Does cytogenetic evolution have any prognostic relevance in myelodysplastic syndromes? A study on 153 patients from a single institution

Paolo Bernasconi, Catherine Klersy, Marina Boni, Paola Maria Cavigliano, Ilaria Giardini, Barbara Rocca, Rita Zappatore, Irene Dambruoso, Celeste Calvello, Marilena Caresana, et al.

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Corresponding Author: Prof. P. Bernasconi,

Corresponding Author's Institution:

First Author: P. Bernasconi

Order of Authors: P. Bernasconi; Catherine Klersy; Marina Boni; Paola Maria Cavigliano; Ilaria Giardini; Barbara Rocca; Rita Zappatore; Irene Dambruoso; Celeste Calvello; Marilena Caresana; Mario Lazzarino, Prof

Abstract: The present study was designed to establish the incidence of cytogenetic evolution (CE), defined as the acquisition of chromosomal defects during the course of MDS, in order to correlate it with the WHO classification and IPSS score; and to assess its impact on overall survival (OS) and risk of MDS/AML evolution (progression-free interval, PFI) by means of Cox models for time-dependent covariates. Adjustments for known risk factors were achieved by performing a bi-variable analysis. The study was carried out in 153 MDS patients who were followed for a median period of 45.2 months. Disease progression occurred in 42.4% of patients after a 65.2 month median PFI, while CE occurred in 30.7% of patients.
Our study shows that i) CE was more common in advanced than in early MDS and advanced MDS presented secondary chromosomal defects distinct from those of early MDS; ii) CE significantly effected OS and PFI independently of other prognostic variables; iii) del(7)(q31q34) was the only secondary chromosomal defect which significantly effected PFI; trisomy 8 had only a moderate influence.

Response to Reviewers: Replies to Reviewers' comments

As requested:

1. FAB classification has been completely omitted from the manuscript (text and tables). However, in table 1 it has been underlined that 11 of the 153 patients, classified as RAEB-t according to FAB classification, were classified as AML according to WHO. Furthermore, in table 2 and 3 it is underlined that 5 of the 40 patients who experienced cytogenetic evolution were classified as RAEB-t according to FAB but as AML according to WHO.
2. Peripheral blood counts have been completely omitted from the tables.
3. The WHO classification at diagnosis and cytogenetic evolution with percentage of marrow blasts has been provided (table 2).
4. IPSS has been deleted from the tables and the manuscript has concentrated on cytogenetic evaluation

5. The number of tables has been reduced to four. In particular:
The previous tab.2 has been fused to tab.1; the previous tab.3 has been omitted and substituted with the new tab.2 which reports the clinical and cytogenetic features of the 40 patients who experienced CE; the previous tables 4 and 5 has been fused together in the new table 3 which maintains their relevant information; the previous table 6, now table 4, has been maintained; the previous tables 7 and 8 has been omitted.

DOES CYTOGENETIC EVOLUTION HAVE ANY PROGNOSTIC RELEVANCE IN MYELODYSPLASTIC SYNDROMES? A STUDY ON 153 PATIENTS FROM A SINGLE INSTITUTION.

Paolo Bernasconi[1], Catherine Klersy[2], Marina Boni[1], Paola Maria Cavigliano[1], Ilaria Giardini[1], Barbara Rocca[1], Rita Zappatore[1], Irene Dambruoso[1], Celeste Calvello[1], Marilena Caresana[1], Mario Lazzarino[1].

[1]Division of Hematology, Fondazione IRCCS Policlinico San Matteo, University of Pavia, Italy; [2]Scientific Direction, Clinical Epidemiology and Biometry Service, Fondazione IRCCS Policlinico San Matteo, Italy.

Running title: Cytogenetic evolution in MDS

Prof. Paolo Bernasconi
Hematology Division,
Fondazione IRCCS Policlinico San Matteo
University of Pavia
27100-Pavia, Italy
Phone +39 0382 503065
Fax +39 0382 502250
E-mail:p.bernasconi@smatteo.pv.it

Summary The present study was designed to establish the incidence of cytogenetic evolution (CE), defined as the acquisition of chromosomal defects during the course of MDS, in order to correlate it with the WHO classification and IPSS score; and to assess its impact on overall survival (OS) and risk of MDS/AML evolution (progression-free interval, PFI) by means of Cox models for time-dependent covariates. Adjustments for known risk factors were achieved by performing a bi-variable analysis. The study was carried out in 153 MDS patients who were followed for a median period of 45.2 months. Disease progression occurred in 42.4% of patients after a 65.2 month median PFI, while CE occurred in 30.7% of patients.

Our study shows that i) CE was more common in advanced than in early MDS and advanced MDS presented secondary chromosomal defects distinct from those of early MDS; ii) CE significantly effected OS and PFI independently of other prognostic variables; iii) del(7)(q31q34) was the only secondary chromosomal defect which significantly effected PFI; trisomy 8 had only a moderate influence.

Keywords: Myelodysplastic syndromes, cytogenetics, prognostic factors

Introduction

Myelodysplastic syndromes (MDS) are heterogeneous clonal stem cell disorders characterized by a hypercellular marrow showing ineffective hematopoiesis which is responsible for one or more peripheral blood cytopenia(s) [1,2]. Currently, MDS is diagnosed according to the World Health Organization (WHO) classification which identifies eight subtypes [3]. This last classification has been validated in a large number of patients [4-8] and is continually subject to improvement [9-14]. The WHO classification system provides relevant prognostic information for a disease with an extremely variable natural course [1-15]. MDS patients experience morbidity and mortality rates higher than those of age-matched normal subjects because of complications related to peripheral cytopenia [16] and AML progression which occurs in about 30% of cases [17]. In 1997, the International Prognostic Scoring System (IPSS) revealed that the number of peripheral blood cytopenias, cytogenetic pattern and blast cell percentage are the most significant prognostic factors in MDS [18]. Additional studies have demonstrated that IPSS power may be improved by better defining the prognostic relevance of the chromosomal defects included within the intermediate IPSS cytogenetic category [19-22] and this assumption has been further validated [23]. Even more recently, the prognostic power of the cytogenetic pattern has been proven by the newly developed WHO classification-based prognostic system (WPSS) [24]. However, all these studies have analysed the clinical relevance of chromosomal abnormalities revealed only at clinical diagnosis (primary defects) [25,26]. In contrast, very few studies have evaluated the incidence and the prognostic significance of cytogenetic evolution (CE), defined as the acquisition of either an abnormal clone in a karyotypically normal patient or additional defects in patients with an already abnormal chromosomal pattern (secondary abnormalities) [27-36]. The majority of these studies, which include small numbers of patients with a short follow-up period, have simply reported that the acquisition of secondary defects occurred at the time of AML progression in approximately 60% of MDS patients, but none have assessed how many patients experience CE before clinical

progression and whether this CE is predictive of overall survival (OS) and progression-free interval (PFI) independently of WHO classification, primary defects and IPSS cytogenetic categories.

These are the goals of the present study, which included 153 patients, observed for a sufficiently long follow-up period, in whom the significant effect of primary cytogenetic defects on disease outcome has already been revealed [21]. An additional goal was to establish which secondary defect significantly predicts leukemic transformation.

Materials and methods

Patients

All 153 *de novo* MDS patients analysed in the present study were included in a previous series and were diagnosed at the Division of Haematology, Foundation IRCCS Policlinico San Matteo, Pavia between January 1990 and December 2004. All patients were classified according to WHO classification. Our study does not include any patient with either secondary MDS or with a white blood cell (WBC) count above $12x10^9$/L. The diagnostic procedures, performed at clinical diagnosis and during follow-up, and clinical monitoring were carried out as already reported [22]. Moreover, since the IPSS was developed for patients undergoing treatments not affecting the natural course of MDS and since the MDS natural course may be significantly changed by intensive chemotherapy and allogeneic bone marrow transplantation, patients submitted to these therapeutic procedures were evaluated only until the time of such treatments.

Cytogenetic studies

Chromosome studies were performed as previously reported [21]. Chromosome identification and karyotype description were performed according to the International System for Chromosome Nomenclature [37]. Karyotypes were defined as complex when they included ≥3 chromosomal abnormalities.

Statistical analysis

Data were described as the mean and standard deviation (SD) or median and 25^{th}-75^{th} percentiles (IQR) if continuous and described as a count and a percentage if categorical. The role of CE on survival and MDS/AML evolution-free survival was assessed by Cox models for time-dependent covariates. A bi-variable analysis was also performed, in order to adjust for known risk factors. In a predefined subgroup analysis the following risk factors were investigated: cytopenias, blast cell percentage, WHO classification, karyotype pattern at clinical diagnosis, and IPSS cytogenetic categories. Time to event or to censoring was computed from diagnosis. The role of specific secondary defects on survival and on MDL/AML evolution-free survival after CE was assessed by Cox regression, with the time to event or censoring computed from CE. Hazard ratios (HR) and their 95% confidence intervals were estimated. The Kaplan Meier method was used to compute the cumulative event-free survival (PFI) and its 95% confidence interval (CI) at two and five years from diagnosis (for all patients) or CE onset (for patients with CE).

Stata 10.1 (StataCorp, College Station, TX, USA) was used for computation. A 2-sided p-value<0.05 was considered statistically significant.

Results

Patients

Patients' clinical and haematological features at clinical diagnosis are listed in table 1. Considering all 153 patients, the median follow-up was 45.2 months (IQR=23.8-75.7), whereas the median survival was not reached (IQR=34.3-not reached). At the time of analysis, 107 (69.9%) patients had survived and 46 (30%) had died. Disease progression occurred in a total of 65 (42.4%) patients, 11 evolved to a more advanced MDS subtype and 54 to AML. The median progression-free interval (PFI) was 65.2 months (IQR 16.60-not reached).

Treatment was as follows: 128 patients received supportive care (transfusions and hematopoietic growth factors); 3 differentiation-inducing agents; 2 immunosuppressive agents; 5 low-dose

chemotherapy aimed at reducing WBC counts and 15 various regimens of intensive chemotherapy. Twelve patients were submitted to allogeneic bone marrow transplantation (allo-BMT): 11 had previously received supportive treatment only, whereas one had been submitted to intensive chemotherapy which induced a partial remission. The decision to administer intensive chemotherapy or allo-BMT was exclusively based on either the patients' age or the fact that progression to a more advanced MDS or to AML had just occurred.

Since our patients were not uniformly treated, in order to avoid any effect of intensive chemotherapy or allo-BMT on our results, the 15 and 12 patients submitted to the respective procedures were censored at the start of both treatments. So, we are quite confident that, by excluding these patients, we did not affect the impact of CE on OS or PFI.

Cytogenetic results

At diagnosis, all 153 patients had successful cytogenetic analyses (tab. 1). Cytogenetic evolution occurred in a total of 47 (30.7%) patients. However, since six patients (one 5q- syndrome, one RA, one RCMD and 3 RAEB-2) developed secondary abnormalities after disease evolution, they were considered chromosomally stable when assessing whether CE was predictive of OS or PFI. Thus, a total of 40 (26.1%) patients acquired secondary abnormalities during the course of the disease, before disease progression. Their WHO subtype and blast cell percentage at clinical diagnosis and at cytogenetic re-evaluation are listed in table 2. In these patients, the median time from clinical diagnosis to the acquisition of secondary defects was 12.9 months (IQR=6.6-27.3). The secondary abnormalities included 5q- in four patients, monosomy 7 in three, del(7)(q31q35) in eight, trisomy 8 in five, del(17)(p13) in four and various defects (single and double abnormalities) in sixteen. The interstitial long arm deletion of chromosome 5 more commonly occurred in patients who did not experience disease progression, instead -7, del(7)(q31q35) and del(17p) more frequently developed in patients with disease progression. Patients surviving with or without CE according to various

parameters and those with either a stable disease or evolving to more advanced MDS/AML with or without CE according to the same parameters are reported in table 3.

Prognostic relevance of cytogenetic evolution

The significant impact of cytogenetic evolution on OS and MDS/AML progression was analysed by time-dependent Cox regression. Patients who experienced CE during the follow-up period increased their risk of dying by seven-fold and their risk of disease progression (PFI) by thirty-six-fold with respect to patients who remained karyotypically stable ($p<0.001$ in both cases) (tab.4). Two- and 5-year survivals were respectively 40% (95% CI: 19-60) and 10% (95% CI: 0.7-35) in patients with CE, versus 93% (95% CI: 86-96) and 70% (95% CI: 58-70) in patients without CE. After adjusting the effect of CE for other known risk factors (as listed in the methods section) in a bi-variable analysis, the prognostic power of CE was proven in all cases for both death and PFI (tab.4). Furthermore, CE had a statistically relevant influence on the OS of patients who at clinical diagnosis were either chromosomally normal or abnormal ($p=0.004$ and $p<0.001$ respectively). In addition, when the impact of CE on the risk of MDS/AML evolution was adjusted for the presence of primary defects, which significantly effected PFI when analysed as a single variable (Hazard ratio= 2.3 [85% CI= 1.07-4.94], $p=0.02$), CE maintained its statistical power ($p<0.001$), whereas primary defects lost their statistical power ($p=0.501$) (data not shown).

Finally, the OS and the risk of MDS/AML evolution for each of the most common single chromosomal defects acquired during the follow-up period before disease evolution were compared to those of the remaining set of cases with secondary defects. This analysis showed that no single secondary defect effected OS. In contrast, del(7)(q31q34) was the only chromosomal abnormality that significantly effected the risk of MDS/AML progression ($p=0.002$), while trisomy 8 had only a moderate effect ($p=0.052$).

Discussion

To the best of our knowledge, this is one of the few studies which evaluates the incidence of secondary chromosomal defects (CE) in an adequate number of MDS patients with a sufficient follow-up period of 45.2 months (IQR=23.8-75.7). These patients have been cytogenetically analysed at least twice before MDS/AML evolution. Former studies, which often included small numbers of patients with short follow-up periods, have established that CE occurs during the course of the disease in about 14-46% of MDS patients, more commonly in those with a sudden evolution to AML [27-35]. In a more recent study, 24% of MDS patients developed disease progression which, in the eight patients cytogenetically examined during transformation, was associated with CE [36]. None of these studies has evaluated the incidence of secondary defects in relation to other biological and clinical parameters. In the present series, 47 patients (30.7%) acquired additional chromosomal defects during the follow-up period, 40 of them (25.4%) before disease progression (tab. 2). The frequency of this event increased with the worsening of the MDS clinical stage (tab. 3). All these data confirm current evidence which suggest that MDS evolution is accompanied by a steady increase in genetic instability and by an expansion of genomic alterations [17, 38-39].

This is the first study which reveals that the acquisition of secondary chromosomal defects during the follow-up period before disease evolution predicts OS and PFI in MDS patients, an issue never addressed by previously published follow-up studies [27-36]. However, the clinical relevance of CE could be affected by the fact that the decision of performing a new bone marrow aspiration along with a cytogenetic re-evaluation might have been dictated by other clinical indicators of disease progression. These last include a worsening of anemia [16], neutropenia [40], thrombocytopenia [41], a sudden need for red blood cell (RBC) transfusions [24] and an abrupt increase in lactic dehydrogenase levels [42]. Recently, a study has evaluated the impact of cytopenias on MDS outcomes [43]. This study has established that in IPSS intermediate-1 and -2 categories, the haemoglobin value only, has an additive prognostic value regarding IPSS for evaluation of OS, but not for time to AML evolution. Another retrospective analysis has pointed out that in low-risk MDS the total number of RBC transfusions is an independent negative prognostic factor for OS and PFI

[24]. In the present study, the 40 patients who experienced CE before disease progression underwent a cytogenetic re-assessment because of a reduction in peripheral blood values, especially haemoglobin values. Thus, most of our patients presented a worsening of anemia that could be due either to an increase in bone marrow apoptosis, the feature dissociating MDS from AML [17, 39], or to an increase in marrow blast cell percentage. However, this last event was excluded. In fact, at the time of CE no patient changed the subtype of MDS diagnosed at the onset of the disease (tab. 2).

In our series, patients who experienced CE presented a risk of dying seven-fold superior and a risk of transformation to advanced MDS and AML thirty-six-fold superior than those of chromosomally stable patients (tab. 4). However, since many studies have demonstrated that primary chromosomal defects and other clinical-biological factors significantly effect MDS natural history [18-23], it was questioned whether after adjusting for these last variables the statistical power of cytogenetic evolution was maintained. In a first step, the bi-variable analysis applied revealed that the impact of CE on OS was independent of the number and type of peripheral blood cytopenias, the blast cell percentage, the WHO classification, the initial chromosomal pattern and IPSS cytogenetic categories.

In the subsequent bi-variable analysis it was revealed that after adjusting for all other prognostic variables, the relevance of CE on PFI was still significant (tab. 4). In particular, the acquisition of secondary defects remained significant even after adjusting for the prognostic influence of the initial chromosomal pattern ($p<0.001$ in patients with either a normal or an abnormal karyotype at clinical diagnosis), which impact on PFI was lost after performing this analysis. We speculate that this effect could be due to the fact that 28 of the 94 patients (30%) with an abnormal karyotype at clinical diagnosis acquired additional defects during the follow-up period (tab. 2).

As far as leukemic evolution is concerned, until now no study has established which numerical or structural abnormality is required for such an event. It has been proposed that some chromosomal alterations may be responsible for each step in the evolution of the disease, but since the pathway of

evolution is not unique, it is very difficult to define which genetic alteration comes first [36]. However, this assumption seems to be disproved by a recent array comparative genomic hybridization study which revealed that in low-risk MDS, CD34 negative cells harbour genetic alterations in addition to those present in CD34 positive cells [44]. Thus, disease progression might be caused by the outgrowth of a clonal CD34 negative cell population already present at clinical diagnosis and by the type of genetic lesion present in these dysplastic cells. This last suggestion is also strengthened by our study. In fact, in our series, the majority of secondary defects revealed in early/low-risk MDS were distinct from those revealed in advanced MDS: 5q- and del(11)(q14q23) prevailed in patients with early disease, -7, del(7)(q31q34) and del(17p) in those with advanced disease. Trisomy 8 was equally distributed. Thus, it is not surprising that del(7)(q31q34) was the only secondary defect significantly effecting the risk of MDS/AML evolution.

In conclusion, i) CE occurs in 30.7% of patients, particularly in those with more advanced MDS; ii) it has a significant influence on OS and PFI independently of other variables with well-known prognostic relevance; iii) del(7)(q31q34) is the only single secondary chromosomal defect which significantly effects PFI, whereas trisomy 8 has a moderate influence.

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Table 1

Tab.1: Patients' clinical, hematological and cytogenetic features at clinical diagnosis.

Total of patients	153 (%)
Median age, years (Inter-quartile range)	60.5 (49.8-68.3)
Sex	
Males	80 (52.3)
Females	73 (47.7)
Cytopenias	
None	14 (9.1)
One	74 (48.3)
Two	45 (29.4)
Three	20 (13.0)
Marrow blast cell percentage	
<5	83 (54.2)
5-10	27 (17.6)
11-20	33 (21.5)
21-30	10 (6.5)
WHO classification	
RARS / RA	17 (11.9) / 21 (14.8)
MDS del(5q)	11 (7.7)
RCMDS / RCMD	7 (4.9) / 27 (19.0)
RAEB-1 / RAEB-2	27 (19.0) / 31 (21.8)
MDS-U	1
AML*	11 (7.2)
Karyotype	
Normal	59 (38.5)
Abnormal	94 (61.4)
Chromosomal defects	
Single	75 (49.0)
Double	14 (9.1)
Complex (with ≥3 defects)	5 (3.2)
Chromosomal defects in each WHO subtype	
RARS / RA	6 (35.3) / 8 (39.0)
MDS del(5q)	11 (100)
RCMDS / RCMD	5 (71.4) / 14 (51.8)
RAEB-1 / RAEB-2	19 (70.3) / 22 (70.9)
AML*	9 (81.8)
IPSS cytogenetic categories	
Good	85 (55.5)
Intermediate	49 (32.0)
Poor	19 (12.4)

WHO classification, World Health Organization classification; RARS, RA with ringed sideroblasts; RA, refractory anemia; MDS del(5q), 5q- syndrome; RCMD, refractory cytopenia with multilineage displasia; RCMDS, RCMD with ringed sideroblasts; RAEB-1, RA with excess of blasts type 1; RAEB-2, RAEB type 2; MDS-U, unclassifiable MDS; * classified as RAEB in transformation by the previously applied FAB classification.

Table 2

Tab.2: Clinical and cytogenetic features of the forty patients who experienced cytogenetic evolution (CE)

Clinical and cytogenetic characteristics	At clinical diagnosis / At CE*
Cytopenias	
None	2 (5.0) / 1 (2.5)
One	16 (40.0) / 6 (15.0)
Two	15 (37.5) / 16 (40.0)
Three	7 (17.5) / 17 (42.5)
Blast cell percentage	
<5	13 (32.5) / 13 (32.5)
5-10	16 (40.0) / 11 (27.5)
11-20	10 (25.0) / 15 (37.5)
21-30	1 (2.5) / 1 (2.5)
WHO classification	
RARS	1 (2.5) / 1 (2.5)
RA	6 (15.0) / 6 (15.0)
MDS del(5q)	1 (2.5) / 1 (2.5)
RCMDS	– / –
RCMD	5 (12.5) / 5 (12.5)
RAEB-1	7 (17.5) / 7 (17.5)
RAEB-2	15 (37.5) / 15 (37.5)
MDS-U	– / –
AML[a]	5 (12.5) / 5 (12.5)
Karyotype	
Normal	12 (30.0) / –
Abnormal	28 (70.0) / 40 (100.0)

*= number of patients with percentages in parentheses; [a]= classified as RAEB in transformation by the previously applied FAB classification.

Table 3

Tab.3: Survival and disease progression in relation to CE according to WHO classification, karyotype and IPSS cytogenetic categories

	Survived without CE	Survived with CE	In disease prog. without CE	In disease prog. with CE	Tot. of pts
Pts‡	86/113 (76.1)	21/40 (52.5)	34/113 (30.1)	31/40 (77.5)	153
WHO‡:					
RARS	16/16 (100)	1/1 (100)	—	—	17
RA	15/15 (100)	5/6 (83.3)	5/15 (33.3)	1/6 (16.7)	21
5q- Syn.	8/10 (80.0)	1/1 (100)	2/10 (20)	1/1 (100)	11
RCMDS	5/7 (71.4)	—	1/7 (14.3)	—	7
RCMD	17/22 (77.3)	2/5 (40.0)	5/22 (22.7)	4/5 (80.0)	27
RAEB-1	11/20 (55.0)	3/7 (42.9)	6/20 (30.0)	6/7 (85.7)	27
RAEB-2	9/16 (56.2)	7/15 (46.7)	10/16 (62.5)	14/15 (93.3)	31
AML*	4/6 (66.7)	2/5 (40.0)	5/6 (83.3)	5/5 (100)	11
Karyotype‡					
Normal	41/47 (87.2)	8/12 (66.7)	9/47 (19.1)	9/12 (75.0)	59
Abnormal	45/66 (68.2)	13/28 (46.4)	25/66 (37.8)	22/28 (78.5)	94
IPSS cyto. cat.‡					
Good	58/67 (86.6)	10/18 (55.6)	15/67 (22.4)	15/18 (83.3)	85
Intermediate	25/37 (67.6)	8/12 (66.7)	15/37 (40.5)	7/12 (58.3)	49
Poor	3/9 (33.3)	3/10 (30.0)	4/9 (44.4)	9/10 (90.0)	19

‡ Numbers and percentages in parentheses represent the true numbers and true percentages of patients either surviving or experiencing disease progression over the total number of patients included in each category analysed; WHO, World Health Organization; RARS, refractory anemia with ringed sideroblasts; RA, refractory anemia; MDS del(5q), 5q- syndrome; RCMDS, refractory cytopenia with multilineage displasia with ringed sideroblasts; RCMD, RCMD refractory cytopenia with multilineage displasia; RAEB-1, RA with excess of blasts type 1; RAEB-2, RAEB type 2; * classified as RAEB in transformation by the previously applied FAB classification.

Table 4

Tab. 4: Cytogenetic evolution (CE) predicts OS and PFI in univariable analysis and maintains its relevance independently of other well-known prognostic parameters in adjusted bivariable analyses.

	Survival						MDS/AML evolution			
	Tot. Pts[a] (%)	Events[b] (%)	2-year surv. (95% CI)	5-year surv. (95% CI)	HR (95% CI)	P-value	Tot. Pts[a] (%)	Events[b] (%)	HR (95% CI)	P-value
Cytogenetic evolution:										
No	113 (100)	27 (24)	93 (86-96)	70 (58-79)	–	–	113 (100)	34 (30)	–	–
Yes	40 (100)	19 (47)	40 (19-60)	10 (0.7-35)	7.1 (3.92-12.86)	<0.001	40 (100)	31 (77)	35.8 (20.68-62.13)	<0.001

Cytogenetic evolution adjusted for	Survival		MDS/AML evolution	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Cytopenias	6.4 (3.54-11.77)	<0.001	29.7 (16.80-52.53)	<0.001
Blast cell percentage	4.4 (2.34-8.60)	<0.001	26.4 (14.38-48.53)	<0.001
WHO classification	4.0 (1.96-8.26)	<0.001	25.1 (12.39-50.91)	<0.001
Chrom. pattern at clin. diagn.	6.6 (3.65-12.06)	<0.001	33.9 (19.16-60.03)	<0.001
IPSS cytogen. categories	6.6(3.65-12.2)	<0.001	32.8 (18.67-57.88)	<0.001

HR= Hazard Ratio; 95% CI= 95% confidence intervals; [a] the percentages are calculated for total number of patients; [b] the percentages are calculated for total number of patients included in each category; WHO, World Health Organization.

Replies to Reviewers' comments

As requested:

1. FAB classification has been completely omitted from the manuscript (text and tables). However, in table 1 it has been underlined that 11 of the 153 patients, classified as RAEB-t according to FAB classification, were classified as AML according to WHO. Furthermore, in table 2 and 3 it is underlined that 5 of the 40 patients who experienced cytogenetic evolution were classified as RAEB-t according to FAB but as AML according to WHO.
2. Peripheral blood counts have been completely omitted from the tables.
3. The WHO classification at diagnosis and cytogenetic evolution with percentage of marrow blasts has been provided (table 2).
4. IPSS has been deleted from the tables and the manuscript has concentrated on cytogenetic evaluation
5. The number of tables has been reduced to four. In particular:
   The previous tab.2 has been fused to tab.1; the previous tab.3 has been omitted and substituted with the new tab.2 which reports the clinical and cytogenetic features of the 40 patients who experienced CE; the previous tables 4 and 5 has been fused together in the new table 3 which maintains their relevant information; the previous table 6, now table 4, has been maintained; the previous tables 7 and 8 has been omitted.