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MYELODYSPLASTIC SYNDROMES WITH MONOCYTIC COMPONENT: HEMATOLOGIC AND CYTOGENETIC CHARACTERIZATION

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ABSTRACT

Background and Objective. Patients with myelodysplastic syndromes (MDS) showing high numbers of abnormally localized immature precursors (ALIP) also display myelomonocytic antigens on immature cells, and it has been suggested that the presence of monocytosis could define a distinct subset of MDS characterized by poorer survival. The objective of this study was to analyze the incidence and significance of monocytosis among our series of patients with MDS and correlate the distributions of these elements with other hematologic features.

Methods. We evaluated the monocytic component in myelodysplastic syndromes in order to clarify the significance of monocytosis in MDS and its relationship with CMMoL and other MDS. Monocytosis was defined as a percentage of blood monocytes greater than 10%.

Results. Among a series of 139 consecutive MDS patients, we describe a group of 29 (20.8%) patients with monocytosis and dysplastic features involving multiple cell lineages which do not fulfill the criteria for diagnosis of CMMoL or aCML. These patients, who do not differ from MDS without monocytosis in the main clinical parameters, are characterized by relatively higher leukocyte (WBC 6.6x10^9/L) and granulocyte counts (PMN 2.5x10^9/L), hypercellular bone marrow and relatively poor prognosis. Among these patients, we observed a particularly high incidence of evolution to CMMoL (34.5%) and AML (17.2%) with monocytic component (FAB M4 and M5). Cytogenetic data demonstrated clonal chromosome changes in 11/13 patients with MDS and monocytosis, while only 19/41 patients without monocytosis showed clonal abnormalities.

Conclusions and Perspectives. The combination of hematologic and cytogenetic features in our study suggests that it is reasonable to consider myelodysplasia with monocytosis as a distinct disease subset of MDS, characterized by multilineage dysplasia along with a higher incidence of karyotype aberrations. The multi-step pathogenetic process in these patients may have reached a more advanced stage at which the relative or absolute increase in the number of monocytes may represent the first event in the subsequent progression of the disease towards acute leukemia.
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Key words: myelodysplastic syndromes, monocytosis, myeloproliferative disorders, chronic myelomonocytic leukemia

The French-American-British (FAB) classification[1] has provided a common language for physicians in the investigation of myelodysplastic syndromes (MDS)[2] and prognostic information allowing the definition of various prognostic scores by different groups.[3]

The incorporation of chronic myelo-monocytic leukemia (CMMoL) among MDS has been largely debated.[4] One point of criticism relates to the poor prognostic power of this subgroup with survival ranging from 11 to 60 months.[5] Another controversial issue arises when it is considered that, despite the fact that this disorder is characterized by ineffective hemopoiesis with resultant peripheral blood cytopenia, it includes by definition cases with peripheral monocytosis (> 1×10^9/L) frequently associated with neutrophilic leukocytosis. Since CMMoL may share some hematologic features with chronic myeloproliferative syndromes, the FAB group recently proposed guidelines for distinguishing chronic granulocytic leukemia, atypical chronic myeloid leukemia (aCML) and CMMoL on the basis of peripheral blood findings,[6] suggesting that it is more appropriate to include CMMoL with significant leukocytosis as a chronic myeloproliferative disorder.

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The importance of a more definite assessment of the monocytic component in MDS emerges from the demonstration, by immunohistochemical methods, that a significant percentage of patients with large numbers of abnormally localized immature precursors (ALIP) display myelomonocytic antigens on immature precursors,[7] suggesting that abnormal stem cells may be capable of differentiating along the granulocytic and the monocytic pathways.

This observation of a possible correlation between monocytic differentiation and an unfavorable prognostic feature in low-grade MDS, such as the presence of ALIP, may provide a biological argument supporting the contention that the presence of monocytosis could define a distinct subset of MDS characterized by poorer survival.[7] It should also be noted that juvenile chronic myelogenous leukemia, a pediatric MDS associated with severe monocytosis, is characterized by an aggressive clinical course with a median survival time lower than 10 months from diagnosis.[8]

Since an increase in monocytes could represent a crucial step in the evaluation of MDS disorders, we analyzed the incidence and significance of monocytosis among our series of patients with MDS and correlated the distributions of these elements with other hematologic features.

Materials and Methods

Patients

One hundred and thirty-nine MDS patients referred to the Institute of Hematology of Ferrara in the period 1990-1995 were retrospectively analyzed. All patients were classified in accordance with FAB criteria.[1] Complete peripheral blood count and differential with special attention to dysplastic features were analyzed. Bone marrow (BM) aspirate was performed in all cases at diagnosis and during the course of the disease. BM cellularity was assessed by evaluating, under low power, the relative proportion of fat and marrow cells in 4-6 particles, as proposed by Tuzuner *et al*.[9] Patients displaying BM cellulariy lower than 30% were defined as hypocellular MDS, while those with cellularity higher than 50% were considered hypercellular MDS. BM biopsy was performed in all the cases showing decreased cellularity on BM aspirate smears in order to rule out hypoplastic acute myeloid leukemia (AML)[10,11] or aplastic anemia. The number of micromegakaryocytes, the myeloid-erythroid ratio, the percentage of blasts and the percentage of monocytes were also assessed. Patients were reviewed at our center at 1-4 month-intervals with a minimum follow-up of 10 months.

MDS with monocytosis

MDS with monocytosis was defined as a percentage of monocytes greater than 10%. This cut-off value was chosen on the basis of the scheme proposed by the FAB group for discriminating chronic myeloid leukemias. In particular, a value of 10% allow us to distinguish between aCML, in which the monocytes represent 3-10% of peripheral leukocytes, and CMMoL in which the monocytic component usually exceeds 10%. Both disorders are characterized by dysplastic hematopoiesis. The monocytic component was estimated on 200 cells by morphologic analysis of May Grünwald-Giemsa stained peripheral blood (PB) smears, and by a dual esterase reaction using ASD-chloroacetate esterase plus alpha-naphthyl acetate esterase (200 cells observed). Those patients with $>1\times10^9$/L monocytes were categorized as CMMoL, whereas the ones with >10% monocytes who did not fulfill the FAB criteria for a diagnosis of CMMoL were classified as MDS with monocytosis.

Cytogenetic analysis

Cytogenetic analysis was performed according to standard techniques as part of a routine diagnostic workup in 59 cases admitted to our Institution since 1993. Chromosomal abnormalities were described according to the *International System for Human Cytogenetic Nomenclature*.[12] Complex karyotypes were defined by the presence of 3 or more events of translocation or non-disjunction occurring in the same clone.

Statistical analysis

One-way analysis of variance (ANOVA) with Bonferroni correction was used in the comparison of continuous variables among the different groups, while the chi-square test was applied for categorical variables. Patient survival was estimated by the Kaplan-Meier method from the date of diagnosis until death from any cause or until the last patient follow-up. Survival curves were statistically compared by the log-rank test. The backward multiple regression method of Cox was used to identify the most significant independent prognostic variables for survival. Logistic regression analysis was employed to explore the relationship between dichotomous outcome variables and other covariables.

Results

Patients and monocytosis

The distribution of the 139 patients according to FAB classification and the presence of monocytosis is shown in Table 1. According to the FAB criteria, 61 patients had refractory anemia (RA), 9 refractory anemia with ringed sideroblasts (RARS), 44 refractory anemia with excess of blasts (RAEB), 10 RAEB in leukemic transformation (RAEB-t), and 15 had CMMoL. Overall, 43 patients (30.9%) showed monocytosis, 14 of whom were classified as CMMoL. Thus 14 CMMoL cases showed more than 10% monocytes in PB smears, whereas less than 10% monocytes were detected in the remaining patient with CMMoL, who by definition had $>1\times10^9$/L blood monocytes.

The clinical characteristics of the patients with and without monocytosis are summarized in Table 2. In order to better identify the characteristics of CMMoL with respect to other MDS with monocytosis, patients with >10% monocytes were subdivided when appropriate into CMMoL and non CMMoL. As shown in Table 2, the majority of hematologic parameters included in this analysis did not differ significantly between those MDS patients with monocytosis who were non CMMoL and the patients without monocytosis. In contrast,

Table 1. FAB distribution according to the presence of monocytosis (≥ 10%) in 139 MDS patients.

FAB	*without monocytosis*	*with monocytosis*
RA	47	14
RARS	9	0
RAEB	32	12
CMMoL	1	14
RAEB-t	7	3
total	96 (69.1%)	43 (30.9%)

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Table 2. Monocytosis and clinical characteristics at presentation.

Parameter	*Without monocytosis*	*With monocytosis*	*CMMoL*
No. of patients	95	29	15
Age yr. (range)	65.9 (23-84)	65.1 (18-83)	69.5 (51-84)
Hb g/dL	10.4 (2.9-16.1)	10.6 (6.7-14.3)	10.3 (6.4-16.4)
WBC 10^9/L*	3.9 (0.9-19.8)	6.6 (1.4-76.3)	26.7 (2.0-159)
PMN 10^9/L*	1.9 (0.18-15.5)	2.5 (0.3-25.2)	17.0 (0.4-117)
PLT 10^9/L	188 (20-1117)	168 (23-284)	153 (30-491)
Liver cm	1 (0-8)	1.5 (0-6)	1.7 (0-10)
Spleen cm	1 (0-15)	1.5 (0-15)	1.7 (0-18)
LDH U/L*	432 (132-1960)	471 (176-1838)	842 (236-2648)
BM bl %	7 (2-30)	9 (2-30)	9 (2-20)

**Significant differences were demonstrated between patients with and without monocytosis versus CMMoL (p=0.0001 on one way ANOVA with Bonferroni correction).*

a significant difference was observed between patients with and without monocytosis versus CMMoL regarding leukocyte and granulocyte counts and LDH values. Patients with monocytosis displayed a higher incidence of hypercellular bone marrow than those without monocytosis: 34/43 (79.1%) patients with monocytosis or CMMoL had > 50% cellularity, compared with 48/96 (50%) cases without monocytosis. In 18 cases with hypocellular BM aspirate, a finding confirmed at bone biopsy, only 3 had monocytosis or CMMoL (Table 3).

Monocytosis and hematologic evolution

Disease progression was observed in 15/29 (51.7%) patients in the group of MDS with monocytosis, compared with 17/95 (17.9%) in the group of MDS without monocytosis. In the former group, 5/29 evolved into AML (17.2%), while in the latter, 13/95 (13.7%) dysplayed AML transformation (Table 3). Out of the 29 non CMMoL patients with monocytosis, 10 developed a hematologic picture of CMMoL during a 2-59-month period (median 17 months), and five of them evolved into AML M4 FAB through a phase of subacute myelo-monocytic leukemia consisting of the presence of >5% peripheral blast cells with 20-30% BM blasts.[13]

Four out of the 96 cases without monocytosis evolved into CMMoL in a 10-36-month period (median 24 months); two of them showed 9% monocytes at presentation, a figure very close to the 10% cut-off value adopted in this analysis. All 11 cases undergoing leukemic transformation in MDS with monocytosis were classified as AML-M4 (10 cases) or AML-M5 (1 case), whereas evolution into AML with monocytic component (M4-M5) was observed in only 1 out of 13 cases undergoing leukemic transformation in MDS without monocytosis.

Table 3. Monocytosis and clinico-biological characteristics.

	without monocytosis	*with monocytosis*	*CMMoL*	
BM cellularity				
hypo	15	2	1	p=0.019
normal	33	5	1	
hyper	47	22	13	
Evolution				
yes	17	15	6	p=0.001
no	78	14	9	
FAB and evolution				
M1	5	–	–	
M2	7	–	–	
M4	1	5	5	
M5	–	–	1	
CMMoL	4	10	–	
Karyotype				
normal	22	2	1	p=0.03
abnorm. (complex)	19 (5)	11 (3)	4 (2)	

Monocytosis and karyotype

As shown in details in Table 4, clonal chromosome changes were detected in 34 of 59 consecutive cases (57.6%) since 1993. Twenty-five cases displayed more than 10 cells with normal karyotype.

Recurrent abnormalities were -5/5q- (10 cases), aberrations of 17p (6 cases), +8 (6 cases), +21 (5 cases) and monosomy 7 (4 cases). Abnormal karyotypes were seen in 11/13 patients with MDS and monocytosis as compared with 19/41 among MDS without monocytosis. Complex karyotypes were found in 10 patients, 3 of whom had MDS and monocytosis (Table 3).

Monocytosis and outcome

Cox's backward multiple regression analysis demonstrated that the absolute number of monocytes (p<0.0001) along with age (p=0.017), BM blasts (p<0.0001), hemoglobin level (p<0.0001) and platelet count (p<0.0001) were the most important independent prognostic factors. Furthermore, monocytosis was a strong prognostic factor among patients with low-risk MDS, as well as within the group of patients with refractory anemia (Figure 1). Logistic regression analysis documented that monocytosis (p=0.0005) and BM blasts (p=0.0013) were the only two independent factors predictive of evolution to CMMoL or AML.

Discussion

The inclusion of CMMoL among MDS is justified by the presence of dysplastic morphology and by the increased percentage of BM blasts (up to 20%). Indeed, this disorder may resemble refractory anemia or refractory anemia with excess of blasts in

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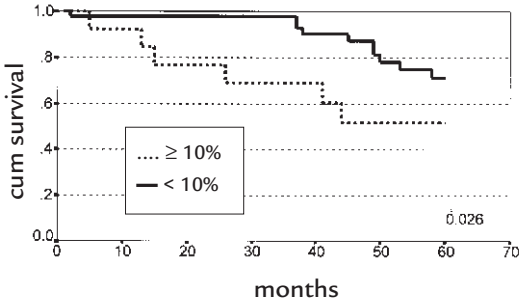

Figure 1. Refractory anemia: survival functions according to the presence of monocytosis ≥ 10%.

most cases, but it can be distinguished on the basis of a monocytic count in excess to 1×10^9/L.[1] On the other hand, prominent hyperplasia of the granulomonocytic lineage can be observed in a significant proportion of cases, making identification of CMMoL and myeloproliferative disorders arbitrary.

The FAB group recently defined guidelines for distinguishing different nosologic entities among the spectrum of manifestations of chronic myeloid leukemias, and proposed considering CMMoL with a significant degree of leukocytosis (>13×10^9/L) as a myeloproliferative disorder (MPD).[5]

The existence of a transitional stage between MDS and MPD has been largely debated over the last several years. Verhoef *et al.*[14] identified a subset of MDS with bone marrow fibrosis and characteristics of both MDS and MPD, with a high incidence of leukemic transformation and a high mortality. Although rare, evolution of MDS into MPD is well documented, as described by Ohyashiki *et al.*[15] and by Verhoef *et al.*[16]

More recently Oscier,[17] debating whether aCML should be regarded as a separate disorder or as part of a spectrum of myeloproliferative disorders with dysplastic features, presented data on 10 cases of MDS developing features of aCML during the course of their disease. He concluded that it would be premature to regard aCML as a distinct entity and hypothesized that aCML may represent a disease initially indistinguishable from refractory anemia that may evolve towards a phase in which myeloid maturation becomes proliferative rather than ineffective.

Table 4. Cytogenetic findings in 34 cases with clonal abnormalities.*

Case	*Diagnosis*	*No. of cells/total*	*Karyotype (no. of cells)*
1	RA mono	[3/10]	47, xy, +10
2	RA mono	[7/55]	47, xx, +mar
3	RAEB mono	[10/10]	46, xx, del(5)(q13;q34) (7) 47, idem +21 (3)
4	RAEB mono	[11/11]	40-45, xx, -7
5	RAEB mono	[2/10]	47, xy, +20
6	RAEB mono	[10/10]	45-47, xy, +8
7	RAEB mono	[2/11]	46, xy, i(17q)
8	RAEB mono	[9/9]	46, xy, del(3)(p11;p25), t(1:7)(p14, p11), add(17)(p12) (4) 47, idem, +1, der(5)t(5;17)(q31;q12) (5)
9	RAEB-t mono	[4/11]	47, xy, +11
10	RAEB-t mono	[22/24]	47, x, -x, +idic(x)(q12), del(16)(q22), +mar1 (9) 47-50, idem, add(4)(q35), +19, +21, +mar2 [cp13]
11	RAEB-t mono	[11/13]	46, xx, der(4)t(4;?)(q21;?), der(5)t(5;15)(q15;q21),der(7)t(7;?)(q22;?), der(8)t(8;17)(q24;q12) [cp11]
12	CMMoL	[5/13]	47, xy, +8 (3) 48, xy, idem, +21 (2)
13	CMMoL	[5/10]	46, xy, i(17q)
14	CMMoL	[10/10]	50-56, xy,+x,der(1)t(1;?)(p35;?), +3, +3, +4, +5, i(7)(q10), +7, +11, +13, +18, i(14)(q10), i(17)(q10) [cp10]
15	CMMoL	[9/9]	44-46, xy, -6, +dic(6;7)(p23;p14), -7, der(16)t(16;11)(q23;q13), -19, +mar [cp9]
16	RA	[13]	47, xx, +8
17	RA	[3/10]	45, xy, -8
18	RA	[13/15]	46, xy, del(20)(q11)
19	RA	[10/12]	45-46, xx, del(5)(q31;q32)
20	RA	[2/10]	45,x,-y +20
21	RARS	[3/10]	47, xy, +15, add(18)(p12)
22	RAEB	[6/12]	47, xy, +17
23	RAEB	[7/11]	47, xy, +8 (4) 46, xy,-5,+8 (3)
24	RAEB	[8/12]	46, xx, del(5)(q13;q32), del(11)(q23;q25)
25	RAEB	[3/10]	45, xx, -21
26	RAEB	[8/8]	46, xx, i(17)(q10),(4), 47, idem +13 (2), 47, idem +19 (2)
27	RAEB	[6/10]	46, xy, +8
28	RAEB	[8/10]	46, xx, del(5)(q31)
29	RAEB	[15/15]	44-47,x,-x, del(3)(q21), del(5)(q31), -8, der(12)t(1;12)(q22;q11), add(17)(q25),-22, +mar1, +mar2 [cp15]
30	RAEB	[8/11]	45, y, -x, +der(x)t(1;x)(q25;q27) (1) 46, idem, +8 (7)
31	RAEB	[10/10]	48, xx, del(4)(q33), del(10)(q24), +12, +21
32	RAEB-t	[10/10]	45, xy, add(16)(p13), -19
33	RAEB-t	[6/13]	44-46, xx, del(5)(q21), del(7)(p11), der(7)t(7;?)(q21;?), del(11)(q11), -12
34	RAEB-t	[8/10]	45-46, xx, -3,del(5)(q13;q22), del(6)(q24), add(17)(p13) [cp8]

*In 25 cases not included in this table, a normal karyotype was found in more than 10 metaphases. FAB diagnoses in these 25 cases with normal karyotype were: MDS with monocytosis: RA=1, RAEB=1, CMMoL=1; total 3. MDS without monocytosis: RA=9, RAEB=11, RAEB-t=2; total 22.

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The FAB classification, as far as the monocytic component is concerned, only recognized the CMMoL sub-group characterized by monocytosis greater than 1×10^9/L. However, it is well documented that there is a significant percentage of MDS with a monocytic component not exceeding 1×10^9/L monocytes for which the existing FAB categories are unsatisfactory. Whether MDS with monocytosis might represent a distinct sub-entity of MDS with proliferative features and could be regarded as a transitional stage between classical MDS and MPD still is an open question.

In this analysis of hematologic features in 139 consecutive MDS, we chose a 10% cut-off value to define monocytosis on the basis of the scheme proposed by the FAB group for distinguishing chronic leukemias. This 10% cut-off enables us to differentiate selected patients from aCML, a condition usually characterized by marked granulocytic dysplasia with a percentage of monocytes varying between 3 and 10%. Thus we could separate a group of patients with dysplastic features and monocytosis who did not fulfill the criteria for a diagnosis of CMMoL or aCML.

Although molecular studies of bcr gene status were not performed in our series, our patients were unlikely to have Ph-negative bcr-positive CML for the following reasons:[18] a) the leukocyte alkaline phosphatase score was normal in all cases, and b) marked splenomegaly or progressive leukocytosis were not observed.

Our patients with MDS and monocytosis did not differ from other MDS in a number of hematologic parameters; however, they were characterized by relatively higher leukocyte count, frequent hypercellular BM and relatively poor prognosis, with a very high incidence of evolution to CMMoL and AML with monocytic component (M4 and M5 FAB). Although we did not perform bone marrow biopsy as a routine diagnostic procedure in this study, it is worth noting that a fairly good correspondence has been reported recently between cellularity as assessed on BM aspirates and on biopsy specimens.[9] In line with these findings, < 30% cellularity was observed on bone biopsies in 18 patients showing hypocellular BM aspirates in our study.

The prognostic relevance of monocytosis was recently highlighted by Cunningham *et al.*[19] on a large series of patients from a single Institution. In the present investigation, we were able to confirm the prognostic relevance of monocytosis together with the other classical parameters applied in the different scoring systems, i.e. age, hemoglobin level, platelet count and bone marrow blasts. Indeed monocytosis and the percentage of BM blasts proved to be independent parameterers predictive of evolution to CMMoL or AML in the present series. Interestingly, the presence of monocytosis as defined in this study also proved to have prognostic significance among RA patients and among patients with a percentage of blasts lower then 10% (low-risk MDS, Figure 2). It is noteworthy that no statistical differences were observed in the percentage of BM blasts, hemoglobin levels or platelet counts between patients with and without monocytosis and CMMoL. These parameters were regarded in several studies as the most important prognostic features in MDS. Evolution of MDS with monocytosis into CMMoL is not infrequent, having been described in 14/125 cases in this series and by Rosati *et al.*[20] among a series of low-risk MDS patients. Moreover, 6/10 patients in Oscier's study developed monocytosis in the course of their disease and the mean leukocyte count of his series was very similar to that of our patients (6.6 Vs 6.5×10^9/L). Thus, although some patients with MDS and monocytosis may clearly represent early stage CMMoL, the presence of monocytosis in MDS may represent a more general phenomenon, possibly identifying a distinct disease subset.

Chromosome findings in this series must be interpreted with the understanding that data were only obtained in consecutive patients who were hospitalized after 1993. For this reason, the majority of high-risk MDS were submitted to cytogenetic investigations, whereas only a few cases with RA were analyzed (17 out of 61 RA in this series). Consequently, a 57.6% rate of clonal chromosomal abnormalities was obtained in our 59 patients, a higher figure that compares favorably with those reported in previous studies of unselected MDS (30%-50% rate of chromosome anomalies).[21-24] Overall, a higher number of MDS with monocytosis in our series showed clonal chromosome changes and complex aberrations than MDS without monocytosis. It is remarkable that, apart from those anomalies largely reported in MDS such as –5/5q–, +8 and –7, aberrations involving the short arm of chromosome 17, which usually lead to total or partial 17p loss, were the most frequent chromosome changes in this series. 17p anomalies, in particular

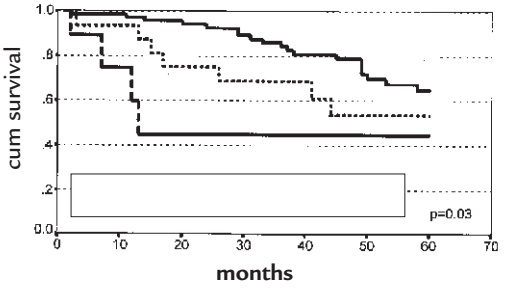

Figure 2. Low-risk MDS (bone marrow blasts < 10%): survival functions according to monocytosis and comparison to CMMoL.

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iso(17q), appear to be very frequent among patients with monocytosis, having been detected in 2/11 patients with MDS and monocytosis and in 2/5 CMMoL, as compared with 2/19 cases with MDS without monocytosis. Interestingly, 17p loss was recently found in association with late-stage or refractory disease in a variety of hematologic malignancies, including MDS.[25-27] The presence of multilineage dysplasia along with genetic instability leading to the acquisition of karyotype aberrations in the majority of MDS with monocytosis may suggest that the multi-step pathogenesis, which is characteristic of MDS, may have reached a more advanced stage in these patients. Persistent cytogenetic abnormalities reflect a high risk of developing myelodysplasia or acute leukemia also in patients with aplastic anemia.[28]

In conclusion, clinical and laboratory data in our patients, in combination with those reported in the literature, raise the possibility that we should consider the existence of a sub-entity of MDS for which the definition of *myelodysplasia with monocytosis* may be appropriate. The recognition of monocytosis at diagnosis of MDS may herald progression towards more aggressive disease, including high-risk MDS and AML.

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