intensive chemotherapy and persisted for months [27]. The infections that the patients could have developed, possibly aggravated the myelodysplasia.
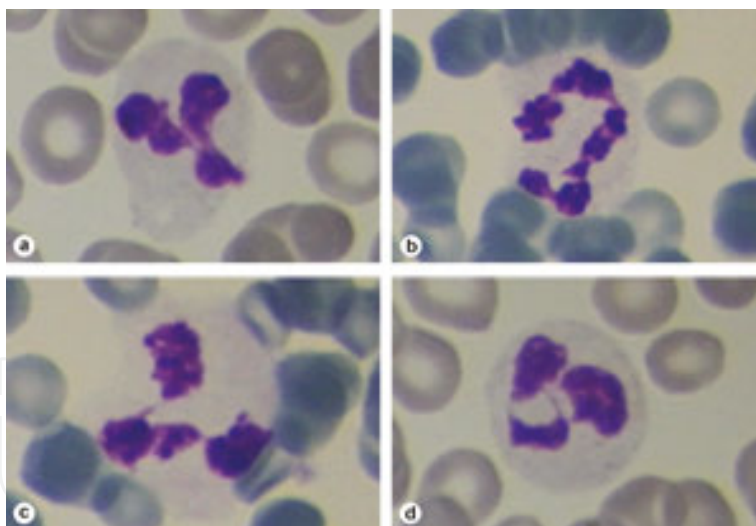

Figure 8. Neutrophils of a pediatric ALL patient while he was on maintenance chemotherapy (a, b), and 6 months after cessation of therapy (c, d) (personal archives). Cytoplasmic agranulation (a–d), large size (a–d), chromatin clumping (b, c), long chromatin string between the nuclear lobes (d) are striking.

On the other hand, these findings closely overlap with those in therapy-related MDS (t-MDS) or therapy-related myeloid neoplasms (t-MN) in the new nomenclature. It was reported that t-MN followed treatment of lymphomas and solid tumors but more rarely leukemias [101]. Appearance of new dysplastic changes after complete remission of leukemia [102] or solid tumors should alert the physician for development of t-MN.

2.9.2. Steroids

Steroids give rise to hyperdiploidy, and therefore macropolycytes in neutrophils [17] in addition to abnormal nuclear lobulation (**Figures 9** and **10**).
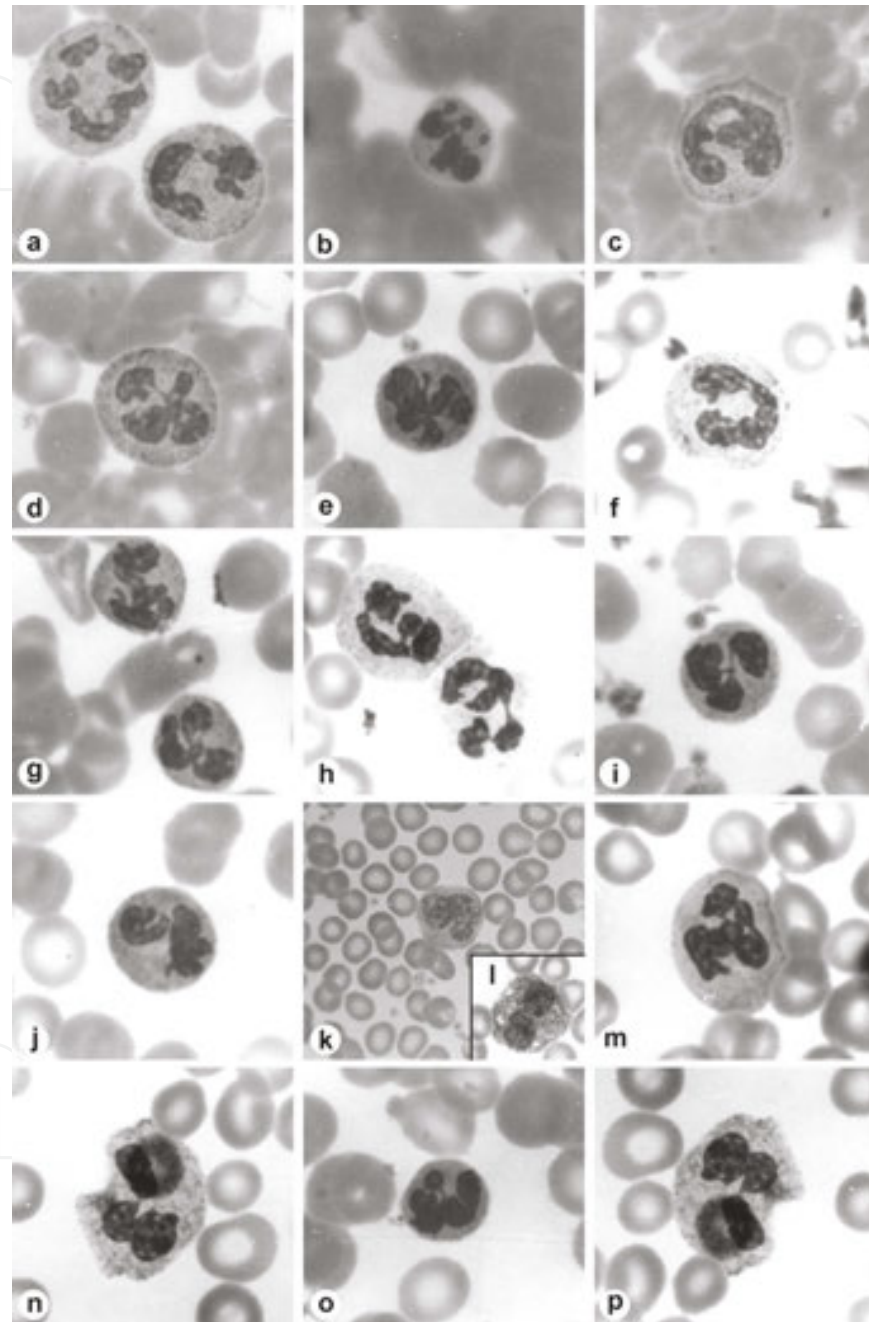

Figure 9. Peripheric blood neutrophils of children who were on high dose or long-term steroid therapy for acute and chronic ITP (personal archives). On the seventh (last) day of mega-dose methyl prednisolone therapy [116] of patient IY (a–g, i, j, l, o) and OI (k); during the phase of tapering down long-term prednisolone therapy of patient YP (n, h, p); 6 months after the last steroid therapy in YP (m). Macropolycytes (neutrophils with >14 μm diameter) in 15–20 μm diameter (a, c, h, m, n, p), pseudo-Pelger-Huet/like cells (a, c, j), chromatin clumping (c, f, k, n, p), bizarre nucleus (b, c, d, e, g, j, o), vacuolated eosinophil with both basophilic and eosinophilic granules (l).

2.9.3. Alcohol

Anemia with/without other cytopenias or pancytopenia were reported in alcohol dependent patients. Anemia was normochromic or macrocytic with round macrocytes (unlike oval macrocytes of megaloblastic anemia) [27, 50]. Vacuolated neutrophils, stomatocytes, sometimes target cells were evident. In hemolytic anemia and hyperlipidemia due to alcoholic liver disease, spherocytes, irregularly contracted cells (Zieve's syndrome) were demonstrated [27].
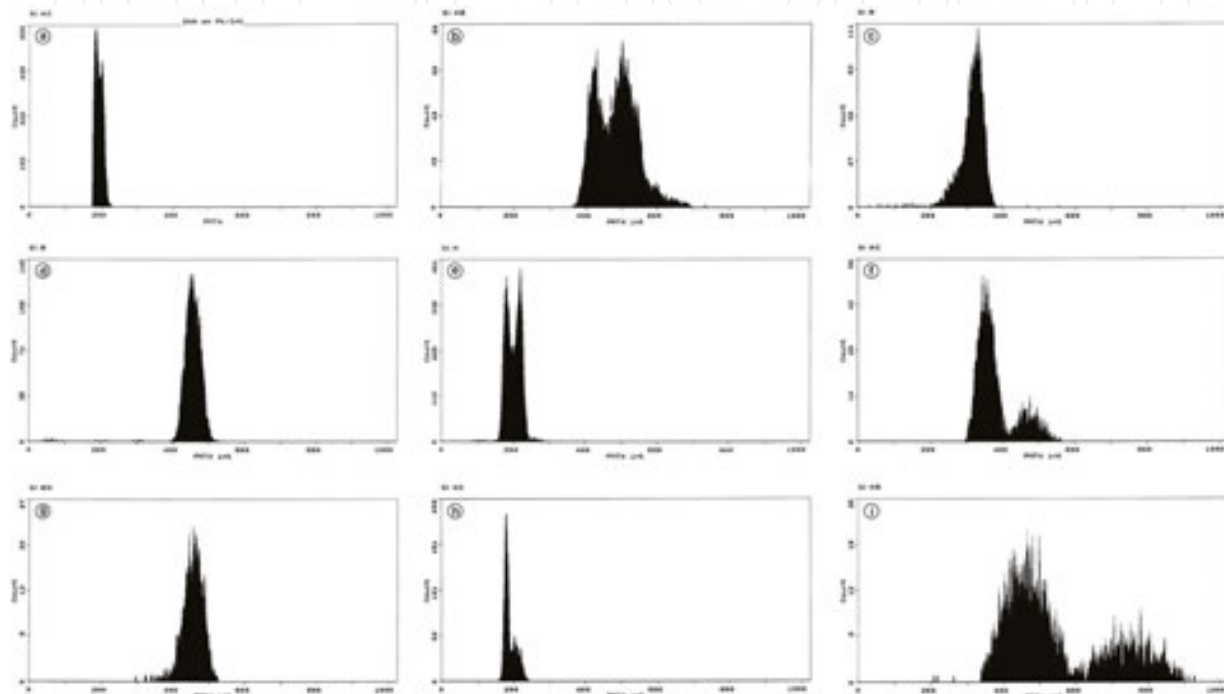
Figure 10. Various cell cycle configurations in patients with myelodysplasia (personal archives). Cell cycle analysis of granulocytic and mononuclear cells of children with acute or chronic immune thrombocytopenic purpura (ITP) before, during and after therapy (a–h), and granulocytes of a non-neutropenic mother of a dygranulopoietic neutropenia patient with dysgranulopoiesis (i). Normal cell cycle of granulocytic cells (G0 phase) (a). Granulocytes of patient YP with chronic ITP while taking oral steroids on different occasions (b, c). Mononuclear cells (d) tested on the same occasion with (c). Granulocytes of patient AT with chronic ITP. Tested 10 days after he received anti-D therapy (e). Granulocytes of IY with acute ITP, before mega dose methyl prednisolone (MDMP) therapy (f, g); 1 week after MDMP therapy ended (h). Granulocytes of non-neutropenic mother of a congenital dysgranulopoietic neutropenia patient who had myelodysplasia (i).

The most striking changes in the bone marrow was in the erythropoietic lineage as erythroid hyperplasia, ineffective erythropoiesis and dysmorphism in erythroid lineage including ring sideroblasts (positive by 75%) and dysmorphic granulopoietic cell lineages. There was no morphologic abnormality in megakaryocytes [50], but the megakaryocytes reportedly increased or markedly decreased [27]. Megaloblastic changes were associated [103] and not associated [50] with folic acid and/or vitamin B12 levels.

Serum iron levels which were elevated in the majority (being the most prominent in those with fatty liver and typical cirrhosis), iron granules in plasma cells [50] macrophages and endothelial cells [27], large numbers of sideroblasts along with ring sideroblasts [50] were other

characteristics of alcohol effect. In Zieve's syndrome, excess iron-laden foamy macrophages were encountered [27]. No correlation was found between serum iron and ring sideroblasts in the bone marrow [50].

The normal colonies of all hemopoietic cell lines and cell culture ratios in alcohol-dependent individuals showed that alcohol exerted its toxic effect not on committed stem cells but peripheral cells also [50]. Reversible bone marrow aplasia due to alcohol was also reported [27].

Cytoplasmic vacuolization was reported to be due to inhibition of acetaldehyde dehydrogenase and thus reduced rate of degradation of acetaldehyde.

Alcohol has toxic effect on cell division giving rise to arrest in cell division and multinucleated erythroblasts; direct antifolate effect on nucleic acid metabolism leading to development of megaloblasts. Additionally, it impairs iron utilization giving rise to large number of sideroblasts and ring sideroblasts through disrupting pyridoxine kinase inhibiting delta aminolevulinic acid synthetase which is necessary for heme synthesis [50].

Alcohol-induced hematopoietic abnormalities closely mimic those of MDS-RARS. That alcohol-induced bone marrow damage is always reversible if the patient stops to drink alcohol, and that cell culture of alcohol dependent people show normal colonies of all hematopoietic cell lines are two important points for differentiation between alcohol-induced cell damage and MDS-RARS [50].

2.9.4. Smoking

Moderate leukocytosis being mainly due to neutrophilia and lymphocytosis was reported. Bone marrow biopsies of 32 smokers showed normal or slightly increased cellularity, modest and mild increase in granulopoiesis and erythropoiesis respectively, right shift of granulopoietic cells (the half being mature segmented neutrophils).

The special appearing macrophages with intracytoplasmic small, polygonal corpuscles showing neutrophils were striking. It was postulated that smoking inhibited locomotion of the segmented neutrophils leading to granulocytopoietic hyperplasia and accumulation of mature neutrophils in the bone marrow. These neutrophils were broken-down when they got senescent and were phagocytosed by these macrophages, resulting in "smokers' dysmyelopoiesis" [51].

On the other hand, both smoking and alcohol intake were shown to constitute risk factors for MDS [104].

2.9.5. Arsenic

Arsenic can cause mono-, bi-, and pancytopenia with marked dyserythropoiesis or megaloblastosis. Pancytopenia with trilineage dysplasia in the bone marrow mimicking MDS was reported. Associated symptoms like long-lasting gastrointestinal and neurological symptoms, arsenic in the urine analysis, favorable response to British anti-Lewisite (BAL) help the clinician distinguish between the two entities, although neurological symptoms may progress [27, 52].

2.9.6. Lead

Lead poisoning leads to sideroblastic anemia, microcytosis in addition to basophilic stippling and hemolytic anemia [27]. It can mimick MDS.

2.9.7. Other drugs

Isoniazid causes sideroblastic anemia. Antibiotiotic linezolid can give rise to vacuolization in elytroid precursors and ring sideroblasts together with anemia or pancytopenia. Chloramphenicol makes mild bone marrow suppression with ring sideroblasts and vacuolation in erythroid and granulocyte precursors. Sodium stibogluconate, prescribed in leishmaniasis causes erythroblast karyorrhexis and severe anemia [27]. Nitrous oxide (laughing gas) which has an anesthetic and recreational use and inactivator of hydroxycobalamin may give rise to megaloblastic anemia and vitamin B12-deficiency related neurological and hematological effects associated with heavy use by healthcare workers who inhalate it in operating rooms or intensive care units [27, 53].

Granulocyte colony stimulating factor (G-CSF) and GM-CSF cause neutrophil vacuolation and dysplastic neutrophils with abnormal lobulation and development of macropolycytes, in addition to neutrophilia, eosinophilia, toxic granulation and blasts in the peripheral blood, the latter mimicking progression of leukemia or MDS [54]. Antiepileptic drugs also can give rise to cytopenia and multilineage dysplasia (personal experience).

2.10. Posttransplantation

Dysplastic findings were demonstrated after solid organ transplantation, like liver, kidney, heart, and lung. Clatch et al. [55] reported bone marrow findings of 17 liver transplantation patients taken before or 1–1288 days after orthotopic liver transplantation (post-OLT) when they developed mono-, pancytopenia or fever. The patients had received cyclosporine A and prednisolone, numerous antibiotics intermittently and a few additionally received Muromonab-CD3 and/or antilymphocyte globulin.

While dysplastic hematopoiesis was completely absent from biopsies of patients with end-stage liver disease obtained before transplantation, significant trilineage dysplasia was a consistent finding in all patients who underwent OLT. Megaloblastic erythropoiesis, the most characteristic finding, macrocytosis, dysynchronous nuclear-to-cytoplasmic maturation and significant nuclear budding or bilobation were striking. Typical megaloblastic changes of the myeloid series were absent. Dysynchronous myeloid maturation, as a left shift giving rise to decreased bands and mature neutrophils, additionally dysmyelopoiesis and dysmegakaryopoiesis were evident [55].

The authors postulated that iatrogenically-induced T-cell dysfunction in transplanted patients which gave rise to alterations in microenvironment, direct pharmacological toxicities and the effects of secondary infections on hematopoietic cells might have had roles in the etiology [55].

After bone marrow or hematopoietic stem cell transplantation, bone marrow was severely hypoplastic and after successful engraftment was achieved, all hematopoietic cells, mostly the erythropoietic cells appeared dysmorphic. During the following months, striking and transient increase in hematogones, mimicking leukemia was observed. During engraftment, bone marrow architecture showed various alterations, some of which were more striking in leukemia patients whose stromal cells had been damaged during previous chemotherapies [27].

Secondary MDS may develop after autologous stem cell transplantation, due to previously damaged stem cells by chemotherapy. Cytogenetic and molecular studies are useful for distinction between secondary MDS and usual secondary myelodysplasia of early posttransplantation [27].

2.11. Other disorders with secondary dysplastic features

Multiorgan failure, autoimmune lymphoproliferative syndrome associated with Fas or Fas ligand deficiency can give rise to secondary myelodysplasia. Hypothermia can lead to sideroblastic anemia with recurrent thrombocytopenia [27].

3. Cases with hypoplastic bone marrow mimicking hypocellular MDS

3.1. Hypoplastic MDS and severe aplastic anemia (SAA)

In childhood MDS, RA (RCC) subgroup constitutes the majority of patients and bone marrow cellularity was reported decreased in nearly 50% [9] and 81% [4] of children with low-grade MDS. On the other hand, hypocellular MDS (H-MDS) in adulthood is encountered in the elderly.

Differentiation between H-MDS/hypocellular RCC and AA may be challenging both in adults and children.

There are many nonhematological factors that can give rise to bone marrow hypoplasia in childhood like any type of infections (vitamin deficiencies, metabolic disorders like mevalonate kinase deficiency, rheumatic disease, mitochondrial deletions (Pearson syndrome)). Moreover, there are many hereditary or nonhereditary hematological disorders that should be differentiated from RCC in the setting of hypoplastic bone marrow like inherited bone marrow failure syndromes [4].

The most recent histopathologic criteria to distinguish RCC from SAA in childhood is presented in **Table 7** [14, 57].

In adulthood, a similar study described standardized criteria to distinguish hypocellular AML from H-MDS and aplastic anemia (AA) (**Table 8**) [59].

	Refractory cytopenia of childhood (RCC)	Severe aplastic anemia (SAA)
Differences	1. Erythroid lineage	1. Erythroid lineage
	-Patchy distribution (clusters consist of ≥10	-Missing or a single and small cluster
	erythroid precursors)	-Clusters consist of <10 erythroid cells
	-Maturation arrest	-Erythroid cells with maturation
	-Increased mitosis	
	-Increased proerythroblasts (left shift)	
	2. Granulocytic lineage	2. Granulocytic lineage
	-Marked decrease	-Missing or marked decrease
	-Left shift	-Very few clusters of granulocytes with maturation
	3. Megakaryocytic lineage	3. Megakaryocyte lineage
	-Marked decrease	-Missing or very few
	-Micromegakaryocytes	-No dysplastic changes
	-Dysplastic findings	-No micromegakaryocytes
Similarities	1. Lymphoid lineage	1. Lymphocyte lineage
	-May be increased focally or dispersed	-May be increased focally or dispersed
	2. CD34+ cells	2. CD34+ cells
	-No increase	-No increase

Table 7. Histopathologic criteria for differential diagnosis of SAA and RCC [4, 14, 57, 105].

Manifestation	Indicative of
Presence of unequivocal blasts in the peripheral blood	MDS or AML
Hypogranular neutrophils or pseudo-Pelger neutrophils (>10%)	MDS or AML
Presence of >1–20% blasts in the bone marrow + dysplasia	MDS
Dysplasia of either granulocytes or megakaryocytes in the bone marrow (if erythroid hyperplasia is the sole finding, dysplasia in erythroid lineage should be moderate to severe)	Inconsistent with AA
Presence of any abnormal sideroblasts (>5 granules around the nucleus or constituted at least 1/3 of the circumference	Excludes AA
1–2 cm core biopsy demonstrating four to five undistorted fields (×100 magnification)	Reliability in diagnosis
Presence of two or more clusters of immature precursors (being minimum three blast per cluster) in bone marrow biopsy	MDS or AML
Consensus diagnosis required by at least 5/7 participants	Reliability in diagnosis

Table 8. Recommendation for standardized approach to distinguish hypocellular AML from hypocellular MDS and AA [59].

In addition to the parameters listed in **Tables 7** and **8**, presence of ALIP, abnormal localization of megakaryocytes, erythroblast clusters, fibrosis, abnormal karyotype were also reported as

parameters to be used to distinguish H-MDS and SAA (**Table 8**) [59]. Nevertheless, further studies are needed to examine the validity of histopathologic approach to hypocellular RCC and AA [60].

3.2. Inherited bone marrow failure (IBMF) disorders in the differential diagnosis of hypocellular RCC

Several children who are diagnosed with hypoplastic RCC, may actually have one of inherited bone marrow failure (IBMF) disorders which have not been diagnosed yet. Hence, 15% of patients diagnosed with hypoplastic RCC and 2, 5, and 10% of patients diagnosed with hypoplastic RCC or aplastic anemia were later diagnosed as Fanconi anemia and heterozygous or homozygous dyskeratosis congenita [4, 9]. Inherited bone marrow failure syndromes with pancytopenia like Fanconi anemia, dyskeratosis congenita, Shwachman Diamond syndrome, amegakaryocytic thrombocytopenia (in progression), and pancytopenia with radio-ulnar synostosis display common manifestations with hypoplastic RCC, such as macrocytosis, elevated HbF, common bone marrow features. Therefore, a careful family and past history, physical examination is essential. Laboratory and molecular studies like chromosome break-age test and telomere length assay should be carried on, since not all children with IBMF syndromes have phenotypic characteristics [4, 9]. These diseases can progress to MDS gaining chromosomal abnormalities specific to MDS and 3q26 segment in Fanconi anemia. However, abnormal clones can also regress in any IBMF syndrome [4].

In childhood, in the setting of hypocellular bone marrow with absence of cytogenetic abnormality [4] or a bone marrow biopsy with topography and cellularity of the local hematopoiesis [9], two bone marrow biopsies at least two weeks apart are necessary [4].

4. Transient chromosome abnormalities in the setting of cytopenia/spontaneous remission in MDS

4.1. Transient MDS with/without chromosomal alterations

Monosomy 7 is an harbinger of poor prognosis and higher risk of transformation to high-risk MDS and AML [4, 61–67, 69, 70] than other chromosomal abnormalities and normal karyotype [4, 105]. The estimated time of progression in children with monosomy 7 was reported as 1.9 years, with a cumulative progression incidence of 80% at the sixth year of diagnosis [4].

In the literature, we found 13 patients who presented with MDS (n:12) or MDS-like features (n:1) and had genetic abnormalities but achieved remission only after symptomatic (n:12) and vitamin B12 and folic acid (n:1) therapies. The patients had abnormalities of chromosome 7 (12 out of 13, as −7, −7q, i7) and 11q23 translocation (1 out of 13), and +21 (1 out of 13, coexistent with −7). Two additional MDS patients (RA, RAEB) with normal karyotype achieved spontaneous remission (**Table 9**).

Spontaneous disappearance of abnormal clones were reported previously in AML, EBV-associated myeloproliferative disorder and Fanconi anemia [65]. Development of cytogenetic

abnormality is only one step (first hit) in the progression of malignant clone. In order to gain growth advantage, the transforming cells need other molecular changes within the cells and in the marrow microenvironment (second hit). The cases who attained spontaneous remission suggest that the development of cytogenetic abnormality might have not been supported by the other cellular and microenvironmental changes [65, 66]. Additionally, the mutation may have developed in a hematopoietic cell with limited self-renewal capacity and may not have involved the whole stem cell pool [66]. Two cases of Bader-Meunier et al. [68] suggest that patients with MDS who do not show any chromosomal abnormality can also achieve complete remission (**Table 9**). These cases show that patients who are stable should be closely observed for some time before potentially toxic therapies are started.

Cases	Diagnosis	Age/sex	Genetic analysis	Management therapy	of Outcome /time elapsed to hematologic /cytogenetic complete remission (hem/cyto rem) (months)	Duration of follow-up after cyto rem attained (months)	Reference
1	De novo MDS[†]	8/12, M	45,XY,−7 [12]	Transfusions	<2 years (hem + cyto rem)	108 months	[61]
2	De novo MDS[†]	15/12, M	46,XY [13]/45,XY, −7 [7]	No	8 months (hem + cyto rem)	14 months	[61]
3	t-MDS[†] (after completing rabdomyo-sarcoma therapy)	10.5, M	46,XY,del (7)(q22q32) [4] (out of 40 metaphases)	No	5 months (hem + cyto rem, hem rem partial)	95 months	[61]
4	t-MDS[†] (after completing medullo-blastoma therapy)	8/M	46 XY,del(7)(q31q36) [5]/46, XY,+der(1)t(1;7)(p11.2;p11.2), -(3)/46,XY,+dic(1;21) (p11.2;p11.2), −21 [4] and other anomalies	ivIgG and transfusion	4 weeks (hem recovery; cyto rem unknown[©])	67 months	[61]
5	RAEB	14/12, M	46,XY [13]/45,XY, −7 [7]	Transfusions /BMT planned	13 months (hem + cyto rem)	21 months	[62]
6	t-MDS[†] (Spina bifida, ESRF[*] ; AZA therapy[**]	19, F	45,XY, −7 [5] (out of 62 metaphases)	AZA stopped, transfusions	17 months (hem + cyto rem)	NS[#]	[63]
7	t-MDS[†***] after completing Hodgkin's disease therapy	15, F	45, XY, −7 [19] (out of 20 metaphases)	NS[#] BMT planned	10 months (hem + cyto rem)	NS[#]	[64]
8	t-MDS[†] (after completing Ewing sarcoma therapy	19, M	46,XY,(11;16)(q23;p13.3) [13] (out of 20 etaphases)	NS[#] BMT planned	12 months (hem + cyto rem)	NS[#]	[64]

Cases	Diagnosis	Age/sex	Genetic analysis	Management of therapy	Outcome/time elapsed to hematologic/cytogenetic complete remission (hem/cyto rem) (months)	Duration of follow-up after cyto rem attained (months)	Reference
9	De novo MDS[†]	13/12, F	46,XX,i(7)(q10) [3]/46,XX [11]	NS[#]	10 weeks (hem + cyto rem)	NS[#]	[65]
10	De novo MDS[†]	3, M	45, XY, −7 [8] (out of 12 cells)	No BMT planned	30 months (hem + cyto rem)	>80 months	[66]
11	Pancytopenia bilineage dysplasia mimicking de novo MDS[‡]	7.5, M	Complex karyotype including del(7)(q11) (in 3 out of 21 cells): [*]58,XY,+2,+3,+6,+inv(7)(p13p22),del(7)(q11),+10,+11[1] [*] + 15,+17,+18,+19,+20,+22:46,XY,del(7)(q11)[1][*] 45,XY, del(7)(q11), −18 [1] (+chromatid, isochromatid breaks)	VB12 and folic acid therapy due to their deficiency	4 weeks (hem + cyto rem)	NS[#]	[67]
12	RA	8, F	Normal (46,XX) [cell no NS[#]]	No	Hem rem: NS[#]	Nearly 72 months	[68]
13	RAEB	3mo, F	Normal (46,XX) [cell no NS[#]]	No	Hem rem: NS[#]	Nearly 66 months	[68]
14	Donor cell-derived MDS after cord blood transplantation (+3 mo) for AML secondary to ALL[†]	4, F	46 XY,-7 [cell no NS[#]]; full donor chimerism; no genetic abnormality in the cord blood and the donor	Transfusions	Nearly 24 mo (hem + cyto rem)	NS[#]	[69]
15	Aplastic anemia on intravenous cyclosporine and androgen therapy	21,M	47,XX, −7,+21,+mar [cell no NS]	Drug switched to oral form. Then abnormality disappeared	Hem improvement as transfusion independence; cyto rem	Minimum 6 months	[70]

[†]The subgroup has not been reported. Probably corresponds to RCC subgroup in childhood and RCMD or MDS-U subgroups in adulthood MDS, according to the WHO 2008 classification.
©Cytogenetic abnormality found sustained on 13th month after hematologic recovery.
[*]End-stage renal failure.
[**]The patient received long-term immunosuppressive therapy with cyclosporine (for 19 months), later azacytidine (Aza) (for 6 years).
[#]Not stated.
[***]The patient had pancytopenia persisting after the 1st course of Hodgkin's disease therapy.

Table 9. Patients with MDS who achieved remission spontaneously, after supportive or vitamin B12 and folic acid therapy [61–70].

4.2. Vitamin B12 (VB12) and folic acid deficiency and transient chromosome abnormalities which mimick MDS

Vitamin B12 and folic acid deficiencies can present as mono-, bi-, and pancytopenia [106], myelodysplasia, genetic abnormalities like increased frequency of spontaneous chromosome breakage and centromere spreading [107, 108], elongation and despiralization of chromosomes [107], multiple rearrangements and deletions of different chromosomes [108] which can mimick that of MDS (**Table 9**, patient 11) [67].

Many of these chromosomal abnormalities were reported to reduce [107], completely disappear [67, 107, 108] or persist up to 6–12 months after hematological remission was attained [107] after therapy.

These cases show that defective synthesis and repair of DNA which were reversed by VB12 and folate plays role in the pathogenesis of genetic abnormalities in megaloblastic anemia.

Increased immature myeloid cells indistinguishable from myeloblasts in the bone marrow of patients with vitamin B12 and folate deficiency make the differentiation between megaloblastic anemia and MDS more difficult [109].

On the other hand, in our clinics, we have encountered considerable number of patients who had VB12 and/or folic acid deficiency coexistent with MDS or leukemia.

5. Mutations in the elderly and other cases

In more than 10% of healthy people older than 70 years, clonal hematopoiesis is present [10]. Additionally, loss of Y chromosome in hematopoietic cells in association with aging was also reported [1]. Therefore, clinicians should keep reserved when abnormalities in molecular genetics and karyotype are found in the elderly when dysplasia is absent [10].

Mutations like del(20q), +8, −Y have been reported in patients with aplastic anemia or other cytopenic syndromes who were good responders to immunosuppressive therapy and/or no evidence of MDS findings in the follow-up [1].

6. Acute myeloblastic leukemia

6.1. AML with low blast cell count

AML is distinguished from MDS by the percentage of the blasts which is higher than 30% in AML in children (>20% in adults) and lower than 30% (lower than 20% in adults) in MDS. However, patients that have blast cells lower than 30%, but cytogenetic features characteristic of childhood de novo AML [t(8;21), inv(16), t(11;17), t(9;11), i(1)] is designated as AML with low blast cell count (AML-LBC).

Those with AML-LBC were significantly younger than MDS cases (3.7 vs 7.4); their dysplasia score was lower and the response to AML type chemotherapy was higher than that of MDS patients. The authors encountered chloroma only in AML-LBC [71].

6.2. AML with myelodysplasia-related changes (AML-MRC)

In a subgroup of leukemia that was introduced by WHO is AML with myelodysplasia-related changes (AML-MRC) which defines AML arising from previous MDS or MDS/MPN, with an MDS-related cytogenetic abnormality and/or AML with multilineage dysplasia (AML-MLD). This group was reported to have worse overall survival when compared with patients with AML-not otherwise specified [110].

6.3. MDS with myelofibrosis, AML-M7, and other disorders

The blast cell percentage in the bone marrow (>30% in children and >20% in adults) and t(1;22) (p13;q13) differentiated AML-M7 from MDS.

Although hypoplastic MDS with increased reticulin ± collagen fibers is rare in childhood [4], hypoplastic myelofibrosis may be encountered in childhood [111].

7. Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of undetermined significance (IDUS)

Patients with persistent (marked constant) cytopenia(s) involving one of more hematopoietic lineages, for at least 6 months, in the setting of absent multiunilineage dysplasia and cytogenetic abnormality except –Y, +8, del(20q) were suggested to be termed as idiopathic cytopenia of undetermined significance (ICUS) [5, 58, 112–116]. Criteria of cytopenia for diagnosis of both MDS and ICUS according to the 2007 Consensus Group are: Hemoglobin (Hb) <11g/dl and/or neutrophils <1500/mm^3, and/or thrombocytes <100,000/mm^3. The cut off levels for Hb is 10 g/dl, for neutrophils 1800/mm^3 according to WHO and International Working Group on Morphology of MDS (IWGM-MDS) [58, 114].

The terms "ICUS-anemia, ICUS-neutropenia, ICUS-thrombocytopenia, ICUS-bicytopenia, or bi/pancytopenia" were also proposed in which the cut-off levels of cytopenia were the same of those in 2007 Consensus Group, except that of neutropenia which was proposed as <1000/mm^3 [114].

For patients with morphological dysplasia (>10% in a major cell line) with/without karyotypic abnormalities but no or mild cytopenia, idiopathic dysplasia of undetermined significance (IDUS) has been proposed [5, 7]. Criteria of mild cytopenia has been reported as Hb ≥ 11 g/dl, neutrophils ≥ 1500/mm^3, thrombocytes ≥ 100,000/mm^3 [113] (**Table 10**).

Patients with both ICUS and IDUS may progress to overt MDS, MPN, MDS/MPN overlap disease, chronic myelomonocytic leukemia, AML after a variable period. ICUS can also reportedly transform to systemic mastocytosis, non-Hodgkin lymphoma, aplastic anemia [5,

113, 114]. There is no proof that every patient with ICUS or IDUS will develop neoplasia [114]. ICUS was reported to resolve spontaneously also [112]. However, the median overall survival in ICUS group was reported 44.3 months, being shorter than RA but longer than RCMD [116].

	Idiopathic cytopenia of unknown significance (ICUS)	Idiopathic dysplasia of unknown significance (IDUS)
Constant marked cytopenia[*]	Present [5, 58, 112–116]	Absent [5, 58, 112–116]
Diagnostic criteria of MDS	Absent [5, 58, 112–116]	Absent [5, 58, 112–116]
Diagnostic cocriteria of MDS[**],[†]	Absent [113]; flow cytometric abnormalities: not known [112]	–?
Dysplasia (>10% of cells)	Absent [112–115]	Present [112–116]
Karyotype typical for MDS	Absent but FISH may reveal a very small clone carrying MDS-related cytogenetic defect [113, 114]	Present in a minority [112]
Clonality by HUMARA	In a minority of patients [112] (CCUS)	Not known [112]
Other diseases leading to cytopenia	No [113]	–
Other diseases leading to dysplasia	–	No [113]
Age	Older [113]	Younger [113]
Erythropoietin level	Low [113]	Adequate [113]
Marked reduction in BFU-E	In a minority of patients [112]	In a minority of patients [112]
Ring sideroblasts >15%	Absent [114]	In a majority of patients [114]

[*]Cytopenia in one or more of hematopoietic lineages lasting for at least 6 months, with Hb <11 g/dl and/or neutrophils <1500/mm^3, and/or thrombocytes <100,000/mm^3.
[**]Cocriteria of MDS: Colony forming cells and reticulocytes in circulation, abnormal immunophenotyping by flow cytometry, monoclonality of myeloid cells detected by molecular markers or mutations, abnormal gene expression profile by mRNA profiling assays.
[†]If one or more cocriteria are found, the disorder should be called 'highly susceptive for a clonal myeloid disease/MDS' [113].

Table 10. Diagnostic criteria for ICUS and IDUS [5, 58, 112–116].

On the other hand, recent reports demonstrated that ICUS had a broad spectrum including patients with both nonclonal and clonal hematopoiesis, the latter being called as clonal hematopoiesis of indeterminate potential (CHIP) [112]. Hence, 35% of ICUS patients were found to carry a somatic mutation or chromosomal abnormality indicative of clonal hematopoiesis [117] called clonal ICUS (CCUS).

Differentiation between MDS and ICUS may be challenging. In ICUS, FISH may reveal a very small clone carrying MDS-related cytogenetic defect [113, 114]. Clonal expansion of such a

small clone in ICUS and occurrence of slight cytopenia in IDUS in the follow-up, points at an imminent transformation to MDS [58, 113, 114].

These patients should be examined regularly, like in low-risk MDS, from the aspect of hematological findings, karyotype, FISH, flow cytometry, and flow FISH, if available [114].

Reduced number of colony forming unit (CFU) progenitor cells like CFU-granulocyte-macrophage (CFU-GM) and burst-forming unit-erythroid (BFU-E) show impaired bone marrow function in MDS [58]. While BFU-E is markedly reduced in MDS, it is reduced only in a minority of patients with ICUS and IDUS [114]. However, reduced numbers of CFU-GM and BFU-E are also found in aplastic anemia, acute leukemia, and in post chemotherapy conditions, but not in nonclonal cytopenias like vitamin B12 deficiency, autoimmune hemolytic anemia and chronic inflammation [58] (**Table 10**).

Screening for molecular lesions by exome sequencing and other "omics-based techniques" can be used, in order to search clonality. However, these techniques are expensive and not practical [58]. Human androgen receptor gene-based assay (HUMARA) which is promising has restrictions, since it can be used only in females and it is positive in other clonal disorders also [58, 116]. Application of flow cytometric tools are of considerable help [58] (see Section 10). Patients with CCUS can be differentiated from low-risk MDS only by lack of dysplasia [112].

The data about patients with ICUS, CCUS, and IDUS are limited. Future studies will enlighten the pathogenesis of ICUS and IDUS which is not well understood yet.

8. Autoimmune disorders

Patients with chronic immune stimulation and autoimmune disorders have a tendency to develop malignant neoplasias and MDS.

On the other hand, MDS and AML can trigger paraneoplastic syndromes and manifestations including inflammatory paraneoplasia, like seronegative rheumatoid arthritis, Sweets syndrome, hemophagocytic lymphohistiocytosis, pyoderma gangraenosum, cutaneous vasculitis, lupus-like symptoms [72, 73], polychondritis [73], Behçet syndrome, inflammatory bowel disease, cryoglobulins, vitiligo, autoimmune hemolytic anemia, peripheral neuropathy, by 12–19% in adults but in lower frequency in childhood [74].

Relevance of autoimmune disorders with prognosis of MDS is disputable. Their response to immunosuppressive therapy is good [72].

In MDS, not only the stem cells but an inflammatory microenvironment is also involved. Therefore, the inflammatory microenvironment aggravates ineffective hematopoiesis and carcinogenesis/tumorigenesis [72].

The majority of acquired AA and some RCC cases can be considered as T-cell-mediated autoimmune disease, resulting in bone marrow failure [74, 76]. Autoantibodies are detected in both conditions. However, their significance and pathophysiological significance in MDS and SAA is unclear [76].

Relative lymphocytosis, oligoclonal T-cell expansion, elevated cytokine levels are common features of AA and MDS, suggesting a common immune defect in the pathogenesis [72] of adults with low-grade MDS.

Decreased CD4 + FOXP3 + Treg cells, increased NK cells/impaired activity of NK cells, suppression of hematopoietic progenitors by cytotoxic CD8+ cells [72, 74], increased cytokines secreted by bone marrow microenvironment, macrophages (IF-, TNF-α which are pro-apoptotic), increased Th17 cells [interleukin ((IL)-17, IL-23, IL-1, and IL-6] which are cytotoxic to bone marrow precursors, decreased dendritic cells, decreased B cells [72], polyclonal hypo-, hypergammaglobulinemia, C3 hypocomplementemia, altered self-reactive antibody repertoires [74] play role in the pathogenesis, some of which change as to the risk of MDS [72].

Some of these abnormalities overlap with those in autoimmune disorders themselves. The dysmorphic features in autoimmune disorders were delineated previously. Patients with autoimmune disorders should always be suspected for being MDS cases and should be evaluated for pathologic and genetic abnormalities.

9. Common features in pathogenesis

9.1. Cytopenia

Inherited bone marrow failure syndromes, MDS and SAA share the same pathogenetic features for involving a driver mutation, overproduction of cytokines and/or suppression of hematopoiesis through cytokines and deregulation of stem cell niche [76].

MDS and severe aplastic anemia share the same pathogenesis, as to abnormalities in T cells, especially in CD8+ cells; autoimmune manifestations and giving good response to immuno-suppressive therapy [76].

9.2. Myelodysplasia in relation to cell cycle and other factors

Myelodysplasia in blood cells arise due to any challenge during the course of normal differentiation in which cells exit the cell cycle and enter G0 phase permanently (**Figure 10a**)

Cell cycle control system depends on cyclically activated cyclin-dependent protein kinases (Cdks) a number of enzymes and other proteins, the most important being cyclins and other genes [118]. Stem cell differentiation is regulated by differentiation specific genes, homeotic genes, tumor suppressor genes, abnormality of which result in restriction in further proliferation giving rise to alteration in normal cell cycle, and dysdifferentiation [119].

Hence, disturbance of expression of iron dependent genes regulating cell cycle in differentiation of hematopoietic cells in iron deficiency (see Section 2.5.2), deletion in human cell division cycle related gene (hCDCrel) in patients with del (22q11.2) are a few examples that lead to aforementioned myelodysplastic findings through genetic abnormalities. That the severity and spectrum of dysmorphic features in myelodysplasia differ according to the underlying secondary or primary pathology like in dysmegakaryopoiesis and thrombocytosis in inv

3(q21q26)/t(3;3)(q21;q26) and 5q-syndrome also reflect the various alterations playing role in different levels of differentiation.

We previously detected various temporary or permanent cell cycle abnormalities in total leukocytes, granulocytes and mononuclear cells from peripheral blood of ITP patients who had received steroids and a child with congenital neutropenia and her non-neutropenic mother all displaying myelodysplasia (**Figure 10**) [17, 45, 99].

The peripheral granulocytes (neutrophils and bands), monocytes, and lymphocytes are expected to be in G0 phase of the cell cycle, since they are terminally differentiated (**Figure 10a**). We interpreted these numerous abnormal cell cycle configurations instead of G0 [99], as an alteration in differentiation, after a stimulus that was sensed as DNA damage, probably through disruption of one or more of these aforementioned enzymes [118]. Increased trilineage apoptosis, a type of cell death in some of these patients [45, 99], all having myelodysplasia are also in accordance of this interpretation.

Overlapping of a number of myelodysplastic features (**Tables 1–4**) closely with those of senescence-like phenotype (SLP) of rapid cell senescence (RCS), another type of cell death, led us to search RCS both in congenital neutropenia [45, 99] and autoimmune disorders (JRA, SLE, and ITP) all having myelodysplasia [17, 28, 30, 31, 79]. We detected that three children with congenital neutropenia and their non-neutropenic mothers [45, 99] and all patients with autoimmune disorders displayed RCS in their leukocytes shown by β-galactosidase (SA-β-gal) positivity [79] (**Figure 1**). In several patients, cell cycle abnormalities accompanied [99] (**Figure 10I**).

The RCS that was detected in autoimmune disorders and those with congenital neutropenia [45] were attributed to increased proinflammatory cytokines and chemokines in autoimmune disorders [79] and congenital neutropenia [45] through giving rise to loss of telomeres by keeping the immune system in a state of low level of activation [79]. Absence of RCS (unpublished data) in iron deficient patients was thought to be due to absence of increase in proinflammatory cytokines, confirming this hypothesis.

Differentiation is controlled not only by intracellular genetic factors but by extracellular factors like extracellular matrix and soluble factors like fibroblast growth factor (FGF), transforming growth factor beta (TGFB), colony stimulating factor-1 (CSF-1), GCSF, GM-CSF, stem cell factor (SCF), Fms-like tyrosine kinase 3 ligand (Flt-3 ligand), ILs as well [119].

The reasons that we previously discussed in secondary myelodysplasia like immune destruction of stromal and hematopoietic cells, inhibitory effects of cytokines and/or other intracellular/extracellular messengers/soluble factors, on the microenvironment and/or hematopoietic cells; decreased differentiation, regeneration and increased clearance of stem cells, reflected by alterations in cell cycle, impairment of heme synthesis and iron utilization also play role in dysdifferentiation.

Therefore, myelodysplasia either primary or secondary is associated with cell death parameters, and cell cycle alterations. It reflects the viability of the cell and can be assumed as a tip of a big iceberg that is an harbinger of a large spectrum of primary (clonal) and secondary

(nonclonal) disorders. We think that the criteria of dysplasia should be revised in the definition of MDS and attention should be exercised to find out practical laboratory means to detect clonality.

10. Differential diagnosis

Discrimination between low-risk MDS and disorders mimicking MDS depends on determining whether hematopoiesis is clonal or not.

To assess clonality and dysplasia, flow cytometric evaluation is promising [58, 120, 121]. The minimal requirements to assess dysplasia by flow cytometry have been defined for adulthood low-risk MDS as in the following [120]:

(a) For immature myeloid and monocytic progenitors: Increased percentage of cells in nucleated cell fraction, lack of/decreased/increased expression of CD45, CD34, CD13 + CD33, homogenous under/overexpression of CD117, lack of/increased expression of HLA-DR, asynchronous expression of CD11b, CD15, expression of CD5, CD7, CD19, CD56 which are lineage infidelity markers.

(b) For maturing neutrophils: Decreased percentage of cells as ratio to lymphocytes, sideward light scatter (SSC) as ratio vs SSC of lymphocytes, altered pattern in relationship of CD13 with CD11b and CD13 with CD16, CD15 with CD10 (like lack of CD10 on mature neutrophils).

(c) For monocytes: Decreased or increased percentage of cells, shift toward immature distribution, altered pattern in relationship of HLA-DR with CD11b and CD36 with CD14, homogenous under or overexpression of CD13 and CD33, expression of CD56 as which is a lineage infidelity marker.

(d) Progenitor B cells: Decreased or absent progenitor B cells when enumeration is performed as fraction of total CD34+ based on CD45/CD34/SSC in combination with CD10 or CD19.

(e) Erythroid compartment: Increased percentage of nucleated erythroid cells, altered pattern in relationship of CD71 with CD235a, decreased expression of CD71 and CD36, increased percentage of CD117-positive precursors.

(f) For megakaryocytes: No standard application of flow cytometry has been described for megakaryocytes yet.

World Health Organization recognized more than three flow cytometric aberrancies as indicative of MDS [120]. It was also reported that two or more aberrancies in only the four parameters as increased percentage of CD34+ progenitor cells in bone marrow, decreased number of progenitor B cells within the CD34+ compartment, decreased or increased CD45 expression on myeloid progenitor cells and decreased SSC of neutrophils, CD10, CD15, CD11b, CD56 being additional useful markers could identify 70% of low-risk MDS cases with 94% specificity [120]. In children, for distinction between SAA and RCC, which generally present with hypocellular bone marrow, a cutoff of 2 flow cytometric abnormalities was found to have

60% sensitivity and 88% specificity which changed as 76% and 84% respectively, when combined with other diagnostic parameters [121]. In nonclonal disorders either no or only one flow cytometric abnormality was found. In low-risk MDS, in rare patients no flow-cytometric abnormality was found [58].

However, abnormal flow patterns were encountered in AML, MPN, and natural aging also [58]. Current knowledge on normal and abnormal patterns in the elderly and in normal controls is still inadequate [120].

The flow cytometric analysis of children with RCC (low-risk MDS in childhood) showed no difference in the relative SSC of granulocytes between those in RCC and healthy controls and absence of lineage infidelity markers on myeloid blasts unlike commonly occurring in adult low-risk MDS. The most frequent abnormality in RCC was reported to be heterogeneous expression of CD71 and CD36 on erythrocytes and aberrant expression of CD56 on monocytes (in 58 and 20%). All other abnormalities were observed in RCC in lower frequency than in adulthood low-risk MDS [121].

However, although flow cytometric evaluation is promising in diagnosis of MDS cases which lacked specific diagnostic markers like ring sideroblasts or karyotypic aberrations, it can only be used as a part of a diagnostic work-up consisting of histopathology and cytogenetic analysis [120, 121].

Flow cytometry can be used to rule out PNH; but minor PNH clones are present in 13–23% of adult MDS, and 41% of children with RCC [121].

11. Future recommendations

We recommend that all the aforementioned disorders be considered in the differential diagnosis of MDS. Patients who do not comply with none of a definite diagnosis should be followed-up for a considerable time period in order to assure a spontaneous remission or progression. Spontaneous remission in children and youngsters were reported as 2.2–30 months after the diagnosis [61–68]. In childhood, in the setting of hypocellular bone marrow with absence of cytogenetic abnormality [4] or a bone marrow biopsy with topography and cellularity of the local hematopoiesis [9], two bone marrow biopsies at least two weeks apart are necessary [4]. For cases with refractory cytopenia and cases with less than 15% ring sideroblasts all of which display unilineage dysplasia without excess blasts, repeated bone marrow examination is recommended after a 6 months' observation [5].

Since morphologic dysplasia can be encountered in both clonal and nonclonal disorders, the criteria of dysplasia should be revised in the definition of MDS and attention should be exercised to find out practical laboratory means to detect clonality. Flow cytometry is a promising means to distinguish between clonal and nonclonal cytopenia when used together with other diagnostic tools.

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