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Myelodysplastic Syndromes: evaluation of different prognostic scores in patients treated with chemotherapy

Myelodysplastische Symdrome: Evaluation des prognostischen Wertes von WPSS und Vergleich mit den Indices IPSS und IPSS-R bezogen auf Patienten mit Chemotherapie

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Meinen Großvätern Giuseppe und Giovanni

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1.INTRODUCTION

1.1 Epidemiology of myelodysplastic syndromes

The expression “myelodysplastic syndromes” (MDS) is referring to a heterogeneous group of hematologic disorders characterized by single or multiple peripheral blood cytopenias. These alterations are linked to the inability of the bone marrow stem cells to produce mature progeny, both in term of quality and quantity.

For many years, they were also known as “preleukemic syndromes” or “smouldering leukemia”, to highlight the tendency of these disorders to evolve into acute myeloid leukemia (AML), but this term has fallen into disuse because it excludes the many indolent forms that can stay stable also for several years. Although the exact nature of the disease remains controversial, it is generally accepted that MDS generate from a clonal disorder.

The MDS include the idiopathic acquired syndromes, also called primary MDS or MDS de novo, and the secondary MDS, which are mostly a consequence of treatments with antineoplastic agents or radiotherapy used to cure a primary malignancy. The last ones are also named “therapy MDS” (t-MDS). Secondary MDS are frequently characterized by a rapid evolution and are oft correlated to a fibrotic or hypoplastic bone marrow. In most of the cases, chromosomal aberrations can be found and the rich clinical scenario of these patients is due to the multiple cytopenias in peripheral blood [1].

Population-based bank data on patients with MDS are still scarce. The incidence of MDS increased significantly every year and its rate in the United States is reported as 3,4 per 100.000 people, translating to over 10.000 new diagnoses annually, in a study of 2010 [2-5]. In Europe the general picture is similar: in a recent study of 2011 the crude incidence rate was 4,15 pro 100.000 persons per year, with a prevalence of 7/100.000 [6, 7]. These apparently rapid increases, in the last decade, seem to be correlated to an aging population and to the increasing recognition of the pathology as a separate entity with an own dignity. Nowadays, though, MDS incidence and prevalence are probably underestimated, because a lot are the clinical cases that are not reported, and, even more are the patients whose apparently unexplained pancytopenia is not properly investigated [5, 8, 9].

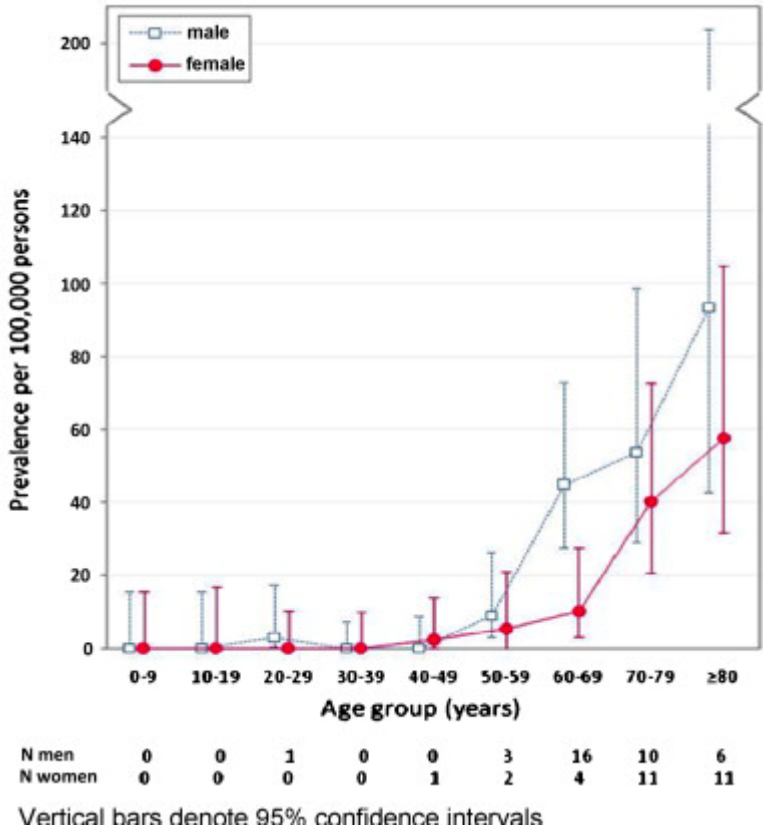

Figure 1: Age- and sex-specific incidence rates of MDS in Düsseldorf (1996–2005) [6]

The MDS de novo are a typical disease of the elderly. Most of cases are diagnosed in individuals, who aged ≥60 years at the time of their diagnosis (Figure 1). The incidence of MDS rose with increasing age both among men and women. Age-specific prevalence has a similar pattern of increasing prevalence with increasing age [6].

Different are the data concerning the t-MDS. In these cases, patients are often younger and the myelodysplasia shows up also after several years from the primary, oft potentially curable, malignancies [6, 11]. Patients, who underwent a therapy for non-Hodgkin's lymphoma (NHL), breast or testicular cancer presented a 2- to 15-fold increased risk of developing t-MDS, this risk grows till to 20- to 40-fold in patients who had a previous Hodgkin’s disease [10].

Another important epidemiological data concerned the sex. MDS are mostly a disease of males. Although there are often no significance differences regarding the prevalence of MDS between males and females, the incidence pro year is significantly more for men. The explanation is probably to find in a generally longer life expectancy of women [6, 11]. The only exception is for the 5q- syndrome, which is a particular case of MDS associate with the deletion of part of the chromosome 5. In fact, it shows a marked female preponderance [11].

Although some studies highlight the role of ethnicity as risk factors for MDS, no firm consensus on its importance has been reached. Whites, for example, seem to have in America a higher incidence rate than other racial/ethnic groups [2]. Surely, most must be done to delineate the geographical and ethical distribution of MDS in the world.

1.2 Pathogenesis

Despite the increasing interest in MDS, a lot remains still undefined about the etiopathogenesis. Several theories have been developed in the attempt to clarify the complex pathways at the basis of MDS.

Surely a key role is played by cytogenetic alterations, which can be proved in about half the cases of MDS [12]. Although this evidence, there are no specific genetic disorders that can be clearly associated with the development and progression of the disease. Probably, like already hypothesized in 1996 by Knudson, speaking about the pathogenesis of cancers in generally, the selective advantage of the neoplastic clone is determined by the accumulation of genetic alterations that together seem to make the myeloblastic cell more incline to cell turnover and cell division [13]. Starting with these bases, it is easy to suppose that the progress of MDS is due to percentage increases of blasts in bone marrow and accumulation of more and more cytogenetic abnormalities.

In most patients, though, hypercellular or normal cellular bone marrows coexist with the typical peripheral cytopenias. That can be considered a paradox, if the phenomenon would not be explained with a counterbalancing increase in apoptosis. The deregulation of apoptosis is due to the alterations of the intrinsic and/or extrinsic pathways.

Furthermore, altered epigenetic mechanisms concurred to the development of some of the severest forms of MDS. The reversal of epigenetic aberrations is the rational of the use of new drugs, like 5-Azacitidin, for the treatment of MDS. This example shows how essentially is to know the pathogenesis of the disease in order to find new appropriate and targeted treatments specific for each patients.

Another element that seems to have abandoned the role of simple spectator, not only in the MDS but also in other cancer diseases, is the microenvironment. It has, in fact, conquered one of the main roles in the genesis of bone marrows disorders and that can be the key for the development of future new treatments.

These three specific mechanisms, involving the increase of apoptosis, epigenetic abnormalities and microenvironment alterations deserve probably a closer description.

1.2.1 Apoptosis

The frequent descriptions of hypercellular bone marrows in patients with MDS, associated with peripheral pancytopenia, guided the science world to a new etiopathological hypothesis based on the alteration of the cell self-renewal. In particular, it seems that, in the earlier phases of the disease, the extrinsic mitochondrial pathway is particularly active and particularly expressed are molecules like TNF-α (Tumor Necrosis Factor-α), Fas-ligand, TNF-related apoptosis-inducing ligand (TRAIL) [14-16]. It is though still unclear if the primary event is the pathway-activation or the amplification of signals originated from the death-receptor stimuli, like TNF-α.

Also the intrinsic pathway can show mutations, like the overexpression of caspase-9 that can be responsible of an excessive apoptosis, especially in the early stage of MDS [17]. Both apoptotic ways converge then in the caspase-3 that can be also susceptible to phenomenon of hyperexpression in the bone marrow. In these cases, the overexpression of caspase-3 is the principle cause of thrombocytopenia [17].

With the progression of the disease, other mutations can occur. For example, alterations of Bcl-2 family proteins may affected the events cascade that can lead to the leukemic transformation [18].

1.2.2 Epigenetic Aberrations

In the last decades, several studies have focused on etiological role of epigenetics in MDS, most of them highlighted the abnormalities of DNA methylation.

Even if it seems today clear that the methylation levels are higher in the DNA of patients with myeloid malignancies, more must be done to understand if these alterations are one of the causes of the disease or if they are mere consequences of them.

Changes in DNA methylation may silence the expression of some genes and their related proteins. In particularly, hypermethylation, observed mostly outside the CpG islands, is responsible of the alterations of various genes, including cell cycle regulators, apoptotic genes, and DNA repair genes. Of great interest is the correlation between chromosomal deletions and the hypermethylation that suggests the cooperation in silencing tumor suppressor genes [19].

1.2.3 Microenvironment

In the pathogenesis of MDS are increasing the studies that demonstrate how the microenvironment interactions can influence the genesis of dysplastic clones.

One example is given by a study that shows how the stromal cells can induce the expression of inducible gelatinase B/matrix metalloproteinase(MMP)-9 in monocytes, enzymes that influence cell regulation [20]. Data in vivo prove a reduction of the MDS derived monocytes' sensibility to stromal stimuli and a consequent reduction of these MMPs [20]. In a similar way, also the expression of MMP-2, which may influence above all the erythroid line, has been proved to be altered in the MDS as well as in the LAM patients [21, 22].

These data, together with others regarding the role of genetic aberrations of stromal cells in MDS bone marrows [23], suggest a cooperation of different proliferative stromal stimuli which may contribute to the selection of a dysplastic clone and lay the groundwork for new targets for treatment of MDS [24].

Promising are the therapies with drugs that have as target the alteration of the interaction between chemokine receptor CXCR4 and its ligand CXCL12. The purpose is the mobilization of MDS malignant cells from the environment in which they originated, to avoid the activating stromal stimuli and possibly make them more responsive to treatments. This hypothesis is actually under evaluation, but more data should be collected to validate it [18].

Some of the drugs used today, like lenalidomide, are already based on their multifactorial actions in the bone marrow microenvironment, including antiangiogenic and anti-inflammatory effects.

Further research is required to develop new etiopathology-based therapies and this can be obtained just with further studies on the unsolved mysteries still linked to the MDS and LAM origins.

1.3 Diagnosis

Signs and symptoms of MDS are mostly unspecific, so that diagnosis can represent a real challenge for physicians. In most cases, there is just an altered blood cell count. In particular, the presence of bi- or pancytopenia together with alterations of the differential cell count is highly suggestive for a MDS. Although, more often the anemia is the only element that physicians identify after routine examinations. In this case, the first step is to rule out the more frequent causes that can provoke anemia (iron deficiency, hemolysis, occult gastrointestinal bleeding etc.). The further workup to a correct diagnosis, though, must include also the karyotype and the cytological analysis on bone marrow aspirate and peripheral blood smears, appropriately stained with, for example, the May-Grünwald-Giemsa.

1.3.1 Cytomorphology

The meticulous microscopic examination of blood and bone marrow aspirate is a simple, ideal initial approach for a correct diagnosis of MDS and for a proper differential diagnosis with other causes of anaemia and/or thrombocytopenia and/or neutropenia.

The cells that can be found in a MDS patient present typically morphological abnormalities. The table 1 presents the most common alterations. However, they are not pathognomonic signs of MDS and they can be observed also in other diseases, like AML, myeloproliferative syndromes and other nonhematological diseases.

Several dysplastic elements, often affecting more than 10% of nucleated cells, are usually together with an increased number of blasts, which by definition can not be more than 20% of cells (if more than 20% we are already in the sphere of AML) [25]. The amount of blasts is not just necessary for the diagnosis, but has also a prognostic relevance.

Besides the May Grünwald-Giemsa staining, there are other methods to prepare a smear. The iron staining called Perls Prussian blue is useful to identify the presence of iron and to highlight the so called ringed sideroblasts (so named because the iron granules are arranged in a ring around the nucleus). Also the periodic acid Schiff reaction is a valid indicator of dyserythropoiesis in MDS.

	Peripheral Blood	Bone Marrow
Erythroid series	• Macrocytic anaemia • Punctate basophilia • Anisopoikilocytosis • Howell-Jolly bodies • Hypochromic fragments	• Ringed sideroblasts • Megaloblastosis • Dyserythropoiesis • Erythroid Hyperplasia
Granulocytic series	• Hyposegmented neutrophils • Dhole bodies • Nuclear Stick • Chromatic Clumping • Dimorphic granules • Pseudo Pelger-Hüet cell **	• Hypogranular myeloid precursors
Megakaryocytic series	• Giant platelets	• Microkaryocytes • Single nuclear lobe megakaryocytes • Pawn ball megakaryocytes
Monocytic series	• Promonocytes	
* Multiple separate nuclei megakaryocytes ** Neutrophils with dumbbell-shaped bilobed nucleiand coarse clumping of the nuclear chromatin.		

Table 1 Dismorphic cellular aspect in MDS

1.3.2 Bone Marrow histology

In the diagnostic workup, together with bone marrow aspirate analysis, histomorphological investigations are useful to identify the eventual topographic distortion of the bone marrow architecture or the presence of fibrosis [26].

In physiological conditions, the erythroblastic islands and the megakaryocytes are placed adjacently to the sinusoids endothelium, in the central cavities of bone marrow; on the other hand pluripotent stem cells appear mostly peripherally from the central, in close relationship with the endosteal surface. The more mature granulocytic forms are randomly distributed in the central intertrabecular areas [27].

The topographic alterations of progenies in MDS were described already in 1984 by Tricot [27], who highlighted the tendency of myeloid precursors to aggregate in cluster in the intertrabecular areas. This histoarchitectural displacement is also known as Abnormal

Localization of Immature Precursors (ALIP). Even if ALIP can be found also in not-severe forms of MDS, it seems to be of prognostic relevance [28].

Furthermore, the biopsy allows to estimate the presence of fibrosis and can define the hematopoietic cellularity that is particularly important when there is not the possibility to obtain a bone marrow aspirate (so called “puntio sicca”) [27, 29].

1.3.3 Genetic Aberrations

The karyotype analysis plays a fundamental role both in the etiopathogenesis and in the workup of a correct diagnosis. There are also studies proving that some genetic aberrations have a prognostic significance in patients with MDS [30-32].

About 50% of MDS patients have a normal karyotype at the first diagnosis, even if it is possible that, within them, there are cases with cytogenetic aberrations still not traceable at conventional cytogenetic analysis. This data is proved by new studies which investigate the importance of the fluorescent in situ hybridization (FISH) for a precise definition of genetic abnormalities [33].

The other 50% of patients presents chromosomal alterations already at the first diagnosis [34-36]. In most of cases, there is a single mutation, to which many others can be added in course of disease. Addictions of new chromosomal aberrations are linked to a higher risk of leukemic evolution [37]. For this reason, it is fundamental to repeat the analysis during the follow up both to monitoring the response to treatment and to evaluate the residual disease.

Patients with secondary MDS have mostly multiple chromosomal aberrations already at the first diagnosis; the finding of ring or dicentric chromosomes is also common, probably due to the previous chemotherapy.

The chromosomal aberrations are not usually related to a specific symptomatic or syndrome. The only exception is the deletion of chromosome 5, that lead to the so called “5q- syndrome”. Detectable in 10-30% of cases, is one of the most frequent genetic alterations in MDS [38, 39]. It regards the long arm of chromosome 5, where are mapped several regulatory genes of haematopoiesis. Two are the regions most frequently altered: 5q33.1, that can be found in all 5q- patients and associated with a better prognosis, and 5q31, in particular in patients with t-MDS, linked to a more aggressive clinical course [40-42].

Some of the most frequent chromosomal alterations found in MDS patients are shown in the Table 2.

Primary MDS	Chromosomal aberrations	Frequency (%)
Not Balanced	+8	10
	-7 / del (7q)	10
	-5 / del (5q)	10
	del (20q)	5
	-Y	5
	i(17p)	3
	-13 /del (13q)	3
	del (11q)	3
	del(12p)/t(12p)	3
	del (9q)	1
Balanced	t(1;3)(p36.3;q21)	1
	t(2;11)(p21;q23)/t(11q23)	1
	inv(3)(q21q26.2)	1
	t(6;9)(p23;q34)	1
Secondary MDS	-7/del(7q)	50
	-5/del(5q)	40

Table 2 From the study of Vardiman, JW, Brunning, RD, Arber, DA, et al. Introduction and overview of the classification of the myeloid neoplasms. In: WHO classification of tumors of hematopoietic and lymphoid tissues, Swerdlow, SH, Campo, E, Harris, NL, et al. (Eds), WHO Press, 2008. p.18.

According to recent studies, the deletion of chromosome 7 is the second more common genetic mutation after the 5q- (respectively 25% and 30%) [34]. The alteration is usually in the long arms of the chromosome 7 and more often in the s-MDS. Less common is the finding of monosomy 7. In any case, alterations of chromosome 7 are linked to a bad prognosis. Trisomy 8, present in circa 10% of cases, is considered by the most an alterations of intermediate risk [30, 32, 43], but there are also others opinions about its prognostic relevance [44].

The International System for human Cytogenetic Nomenclature (ISCN) defines “complex” a karyotype with three or more chromosomal aberrations [45]. It is associated to a bad prognosis and a rapid leukemic progression.

1.4 Classification

1.4.1 French-American-British (FAB) Classification

One of the first attempts of MDS classification was published in 1976, and revised in 1982, by the FAB group (French-American-British) [46]; it is based exclusively on morphological criteria and, in particularly, on proportion of blast cells in the peripheral blood and/or bone marrow and on type and grade of cellular dysplasia. This classification was immediately accepted by the scientific community, but its worldwide application has revealed the inherent limits.

1.4.2 World Health Organization (WHO) Classification

Nowadays, the probably more used morphological classification is the one proposed by the WHO (World Health Organization) Clinical Advisory Committee [47]. First introduced in 1999, its original version was later re-update. The following table shows a revised edition published in 2008 [48].

Compared to the previous classifications, the WHO excludes the RAEB in transformation (RAEB-t) from the MDS frame, because of the poor median survival and the scarce response to therapy. Today, patients who present 20 to 30% blasts in the bone marrow are already in the category of AML and, therefore, should be treated as AML.

In response to the critics against the FAB classification, on the one hand the WHO introduced the concept of “multilineage dysplasia”, highlighting the better prognosis of patients with only dyserythropoiesis when compared to those with pancytopenia and dyplasia in two or more myeloid lineage. On the other hand, the RAEB category proposed by the FAB has been divided into two subtypes: RAEB -1 and RAEB-2, according to the percentages of blasts in blood and bone marrow. Moreover, patients with 2-4% blasts in the peripheral blood and less than 5% in the bone marrow should be considered RAEB -1 if there are clinical or laboratory evidences supporting the diagnosis of MDS.

The CMML is still today a controversial entity. Some patients may no present dysplastic abnormalities, but leukocytosis and splenomegaly which are more typical for MPN. Open was the debate whether to consider it a MPN or MDS. The solution proposed by the WHO is to place it in a separate group overlapping both MDS and MPN. This was called MDS/MPN category and included not only the CMML, but also similar entities like the atypical chronic myeloid leukemia (aCML), juvenile myelomonocytic leukemia (JMML), and the refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) [49]. In contrast to the FAB

classification, the WHO proposed also a distinction regarding the count of blasts in blood and bone marrow. The reason is to be found in their prognostic value, so today there are two classes: CMML-1, with less than 5% blasts in blood and less than 10% in bone marrow, and CMML-2, with a percentage between 5-19% in blood or 10-19% in bone marrow [50].

Compared with the FAB classification, the WHO seems to have a better predictive value [51] and several are the studies confirming its importance for prognosis and therapy [32, 49, 52, 53].

WHO Class	Peripheral Blood	Bone Marrow
Refractory anemia (RA) or Refractory Neutropenia (RN) or Refractory Thrombocytopenia (RT)	• Anemia • No Blasts	• Dysplasia in ≥ 10% of cells in just one myeloid lineage • < 5% blasts • < 15% of ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	• Anemia • No Blasts	• ≥ 15% of ringed sideroblasts • Isolated erythrocytes' dysplasia • < 5% of blasts
Refractory Cytopenia with Multilineage Dysplasia (RCMD)	• Bi- or pancytopenias • No or rare blasts • No Auer rods • $<1x10^9$/L monocytosis	• Dysplasia in two or more myeloid lineages (>10% of cells in each affected lineage) • < 5% blasts • No Auer rods • May have 15% or more ringed sideroblasts
Refractory anemia with Excess Blasts (RAEB-1)	• One or more cytopenias • < 5% blasts • No Auer rods • $<1x10^9$/L monocytosis	• Dysplasia in one or more myeloid lineages • 5-9% blasts • No Auer rods
Refractory anemia with Excess Blasts -2 (RAEB-2)	• One or more cytopenias • 5-19% blasts • ± Auer rods • $<1x10^9$/L monocytosis	• Dysplasia in one or more myeloid lineages • 10-19% blasts • ± Auer rods
MDS, unclassified (MDS-U)	• One or more cytopenias • < 1% blasts	• Dysplasia in one or more myeloid lineages with MDS specific genetic abnormality • < 5% blasts
MDS with isolated del(5q) (5q-syndrome)	• Anemia with or without other cytopenias • Normal or increased platelet count • < 1% blasts	• Normal or increased hypolobulated megakaryocytes • < 5% of blasts • Isolated del(5q) • No Auer rods

Table 3 WHO Classification (2008**)**

1.5 Prognosis

About one-third of patients with MDS develop secondary acute myeloid Leukemia (AML). [54, 55]. The leukemic transformation is a severe consequence, because these patients are often resistant to the standard therapeutic options and only less than 10% of the treated cases has a long-term survival rate [56]. In the remaining two-thirds, the *exitus letalis* is due to bleeding, grave infections or anemia caused by the progressive bone marrow failure [54, 56].

The course of the pathology is, though, extremely varied, so it is fundamental to define different prognostic classes in order to obtain a correct evaluation of the risk, in terms of survival and leukemic progression, and to choose the appropriate therapeutic treatments.

To decide the correct medical approach, an oversimplified distinction between high- and low-risk forms was used for several years considering the amount of blasts. Later, also the morphological classifications, in particularly the WHO, proved to have prognostic relevance.

Nowadays, two are the most used prognostic scores: international prognostic scoring system (IPSS) and WHO adapted prognostic scoring system (WPSS). Moreover, the attempt to update the IPSS, providing a better risk stratification, gave born in 2013 to the new IPSS-R, which is slowly replacing the IPSS in the clinical routine. These three scores are presented hereunder.

1.5.1 International Prognostic Scoring System (IPSS)

The IPSS (International Prognostic Scoring System) has been proposed by Greenberg and coll. in 1997 [30]. It distinguishes four risk groups according to a specific score obtained adding the points associated with the prognostic variables shown in the following table (Table5).

PROGNOSTIC VARIABLE	IPSS SCORE				
	0	**0,5**	**1**	**1,5**	**2**
BONE MARROW BLAST (%)	0 - 4	5-10	-	11-20	21 - 30
CYTOGENETIC RISK GROUP	Low	Intermed.	High		
NUMBER OF CYTOPENIAS*	0 - 1	2-3			
* ANC<1800/mmc, Hb<10 g/dl, piastrine<100x103/mmc					

Table 4 International Prognostic Scoring System (IPSS)

This classification highlights the prognostic importance of karyotype, proposing three risk groups:

- Low Risk: normal karyotype, -Y, del(5q), del(20q)
- High risk: complex karyotype (≥ 3 anomalies), chromosome 7-anomalies
- Intermediate risk: all other aberrations

In this classification, morphological differences are not included. The IPSS was broadly accepted in the scientific community and for a long period considered the gold standard for the everyday clinical routine.

1.5.2 WHO adapted prognostic scoring system (WPSS)

The WPSS has been developed considering the important prognostic value of the WHO morphological classification [52, 57] and that of the IPSS cytogenetic risk stratification [58], together with the identification of frequent transfusion requirements as negative prognostic sign in MDS patients [32]. Cytogenetic aberrations became more prognostic weight as

compared to the It is a dynamic scoring system, able to identify five different classes of risk and to provide at any time in the course of disease information about the survival expectancy and the probability of an evolution to AML. The score system is shown in the following table (Table 6).

PROGNOSTIC VARIABLE	WPSS SCORE			
	0	1	2	3
WHO SUBTYPES	RA/RARS/5q-	RCMD/RSCMD	RAEB I	RAEB II
CYTOGENETIC GROUPS*	Low	Intermediate	High	
TRANSFUSION REQUIREMENTS	No	Yes		
*same cytogenetic risk groups used for the IPSS ** at least a transfusion every 8 weeks over a period of 3 months				

Table 5 WHO adapted Prognostic Scoring System (WPSS)

1.5.3 International Prognostic Scoring System-Revised (IPSS-R)

In the attempt to revised and update the IPSS, a group of experts analyzed more than 15 databases from different international institutions and, focusing on patients in best supportive care, created the so called IPSS-Revised (IPSS-R) [31]. The most relevant innovation is the cytogenetic risk stratification in five classes (Table 8) highlighting the prognostic value of this variable.

The table 3 shows the prognostic variables used in this score and the related scores.

Even if more studies have to confirm the prognostic value of the IPSS-R, it is has already took a relevant place in the daily practise in hematologic departments [59].

PROGNOSTIC VARIABLE	IPSS-R SCORE						
	0	**0,5**	**1**	**1,5**	**2**	**3**	**4**
BONE MARROW BLAST (%)	≤2		3-4%		5-10%	>10%	
CYTOGENETIC CATEGORY	Very Poor		Poor		Interm.	High	Very Good
HEMOGLOBIN	≥ 10		8 to 9,9	< 8			
PLATELETS	≥ 100	50 to100	< 50				
ABSOLUTE NEUTROPHIL COUNT	≥ 800	< 800					

Table 6 IPSS-Revised (IPSS-R)

CYTOGENETIC CATEGORY	Cytogenetic Abnormalities
VERY GOOD	del(11q), -Y
GOOD	Normal karyotype, del(5q), del(12p), del(20q), double including del(5q)
INTERMEDIATE	del(7q), +8, +19, i(17q), any other single or double independent clone
POOR	inv(3)/t(3q)/del(3q), -7, -7/del(7q), *complex* with 3 abnormalities
VERY POOR	Complex: > 3abnormalities

Table 7 Cytogenetic Category according IPSS-R

1.6 Clinical Manifestations

The clinical manifestations of MDS are consequences of inadequate hematopoiesis. Thanks to the body's adaptive capacity, about 50% of patients can be totally asymptomatic at the first diagnosis [60]. These are fortuitous cases of MDS that followed a routine blood control. The other 50% presents light to severe clinical manifestations due to anemic conditions, thrombocytopenia and/or neutropenia [60, 61].

The clinical suspect is linked to the age of the patients. A myelodysplastic condition is rare under 30 years old, but the incidence increases with the aging [62, 63]. Considering though the frequent co-morbidities and the iatrogenic treatments in old age, an accurate diagnosis is need to differentiate MDS from the several causes of peripheral mono-, bi- or pancytopenia.

1.6.1 Cytopenia

The anemia with its related symptoms is often the first clinical manifestation leading to MDS diagnosis. It is generally macrocytic or normocytic, only rarely microcytic. The 95% of MDS patients are anemic. In about 80% of cases the value of hemoglobin (Hb) is less than 10g/dl, already at first presentation [64].

The reduction in thrombocytes count is frequent in MDS patients [65, 66]. Numerous studies showed that the prevalence of thrombocytopenia in MDS ranged from 40% to 65% [61, 67-69]. Considering that a reduction of platelets can be also drug-induced, the debate on the correct treatments for these patients is still open [30, 31, 70, 71]. Only the minority of MDS patients (<10%) presents severe bleedings as first manifestation of disease [60]. However, thrombocytopenia is one of the major causes of death in MDS patients with a frequency of lethal hemorrhagic episodes that ranges from 14% to 24% [65, 72] [67].

The leukopenia is responsible of a lack of immune body reactions to viral or microbial agents. This is the reason why infections are one of the most frequent causes of complications and death in MDS patients. In the case of MDS, the neutrophils are mostly altered. This is the reason why bacterial infections are more common than viral ones. The first clinical manifestations of a neutropenia come when the ANC is under 800/mm^3. For values under 500/mm^3 the neutropenia is severe and associated to recurrent infections.

Functional granulocyte alterations, such as deficient chemotaxis, reduced phagocytosis and enzyme content as well as decreased microbicidal capacity, could be causes of a major risk of infections, even when the ANC is in the normal range [73].

1.6.2 5q- Syndrome

As above mentioned, the 5q- syndrome is the only known case in which a mutation determined a specific symptomatology. Contrary to the other MDS forms, it is typically a female disease. On the other hand, it remains a pathology of the elderly with a peak of incidence between 65-70 years old. At the first diagnosis, patients present usually a refractory macrocytic anemia and normal or increased thrombocytes; neutropenia is generally absent [74].

The lack of thrombocytopenia and the normal leucocytes count explain the absence of bleeding or infectious manifestations in these patients, who are though dependent on transfusion-therapy.

The prognosis is generally good, but only if the mutation remains isolated, namely if there are no other chromosomal aberrations in the following bone marrow analysis, which should be done regularly during patients follow-up.

1.7 Therapy of myelodysplastic syndromes

Being typical diseases of elderly, patients with MDS present themselves together with other comorbid illnesses. This evidence determines often a limitation as far as the therapy is concerned. To guarantee the appropriate treatment, the best approach is to divide the patients in prognostic classes, performance status, age and comorbidities.

Lot of diagnostic algorithms have been proposed in the years, the majority are based on the IPSS and the WPSS scores (Figure 2) [75-78].

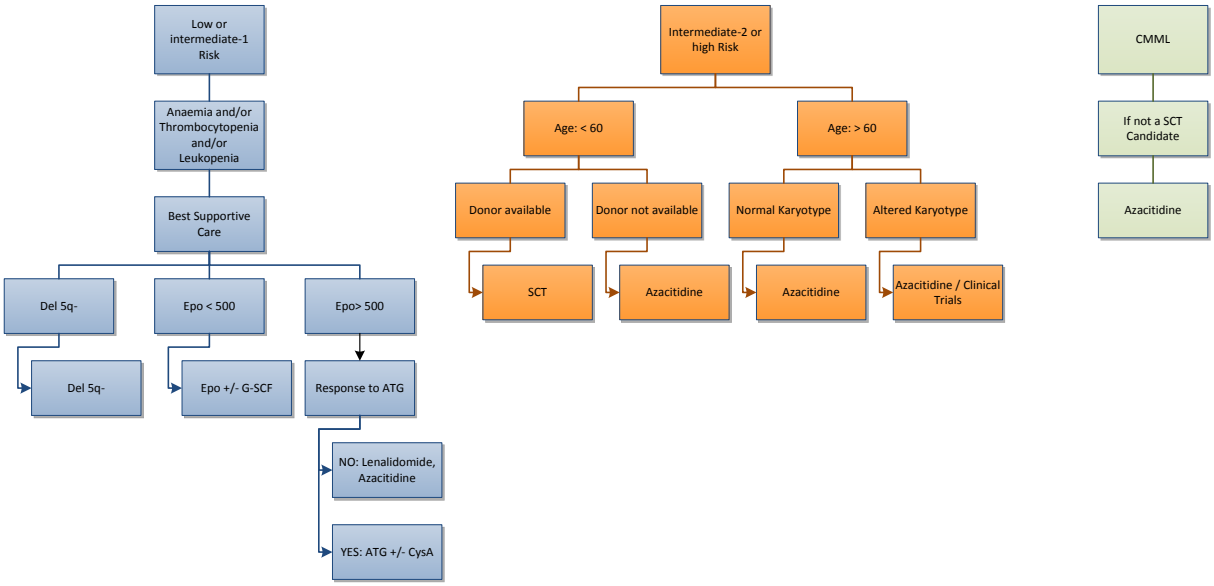

Figure 2 Therapeutic algorithms of MDS

Not all the patients need though a therapy, for those who are asymptomatic, with a low risk of leukemic evolution or dead for complications, the “watch and wait” approach is the gold standard.

1.7.1 Best Supportive Care

The so called “Best Supportive Care” (BSC) is the first choice therapy in patients classified as “low risk” or “intermediate-1-risk” according to the IPSS [75]. Even if the probability of healing thanks to a allogeneic hematopoietic cell transplantation is high (circa 60% of patients with low risk and 40% of patients in the intermediate-1 risk class), mortality and morbidity linked with the procedure and the risk of a relapse in five years do not improve the overall survival [79, 80]. Besides the BSC is indicated for patients, who can not receive more aggressive therapies because of age, low performance status or comorbidities.

BSC includes the use of erythrocyte and thrombocyte concentrates, grow factors and antibiotics as well as iron chelation therapy in patients who are chronically dependent to transfusions, and antibiotic therapy.

1.7.2 Immunomodulating Drugs

As mentioned in the pathogenesis paragraph, the rationale of the use of lenalidomide in the MDS therapy is based on its capacity to interrupt the interactions between cancer cells and bone marrow microenvironment together with its anti-inflammatory action. It is a derivate of talidomide and the both are included in the category of immunomodulating drugs. Whereas talidomide is not more considered in MDS international guidelines, due to its important side effects (neuropathy, thrombosis, constipation and teratogenicity), the efficacy and the benefits of lenalidomide have been proved not only in MDS therapy, but also in multiples myeloma and several subtypes of non Hodgkin Lymphoma.
Referred to MDS, patients with a del(5q) are particularly responsive to lenalidomide [81-83].

1.7.3 Epigenetic Therapy

The inhibitors of DNMTs are the so called DNA hypomethylating agents. Although it is not clear if their efficacy is just inked to their demethylating properties, they are usually classified as epigenetic drugs [77].
They were produced more than half a century ago, as cytosine analogs, with the idea of creating a cytotoxic agent to use in chemotherapy. The cytotoxic effect, though, did not show better responses if compered with conventional chemotherapy, on the contrary the new drugs had more side effects. They tried then to reduce the doses, noting that if on the one hand it provokes a reduction of the cytotoxic effect, the demethylating power increases significantly.
The two most important drugs of this category are 5-azacytidine (azacitidine - AZA) and 5-aza-2′-deoxycytidine (decitabine - DAC). Both of them have shown a reduction of leukemic

progress risks and an increasing overall survival (OS) in MDS patients when used in low doses [84, 85].

1.7.4 Conventional chemotherapy

The conventional chemotherapy is an AML-like chemotherapy with standard or high-dose cytosine arabinoside combined with an antracycline. It has been used mainly for young patients with higher risk MDS, but it improves the OS of treated patients just when associated to allogeneic HSCT [86]. Due to the high morbidity and mortality associated with chemotherapy, the first line therapy does not include the use of chemotherapy in MDS patients, if not specifically part of clinical trials. The only suitable candidates for traditional chemotherapy are those who had no significant responses after 6 cycles AZA or are not eligible to a therapy with hypomethylating agents and those who need an induction therapy before HSCT.

1.7.5 Allogeneic Hematopoietic Stem Cell Transplantation

Whereas the allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the only treatment that provides a cure for MDS, these are pathology of the elderly with high rates of treatment-related morbidity and mortality. Nowadays, the new guidelines and therapies for the management of MDS patients and the gradual course of the disease guarantee a longer life expectancy together with a better quality of life. Thus the HSCT is an individual healing try, which must be reserved as first-line-therapy to intermidiate-2 or high risk patients younger as 65 years without severe comorbidities [77].

2. AIM OF THE STUDY

The *International Prognostic Scoring System* (IPSS) has been the most used method to predict outcome and risk of AML-evolution for all MDS patients.

The introduction of WHO-classification and the discovery of other possible risk variables in the prediction of the outcome of MDS-patients lead to the definition of the *WHO-adapted Prognostic Scoring System* (WPSS). It has already been proved to be a better prognostic score both in untreated patients and in those undergoing allogeneic hematopoietic stem cell transplantation (allo-SCT) [32, 49]. Though, until now, the WPSS has not been proved to be able to predict the Overall Survival (OS) or the risk of leukemic progression in patients affected by MDS who underwent an induction chemotherapy or a therapy with 5-Azacitadine. The initial aim of the study was to investigate the prognostic value of WPSS in this particular group of patients.

Moreover, after the awareness of the attempt to refine the IPSS score with the so called *Revised International Prognostic Scoring System* (IPSS-R) [31], the study was extended to the comparison of the well-known IPSS, the WPSS and the new IPSS-R in the prediction of prognosis, in terms of overall survival (OS) and leukemic progression, for this kind of patients, treated with chemotherapy or with 5-Azacitadine.

3. MATERIALS AND METHODS

The study included 375 patients, 315 from the MDS-Registry of the *Universitätsklinikum* of Düsseldorf and the rest from three different Sicilian Hospitals: *Azienda Ospedaliero-Universitaria Policlinico "Paolo Giaccone"* of Palermo, *Azienda ospedaliera Ospedali Riuniti Villa Sofia-Cervello* of Palermo, *Azienda Ospedaliero-Universitaria "Policlinico Vittorio Emanuele"* of Catania.

A collaborative international research protocol and minimal data set was developed and provided to achieve a homogeneous enrolment of patients. Including patients from multiple international institutions enables the analysis of an extended number of patients and reduces the statistical bias due to genetic or geographic features, which may sway the results. Fundamental inclusion criterion was a treatment with chemotherapy and/or 5-Azacitidine. Just 40 patients received a therapy with only 5-Azacitidine. All patients were documented following the minimal data set. The collection of data has been approved by the Ethics committee of the Heinrich Heine University, Germany (Nr. 3008 of 15.1.2008).

A specific informed consent was not required due to the retrospective nature of the study. The clinical variables under examination were collected at the moment of the first diagnosis, before any therapy. Within the patients, 116 underwent allogeneic stem cell transplantation (allo-SCT) after the induction therapy. These patients were censored at the date of allo-SCT. Transfusion requirement has been defined according the WPSS.

The morphological diagnosis was made according to the French-American-British (FAB) criteria and the proposals of the Word Health Organization (WHO) 2008 classification. In particular, FAB criteria were used until 2001. In 2009, the sample was retrospectively re-classified according the WHO classification of 2008.

Cytogenetic analysis at the time of diagnosis was performed by Dr. rer. nat. B. Hildebrandt, at the Institute of Human Genetics, Heinrich-Heine University, Düsseldorf, by Dr. C. Consoli, from the University of Catania and by Dr. V. Calò from the University of Palermo. The cytogenetic data were subdivided according to the International System for human Cytogenetic Nomenclature (ISCN) [45]. A minimum of ten metaphases was required for the analysis.

For every patient, investigations about survival and progression to leukemia have been conducted until the 31 may 2012. The Overall Survival (OS) has been calculated with the non-parametric method of Kaplan-Meier [87], considering the date of the first diagnosis and either the time of death (complete data), independently from the cause, or the time of the last

follow up (censored data). The same method was used to define the leukemic progression. The Long-rank test together with the Breslow- and Tarone-Ware-testes were important elements to compare different Kaplan–Meier curves.

The independent prognostic features have been analyzed using uni- and multivariate models [88]. The clinical and hematological data, at the first diagnosis, have been compered with Chi-Square test, also referred to as χ^2 test. When including the WPSS in the comparison, patients with CMML and RAEB-T could not be included in the calculations. Statistically significance was defined for a p-value less than 0.05. All data were analyzed with IBM SPSS Statistics Version 20.

4. RESULTS

4.1 Descriptive Statistic

We studied 375 patients, including 227 males (60,5%) and 148 females (39,5%). 64% of analyzed patients were older than 55. The median age at MDS diagnosis was 58 years (range: 14-92) and median survival of the entire group was 26 months.

Patients in the database were grouped according to the WHO criteria into: 15 (4%) with refractory anemia (RA); 48 (12,7%) refractory cytopenia with multilineage dysplasia (RCMD); 12 (3,2%) with RCMD and ringed sideroblasts (RSCMD); 2 (0,5%) with refractory anemia with ringed sideroblasts (RARS); 4 (1,1%) with MDS associated with del(5q); 64 (17,1%) with RA with excess blasts-1 (RAEB-1); 110 (29,3%) with RA with excess blasts-2 (RAEB-2). The remaining patients, not compatible with the WHO2008 classification, included: 17 (4,5%) with chronic myelomonocytic leukemia-1 (CMMLI), 18 (4,8%) with chronic myelomonocytic leukemia-2 (CMMLII) and 85 (22,7%) with refractory anemia with excess blasts in transformation (RAEB-t). Further characteristics of the patients are summarized in Table 8.

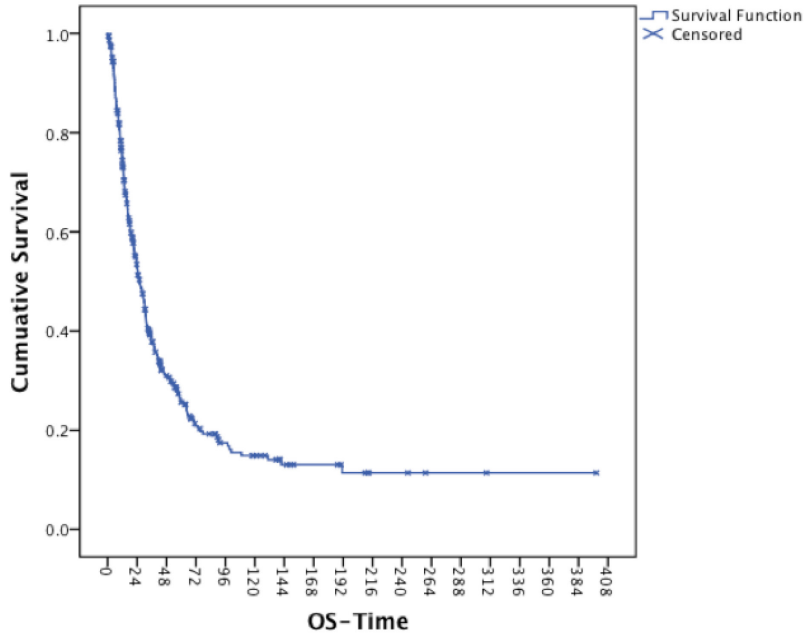

Figure 3 Overall Survival of patients under analysis

There is no significant difference in OS between the two centers (Sicily VS Düsseldrof). The 5-years OS was 25%, whereas 5-years probability of AML-evolution was 67%.

The IPSS could be defined in 364 of 375 patients: 120 as intermediate-1 risk (32%), 135 as intermediate-2 risk (36%) and 109 as high risk (29,1%). None of our patients was classified as low risk according the IPSS classification (Figure 4).

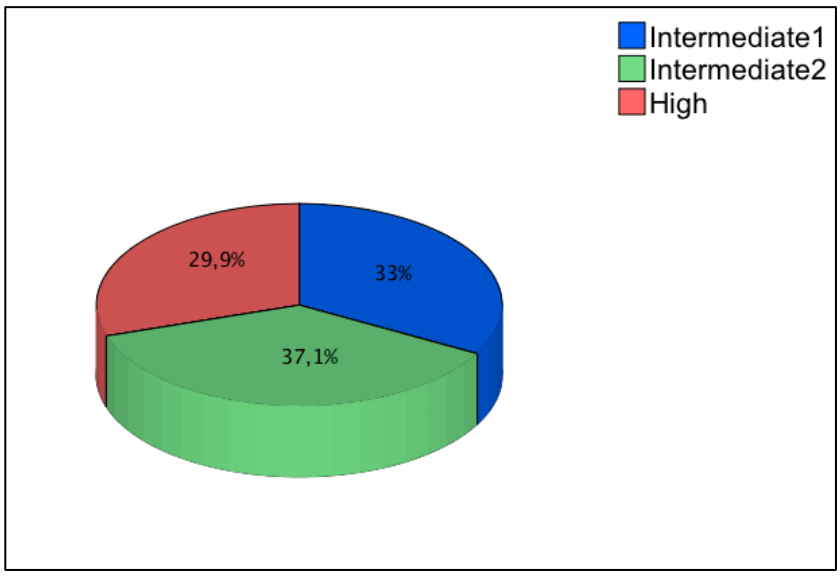

Figure 4 Patients classification according IPSS-Score

WPSS score was calculated for 222 patients. Among these, 15 were classified as low risk (6,8%), 25 as intermediate risk (11,3%), 125 as high risk (56,3%) and 57 as very high risk (25,7%).

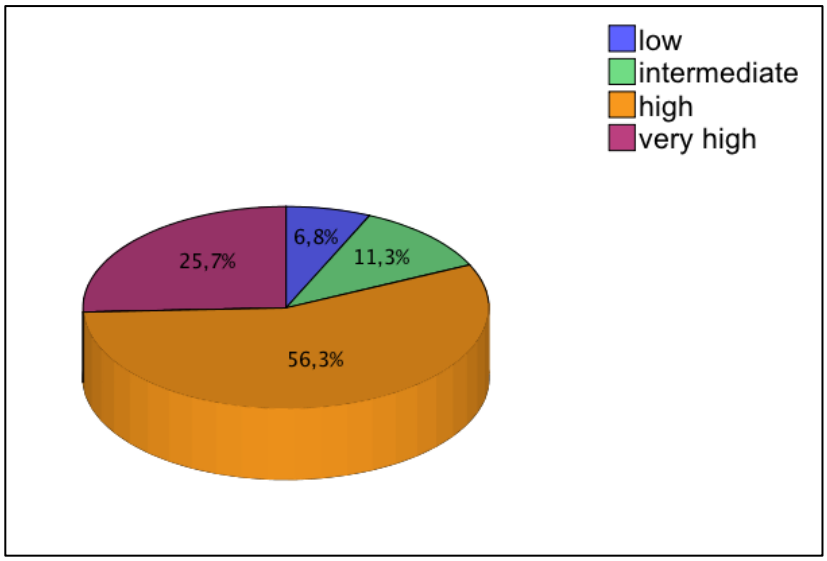

Figure 5 Patients classification according WPSS-Score

Patients classified according to the IPSS-R were 370: 9 very low risk (2,4%), 29 low (7,7%), 103 intermediate (27,5%), 115 high (30,7%) and 114 very high risk (30,4%). Due to the low percentages, we excluded patients with very low IPSS-R risks.

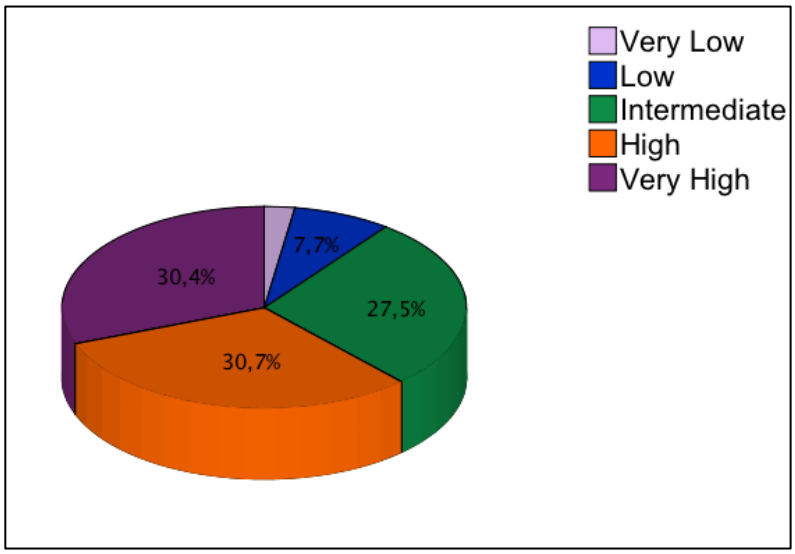

Figure 6 Patients classification according IPSS-R-Score

4.2 Univariate Analysis on overall survival (OS) and acute myeloid leukemia (AML) evolution

To identify which patient's characteristic and which IPSS-, WPSS- or IPSS-R-related criteria had a prognostic value, a univariate analysis has been performed. Based on this analysis, age, transfusion dependency, AML-evolution and chromosomal aberrations (stratified into risk groups according to IPSS, WPSS and IPSS-R indications) were significant predictors of patients' outcome (Table 8).

In particularly, patients older then 55 years had, with a median survival of ca. 24 months, a significant reduced OS than the younger patients (*p*: 0,001).

WHO classification at first diagnosis had a borderline influence on OS (*p*: 0,055). On the other hand, it proved to be a significant predictor of AML-evolution risk (*p*: 0,006).

A non-significant difference in OS was seen between patients with different transfusion needs at first diagnosis (*p*: 0,886), whereas significant for the OS is the need of transfusions in the follow up (*p*: 0,018).

Altered blood count (ANC and/or PLT and/or Hb) at first diagnosis failed to show effects on OS in the univariate analysis, both when considered subdivided according the IPSS- or the IPSSR-stratifications.

Bone marrow blasts classification seems not relevant both according the IPSS- and IPSSR-schemas (*p*: 0,202 and *p*: 0,067, respectively). Even if, significant is the difference in OS between patients with less than two blasts (Median Survival-MS: 38,6 months) and patients with more than ten blasts (MS: 21,87 months) at the first diagnosis (*p*: 0.009).

Chromosomal aberrations organized both in IPSS/WPSS and IPSSR classes were significant risk factors for OS (both $p < 0{,}001$).

Among the patients under analysis, 201 (53,6%) developed an AML. This data is not surprising considered that our pool of patients is per definition a "high-risk-pool of MDS patients". The median survival (MS) of AML-patients is halved when compered to those who had not an AML-evolution (MS: 41,63 VS MS: 22,40 months, $p < 0{,}001$).

Variable	N	%	Median	Log-rank	*p*
CENTRE					
Düsseldorf	315	84,0	24,47	3,270	0,071
Sicily	60	16,0	34,00		
GENDER					
Male	227	60,5	25,90	0,938	0,333
Female	148	39,5	26,83		
AGE					
<55	132	35,2	30,17	10,837	0,001
>55	243	64,8	24,73		
WHO TYPES					
RA	16	4,2	53,97	16,607	0,055
RARS	2	0,5	25,90		
RCMD	48	12,7	32,73		
RSCMD	12	3,2	13,53		
RAEB I	66	17,5	31,13		
RAEB II	110	29,1	27,53		
5q-	2	0,5	n.r.		
RAEB-T	85	22,5	16,43		
CMML I	17	4,5	27,00		
CMML II	18	4,8	21,40		
TRANSFUSION AT 1st DIAGNOSIS					
No	142	42,0	30,37	0,020	0,886
Yes	196	58,0	24,87		
TRANSFUSION IN FOLLOW UP					
No	69	23,7	38,20	5,592	0,018
Yes	222	76,3	24,10		
ANC IPSS					
>1800	112	33,0	23,50	0,321	0,571
<1800	227	67,0	30,17		
ANC IPSS-R					
> 800	215	63,4	28,50	0,860	0,354
<800	124	36,6	23,87		
PLT IPSS					
>100	142	38,1	31,13	0,606	0,436
<100	231	61,9	23,07		
PLT IPSS-R					
>100	142	38,1	31,13	1,082	0,582
50-99	103	27,6	26,83		
<50	128	34,3	21,10		
HB IPSS					
>10	151	40,4	32,73	3,260	0,071
<10	223	59,6	21,40		
HB IPSS-R					
>10	149	39,9	32,73	3,165	0,205
8-9,9	125	33,4	23,50		
<8	100	26,5	16,50		
BLASTS IPSS					
≤4	87	23,2	30,50	4,617	0,202
5-10	101	26,9	30,37		
11-20	106	28,3	26,27		
>20	81	21,6	17,40		
BLASTS IPSS-R					
≤2	52	13,9	38,60	7,145	0,067
3-4	35	9,3	24,47		
5-10	101	26,9	30,37		
>10	187	49,9	21,87		
CHROMOSOMAL CATEGORIES ACCORDING IPSS AND WPSS					
Low	199	54,5	32,80	33,645	<0,00005
Intermediate	64	17,5	26,07		
High	102	28,0	12,47		
CHROMOSOMAL CATEGORIES ACCORDING IPSS-R					
Very Low	5	1,4	28,93	47,660	<0,00005
Low	203	55,6	32,73		
Intermediate	54	14,8	27,53		
High	42	11,5	21,00		
Very High	61	16,7	10,10		
AML-EVOLUTION					
No	174	46,4	41,63	31,261	<0,00005
Yes	201	53,6	22,40		

Table 8 Univariate analysis of prognostic factors

4.3 Multivariate analyses

The proportional hazard regression multivariate analysis was used to investigate the prognostic value of the parameters, taking now into account the effects of all variables on the OS and AML-Transformation.

We included in this model the following variables: sex, age, WHO-classification, need of transfusion at the first diagnosis and in follow up, the absolute neutrophils count (ANC), the number of platelets (PLT), the value of hemoglobin (Hb) and the blast-count according IPSS- and IPSS-R-classification as well as the chromosomal aberrations both according IPSS/WPSS- and IPSS-R-classifications.

The most significant independent variables for predicting the outcome of our patients were age, Hb according to IPSS classification, blast percentage and chromosomal aberrations stratified according to IPSS-R criteria (Table 9).

PROGNOSTIC VARIABLES	**Wald**	**df**	***p***	**Exp(B)**
AGE	8,365	1	0,004	1,666
HB IPSS	5,232	1	0,022	1,463
BLASTS IPSS-R	3,942	1	0,047	1,154
CHROMOSOMES IPSS-R	31,583	1	<0,0005	1,462
The categories of variables used in the equation for the multivariate analysis are showed in Table 8, just the variables “sAML evolution” and “centre” were not included in the calculation.				

Table 9 Significant prognostic parameters for the OS in multivariate analysis

In the performed multivariate analysis on the variables that may be important to define the risk of AML-Transformation, the chromosomal aberrations, this time both according IPSS/WPSS- and IPSS-R-classifications, showed to have a significant prognostic value. Moreover, the WHO-Types and the need of transfusions in follow up seemed to have a prognostic relevance in this model (Table 10).

Variable	**Chi-Square**	***p***
GENDER	0.143	.705
AGE	2.928	.087
WHO TYPES	26.333	.002
TRANSFUSION AT 1st DIAGNOSIS	0.158	.691
TRANSFUSION IN FOLLOW UP	7.133	.008
ANC IPSS	0.447	.504
ANC IPSS-R	0.512	.474
PLT IPSS	0.0	.
PLT IPSS-R	1.280	.258
HB IPSS	0.0	.
HB IPSS-R	0.200	.655
BLASTS IPSS	0.697	.404
BLASTS IPSS-R	1.092	.296
CHROM IPSS AND WPSS	9.059	.011
CHROM IPSS-R	9.975	.041

Table 10 Prognostic parameters on AML-Transformation in multivariate analysis

4.4 Prognostic Scoring Systems to evaluate the overall survival (OS)

In the following chapter are presented the Kaplan-Meier curves stratified according to the three different scores.

Using the IPSS score, median OS for each group was of 32 months in the intermediate-1-risk group, 27 months in the intermediate-2-risk group and 15 months for the high-risk group (*p*=0,003). There is significant difference when compering the intermediate-1-risk with the intermediate-2-risk, but not between intermediate-2-risk and high-risk (Figure 9).

OS curves evaluated with Kaplan-Meier method are shown in Figure 5, while the Overall Comparison (OC) is shown in Table 11.

Overall Comparisons IPSS

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	10,833	2	0,004
Breslow (Generalized Wilcoxon)	22,205	2	0,0005
Tarone-Ware	17,457	2	0,0008

Table 11 Test of equality of survival distributions for the different levels of IPSS

Overall Comparisons WPSS

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	19,509	3	<0,0005
Breslow (Generalized Wilcoxon)	17,501	3	0,001
Tarone-Ware	18,421	3	0,0008

Table 12 Test of equality of survival distributions for the different levels of WPSS

Overall Comparisons IPSS-R

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	37,498	3	0,001
Breslow (Generalized Wilcoxon)	51,870	3	<0,0005
Tarone-Ware	46,945	3	<0,0005

Table 13 Test of equality of survival distributions for the different levels of IPSS-R

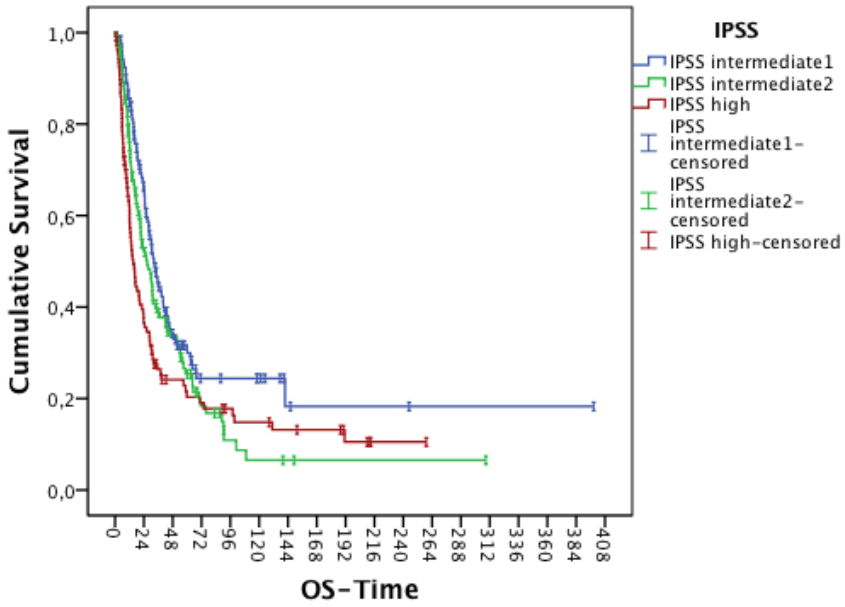

Figure 7 Kaplan-Meier OS curves for patients of each IPSS group

Considering the WPSS, median survival was 68, 40 and 27 months for the low-risk, intermediate-risk and high-risk WPSS groups, respectively (p<0,00005). For patients with very high WPSS it was 20 months (p<0,00005). OS curves evaluated with Kaplan Meier method are shown in Figure 8.

No statistically significant difference was evidenced between low and intermediate risk groups (*p*=0,427) and between intermediate and high risk groups (*p*=0,052). In contrast there is a significant difference between high and very high groups (*p*=0,009).

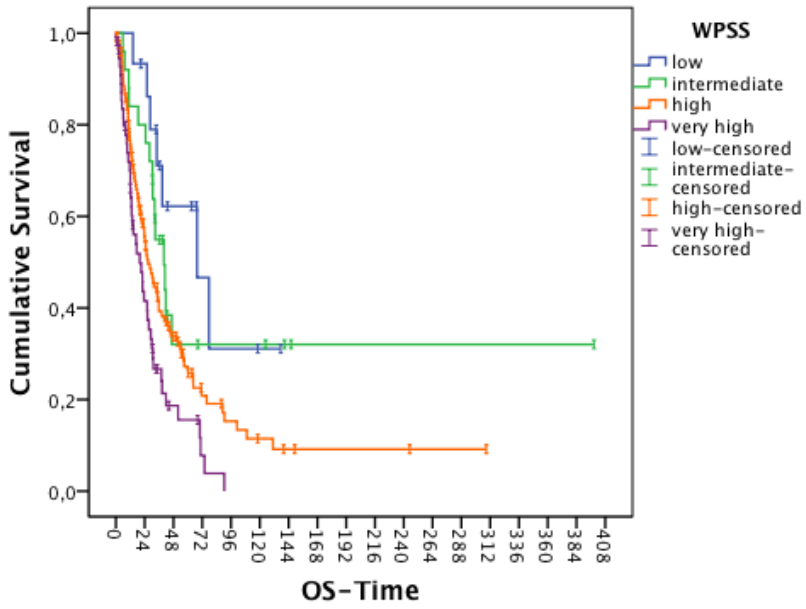

Figure 8 Kaplan-Meier OS curves for patients of each WPSS risk group

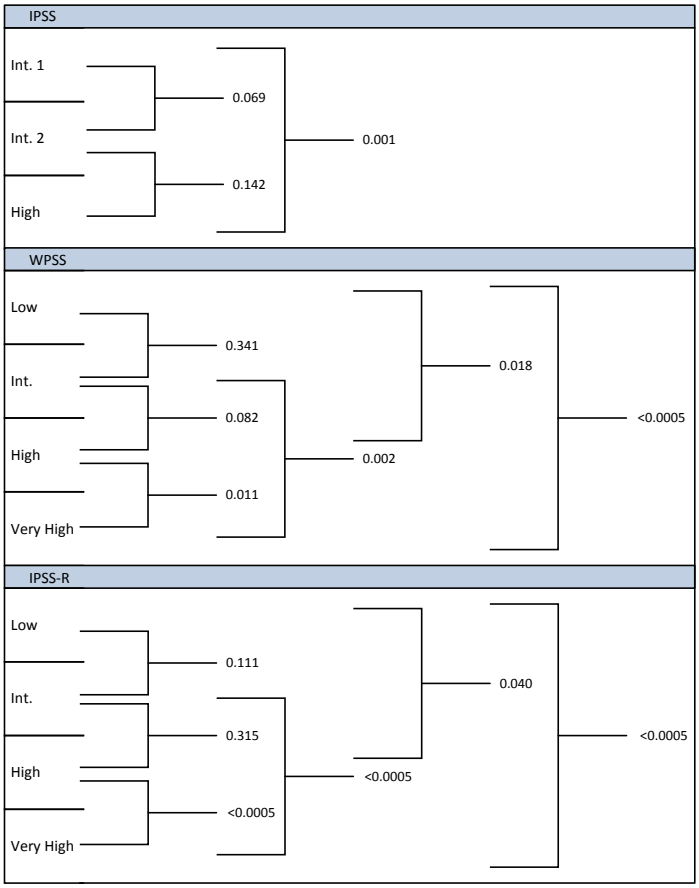

Figure 9 - Mantel-Cox (p) in the OS-analysis between different groups

Median survival time was 42, 32, 24 and 13 months for the low-risk, intermediate-risk and high-risk IPSS-R groups, respectively (p<0,00005). Statistical differences between prognostic groups are shown in Figure 9.

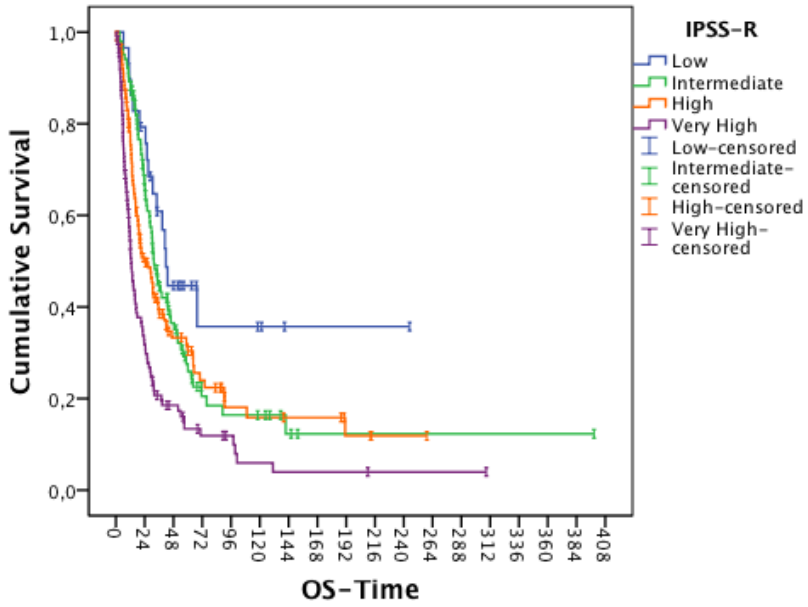

Figure 10 Kaplan-Meier OS curves for patients of each IPSS-R risk group

Very interesting are the statistically significant differences between: low-high risk (p=0,040) and high-very high risk (p<0,0005). No statistically significant difference was detected between intermediate and high risk groups (p=0,315).

4.5 Scoring Systems to evaluate the risk of acute myeloid leukemia (AML) evolution

As far as the AML evolution is concerned, we analyzed the cumulative risk of AML-transformation according the three different scores. In particularly, we calculated the risk after 2 and 5 years from the first diagnosis.

Using the IPSS-score, the risk of progression to AML in 2- or 5-years is respectively of 35% and 63% for patients classified as “intermediate-1”, but 65% and 75% for patients in IPSS-high risk group (Table 14). The cumulative incidence curve is shown in Figure 11.

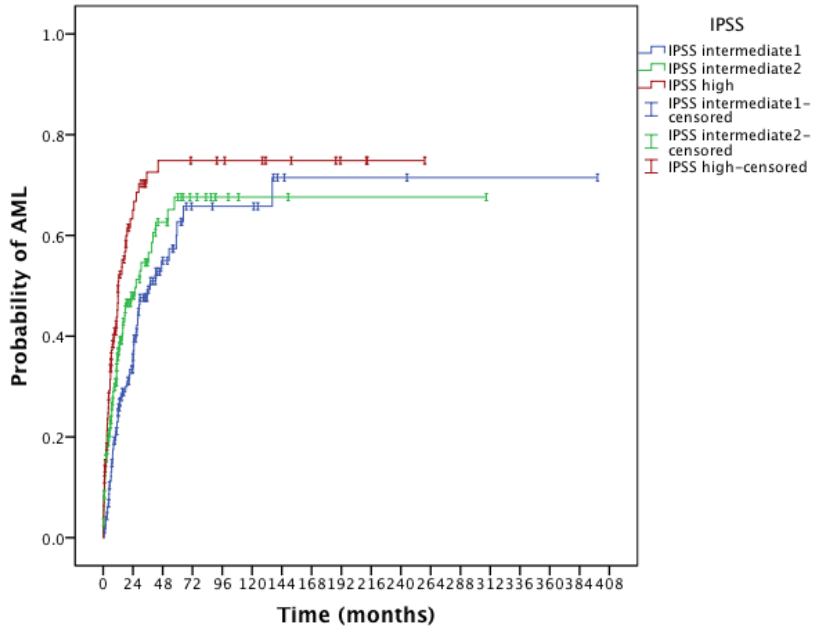

Figure 11 Cumulative incidence of AML-evolution according IPSS-Score

IPSS	n (%)	2 Years	5 Years		p
int.1	118 (34,5%)	35%	63%	p:0,09	0,003
int.2	129 (37,7%)	48%	68%	p:0,07	
high	95 (27,8%)	65%	75%		

Table 14 Cumulative risk of evolving in AML according IPSS-Score

The WPSS-score failed to shown significant differences in classifying the risk of AML transformation according to the initial stratification (Figure 12).

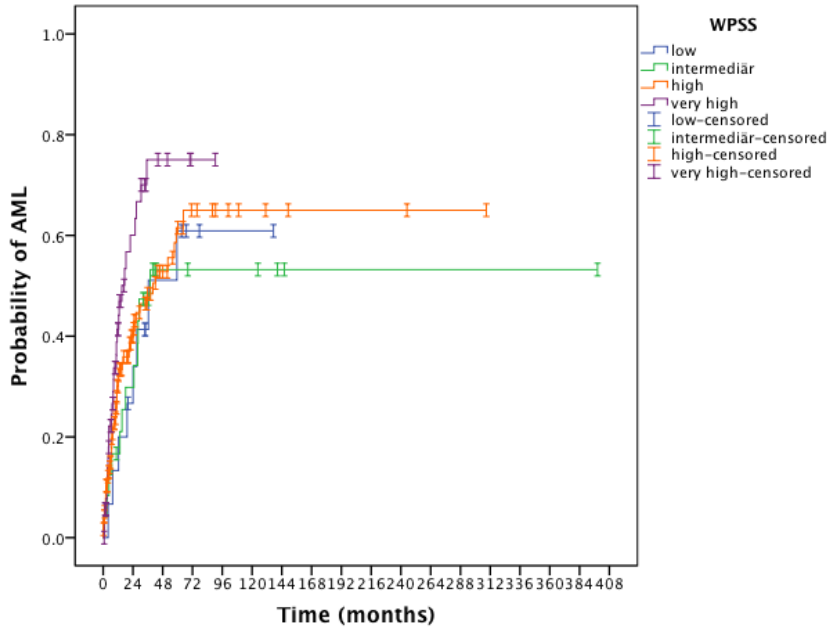

Figure 12 Cumulative incidence of AML-evolution according WPSS-Score

WPSS	n	2 Years	5 Years		p
Low	15 (7,1%)	27%	61%	*p*:0,951	0,101
Intermediate	24 (11,3%)	30%	54%	*p*:0,559	
High	119 (56,1%)	40%	62%	*p*:0,052	
Very high	54 (25,5%)	61%	75%		

Table 15 Cumulative risk of evolving in AML according WPSS-Score

The score that seems to help more in a stratification of patients according the risk of developing an AML is the IPSS-R-Score. With this score, the probability of AML-evolution in 2 years is of 28% in patients with IPSSR-low risk, in contrast with 40-44% probability for the ones in intermediate/high risk and 69% in those who had initially an IPSSR-very high risk (Table 16). The sample size of the very low risk group is too little to gain significance. The cumulative incidence curve is shown in Figure 13.

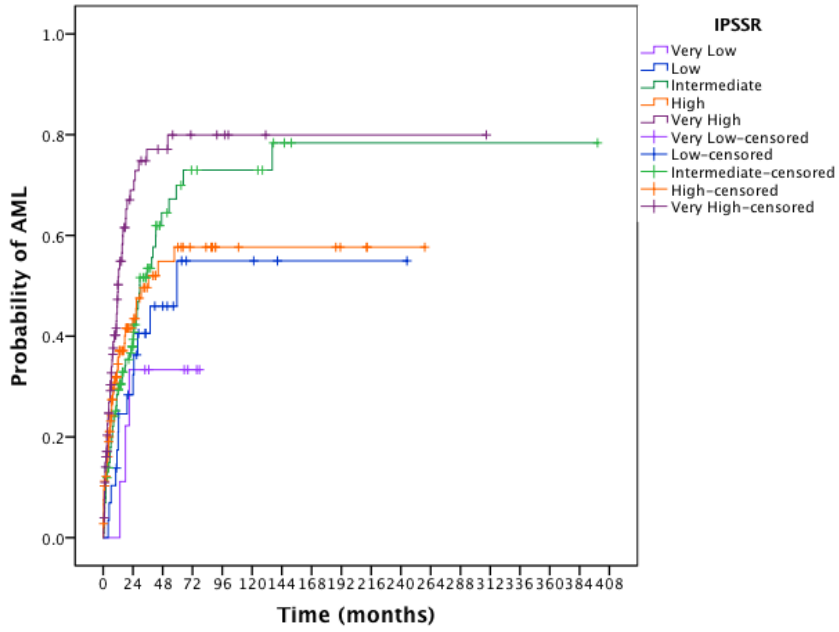

Figure 13 Cumulative incidence of AML-evolution according IPSS-R-Score

IPSSR	n	2 Years	5 Years		*p*
Very low	9 (2,6%)	34%	-	*p*:0,42	
Low	29 (8,3%)	28%	55%	*p*:0,14	
Intermediate	101 (29%)	40%	70%	*p*:0,74	0,001
High	108 (31,1%)	44%	58%	*p*:0,005	
Very high	101 (29%)	69%	80%		

Table 16 Cumulative risk of evolving in AML according IPSS-R-Score

5. DISCUSSION

For more then 10 years, the IPSS score has been used as an essential method to predict the outcome and the risk of AML-evolution for all MDS patients. The introduction of WHO-classification and the discovery of other possible risk variables lead to the definition of the WPSS, which has already been proved to be a valid prognostic score in untreated patients and in those undergoing an allo-SCT [32, 49]. Moreover, a IWG-PM project has recently asked to a group of experts to evaluated more than 15 MDS databases from different international institutions in the attempt to refine the IPSS score, the result is the so called IPSS-R [31]. The major change in the definition between IPSS and IPPS-R is a new classification of chromosomal findings. This new score has been validated using an independent data set [89], both to predict overall survival (OS) and to choose the correct risk-associated management.

The treatment of MDS underwent some important changes in the last decade and new drugs can partly modify the natural history of disease. For this reason, we felt the need to compare these three prognostic scores (WPSS, IPSS, IPSS-R) in a new pool of patients, who did not underwent neither induction-therapy or in best supportive care and, for this reason, still not analyzed with WPSS or with IPSS-R. Together with patients who received a chemotherapy with the intent to prepare the patients to allo-SCT, we recruited both over-65-patients treated with 5-Aza or AML-like chemotherapy and young patients, with high risk of evolution to AML, treated in absence of a suitable donor. These are generally patients with adverse karyotype, high percentage of BM blasts and/or important cytomorphological dysplasia. Consequentially, the vast majority of our patients was classified as high risk patients according to IPSS, WPSS and IPSS-R scores. On the other hand, only a very small number of patients within low-IPSS, very-low-WPSS and very-low-IPSSR categories underwent disease-modifying treatments.

Karyotype has already been proved to be prognostically relevant in all MDS patients [30, 31, 58, 90, 91]. Our data highlight that cytogenetic alterations play a fundamental role in the pathogenesis of MDS and in the development of BM failure, typical of higher risk forms. In particularly, cytogenetic risk groups defined by IPSS/WPSS and IPSS-R have been confirmed as important prognostic scores in the univariate analysis in our cohort. Moreover, karyotype analysis showed to be significantly important parameters in the multivariate analysis of predictive factors for AML evolution. The cytogenetic aberrations classified according the IPSS/WPSS failed though to have a prognostic significant influence in the multivariate analysis for the OS, when compered with IPSSR-cytogenetic categories. On the

other hand, we validated the stratification of karyotype aberrations according to IPSS-R as an independent predictor of OS both in a uni- and a multivariate analysis.

Another important parameter that predicts the overall survival of patients was the age at first diagnosis, as age was shown to be an independent prognostic parameter in the multivariate analysis. In contrast, higher age was not associated with AML-evolution.

The WHO classification failed to maintain a significant role in our analysis as far as the OS is concerned, but had an important role in the multivariate analysis for AML-evolution. In contrast to that finding, the stratification of BM blasts defined according IPSS-R and the hemoglobin according to IPSS and cytogenetic score showed to independently influencing the outcome in the Cox regression analysis.

Parameters reflecting hematopoietic insufficiency such as low PLT and ANC, did not influence significantly the OS in the univariate analyses. In contrast, transfusion dependency, during and after the treatment, significantly impaired OS.

The evaluation of survival curves, according the Kaplan-Meier method, showed that the new IPSS-R score has a better capacity of stratification for patients at risk both for OS and AML-transformation as compared to the IPSS and WPSS. The cumulative risk of AML-evolution after 5 years was 80% in the very high risk group as compered to 55% in low risk group. Accordingly, after 5 years only 15% of the patients in the very high risk group was still alive, as compared to 48% in the low risk group. This result should be an incentive to plan prospective clinical trials based on stratification according to the IPSS-R rather than to the IPSS.

We have the awareness of the limitation of our study due to the moderate number of patients considered. Despite the attempt to avoid any potential confounding factors, the strict inclusion criteria and the long period of recruitment could be potential sources of bias. In fact, we excluded a certain number of patients with missing data for the impossibility of prognostic scores calculations. On the other hand, the multicentric international character of the study allowed a wide sampling design, reducing the environmental bias.

In any case, we are confident that our results could be useful in the future for stratification of risk in MDS and, above all, can be a source of data in the development of more and more personal therapeutic and risk-associated approaches.

6. ABSTRACT

Myelodysplastic syndromes (MDS) are a heterogeneous group of pathologies that may have extremely different clinical developments. A proper classification score is useful not only for defining prognosis, but also to identify the best therapeutic approach. This was a multicenter study involving 375 patients from Germany and Italy. Our study started with the intent to investigate the validity of the WHO classification-based Prognostic Scoring System (WPSS) on the outcome of patients who underwent chemotherapy and/or treated with 5-Azacitidine. We extended then the analysis to compare this score with the well-known International Prognostic Scoring System (IPSS) and the most recently proposed Revised International Prognostic Scoring System (IPSS-R). The general overall survival (OS) was of 26 months. Based on the univariate analysis age, transfusion dependency, AML-evolution and chromosomal aberrations were significant predictors of patients' outcome. Using proportional hazard regression multivariate analysis, the most significant independent variables for predicting the outcome of our patients were age, Hb according to IPSS classification, blast percentage and chromosomal aberrations stratified according to the new IPSS-R criteria. The WHO-classification showed a significant role to define the risk of AML-Transformation, together with chromosomal aberrations and the need of transfusions in follow up. Moreover, the evaluation of survival curves, according to the Kaplan-Meier method, shows that, even if the three scores have all a prognostic significance on OS, the new IPSS-R score provides a better stratification of patients as far as prognosis and risk of AML-evolution is concerned.

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9. EIDESSTATTLICHE VERSICHERUNG

Ich versichere an Eides statt, dass die Dissertation selbstständig und ohne unzulässige fremde Hilfe erstellt und die hier vorgelegte Dissertation nicht von einer anderen Medizinischen Fakultät abgelehnt worden ist.

14.12.2015 Samuela Martino