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Original Article

Idiopathic bone marrow dysplasia of unknown significance (IDUS): definition, pathogenesis, follow up, and prognosis

Peter Valent[1,2], Eva Jäger[3], Gerlinde Mitterbauer-Hohendanner[3], Leonhard Müllauer[4], Ilse Schwarzinger[3], Wolfgang R. Sperr[1,2], Renate Thalhammer[3], Friedrich Wimazal[5]

[1]Department of Internal Medicine I, Division of Hematology & Hemostaseology, Medical University of Vienna, Austria; [2]Ludwig Boltzmann Cluster Oncology, Vienna, Austria; [3]Department of Laboratory Medicine; [4]Institute of Pathology, and [5]Department of Obstetrics and Gynaecology, Medical University of Vienna, Austria.

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Abstract: Minimal diagnostic criteria for myelodysplastic syndromes (MDS) include constant cytopenia recorded for at least 6 months, dysplasia, and exclusion of other causes of cytopenia and dysplasia. However, there are patients with dysplastic bone marrow features with or without a karyotype, who have only mild if any cytopenia. This condition has been termed idiopathic dysplasia of unknown significance (IDUS). Out of a series of 1,363 patients with suspected MDS or mild cytopenia seen between 1997 and 2010, we have identified 10 patients with IDUS, and analyzed their clinical course and outcome as well as features potentially involved in disease-evolution. Follow-up ranged between 2 and 13 years. Progression to an overt myeloid neoplasm was observed in 4 patients: two progressed to frank MDS, one to chronic myelomonocytic leukemia, and one to a myelodysplastic/myeloproliferative neoplasm exhibiting 5q- and *JAK2* V617F. Consecutive studies revealed that most IDUS patients have an adequate production of erythropoietin (EPO) and sufficient numbers of EPO-responsive erythroid progenitors, features rarely seen in MDS. The erythropoiesis-promoting *JAK2* mutation V617F was only detectable in one case. We hypothesize that the dysplastic clone in IDUS cannot manifest as frank MDS because i) the clone retains responsiveness against EPO, and ii) an adequate EPO-production counteracts anemia. Evolution of IDUS to low risk MDS may thus depend on the biological properties of the clone as well as patient-related factors such as EPO production. The latter often decreases with age and may thus explain why MDS often manifests in the elderly.

Keywords: MDS, diagnostic criteria, IDUS, EPO, BFU-E

Introduction

Myelodysplastic syndromes (MDS) are hematopoietic stem cell disorders defined by a maturation defect in myeloid progenitor cells, peripheral cytopenia, and clonal instability with enhanced risk to transform into acute myeloid leukemia (AML) [1-4]. In the past, i.e. before 2001, MDS have been classified according to criteria provided by the French-American-British (FAB) cooperative study group [5]. In 2001 and 2008, the World Health Organization (WHO) has extended the FAB classification and has introduced additional criteria for defining subvariants [6,7].

However, although solid criteria for the discrimination of MDS-variants from each other have been provided by the FAB group and by the WHO, it is often difficult to decide whether a patient with mild cytopenia is suffering from low risk MDS, from an unrelated hematopoietic or non-hematopoietic disease, or has abnormally low blood counts without an underlying disorder [8]. In other patients, it may be difficult to discriminate between MDS and a myelodysplastic/myeloproliferative (MDS/MPN) overlap neoplasm [8]. To overcome the problem of the "diagnostic interface", minimal diagnostic criteria for MDS have been discussed and proposed by several working-groups [7-9]. In resulting proposals, constant cytopenia was considered to be a pre-requisite for the definition of MDS [7-9].

There are two conditions that do not meet minimal diagnostic criteria for MDS although cytopenia or dysplasia is recorded: idiopathic cyto-

penia of unknown significance (ICUS) and idiopathic dysplasia of unknown significance (IDUS) [8-10]. Both conditions may progress to frank MDS over time [8-10]. Therefore, once diagnosed, these patients should have a hematologic follow up.

Patients with IDUS are younger or older patients in whom clonal hematopoiesis is established and may replace normal marrow, but blood counts still remain normal or near-normal over years [11,12]. Although a hypothesis about cytokine-regulation has been proposed, little is known about the pathogenesis and factors that keep clonal hematopoiesis in an 'IDUS-state' without cytopenia and thus without transformation to an overt MDS. It has been hypothesized that at least in some of these patients, the response of clonal erythropoietic progenitor cells as well as erythropoietin (EPO) production remain 'normal' or at least adequate over time, and that both factors act together to keep the hemoglobin level within a normal or subnormal range, so that no frank anemia and thus no overt MDS develops [12]. However, this hypothesis has not been tested formally, and no data documenting EPO-responses have been presented so far. In the present study, we have tested the 'EPO-hypothesis' in a cohort of patients with IDUS. Independent of age, adequate EPO production as well as a measurable response of burst- or colony-forming erythropoietic progenitor cells to EPO could be documented in all cases with IDUS.

Patients and methods

Identification of patients with IDUS

In a series of 1,363 consecutive patients with cytopenia of unknown etiology or/and suspected MDS seen in our department between 1997 and 2010, we have identified 10 well documented cases with IDUS. Of these 10 individuals, 7 were females and 3 were males (**Table 1**). At diagnosis, the age in this group ranged between 33 and 83 years (median: 69 years). The follow-up ranged between 2 and 13 years. The patients' characteristics are shown in **Table 1**. Two illustrative cases are described below.

Investigations at diagnosis and in the follow Up

All individuals were referred because of suspected MDS with or without cytopenia. In case #9, transient leukocytopenia, Pseudo-Pelger-Huet cells in the blood, and an elevated serum tryptase level (29.1 ng ml^{-1}) were known before bone marrow biopsy; this patient was referred because of suspected mastocytosis or/and MDS. At the time of diagnosis, the following investigations were performed: a bone marrow biopsy and aspiration, complete blood counts and differential counts, serum chemistry including tryptase and lactate dehydrogenase (LDH), and erythropoietin (EPO). In addition, the numbers of circulating and bone marrow-derived colony-forming progenitor cells (CFU-GM, BFU-E, CFU-GEMM) were determined in a methylcellulose assay according to published protocols [13,14]. Bone marrow cells were examined for signs of dysplasia on Wright-Giemsa stained smears. The percentage of dysplastic cells (erythroid, neutrophilic, megakaryocytic) as well as cell atypia in the monocytic, eosinophilic, and mast cell compartments, were recorded. The percentage of ring sideroblasts was determined on Prussian blue-stained bone marrow smears. In addition, standard cytogenetic analysis and FISH were performed. Flow cytometry was performed to determine the percentage of CD34+ cells in most cases, and abnormal expression of PI-linked antigens in patient #1 (suspected Coombs-negative hemolysis). Bone marrow sections were examined using standard histologic and immunohistochemical parameters [8,15,16]. Immunohistologic parameters included CD34, tryptase, KIT, CD14, and one platelet-related antigen (CD42 or CD61). The Gomori-stain was also applied in all cases. Molecular analyses included PCR assays for the detection of JAK2 V617F (all patients) and KIT D816V (in case of mast cell atypia). Further staging investigations included an ultrasound of the abdomen (size of liver and spleen), virus serology, and serum vitamin B12- and folate concentrations. Follow-up paramters included a complete blood count and serum chemistry. Depending on the course, all parameters recorded initially (i.e. at first presentation) were repeated in the follow-up. In case of suspected disease-manifestation (MDS), bone marrow investigations were repeated. All patients gave written informed consent before bone marrow biopsy and before blood samples were analyzed.

Case Reports

Case #1: An 83 year old female patient was referred because of mild macrocytic anemia in

Table 1. Patients' Characteristics and Outcome in the Follow-Up (FU)

Case No #	f/m	Age yrs	FU yrs	Karyotype	BFU-E /µl PB	CFU-GM /µl PB	WBC G L^{-1}	PLT G L^{-1}	Hb d dL^{-1}	EPO U L^{-1}	MCV fL	BM Dysplasia Gran	Ery	Mega	Outcome in FU (time to progress)
#1	f	83	03	normal	275	11	6.40	231	11.1	31.2	103.5	+	++	-	MDS/RARS (2 yrs)
#2	f	74	08	5q-	53	98	5.40	385	11.0	71.5	104.6	-	+	+	JAK2 V617F+ MDS/MPN-U (6 yrs)
#3	m	63	11	normal	97	217	2.60	115	12.4	33.1	81.5	++	-	+	CMML (6 yrs)
#4	m	59	03	normal	412	69	6.00	198	11.3	89.5	109.1	-	++	-	MDS/RARS (2 yrs)
#5	m	67	04	normal	76	44	3.22	133	12.3	18.0	90.8	++	+	-	IDUS
#6	f	36	13	complex	110	106	4.67	143	12.8	n.a.	92.5	+	++	-	IDUS
#7	f	80	07	normal	219	189	6.05	195	11.4	55.5	98.2	-	+	-	IDUS
#8	f	33	07	normal	436	46	4.00	124	10.8**	27.0	87.6	-	+	+	IDUS
#9	f	69	02	n.a.	747	35	5.56	186	13.3	09.7	91.4	+	-	-	IDUS
#10	f	73	11	normal	173	39	2.90	136	11.5	10.0	83.1	-	+	-	IDUS

f, female; m, male; FU, follow-up; yrs, years; BFU-E, burst forming erythroid progenitor cells; CFU-GM, granulocyte-macrophage colony-forming progenitor cells; MDS, myelodysplastic syndrome; WBC, white blood count; PLT, platelet count; Hb, hemoglobin; MCV, mean corpuscular volume; PB, peripheral blood; BM, bone marrow; g, gram; L, liter; RARS, refractory anemia with ring sideroblasts; IDUS, idiopathic bone marrow dysplasia of unknown significance; CMML, chronic myelomonocytic leukemia; MPN, myeloproliferative neoplasm; n.a., not available. *BM dysplasia score: +, dysplasia found in 11-30% of all cells in a given lineage on BM smears; ++, dysplasia found in more than 30% of all cells in a lineage. **In this patient, Hb was <11 g/dL for a very short time, and then ranged between 11.9 and 13.4 in the follow-up. n.a., not available.

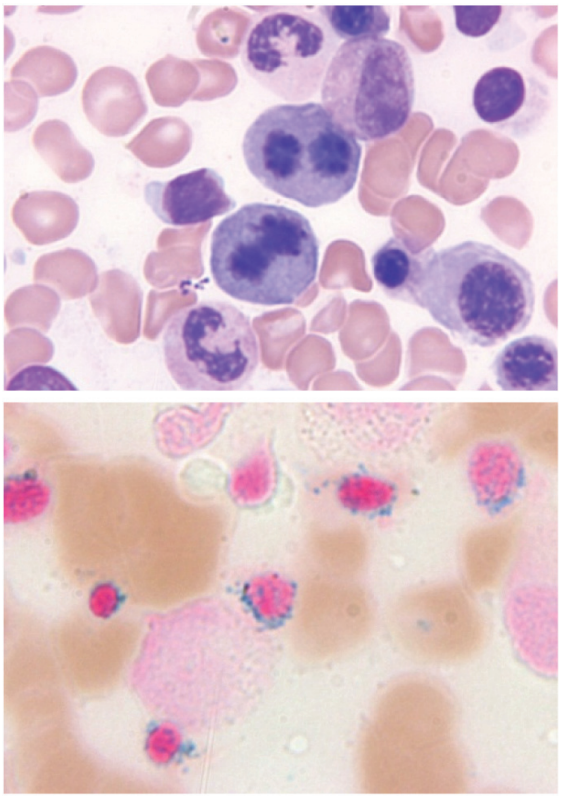

Figure 1. Dysplasia recorded in bone marrow smears. The upper panel shows a Wright-Giemsa-stained bone marrow smear obtained at diagnosis in patient #1. The lower panel shows numerous ring sideroblasts (50% counted) in the iron stain. Magnification of objective-lens: x 60.

July 2007. The case history revealed osteoporosis, chronic gastritis, and polyarthritis. No mutagenic event was reported. Physical examination disclosed normal results. No hepatosplenomegaly and no enlarged lymph nodes were found. The blood count was: erythrocytes 3.2 million μL^{-1}, hemoglobin 11.1 g dL^{-1}, MCV 103 fL, leukocytes 6,400 μL^{-1}, platelets 231,000 μL^{-1}, and reticulocytes 36,100 μL^{-1}. The differential blood count showed 55% segmented neutrophils, 5% band forms, 6% eosinophils, 1% basophils, 30% lymphocytes, and 3% monocytes. Some of the band forms exhibited a Pseudo-Pelger-Huet morphology. The bone marrow smear was normocellular with marked dysplasia in the erythroid lineage, mild dysplasia in the neutrophil lineage, and 50% ring sideroblasts (**Figure 1**). The blast cell count was 1%. Numerous eosinophils and in part atypical mast cells were recorded. Corresponding results were obtained in a bone marrow biopsy: again, the marrow was normocellular without an increase in CD34+ blasts. No focal accumulations of mast cells were found. Megakaryopoiesis was normal on smears and in bone marrow biopsy sections. Chromosome analysis revealed a normal karyotype (46,XX). In addition, no molecular marker of monoclonality was found. The serum tryptase level amounted to 8.1 ng mL^{-1}. The serum ferritin level was 788 ng mL^{-1}, transferrin saturation 51%, bilirubin 1.9 mg dL^{-1}, and lactate dehydrogenase (LDH) level 386 U L^{-1}. The Coombs test was negative and haptoglobin was decreased. All other laboratory parameters were within the normal range. A provisional diagnosis of imminent MDS (subtype: refractory anemia with ring sideroblasts = RARS) with concomitant mild hemolysis was established and the patient´s course observed. During the following months, the blood counts remained stable without drop in hemoglobin. In March 2009, hemoglobin was 11.2 g dL^{-1} (**Table 2**). Finally, despite dysplasia and the presence of ring sideroblasts, and because of the completely stable blood counts, the diagnosis IDUS was established. No transfusions and no therapy was required until 2010. However, in 2010, her hemoglobin level dropped below 11 g/dL for more than 6 months (range: 10.3-10.6) so that the diagnosis RARS was finally established. Currently, the patient is still transfusion-independent, although her hemoglobin is further decreasing.

Case #2: A 74 year old female patient was referred in September 2002 because of mild macrocytic anemia (hemoglobin 11-12 g dL^{-1}) and slightly elevated platelets (385,000 μL^{-1}). Detailed results obtained at diagnosis (IDUS) in this patients have been published elsewhere [12]. In brief, bone marrow investigations revealed mild erythroid dysplasia and 46,XX with del(5)(q13q31) in 12 of 18 metaphases. Correspondingly, bone marrow sections and smears revealed dysplastic megakaryopoiesis with dwarf forms and monolobed nuclei. The serum EPO level amounted to 71.5 units per liter. Numbers of colony-forming progenitor cells were slightly decreased (**Table 1**). During the next few years, hemoglobin levels increased spontaneously and reached normal range. In addition, the MCV decreased to normal, and the levels of CFU-GM and BFU-E also increased to normal values (**Table 3**). The blood count in 2007 was: WBC 6220 μL^{-1}, Hb 13.3 g dL^{-1}, MCV

Table 2. MDS-related parameters recorded during the observation period in patient #1

	Time of Investigation				
Parameter	August 2007	January 2008	March 2009	June 2009	normal range
Ery (T L^{-1})	3.2	3.4	3.2	3.0	3.8-5.2
Hb (g dL^{-1})	11.1	11.5	11.2	10.6	12-16
MCV (fL)	103	103.6	105.3	105.3	78-98
WBC (G L^{-1})	6.4	6.9	6.63	6.78	4-10
PLT (G L^{-1})	231	258	243	227	150-350
EPO (U L^{-1})	31.2	n.t.	51.2	34.7	-
BFU-E (μL^{-1})	275	n.t.	n.t.	101	120-1862
CFU-GM (μL^{-1})	11	n.t.	n.t.	45	50-936
CFU-GEMM (μL^{-1})	0	n.t.	n.t.	0	4-77
LDH (U L^{-1})	386	352	305	359	<250

MDS, myelodysplastic syndrome; Hb, hemoglobin; MCV, mean corpuscular volume; WBC, white blood count; PLT, platelet count; EPO, erythropoietin level; BFU-E, burst-forming unit (cell) giving rise to erythroid cells; CFU-GM, colony-forming cell giving rise to granulocytes and macrophages; CFU-GEMM, multilineage colony-forming progenitor cell; LDH, lactate dehydrogenase; n.t., not tested.

Table 3. MDS-related parameters recorded during the observation period in patient #2

	Time of Investigation				
Parameter	September 2002	March 2004	August 2006	April 2008	normal range
Ery (T L^{-1})	3.1	3.0	3.9	4.6	3.8-5.2
Hb (g dL^{-1})	11.0	11.4	12.8	12.9	12-16
MCV (fL)	105	112	97	85.2	78-98
WBC (G L^{-1})	5.4	5.3	6.63	6.75	4-10
PLT (G L^{-1})	385	450	459	585	150-350
EPO (U L^{-1})	71.5*	n.t.	n.t.	4.6	-
BFU-E (μL^{-1})	53	n.t.	173	510	120-1862
CFU-GM (μL^{-1})	98	n.t.	186	437	50-936
CFU-GEMM (μL^{-1})	0	n.t.	0	0	4-77
LDH (U L^{-1})	150	169	n.t.	266	<250

MDS, myelodysplastic syndrome; Hb, hemoglobin; MCV, mean corpuscular volume; WBC, white blood count; PLT, platelet count; EPO, erythropoietin level; BFU-E, burst-forming unit (cell) giving rise to erythroid cells; CFU-GM, colony-forming cell giving rise to granulocytes and macrophages; CFU-GEMM, multilineage colony-forming progenitor cell; LDH, lactate dehydrogenase; n.t., not tested. *EPO level from early October 2002.

88.5 fl, and platelets 534,000 μL^{-1}. The EPO level decreased to 4.6 units L^{-1}, confirming adequate EPO-response in 2002 (IDUS). Most impressively, however, the platelet counts increased further, and in 2010, platelet counts had reached 734,000 μL^{-1}. In 2007, the patient developed a transient ischemic attack. She received warfarin, and later aspirin. A mutation analysis revealed the *JAK2* mutation V617F. The diagnosis of an unclassifyable overlap-neoplasm (arising from IDUS) with *JAK2* V617F and 5q-, and signs of dysplasia and myeloproliferation, was established. The patient currently receives aspirin and low-dose hydroxyurea.

Results

Bone marrow findings

Wright-Giemsa-stained bone marrow smears revealed signs of dysplasia in at least one major hematopoietic cell lineage in all patients. The percentage of dysplastic cells and the number of lineages affected (with at least 10% dysplastic cells) varied from patient to patient (**Table 1**). Multilineage dysplasia was found in 6 cases. Megakaryopoiesis was also examined in bone marrow smears. However, in all patients, bone marrow histology and immunohistology were employed to confirm/reveal or exclude megakaryocyte dysplasia. In two patients, an increase in ring sideroblasts was detected, and both patients (#1 and #4) developed RARS during the follow up. In none of the patients examined, bone marrow fibrosis was recorded. An increase in CD14+ cells was found in patient #2, who later developed overt CMML. We also found varying numbers of diffusely scattered tryptase-positive cells in bone marrow sections in most of the patients examined. However, no focal mast cell infiltrates or other signs indicative for a primary mast cell disease were found in any of the patients examined. Similarly, no marked increase or focal accumulation of CD34+ progenitor cells (blast cells) was found in the patients analyzed.

Cytogenetic findings

Karyotyping and FISH were performed successfully in 9/10 patients. An abnormal karyotype was found in two patients. In patient #6, a complex karyotype involving chromosomes 7, 13, 20, and 22, was detected in all metaphases tested. A detailed description of the karyotype in this patient has been published elsewhere [11]. In patient #2, an isolated loss of 5q was detected: del(5)(q13q31). In patient #9, no sufficient karyogram or FISH was obtained, and in all other patients with IDUS, no cytogenetic abnormalities were recorded (**Table 1**).

Evaluation of serum EPO levels

A remarkable finding was that serum EPO levels were 'adequately increased' in most patients with IDUS (exception: patient #10: serum EPO concentration: 10 units per liter despite a hemoglobin of less than 12 g dL^{-1}). In fact, even in the older or very old patients (up to 83 years) with IDUS examined, substantial serum concentrations of EPO were measured (**Table 1**). The hypothesis that the mild anemia seen in IDUS is indeed responsible for the increased EPO production was demonstrable in patient #2. In fact, in this patient serum EPO decreased from 71.5 (at the time of IDUS) to 4.6 units L^{-1} at the time when the erythropoiesis-triggering effect of JAK2 V617F+ had led to a complete normalization of the hemoglobin concentration (**Table 3**). In all other patients examined, EPO levels remained fairly stable during the observation period.

Response of progenitor cells to EPO

The numbers of (cytokine-inducible) circulating colony-forming progenitor cells are typically reduced in patients with MDS [8]. Although not widely applied, this typical biological feature is an established co-criterion for the diagnosis of MDS [8] and is routinely recorded in our center. A remarkable and unexpected observation was that in all patients with IDUS, the (presumably) clonal blood-derived progenitor cells were responsive to cytokines and formed erythroid colonies in response to recombinant EPO (**Table 1**). A similar response was found when bone marrow progenitor cells were examined (not shown). In several patients, including those who later developed overt MDS, colony numbers were even found to be within normal range (**Tables 1** through **3**). However, in some of the patients, red cells in erythroid colonies showed poor hemoglobinization. In addition, in several cases, small-sized colonies or micro-clusters were detected, or CFU-GM levels were markedly decreased, such as in patient #1. However, the key feature in IDUS found in this study was that erythroid progenitor cells retained a good or even normal response to EPO. Such 'normal' response of erythroid progenitor cells to EPO and other cytokines is usually not found in MDS.

Evaluation of molecular markers

Because clonal erythroid progenitor cells apparently retain at least some response to (endogenous and exogenous) EPO, which is unusual in MDS, we asked whether clonal bone marrow cells would display any molecular features that could explain their cytokine-responsiveness. Since the *JAK2* mutation V617F has been implicated in erythrocytosis in patients with polycythemia vera and was also

Table 4. Definition of IDUS and comparison to minimal diagnostic criteria for MDS*

IDUS		Minimal Criteria for MDS	
No constant marked cytopenia		Constant cytopenia > 6 months	
	- haemoglobin ≥11 g dL-1		- haemoglobin <11 g dL-1
and	- ANC ≥1,500 µL-1	or	- ANC <1,500 µL-1
and	- platelets ≥100,000 µL-1	or	- platelets <100,000 µL-1
Dysplasia** and/or ring sideroblasts >15% and/or karyotype typically found in MDS		Dysplasia**, ring sideroblasts >15% and/or typical MDS-karyotype	
No other disease as reason for dysplasia/karyotype found		No other disease as reason for dysplasia/karyotype found	

IDUS, idiopathic dysplasia of unknown significance; MDS, myelodysplastic syndrome; *criteria according to the available literature [8,9]; **diagnostic dysplasia: ≥10% of cells in at least one lineage

found in patient #2, we examined all patients with IDUS for the presence of JAK2 V617F. However, in the other patients with IDUS, no *JAK2* mutation could be detected. We also screened for other molecular markers potentially associated with a myeloproliferative neoplasm. However, all mutation analyses performed (AML-related fusion genes by multiplex PCR, KIT D816V, BCR/ABL, FIP1L1/PDGFRA) disclosed negative results.

Follow-up and evolution to MDS in patients with IDUS

The follow-up in our patients ranged between 2 and 13 years (median: 7 years, **Table 1**). During this time-period, 4 of 10 patients developed an overt myeloid neoplasm: two developed an overt MDS and two an MDS/MPN overlap disease (**Table 1**). In both patients with MDS, the subtype RARS was diagnosed, and ring sideroblasts had already been detected at the time of first presentation. In one patient, CMML was diagnosed after 6 years. This patient had already a relative monocytosis at the time of IDUS. However, no cytogentic abnormality was detected in this patient or in the two patients with RARS. However, in the other patient who developed an MDS/MPN overlap disease, a 5q- had been detected initially. Interestingly, in this patient, erythrocytes, hemoglobin levels as well as the platelet counts increased constantly after a few years, so that the initially suspected diagnosis (incipient 5q- syndrome) had to be revisited. Indeed, leukocytes were found to express JAK2 V617F, leading to the final diagnosis of MDS/MPN-U with 5q- and JAK2 V617F. Another remarkable course was recorded in patient #6. In this patient, initial bone marrow investigations revealed multilineage dysplasia and a complex karyotype in all metaphases examined [11]. However, no cytopenia developed. After 1 year, bone marrow investigations were repeated and disclosed identical results. Until today, blood counts remained normal with the exception of Pseudo-Pelger-Huet cells in her differential counts. In other words, this patient remained in an IDUS state for more than 13 years despite a complex karyotype and major bone marrow dysplasia. All in all, 6 patients remained in an IDUS state until 2010, and 5/6 of these patients exhibited a normal karyotype.

Discussion

Minimal diagnostic criteria for MDS include i) marked and constant cytopenia, ii) MDS-specific bone marrow features, i.e. dysplasia, ring sideroblasts, increase in myeloblasts, or a MDS-related karyotype, and iii) exclusion of all other hematopoietic and non-hematopoietic disorders as primary reason for dysplasia and/or cytopenia (**Table 4**) [8,9]. The so called MDS/MPN overlap disorders exhibit additional features including signs of myeloproliferation and increased blood cell production [17]. The prerequisite to call a condition MDS is constant marked cytopenia over 6 months. However, during the past few years, several patients with dysplastic (apparently clonal) bone marrow cells with or without a karyotype, in whom no or only slight cytopenia developed, have been described [9,11,12]. For these patients, the term IDUS has been proposed [9]. However, so far

little is known about the incidence and clinical course of IDUS, and the rate of transformation to overt MDS. In the present study, we report on 10 patients with IDUS, in whom key laboratory and clinical features were recorded at diagnosis and in the follow up. Based on these investigations, IDUS is a potential pre-phase of MDS or MDS/MPN. On the other hand, IDUS may be recorded with stable features over many years or even decades without disease-manifestation. Therefore, we believe that the term ".. of unknown significance" is indeed justified and appropriate. Since these are otherwise healthy persons, and may be stigmatized by a prompt diagnosis of MDS or may then even receive unjustified therapy, we also believe that the IDUS-concept is important and relevant.

Although a hypothesis about the potential pathogenesis underlying IDUS has been proposed [9,12], the exact underlying defect(s) and predisposing factors remain unknown. One hypothesis is that IDUS is a long-lasting pre-phase of MDS in which clonal progenitor cells retain normal function and normal responses to growth factors over many years [9]. This concept has been tested in the current study. Based on our results, several of the above mentioned assumptions could indeed be confirmed. First, the latency period of evolution from IDUS to MDS is variable and may sometimes take several years or even decades. Second, cytokine responses of progenitor cells to growth factors such as EPO could be documented for all IDUS patients in a colony assay. Finally, we were able to document that at least some of these patients with IDUS progress to (manifest as) an overt MDS or an MDS/MPN.

An important question is what features and factors may predict evolution to MDS or MDS/MPN in patients with IDUS. In our patients who progressed to an overt MDS or MDS/MPN, ring sideroblasts, relative monocytosis, or an abnormal karyotype were recorded. However, not all patients with IDUS who have an abnormal karyotype or ring sideroblasts, may progress to a myeloid neoplasm. Likewise, in patient #6, a complex karyotype had been detected in all metaphases [11], but after 13 years of observation, the patient is still in an IDUS-stage without signs of evolution to MDS or an MDS/MPN. Another potentially predictive factor may be age. In fact, evolution of a clonal prephase to overt MDS may be facilitated by an advanced age through several different mechanisms, such as decreased immune surveillance, comorbidity, decreased production of EPO, or accumulation of more decisive hits over time. However, the exact contribution of age and other factors to the prognosis in IDUS remains speculative because of the small number of cases examined and the retrospective way of analysis. Here, larger prospective studies with more patients are certainly required to provide more detailed answers.

Another important aspect is that IDUS may be present for several decades before an overt MDS is diagnosed. This may not only apply to IDUS but may be a general phenomenon of most if not all pre-neoplastic conditions where clonal stem cells persist and produce laboratory abnormalities such as a paraproteinemia in MGUS. However, in contrast to MGUS and ICUS, the IDUS-clone has already replaced (or almost replaced) normal hematopoiesis. In fact, in patient #6, all metaphases examined showed a complex karyotype, and in patient #2, 12 out of 18 metaphases were found to display the 5q-anomaly. In other words, clonal ('neoplastic') hematopoiesis sometimes can mimic normal blood cell production for many years or even decades without progression to an overt neoplasm. This may be explained by a low rate of additional mutations (clonal stability) that are acquired by neoplastic stem cells, an extremely robust immunosurveillance, or by the fact, that no key pro-oncogenic molecules or pathways were activated by the primary stem cell-immortalizing defect. In this regard it is also noteworthy that in most of our IDUS patients, no cytogenetic or molecular defects could be identified even if bone marrow cells were highly dysplastic or an increase in serum tryptase was found. Thus, the primary molecular defect(s) that lead(s) to an IDUS state remain to be identified. Another important fact is that the IDUS clone retains responsiveness against various ´physiologic´ regulators including growth promoting and growth-inhibitory cytokines.

In the elderly population, renal EPO production is often impaired. Sometimes, it turns out that renal insufficiency develops. However, even in otherwise healthy persons with normal (excretory) kidney function, the EPO production may decrease with age [12,18,19]. This condition has also been termed anemia of the elderly, and may be responsible for anemia in a subgroup of patients with ICUS [18]. By contrast, in patients with IDUS, EPO responses to

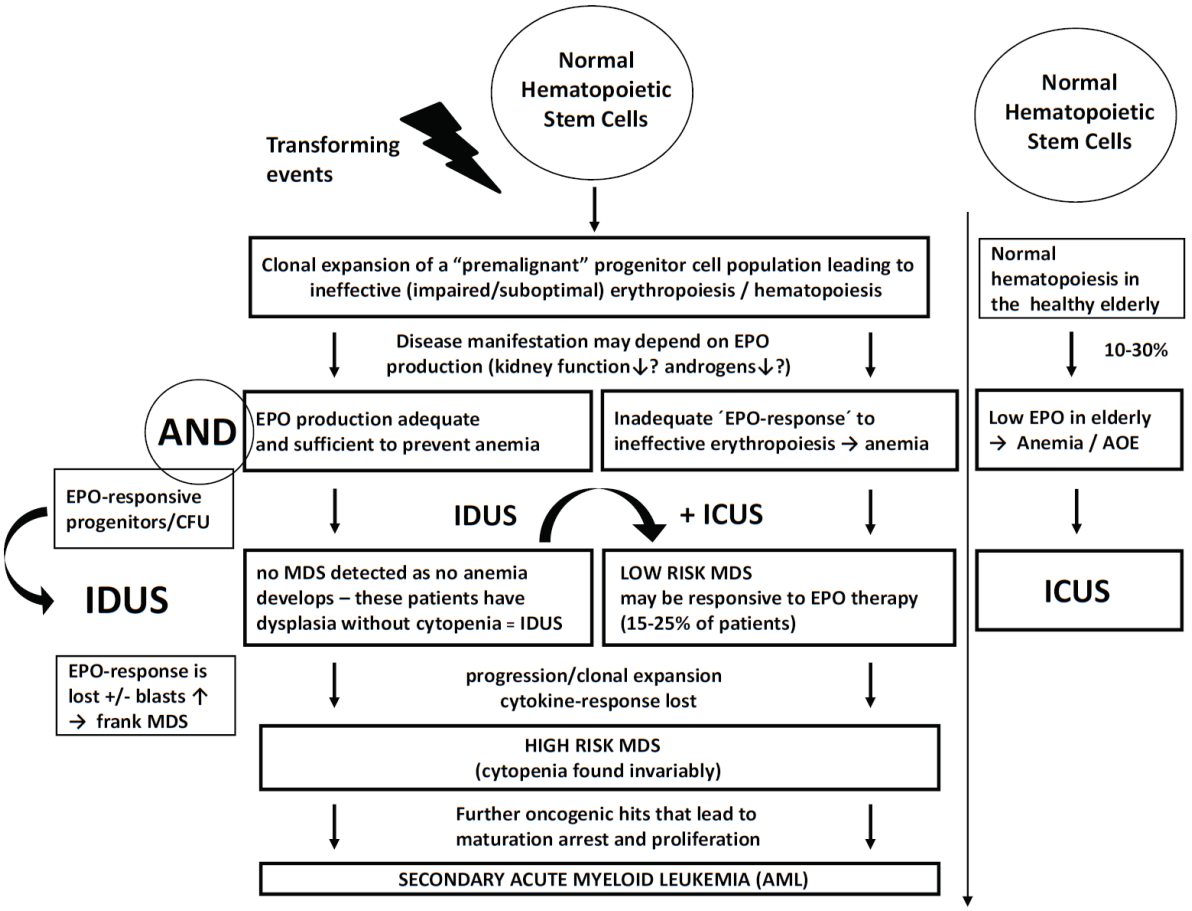

Figure 2. Role of erythropoietin (EPO) production and BFU-E responses in disease manifestation in low risk myelodysplastic syndromes (MDS). MDS arise from hematopoietic stem cells and develop in a step-wise process. Both, clone-specific and patient-related factors supposedly play a role in disease manifestation. In a pre-phase of MDS, the neoplastic clone first replaces normal hematopoiesis, but still may not produce anemia, because clonal (sometimes also residual non-clonal) erythroid progenitor cells (BFU-E) remain responsive to EPO. As long as EPO production is normal in these patients (adequate and able to counteract severe anemia) they will not develop anemia, and no frank MDS is diagnosed. These patients have idiopathic dysplasia of uncertain significance (IDUS). Later, when EPO production declines or clonal progenitor cells no longer respond to EPO, patients develop frank MDS. EPO production may decrease in the elderly, and may then lead to anemia of the elderly (AOE=ICUS). If in these patients, a dysplastic clone develops, the patient develops overt MDS following the equation: IDUS+ICUS=MDS, and when BFU-E remain responsive, exactly these MDS patients will respond to EPO therapy.

anemia are adequate and may prevent anemia-formation. This was nicely demonstrated in patient #2, in whom the initially elevated EPO level decreased back to normal (<10 U L^{-1}) when hemoglobin levels increased because of the erythopoiesis-stimulating effect of the JAK2 V617F mutation. This observation also predicts that all IDUS patients will transform into an overt MDS when EPO levels decrease, i.e. the 'EPO-response' (to decreasing hemoglobin levels) is no longer rescuing from severe anemia. Since EPO responses usually decline in the elderly, this may also explain why low risk MDS is often detected in advanced age. Another confirmation of this concept is the response of low risk MDS patients to recombinant EPO. Notably, in these responding MDS patients, endogenous EPO levels typically are inadequately low [20]. All these observations lead to the biologic equation: IDUS+ICUS=MDS (**Figure 2**) and suggest that both disease-related factors (EPO response) and patient-related factors (EPO production) contribute to the manifestation of MDS in IDUS patients.

The numbers of erythroid (burst forming) colonies (BFU-E, CFU-E) and myeloid cell-forming colonies (CFU-GM, CFU-GEMM) are typically low or not detectable in MDS. In our center, this typical feature is employed as a co-criterion to diagnose MDS. We here show that patients with IDUS have normal or near normal numbers of CFU-GM and BFU-E, which is a remarkable finding. In fact, even if some patients had poorly hemoglobinized red cell colonies or microclusters or even decreased numbers of CFU-GM, this observation clearly points to the fact, that unlike in overt MDS, neoplastic progenitor cells in IDUS retain their response against growth factors such as EPO. Another potential explanation would be that colonies were derived from normal non-clonal cells that were (also) present in the samples analyzed. However, at least in those patients in whom erythroid colonies showed poor hemoglobinization, this possibility seems unlikely. Unfortunately, we were unable in this study to pick out colonies and to examine whether colony-derived cells are indeed clonal cells in our IDUS patients. Such studies are planned but can only be performed in those patients in whom an abnormal karyotype is detectable.

Another hypothesis that has been raised in IDUS is that certain molecular features related to abnormal signalling in clonal cells, counteract anemia in these patients. One obvious candidate is the JAK2-STAT pathway that leads to erythrocytosis in patients with myeloproliferative disorders. Notably, most patients with polycythemia vera carry the transforming and erythropoiesis-stimulating *JAK2* mutation V617F [21]. A most interesting finding in our study was that in one patient with IDUS, neoplastic cells also acquired JAK2 V617F. However, in this particular case, erythrocytes and hemoglobin even increased over time, and thrombocytosis also developed. Therefore, we do not believe that in this patient, JAK2 V617F was the primary defect that kept erythroid progenitor cells responsive to EPO. In line with this assumption, we were also unable to detect JAK2 V617F in the other patient with IDUS examined.

An important question is how to manage individuals with IDUS in the follow-up. In general, management and follow-up-investigations should be the same as that performed in low risk MDS without transfusion-dependence. However, there are certain situations where IDUS cases should have a closer follow-up, or even should be considered for therapy. Likewise, patient #6 in whom a complex karyotype was detected at relatively young age, we recommended a close follow-up and had initiated an urgent search for a bone marrow transplant donor. In other patients, it may be sufficient to control blood counts one or two times a year. When cytopenia develops or progresses in these cases, a full re-evaluation of the condition including a bone marrow biopsy and aspiration is always required in order to establish the diagnosis, and to detect progression to (manifestion of) MDS and to define the MDS-subtype.

In summary, our study describes the clinical course and outcome of patients with IDUS. Based on our data, IDUS is a potential prephase of MDS or an MDS/MPN overlap disorder. Although the pathogenesis of IDUS is not fully understood, our data suggest that an intact interaction between the cytokine network and (presumably clonal) progenitor cells is essential to keep the condition in an IDUS state for years. Finally, our data predict that MDS will manifest in elderly IDUS-patients, as EPO production and EPO responses decrease more frequently in advanced age. Some of these patients may then still respond to exogenous EPO therapy.

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Please address correspondence to: Peter Valent, MD, Department of Internal Medicine I, Division of Hematology & Hemostaseology, Medical University of Vienna, Waehringer Guertel 18-20, A-1090 Vienna, Austria. Tel:+ 43 1 40400 -5488, -6085, Fax:+43 1 40400 4030, E-mail: peter.valent@meduniwien.ac.at

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