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Original Research Paper

Pathology

HEMATOLOGICAL , BONE MARROW AND CYTOGENETICS CHARACTERIZATION IN MYELODYASPLASTIC SYNDROMES

Bibhuti Bhusan Prasad — Assistant Professor, Department of Pathology, Anugrah Narain Magadh Medical College, Gaya , Bihar

Subhash Chandra Jha — Assistant Professor , Department of Pathology, Government Medical College, Bettiah, Bihar

Guari Shanker Lal* — Assistant Professor, Department of Pathology, Anugrah Narain, Magadh Medical College, Gaya , Bihar. *Corresponding Author

ABSTRACT Myelodysplastic syndromes (MDS), also known as myelodysplastic neoplasms, are clonal disorders characterized by a maturational defect in bone marrow progenitor cells,ineffective hematopoiesis with peripheral cytopenia(s), and clonal cytogenetic change with an enhanced risk of transformation to secondary acute myeloid leukemia. CBC , PBS , Bone marrow aspiration , bone marrow biopsy and cytogenetic findings of 10 cases of MDS were collected and studied. MDS is major health problem in old population and diagnosis is challenge because hematopathologist could not give even provisional diagnosis unless until proper clinical note and differential diagnoses are not written in prescription. CBC , PBS , bone marrow aspiration, biopsy and cytogenetics are complimentary to each other.

KEYWORDS : MDS, Pancytopenia, Ineffective hematopoiesis, dysplasia , cytogenetics

INTRODUCTION:

Myelodysplastic syndromes (MDS), also known as myelodysplastic neoplasms, are clonal disorders characterized by a maturational defect in bone marrow progenitor cells,ineffective hematopoiesis with peripheral cytopenia(s), and clonal cytogenetic change with an enhanced risk of transformation to secondary acute myeloid leukemia (sAML) [1-3]. MDS are classified according to criteria of the World Health Organization (WHO) proposal [4-6]. The WHO classification of MDS is updated in 2016 and provides criteria for the discrimination of MDS variants from each other [6].The WHO classification relies on the degree of dysplasia and blasts percentage for disease classification and specific cytopenias have minor impact on classification of MDS(Table1). So in recent 2016 revision, refractory anemia and refractory cytopenia are removed. In addition, minimal diagnostic criteria for MDS have been proposed [6,7]. These criteria allow discrimination of MDS from all other neoplastic or reactive disorders that can also produce cytopenia(s) or/and dysplasia. One of the biggest challenge is to separate MDS from reactive causes of cytopenia and dysplasia Diagnostic criteria for MDS include a) persistent significant cytopenia(s), b) bone marrow or cytogenetic evidence of myelodysplasia, and c) exclusion of all other conditions and disorders producing cytopenia(s) and/or dysplasia [7].The dysplastic cells in any lineage must be at least 10%.

The diagnosis MDS is established in a step-wise procedure In a first step, minimal diagnostic criteria need to be fulfilled. Then, the subtype of MDS according to WHO criteria should be defined. Next, the patient is examined for individual risk factors and scores, in order to establish the overall risk profile, preferably by the international prognostic scoring system (IPSS) [8] and WHO-prognostic scoring system (WPSS) [9].

In most patients with MDS, peripheral blood smears (PBS) shows either refractory anemia or variable cytopenia and the bone marrow (BM) smear reveals marked dysplasia in one or more major hematopoietic lineage/s (erythroid, granulocytic, mega-karyocytic)[10,11]. However thrombocytosis may be found in **5q deletion.** Monocytosis in peripheral blood or/and an increase in blast cells may also be detected in BM smears [10,11]. Typical complete blood count abnormalities found in MDS include macrocytic anemia, bi- or pancytopenia, and signs of dysplasia such as abnormal hypogranulated or/and hypolobated neutrophils (e.g. Pseudo-Pelger-Huet cells). In most patients, a provisional diagnosis of MDS can be established on examination of blood and BM smears.

In other patients, no prominent dysplasia is found but an abnormal karyotype is detected in refractory cytopenia, leading to the posibility the patient may suffer from MDS [7]. However, there are also patients with normal karyotype in whom it is difficult to define whether cytopenia or dysplasia would indeed result from an underlying MDS, a prephase of MDS, or from another hematologic or even non-hematologic disease [7]. In other patients, it is difficult to discriminate between advanced MDS and AML, or a myelodysplastic/myeloproliferative overlap disease (MDS/MPN)

In all these instances, histological and immunohistochemical examination of BM biopsies, a diagnostic approach which was often underestimated in the past, contributes essentially to the diagnosis, classification, and prognostication of (suspected/provisional) MDS [7,12,13]. In order to discuss current standards in the evaluation of MDS by histology and immunohistochemistry, a Working Conference was organized in June 2010. The participating faculty discussed current and novel diagnostic procedures and markers, related criteria, and diagnostic algorithms. The outcome was formulated into consensus statements. The level of consensus was defined as percent agreement (percent of faculty members agreed). A summary of consensus statements and related recommendations are presented in this article.

Table1. 2016 Revision to the WHO classification of Myelody splastic Syndrome

Name	Dysplastic lineage	cytopenias	Ring sideroblasts	BM & PB blasts	cytogenetics
MDS-SLD	1	1 or 2	<15%/<5%	BM<5%,PB<1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated deletion(5q)
MDS-MLD	2 or 3	1-3	<15%/<5%	BM<5%,PB<1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated deletion(5q
MDS-RS-SLD	1	1 or 2	>15%/>5%	BM<5%,PB<1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated deletion(5q
MDS-RS-MLD	2 or 3	1-3	>15%/>5%	BM<5%,PB<1%, no Auer rods	Any, unless fulfils all criteria for MDS with isolated deletion(5q
MDS(del5q)	1-3	1-2	None or any	BM<5%,PB<1%, no Auer rods	Del(5q) alone or 1 additional change except -7 or del7q
MDS –EB1	0-3	1-3	None or any	BM 5-9%,PB2-4%,no Auer rods	ANY
MDS-EB2	0-3	1-3	None or any	BM 10-19%,PB5-19% or Auer rods	ANY
MDS-U with 1% blast	1-3	1-3	None or any	BM<5%, PB<1%, no Auer rods	ANY
MDS-U with SLD & Pancytopenia	1	3	None or any	BM<5%, PB<1%, no Auer rods	ANY
Defining cytogenetic	0	1-3	<15%	BM<5%, PB<1%, no Auer rods	MDS defining abnormality
RCC	1-3	1-3		none	Any

Cytopenias defined as: hemoglobin <10gm/dl, platelet<100x10^9/L, ANC<1.8x10^9/L,PB monocyte<1x10^9/L

MATERIALS AND METHODS:

CBC , PBS , Bone marrow aspiration , bone marrow biopsy and cytogenetic findings of 10 cases of MDS were collected from tertiary teaching institutes of Bihar and data are analyzed .The findings were correlated with recent 2016 revision of MDS classification and only those cases were included in this study that morphologically and cytogenetically fit for diagnosis as per WHO recent classification. The age and sex of patients were also recorded.

RESULT:

All cases in this study were >50 years and male outnumbered the female. The male female ration was 2:1. The 3 cases had refractory pancytopenia (multilineage dysplasia) and one had bicytopenia with normal platelet count with 5q deletion. Three patients had multilineage dysplasia and other 4 had bilineage dysplasia. Two patients had single lineage dysplasia and one had no dysplasia in peripheral blood smear or bone marrow smear or biopsy. One patient presented with bicytopenia with normal platelet count with 5qdeletion on FISH interphase. Other patients presented with different types of cytogenetic of FISH aberrations (Table 2)

Table 2.CBC , Bone marrow or Cytogenetic findings in MDS

CASE NO.	TLC	HB	PLATELETS	MCV	DYSPLASIA	PB BLASTS	BM BLASTS	Cytogenetic	Diagnosis
1	1.5	4	10	100	+++	<1%	<5%	-7	MDS-MLD
2	2.2	5	35	105	+	<1%	<5%		MDS-SLD
3	0.9	3	15	110	+++	<1%	<5%		MDS-MLD
4	1.6	5	180	104	++	<1%	<5%	5q deletion	MDS with del5q
5	1.9	7	158	85	+	<1%	<5%	-Y	MDS-RS-SLD
6	2.1		25	105	++	<1%	<5%	+8	MDS-MLD
7	2		10	100	++	2%	7%		MDS-EB-1
8	1.5		12	105	++	10%	15%		MDS-EB-2
9	2.4		30	108	+++	<1%	<5	20q-	MDS-MLD
10	3.0		45	80		<1%	<5%		MDS-RS-MLD

+=number of lineage dysplasia, MDS= myelodysplastic syndrome, SLD= single lineage dysplasia , MLD= multilineage dysplasia, RS= ringed sideroblasts , EB= excess of blasts

Fig.1 BM with megaloblastic reaction

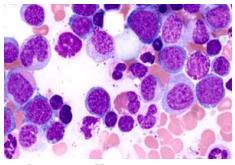
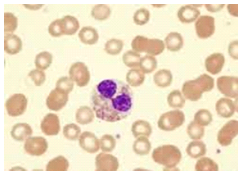


Fig.2. PBS with pelget heut cell & pancytopenia



Review of Literature and Discussion:

The application of immunohistochemical (IHC) markers is recommended in all patients with (suspected) MDS [7,12,14]. Because of subclone-formation and phenotypic diversity, it may sometimes be necessary to apply multiple markers for one lineage (cell type) even in the same patient. The participants agreed that all major BM lineages should be examined by immunohistochemistry in (suspected/provisional) MDS. The minimal panel recommended for all patients includes CD34 (progenitor/precursor cells), CD117/KIT (progenitor/precursor cells, mast cells), tryptase (mast cells, immature basophils), one megakaryocyte marker (CD61 or CD42b), CD20 (B-lineage), CD3 (T cells), and glycophorin-A or -C . Additional (lineage-specific) markers are applied depending on initial staining results and further clinical and laboratory parameters. Such additional markers are essential when the diagnosis MDS is in question or another co-existing neoplasm is suspected. Sometimes, the application of an antibody against myeloperoxidase (MPO), CD25, CD33, or lysozyme is helpful [12]. Using the minimal marker-panel proposed, the pathologist can also study endothelial cells (CD34+/CD31+) and may report on microvessel density [17].

The CD34 stain is useful for the detection of clusters and/or aggregates of immature myeloid cells which should be reported if present [18,19]. In instances where blast cells are CD34-negative cells, KIT is recommended as an alternative (additional) marker [7,12]. However, because KIT is also expressed by a proportion of proerythroblasts [20], evaluation may be difficult in erythroid-rich cases. The faculty agreed that it is essential to report on the estimated percentage of CD34+ cells (percent of all nucleated cells) in each case of (suspected or overt) MDS. The faculty also agreed that any multifocal accumulation (abnormal clustering) of CD34+ cells ,must be regarded abnormal and potentially indicative of an MDS. Without immunostaining, i.e. by morphology alone, it is quite difficult to identify an abnormal localization of immature precursor cells (ALIP) [21], in particular when there is sub-optimal fixation of the trephine. An easier and probably more accurate feature to describe and record in (suspected/provisional) MDS is the 'Abnormal Multifocal Accumulation (clustering) of CD34+ precursor cells' (AMA-CD34) (Figure 1,1A), which should thus replace the reporting on ALIP [7,12]. Nevertheless, histologic blast cell recognition remains important because subpopulations or (rarely) the entire population of blasts may be CD34-negative cells. If an increase of blast cells can neither be documented by histomorphology nor in bone marrow smear.

Megakaryocyte-reactive antibodies are useful for the visualization of normal and abnormal megakaryocytes, and their (abnormal) accumulation in the BM [12,14]. Both small-sized megakaryocytes (dwarf forms including micromegakaryocytes) and megakaryoblasts can be identified using this approach . Notably, in almost all patients with MDS, megakaryocytes show both atypical cytologic features and abnormal distribution [7,12]. The faculty agreed that CD61 and CD42b can be considered as 'standard megakaryocyte markers' in MDS . The linker for activation of T cells (LAT), van Willebrand factor (vWF, factor VIII antigen), CD25, and CD31 are also expressed in megakaryocytes. Furthermore, the CD34 antigen may be detectable in (immature) megakaryocytes and megakaryoblasts in MDS. However, CD34-expression is not a specific feature of MDS-megakaryocytes. On the other hand, most megakaryocytes in the normal/reactive BM usually are CD34-negative, so that a clear-cut expression of CD34 in a majority of megakaryocytes must be regarded as phenotypic aberrancy supporting the conclusion the patient suffers from a myeloid neoplasm such as MDS.

The diagnosis of MDS is primarily based on the presence of persistent (of at least 6 months duration) cytopenia(s), cytomorphologic dysplasia (10% or more) in one or more major BM lineages (Fig.1 & 2) (erythroid, granulocytic, megakaryocytic), and exclusion of other potential disorders that can produce cytopenia and dysplasia [7]. To address these criteria and thus establish the exact diagnosis, it is essential to examine a representative bone marrow(BM) smears ,biopsy and immunohistochemistry. First, the BM histology may reveal a myeloid neoplasm other than MDS, or MDS with a coexisting neoplasm (hematopoietic or non hematopoietic). Likewise, in patients with provisional RAEB-2, the BM biopsy may reveal a final diagnosis of AML , by demonstrating sheets of CD34+ cells focally. In other cases of (provisional) MDS, a co-existing systemic mastocytosis (SM) will be detected, leading to the final diagnosis of SM-MDS [12]. Another example is the discrimination between aplastic anemia, hypoplastic MDS, and hypoplastic AML [3]. Again, the final diagnosis in these patients cannot be established without a thorough investigation of BM sections. Finally, the BM histology may reveal a myeloproliferative neoplasm or an MDS/MPN overlap disease, which can be accompanied by the *JAK2* mutation V617F [25].

After having excluded other (differential) diagnoses in a cytopenic patient, the pathologist will examine the BM smears for features of dysplasia in detail. Whereas dysplasia of erythroid cells and neutrophils is examined preferentially in BM and PB smears, megakaryocyte dysplasia can often be assessed more accurately in BM sections [3,12,7]. This is often essential, especially when BM smears contain only a few megakaryocytes. Dysplasia should count

as an MDS-specific criterion, when ≥ 10% of cells in a given lineage show clear signs of dysplasia, as has been proposed by the WHO and other working groups [5,7]. However, as mentioned above signs of dysplasia in one or even more lineages may also be recorded in a variety of other hematopoietic and even non-neoplastic conditions, such as vitamine B12 or folate deficiency, viral infections, or chronic inflammation.

It may be essential to detect megakaryocyte dysplasia in the BM histology. Thus, the diagnosis of multi-lineage dysplasia, a major diagnostic determinant in the WHO classification [5] often depends on assessment of megakaryocytes in BM sections because BM smears often contain only low numbers of megakaryocytes in MDS. The presence of micromegakaryocytes) and abnormalities in their distribution as frequently seen in MDS can be best established on BM biopsy immunohistochemistry with one or more megakaryocyte marker(s) such as CD61, CD42b, or CD31. Small megakaryocytes with markedly hypolobated nuclei ('mononuclear megakaryocytes') are typically found in patients with the 5q- anomaly. However, there is no absolute correlation between a particular megakaryocyte-morphology and a certain cytogenetic abnormality in MDS.

An important diagnostic approach in MDS is the evaluation of CD34+ progenitor/precursor cells in BM histologic sections. This approach is helpful (often essential) for the delineation between low risk MDS (MDS-SLD, MDS RS-SLD, MDS-ML D) and high risk MDS (RAEB-1, RAEB-2) [18,19]. In each case, the estimated percentage of CD34+ cells (called blast cells when BM smears confirm blast cell morphology) should be reported. Abnormal multifocal accumulation of CD34+ cells (AMA-CD34) is only seen in patients with high risk MDS [3,7,12]. In case of CD34-negative progenitor cells (blasts), KIT/CD117 can also be employed as alternative marker antigen. However, KIT is also expressed on other BM cells including mast cells and a subset of (immature) erythroblasts [20].

Another proposed subtype of MDS that can only be diagnosed by histology is hypoplastic MDS [3,11]. The faculty agreed that this subtype should be recognized as a separate variant of MDS and should be defined by robust criteria. In fact, MDS should be called hypoplastic MDS when a) minimal diagnostic criteria for MDS [7] are fulfilled, b) the BM section is hypocellular compared to age-matched normal BM cellularity [16], and c) causes and therapies producing transient cytopenia have been excluded. Cases of therapy-related MDS can also present with hypocellular marrows. Sometimes, it may be difficult to differentiate between hypoplastic MDS and hypoplastic AML [3,13]. Histologic or immunohist ochemical identification of immature precursor/blast cells (by antibodies against CD34 and KIT) is essential in identifying these cases, and to established the final diagnosis of hypoplastic MDS or hypoplastic AML [12,24].

There are a number of important prognostic parameters that should be addressed and reported by the hematopathologist when evaluating BM sections in MDS patients. Most important prognostic histopathologic variables in MDS are the presence of AMA-CD34 (increase in CD34+ cells), marked BM fibrosis, and an overt MDS/MPD overlap disease, including myelomastocytic transformation.

A diagnostic challenge are patients who do not fulfil minimal diagnostic criteria for MDS but are suffering from persistent (> 6 months) cytopenia or exhibit unexplained dysplasia without marked cytopenia. In these patients, repeated BM investigations and an extensive search for an underlying disease are usually initiated. Repeated tests in the follow up may reveal an underlying hematologic or non-hematologic disease or imminent MDS. If this is not the case, a provisional diagnosis should be established: in those with marked and persistent cytopenia (hemoglobin <10 g/dL and/or neutrophils <1,000/µL and/or platelets <100,000/µL) but no evident dysplasia (<10% of cells in major BM lineages) the diagnosis Idiopathic Cytopenia of Undetermined (Uncertain) Significance (ICUS) is established [7]. In those patients who have marked dysplasia (≥ 10% in at least one major lineage) with or without an MDS-related karyotype but no or only mild cytopenia, the term Idiopathic Dysplasia of Undetermined (Uncertain) Significance (IDUS) should be applied . By definition the presence of both ICUS and IDUS is exclusive since coexistence of these conditions is diagnostic and meets criteria for MDS [57]. Some of these IDUS patients progress to frank MDS over time, whereas others may progress to a myelodysplastic/myeloproliferative neoplasm. All patients with ICUS and IDUS should have a hematologic follow-up in order to document or exclude evolution to MDS. One of the most important diagnostic investigations in patients with IDUS and ICUS is the histopathological examination of the BM. In fact, the diagnosis ICUS can only be established when the hematopathologist confirms the absence of dysplasia, and excludes all other BM disorders including aplastic anemia and hairy cell leukemia. For the same reason, the diagnosis IDUS is also dependent on the final report of the hematopathologist who has to exclude a number of differential diagnoses and can confirm multilineage dysplasia. The faculty also discussed minimal diagnostic criteria for IDUS, and concluded that the presence of dysplasia in at least two BM lineages would allow for a more proper diagnosis of IDUS than has been proposed before, where mild dysplasia in only one BM lineage might still be a questionable condition, not fulfilling the criteria of a clearly dysplastic myelopoiesis.

An important diagnostic approach in patients with ICUS, IDUS, and MDS, is fluorescence in situ hybridization (FISH) of BM interphase cells, especially when conventional chromosome analysis showed a normal karyotype or yielded unclear results. In several of these patients, FISH may reveal the presence of a small population of clonal cells carrying an MDS-related cytogenetic defect [15]. Sometimes, when recorded over time, the size of the clone (number of "FISH-positive" cells) may increase, BM function (i.e. the number of colony-forming progenitors) decreases, and MDS can then be diagnosed. Although IDUS may not be a rare condition, the number of well-documented cases is very low. Similar to patients with ICUS, patients with IDUS should have a hematologic follow up in order to document or exclude evolution to MDS. BM studies should be repeated when cytopenia develops or other signs for evolution to frank MDS are found. However, not all patients with ICUS or IDUS develop MDS even when recorded over many years.

CONCLUSION:

MDS is major health problem in old population and diagnosis is challenge because hematopathologist could not give even provisional diagnosis unless untill proper clinical note and differential diagnoses are not written in prescription. CBC , PBS, bone marrow aspiration and biopsy are complimentary to each other , but in few cases one of the modality of diagnosis independently could clinch the final diagnosis. Clinicaly ,shortness of breath with refractory cytopenia , lasting more than 6 months give provisional diagnosis of MDS that can be approved with bone marrow findings and cytogenetics. . A recommended approach is to proceed in a step wise fashion. In a first step, the diagnosis MDS should be confirmed by minimal diagnostic criteria. In a second step, the WHO classification is applied to define the disease subtype. Important prognostic markers which should always be integrated in the report include the presence and grade of BM fibrosis and the AMA-CD34. Then, the IPSS or WPSS are applied for prognostication.

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