Universitat Autònoma de Barcelona

IMPROVEMENT STRATEGIES FOR THE PROGNOSTIC EVALUATION OF MYELODYSPLASTIC SYNDROMES PATIENTS

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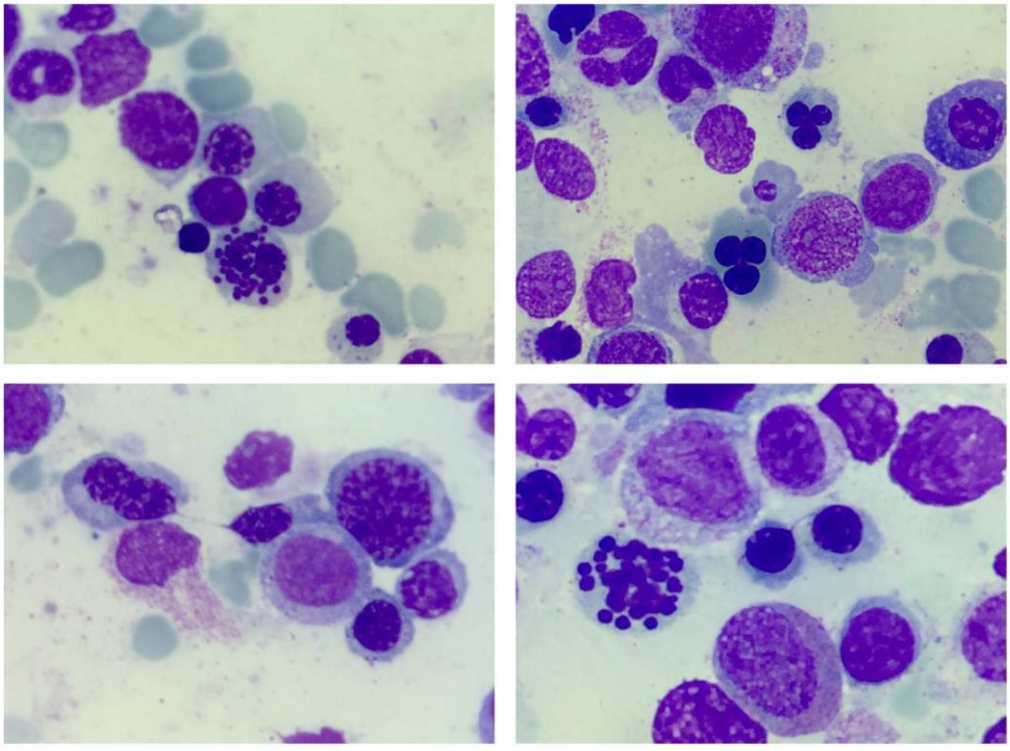

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Doctoral Dissertation by

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ABBREVIATIONS

Abbreviations

AD: Autoimmune disease
AML: Acute myeloid leukemia
ANA: Antinuclear antibodies
ANC: Absolute neutrophil counts
BM: Bone marrow
CG: Cytogenetics
CH: Clonal hematopoiesis
CI: Confidence intervals
CHIP: Clonal hematopoiesis of indeterminate potential
CMML: Chronic myelomonocytic leukemia
DAMP: Damage associated pattern
Del : Deletion
ECOG: Eastern Cooperative Oncology Group
ELN: European Leukemia Net
EPO: Erythropoietin
ESA: Erythropoiesis-stimulating factors
FAB: French-American-British
GESMD: Grupo Español de Síndromes Mielodisplásicos
HR: Hazard ratios
HSC: Hematopoietic stem cells
HSCT: Hematopoietic stem cell transplantation
HSPC: Hematopoietic stem and progenitor cell
Hb: Hemoglobin
I/H-R MDS: Intermediate/high risk myelodysplastic syndromes
IL-1: Interleukin-1
IPSS: International Prognostic Scoring System
IPSS-R: Revised International Prognostic Scoring System
IRAk1: Receptor-associated kinase-1
IRAk4: Receptor-associated kinase-1

IWGM-MDS: International Working Group on Morphology of Myelodysplastic Syndromes

LDH: Lactate dehydrogenase

LRPSS: Lower risk Prognostic Scoring System

MD: Myelodysplastic

MDS: Myelodysplastic syndromes

MDS-AD: Myelodysplastic syndromes with autoimmune disorders

MDSC: Myeloid-derived suppressor cells

MDS EB 1: Myelodysplastic syndromes with excess blasts type 1

MDS EB 2: Myelodysplastic syndromes with excess blasts type 2

MDS MD: Myelodysplastic syndromes with multilineage lineage dysplasia

MDS-noAD: Myelodysplastic syndromes without autoimmune disorders

MDS RS MLD: Myelodysplastic syndromes with ring sideroblasts and multilineage lineage dysplasia

MK: Megakaryocytes

MP: Myeloproliferative

MPSS: MD Anderson Global Prognostic Scoring System

MSC: Mesenchymal stem cells

MDS SLD: Myelodysplastic syndromes with single lineage dysplasia

MDS, U: Unclassifiable myelodysplastic syndrome

NCCN: National Comprehensive Cancer Network

NGS: Next-generation sequencing

NLR: Nod-like receptors

NLRP3 Nod-like receptor pyrin domain-containing-3

NK cell: Natural killer cells

OS: Overall survival

PB: Peripheral blood

PD1: Programmed death-1 receptor

PDL-1: Programmed death-1ligand

RA: Refractory anemia

RARS: Refractory anemia ring sideroblasts

RAEB: Refractory anemia with excess blasts

RAEB-1: Refractory anemia with excess blasts type 1

RAEB-2: Refractory anemia with excess blasts type 2

RAEB-T: Refractory anemia with excess blasts in transformation

RCMD: Refractory cytopenia with multilineage dysplasia

RCMD-RS: Refractory cytopenia with multilineage dysplasia and ring sideroblasts

RCUD: Refractory cytopenia with unilineage dysplasia

RF: Rheumatoid factor

ROC: Receiver operating characteristic

Sbds: Shwachman-Diamond syndrome

TGF-β: Transforming growth factor beta

Th17 lymphocytes: T-helper 17 lymphocytes

TIFAB: TRAF-interacting protein with forkhead-associated domain B

TLR: Toll-like receptors

TNFα: Tumor necrosis factor alpha

TPO: Thrombopoietin

TRAF6: TNF receptor-associated factor 6

Tregs: T-regulatory lymphocytes

U.S.: United States

WHO: World Health Organization

WPSS: WHO classification-based Prognostic Scoring System

WPSS-R: Revised WHO classification-based Prognostic Scoring System

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ABSTRACT

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of myeloid disorders with heterogeneous clinical manifestations and outcomes, ranging from those asymptomatic patients with long-life expectancy, to those with profound cytopenia and high-risk to evolution to acute myeloid leukemia. Treatment options are based on risk prognostic stratification according to the International Prognostic Scoring System (IPSS), being conservative in lower-risk: IPSS low and intermediate-1 (IPSS < 1.5 points), whereas intensive in higher-risk: intermediate-2 and high (IPSS > 1.5 points) patients. Therefore, a proper prognostic assessment is mandatory. Autoimmune disorders (AD) have been described in MDS patients, however, its real incidence and prognostic effects in MDS are not completely understood. Against this background, we have analyzed the prevalence, clinical characteristics and outcomes of AD in MDS patients, and which is the best IPSS-R cut-point that dichotomizes MDS patients in low vs. high-risk. According to our results, AD are frequent in MDS and confers an adverse prognostic impact, and an IPSS-R of 3 points is the cut-point that best to divide patients into low and high-risk subgroups. Altogether, MDS with AD and those with an IPSS-R > 3 should be considered as high-risk patients and treated with intensive treatments.

Lo síndromes mielodisplásicos (SMD) son un grupo heterogéneo de enfermedades mieloides con unas manifestaciones clínicas y un pronóstico muy diferente, con pacientes asitomáticos con una larga expectativa de vida, y otros con citopenias severas y un riesgo elevado de evolucionar a una leucemia mieloide aguda. Las opciones terapéuticas se basan en la estratificación pronóstica del riesgo según el International Prognostic Scoring System (IPSS), siendo conservador en los pacientes de bajo riesgo: IPSS bajo and intermedio-1 (IPSS < 1.5 puntos), e intensivo en los de alto riesgo: intermedio-2 y alto (IPSS > 1.5 puntos). Por lo tanto, la valoración adecuada del riesgo pronóstico en los SMD es fundamental. La presencia de enfermedades autoinmunes se ha descrito en los pacientes con SMD, sin embargo, la incidencia real y el valor pronóstico en estos pacientes se desconoce. Con todo lo anterior, hemos analizado la prevalencia, las características clínicas y el impacto pronóstico de las enfermedades autoinmunes en los pacientes con SMD, y cuál es el punto de corte del IPSS-R que mejor estratifica a los pacientes en alto y bajo riesgo. De acuerdo a nuestros resultados, la presencia de enfermedades autoinmunes es frecuentes y confiere un peor pronóstico a los pacientes con SMD, y un IPSS-R de 3 puntos es el punto de corte que mejor divide a los pacientes en alto y bajo riesgo. Como conclusión, los SMD con enfermedades autoinmunes y con un IPSS-R > 3 se deben considerar como pacientes de alto riesgo y tratarlos de manera intensiva.

I. INTRODUCTION

1. Myelodysplastic syndromes

1. Definition

Myelodysplastic syndromes (MDS) comprise a group of hematopoietic stem cell neoplasms characterized by ineffective hematopoiesis resulting in normocellular or hypercellular bone marrow (BM), cytopenias and dysplastic features. The hallmarks of these disorders include incremented apoptosis of hematopoietic precursors in the marrow, recurrent chromosomal abnormalities, frequent somatic mutations, and a variable predilection to evolution into acute myeloid leukemia (AML) (1).

1.2 Epidemiology

MDS is mainly a disease of the elderly with an incidence that increases progressively with age. As a result of the difficulties in the diagnosis of MDS, the true incidence is not well known and it is probably underreported. Based on recent registry-based studies in the United States (U.S.), median age at diagnosis is 77 years with 85% of patients being diagnosed at age 60 years or older (Figure 1) (2). Estimated incidence rates for the population younger than 40 years is only 0.1 cases per 100,000 person-years, with a progressive increase until an incidence of 56.8 cases per 100,000 population person-years (3). Studies in European countries have shown similar incidence figures (4–6). Given the continuous aging of the population and the improvements on the diagnostic approaches, the number of MDS patients is expected to grow in the following years.

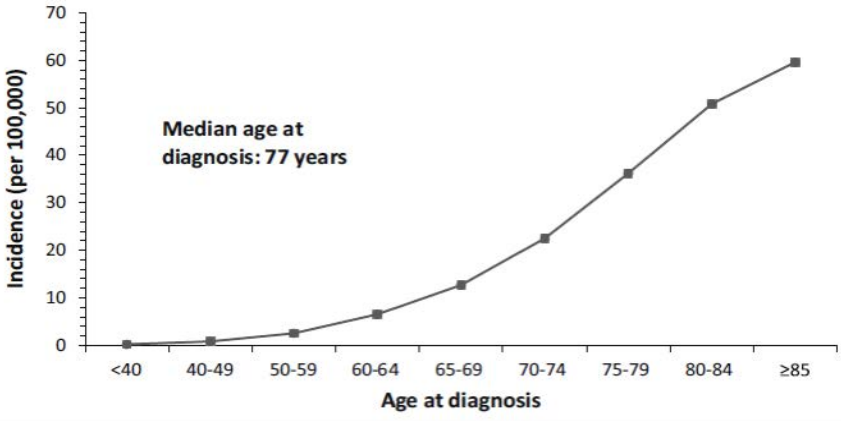


Figure 1. Incidence of patients with MDS by age in the U.S. (Surveillance, Epidemiology, and End Results data, based on the November 2017 submission) (adapted from Zeidan *et al*, 2019) (2).

1.3 Etiology and risk factors

Primary or *de novo* MDS account for 80% of all MDS. Possible etiologies for primary MDS include environmental chemical exposure such as benzene, pesticides, alcohol or cigarette smoking (7). Some inherited hematological disorders, such as Fanconi anemia, Dyskeratosis congenita, Scwachman-Diamond syndrome and Diamond-Blackfan anemia, are also associated with an increased risk of MDS (8). Furthermore, patients with acquired aplastic anemia and myeloid neoplasms with germline predisposition are more prone to develop MDS (9). Nearly 20% of MDS are secondary to prior exposure to cytotoxic agents (chemotherapy and/or immunosuppressive drugs) and radiation therapy (10,11). However, due to its distinct clinical behavior, morphologic features and genetic abnormalities, these patients are included in a separated group recognized by the World Health Organization (WHO), named therapy-related myeloid neoplasms (1).

1.4 Clinical presentation

Clinical manifestations in MDS are nonspecific and vary considerably depending on the subtype and severity of cytopenias, ranging from asymptomatic to life-threatening symptoms. Anemia, typically macrocytic, is the most common

peripheral blood abnormality, occurring in approximately 90% of patients, which is severe at diagnosis in many cases; 60% with hemoglobin (Hb) of < 10 g/dL and 27% with Hb of < 8 g/dL (12). Symptoms related to anemia include fatigue, weakness and dyspnea. Some patients also present thrombocytopenia (platelets < 100.000/L) and neutropenia (absolute neutrophil counts (ANC) < 1.500/L) while pancytopenia is seen in approximately half of MDS patients in different degrees (13). However, isolated thrombocytopenia and neutropenia are much less frequent with an incidence of 12% and 9%, respectively (14). Bleeding and bruising are indicative of severe thrombocytopenia, whereas infections correlates with severe neutropenia. Ten to 30% of patients with MDS display signs of autoimmune disease, the most frequent being hypothyroidism, inflammatory arthritis and vasculitis (15,16). Organomegaly in the form of hepatomegaly, splenomegaly, and lymphadenopathy is uncommon, and its presence should warn for other diagnosis.

1.5 Diagnosis

Myelodysplastic syndromes lack pathognomonic findings; therefore, an accurate diagnostic process is mandatory. Diagnostic evaluation relies on three cornerstones: morphologic analysis of the peripheral blood and BM, cytogenetics, and exclusion of secondary causes of dysplasia.

Morphologic evaluation

The minimal morphologic criterion for the diagnosis is the presence of BM dysplasia in at least 10% of cells based on an evaluation of 200 mature erythroid and granulocytic cells and 30 megakaryocytes. Dysplastic features characteristics of MDS are detailed in Table 1. Dysplasia may be accompanied by an increase in myeloblasts in peripheral blood and/or BM, but blast percentage is always < 20%, which is the recommended threshold for the diagnosis of AML. To determine blast percentage, a 500-cell differential count of all nucleated cells in BM smears and a 200-leukocyte differential count in peripheral blood is recommended. Blasts and dysplastic features must be evaluated in marrow smears, where the morphology of the cells is unharmed. BM biopsy is desirable, especially when suspected fibrosis

(dry-tap) or in hypocellular specimens. Also, when smear evaluation is not conclusive of MDS, biopsy can show a distorted architecture, dysplastic megakaryocytes and high CD34+ cells (17).

Table 1. Dysplastic features described in the 2017 WHO classification and according to the International Working Group on Morphology of Myelodysplastic Syndromes (IWGM-MDS) (1,18–21).

Dyserythropoiesis	Dysgranulopoiesis	Dysmegakarypoiesis
Nuclear	Small or unusually large size	Micromegakaryocytes
Nuclear budding	Nuclear hypolobation (pseudo Pelger-Huët)	Nuclear hypolobation o monolobated
Internuclear bridging	Irregular hypersegmentation	Multinucleation (binucleated or widely-separated nuclei)
Karyorrhexis	Decreased granules or agranularity	Cytoplasmic blebs*
Multinuclearity	Pseudo Chediak-Higashi granules	
Nuclear hyperlobation	Auer rods	
Megaloblastic changes	Macropolocyte*	
Cytoplasmic		
Ring sideroblasts		
Vacuolization		
Periodic acid-Schiff positivity		

*Dysplastic features included in the IWGM-MDS but not in the 2017 WHO classification

Cytogenetics

Conventional cytogenetic analysis plays a major role in the diagnosis and prognosis of MDS. Cytogenetic abnormalities occur in roughly half of patients; while all chromosomes can be involved, the most frequent abnormalities found are single del(5q), monosomy 7 or del(7q), trisomy 8, and del(20q) (22). Recurrent chromosomal abnormalities frequently seen in MDS are detailed in Table 2.

Importantly, some cytogenetics correlates with morphological and clinical features, as for example, MDS with isolated del(5q) is associated with small monolobated megakaryocytes, macrocytic anemia, increased platelet count and favorable prognosis, loss of 17p is associated with pseudo-Pelger-Huët anomaly, small vacuolated neutrophils, *TP53* mutations and an unfavorable prognosis, and complex karyotypes (≥ 3 abnormalities), typically including abnormalities in chromosomes 5 and/or 7, are common in secondary MDS and confer poor prognosis (22). Conversely, isolated del(20q), along with loss of Y chromosome and gain of chromosome 8, are not considered MDS-defining abnormalities in the absence of morphological criteria, as they have been reported to occur in other myeloid malignancies and in non-neoplastic conditions (1). In contrast to primary MDS, 70% of therapy-related MDS are associated with abnormal cytogenetics, typically complex karyotypes with deletions involving either chromosome 5 or chromosome 7 or both (23). Remarkably, since the publication of the WHO classification 2017, mutational status of the *SF3B1* gene in those cases with 5–14% of ring sideroblasts and low blast count is mandatory to diagnose the subtype MDS with ring sideroblasts.

Table 2. Recurrent chromosomal abnormalities and their frequencies in MDS at diagnosis (1).

Chromosomal abnormality	Frequency
Unbalanced	
+8*	10%
-7 or del(7q)	10%
-5 or del(5q)	10%
del(20q)*	5-8%
-Y* 5%	5%
i(17q) or t(17p)	3-5%
-13 or del(13q)	3%
del(11q)	3%
del(12p) or t(12p)	3%
del(9q)	1-2%
idic(X)(q13)	1-2%
Balanced	
t(11;16)(q23;p13.3)	< 1%
t(3;21)(q26.2;q22.1)	<1%
t(1;3)(p36.3;q21.2)	1%
t(2;11)(p21;q23)	1%
inv(3)(q21q26.2)	1%
t(6;9)(p23;q34)	1%

*Chromosomal abnormalities not considered definitive evidence of MDS when occur as a sole abnormality in the absence of defining morphological criteria.

Secondary causes of dysplasia

Dysplastic features can be seen in other clonal disorders and benign conditions that can mimic MDS, therefore it must be excluded before a definitive diagnosis of MDS is established (Figure 2) (24–26).

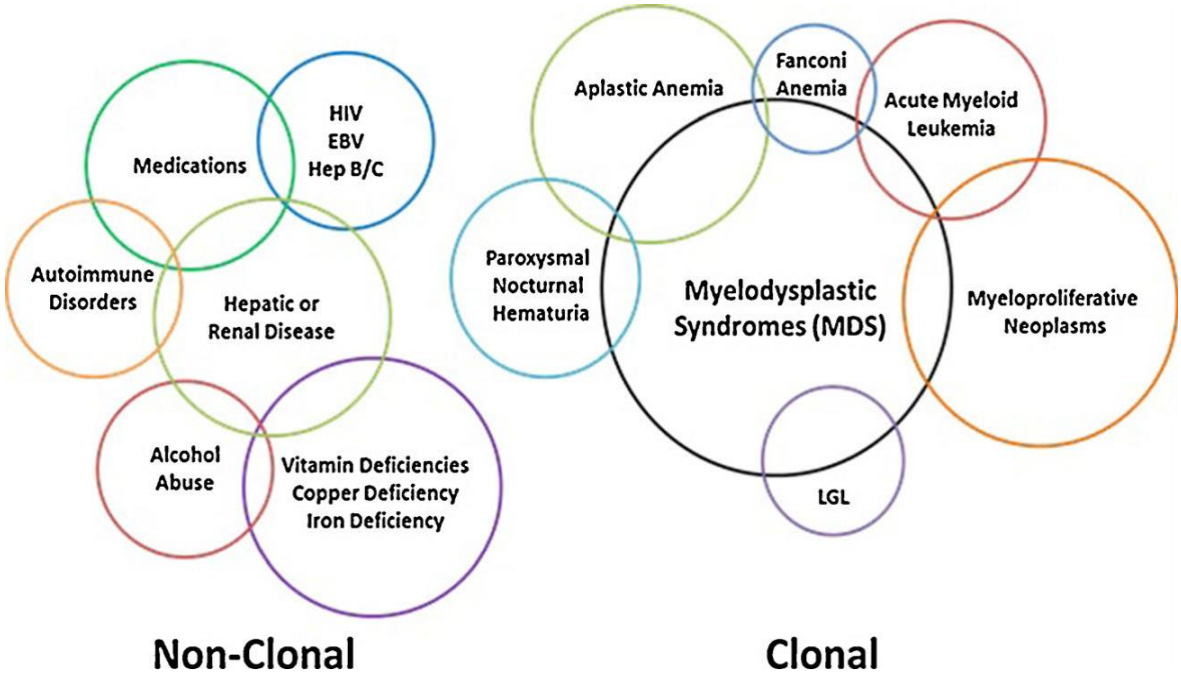


Figure 2. Diagnostic overlap between MDS and other clonal disorders and benign conditions that can mimic MDS (adapted from Bejar *et al*, 2015) (24).

Consequently, an accurate study including complete clinical information, exhaustive morphological evaluation and proper genetic analysis is recommended. It is of utmost importance to consider non-clonal disorders in the differential diagnosis, especially in those cases with mild dysplasia, normal karyotype and without blast increase. Some cases may require hematological follow-up over a period of several months with repeated BM assessment.

In the recent years, immunophenotype analysis have been demonstrated to be an effective tool for MDS diagnosis. Immunophenotypic abnormalities described in MDS are abnormal quantity and aberrant phenotypes of hematopoietic progenitor cells, aberrant immunophenotypic profiles of maturing granulocytic, erythroid and

monocytic cells, and a decrease in hematogones (27,28). However, flow cytometry findings alone are not sufficient to establish a diagnosis of MDS in the absence of definitive morphological and/or cytogenetic findings, and importantly BM blasts determined by flow cytometry cannot replace the morphological count. Furthermore, there are not universal standardized protocols. In this regard, a series of consensus guidelines about the diagnosis work-up of patients with MDS has been recently published by the European Leukemia Net MDS working group (29).

1.6 Classification

Classification of the MDS has undergone modifications over time in relation to a better understanding of the disease.

1.6.1 The French-American-British (FAB) Classification

In 1982, the FAB cooperative group proposed the first MDS classification based on morphologic features and blast percentage in blood and BM, the percentage of ring sideroblasts, the presence peripheral blood monocytes and the existence Auer rods. Five subgroups with significantly different prognoses were established (Table 3) (30). For nearly two decades this classification served as the standard for the evaluation of MDS.

Table 3. The FAB Classification of MDS (30).

	PB Blast (%)	BM blasts (%)	PB Monocytes ($x10^9$/L)	BM Ring sideroblasts (%)
RA	<1	<5 No Auer rods	<1x10^9/L	≤15
RARS	<1	<5 No Auer rods	<1x10^9/L	>15
RAEB	<5	5–19 No Auer rods	<1x10^9/L	Indifferent
RAEB-T	>5	20–29 +/- Auer rods	<1x10^9/L	Indifferent
CMML MD/MP	<5	0–20	>1x10^9/L	Indifferent

RA: Refractory anemia; RARS: Refractory anemia with ring sideroblasts; RAEB: Refractory anemia with excess blasts; RAEB-T: Refractory anemia with excess blasts in transformation; CMML: Chronic myelomonocytic leukemia; MD: Myelodysplastic variant, < 13x10^9/L; MP: Myeloproliferative variant, ≥ 13x10^9/L; PB: Peripheral blood; BM: Bone marrow.

1.6.2 The 2001 WHO Classification

The first modification of the MDS classification was in 2001. This classification reflected a paradigm shift from previous schemes in that, for the first time, genetic information was incorporated with morphologic, cytochemical, immunophenotypic, and clinical information into diagnostic algorithms to define clinically significant disease entities. Thus, based on number of cytopenias, significant dysplastic cell lineages, presence of Auer rods, percentage of peripheral blood and BM blasts and ring sideroblasts, and the identification of isolated del(5q) chromosomal abnormality, 8 categories were defined (Table 4) (31).

Table 4. The 2001 WHO Classification of MDS (31).

	Cytopenias	PB Blast (%)	BM blasts (%)	BM Ring sideroblasts (%)	Dysplasia
RA	Anemia	<1	<5	<15	Erythroid
RARS	Anemia	0	<5	≥15	Erythroid
RCMD	2 or 3	<1	<5	<15	≥2 cell lines
RCMD-RS	2 or 3	<1	<5	≥15	≥2 cell lines
RAEB-1	1 or more	<5	5-9	Indifferent	Indifferent
RAEB-2	1 or more	5–19	10–19	Indifferent	Indifferent Auer rods*
MDS del(5q)	Anemia normal/↑ platelets	<5	<5	Indifferent	Monolobated MK
MDS, U	2 or 3	≤1	<5	Indifferent	1 cell line

*Auer rods designate any case as RAEB-2, independently of the other characteristics.
RA: Refractory anemia; RARS: Refractory anemia with ring sideroblasts; RCMD: Refractory cytopenia with multilineage dysplasia; RCMD-RS: Refractory cytopenia with multilineage dysplasia and ring sideroblasts; RAEB- 1: Refractory anemia with excess blasts type 1; RAEB-2: Refractory anemia with excess blasts type 2; MDS, U: myelodysplastic syndrome, unclassified; MK: Megakaryocytes; PB: Peripheral blood; BM: Bone marrow.

The main modifications respect to the FAB classification were:

- Decrease the level of myeloblasts required for the diagnosis of AML to 20%, therefore the RAEB-T category falls into the AML category.
- Placing CMML into a new category of myeloid neoplasms that have both myelodysplastic and myeloproliferative features.
- MDS with isolated del(5q) with low blast count is recognized as distinctive subtype.

- Divided RAEB categories into RAEB-1 (2–4% and/or <5–9% blasts in peripheral blood and BM, respectively) and RAEB-2 (5–19 and/or 10–19% blasts in peripheral blood and BM, respectively).
- The finding of Auer rods is considered to be RAEB-2 defining, regardless of the blast percentage.
- The presence of t(8;21)(q22;q22), inv(16)(p13.1;q22), t(16;16)(p13.1;p22) or t(15;17)(q22;q12) are considered to be AML defining, regardless of the blast percentage.

1.6.3 The 2008 WHO Classification

The 2008 document maintain the rationale of defining clinico-pathological entities and included the new insights available since the publication of the 2001 edition (Table 5) (32).

Table 5. The 2008 WHO Classification of MDS (32).

	Cytopenias	PB Blast (%)	BM blasts (%)	BM Ring sideroblasts (%)	Dysplasia
RCUD	1 or 2	<1	<5	<15	1 cell line
RARS	Anemia	0	<5	≥15	Erythroid
RCMD	Cytopenia/s	<1	<5	Indifferent	≥2 cell lines
RAEB-1	Cytopenia/s	<5	5–9	Indifferent	Indifferent
RAEB-2	Cytopenia/s	5–19	10–19	Indifferent	Indifferent Auer rods*
MDS del(5q)	Anemia Normal/↑ platelets	<1	<5	Indifferent	Monolobated MK
MDS, U	2-3	≤1	<5		<10% in ≥1 cell line Cytogenetic abnormalities

*Auer rods designate any case as RAEB-2, independently of the other characteristics.
RCUD: Refractory cytopenia with unilineage dysplasia; RARS: Refractory anemia with ring sideroblasts; RCMD: Refractory cytopenia with multilineage dysplasia; RAEB-1: Refractory anemia with excess blasts type 1; RAEB-2: Refractory anemia with excess blasts type 2; MK: Megakaryocytes; MDS, U: myelodysplastic syndrome, unclassified; PB: Peripheral blood; BM: Bone marrow.

The main modifications respect to the 2001 WHO classification were:

- Patients with refractory cytopenia(s) suspected to have MDS, but who lack diagnostic morphologic features may be considered to have presumptive evidence of MDS if they have specific MDS related cytogenetic abnormalities (Table 2).
- The category “refractory *anemia* with unilineage dysplasia” was replaced by “refractory *cytopenia* with unilineage dysplasia” to incorporate patients who exhibited unilineage dysplasia associated with refractory anemia (unilineage erythroid dysplasia), refractory neutropenia (unilineage dysgranulopoiesis), or refractory thrombocytopenia (unilineage dysmegakaryocytopoiesis).
- The category of RCMD is no longer subdivided according to the presence of ≥ 15% of ring sideroblasts, that is, the former category of RCMD-RS is now incorporated in RCMD.
- Patients with 2%–4% blasts in the blood and < 5% blasts in the BM should be diagnosed with RAEB-1 if other clinical and laboratory finding of MDS are present.
- A provisional entity, refractory cytopenia of childhood, was incorporated.

1.6.4 The 2017 WHO Classification

In 2016, Arber and colleagues published the main modifications that were included in the recently published revision of the 2017 WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Table 6) (33).

Table 6. The 2017 WHO Classification of MDS (33)

	Dyplastic lines	Cytopenias	RS (%)	PB Blasts (%)	BM Blasts (%)	CG
MDS UD	1	1 or 2	<15 or <5*	<1	<5	Any§
MDS MD	>1	1-3	<15 or <5*	<1	<5	Any§
MDS RS:						
-UD	1	1 or 2	≥15 or ≥5*	<1	<5	Any§
-MD	>1	1–3	≥15 or ≥5*	<1	<5	Any§
MDS isolated del(5q)	1-3	1 or 2	Indifferent	<1	<5	Del(5q) +/- 1 (except -7 or del(7q)]
MDS EB:						
-MDS EB1	0-3	1–3	Indifferent	2–4	5–9	Any§
-MDS EB2	0-3	1–3	Indifferent	5–19	10–19 or Auer	Any§
MDS, U:						
-1% blasts PB	1–3	1–3	Indifferent	1	<5	Any§
-Pancytopenia + UD	1	3	Indifferent	<1	No Auer	Any§
-Chromosomal abn without dysplasia	0	1–3	<15#	<1		MDS-like

*If SF3B1 mutation is present. §No characteristics of MDS isolated del(5q). #If ≥ 15% of RS, is consider significant dysplasia→ MDS RS UD.
UD: Unilineage dysplasia; MD: Multilineage dysplasia; RS: Ring sideroblasts; EB: Excess blasts; PB: Peripheral blood; BM: Bone marrow; CG: Cytogenetics; Abn: Abnormalities; MDS, U: Myelodysplastic syndromes, unclassified.

These modifications are:

- The term “refractory cytopenia” has been replaced by the more general term “myelodysplastic syndrome”, avoiding the situation in which the lineage manifesting significant morphologic dysplasia do not correlate with the specific cytopenia.
- For the diagnosis of the MDS, unclassifiable, the presence of 1% blasts in peripheral blood has to be demonstrated on at least 2 separate occasions.
- Based on published data in which acute erythroid leukemias (AEL) had a closer relationship to MDS-EB than with de novo AML in terms of

morphologic and genetic features (34–37) the denominator used for calculating blast percentage should include all marrow cells irrespective of the marrow erythroid percentage, in contrast to the former classification in which the presence of ≥ 50% erythroid precursors avoided its inclusion in the estimation of the blast percentage. This result in most cases previously diagnosed as AEL now being classified as MDS-EB.

- Based on recent data showing no adverse effect of one chromosomal abnormality in addition to the del(5q) (40), the entity MDS with isolated del(5q) may be diagnosed if there is one additional cytogenetic abnormality, unless that abnormality is monosomy 7 or del(7q).
- Patients with at least 15% ring sideroblasts without excess of blasts or del(5q), are diagnosed as MDS with ring sideroblasts, and two types are defined, with unilineage dysplasia and with multilineage dysplasia. Whereas, in those patients in which *SF3B1* mutation is present, the diagnosis can be made when marrow ring sideroblasts represent ≥ 5% of the erythroid precursors.

The major characteristics of the different subcategories of MDS in the 2017 WHO Classification are outlined below (1).

<u>MDS with single lineage dysplasia</u>

This category accounts for 7–20% of all cases of MDS. Is defined by the presence of cytopenia or bicytopenia, with ≥ 10% dysplastic cells in one myeloid lineage (irrespective if the lineage cytopenia and lineage dysplasia do not correlate), < 5% of marrow blasts and < 15% of ring sideroblasts. Auer rods are absent.

<u>MDS with ring sideroblasts</u>

This subgroup is characterized by the presence of ring sideroblasts, defined as erythroblasts in which there are a minimum of 5 siderotic granules covering at least one third of the nuclear circumference (38), usually constituting ≥ 15% of the BM erythroid precursors. Secondary causes of ring sideroblasts must be excluded

(i.e. alcohol, lead, benzene). Myeloblasts account for < 5% of the marrow cells and < 1% of the peripheral blood leukocytes. Morphologically, BM is usually hypercellular with erythroid hyperplasia and severe dyserythropoiesis. Two categories are recognized: MDS with ring sideroblasts and single lineage dysplasia (MDS RS SLD), that account for 3–11% of all MDS, and MDS with ring sideroblasts and multilineage dysplasia (MDS RS MLD), that account for 13% of MDS. In the former, patients present anemia and dysplasia is limited to the erythroid lineage, whereas in MDS RS MLD patients present with any number of cytopenias and dysplasia is present in ≥ 2 hematopoietic lineages. Mutation in the spliceosome gene *SF3B1* accounts for 80–90% of MDS RS SLD and 30-70% of MDS RS MLD (39–41). A diagnosis of MDS with ring sideroblasts can also be made if ring sideroblasts are less than 15% (but ≥ 5%) of erythroid cells and *SF3B1* gene is mutated. There should not be Auer rods.

MDS with multilineage dysplasia

This category represents around 30% of all cases of MDS. Is characterized by one or more cytopenias and significant dysplastic changes in two or more cell lineages, < 1% peripheral blood blasts and < 5% marrow blasts. Cases with ≥ 5% but < 15% ring sideroblasts must have *SF3B1* wild type. There should not be Auer rods.

MDS with excess blasts

This is the commonest MDS group, accounting for 40% of patients. Two subgroups, with differences in survival and incidence of evolution to AML have been defined. MDS with excess blasts type 1 (MDS EB 1) is defined by 2–4% blasts in the peripheral blood or 5–9% blasts in the BM, and MDS with excess blasts type 2 (MDS EB 2) is defined by 5–19% blasts in the peripheral blood or 10–19% blasts in the BM. The presence of Auer rods designates any case as MDS EB 2, irrespective of the blast percentage.

MDS with isolated del(5q)

This category is defined by the presence of an interstitial deletion of the long arm of the chromosome 5, either isolated or with one additional cytogenetic abnormality, other than monosomy 7 or del(7q), and blasts < 1% in peripheral blood and < 5% in BM. The most common cytopenia is anemia which is often severe and usually macrocytic. Thrombocytosis is present in almost half of the patients whereas pancytopenia is rare. Indeed, those cases that fulfill the criteria for MDS with isolated del(5q) but with pancytopenia, must be categorized as MDS, unclassifiable. Morphologically, BM exhibits erythroid hypoplasia and an increased number of small megakaryocytes with eccentric monolobulated nuclei. Some patients may present ring sideroblasts, even at high percentages; these cases should be considered also within this category, but once again there should not be Auer rods.

MDS, unclassifiable

The diagnosis of MDS, unclassifiable can be made in any of the following settings:

1. Patients with characteristics of MDS SLD, MDS MLD, MDS RS, or MDS isolated del(5q) with 1% blasts in peripheral blood measured on at least two separate occasions.
2. Patients with characteristics of MDS SLD, MDS RS SLD, or MDS isolated del(5q) with pancytopenia.
3. Patients with persistent cytopenia with < 2% blasts in the blood and < 5% in the BM, no significant (< 10%) dysplasia in any myeloid lineage, and the presence of a cytogenetic abnormality considered presumptive evidence of MDS (Table 2).

Special forms of MDS

Hypoplastic MDS and MDS with fibrosis do not constitute a specific MDS subtype in the WHO classification. However, exhibit distinctive clinical and morphological characteristics.

Hypoplastic MDS

In approximately 10% of MDS the BM is hypocellular according to age. Hypocellularity may lead to difficulties in the differential diagnosis with aplastic anemia (42,43). Furthermore, it is important to exclude toxics, infections and autoimmune diseases. BM biopsy is mandatory for the diagnosis.

MDS with fibrosis

In approximately 15% of MDS significant fibrosis, that means grade 2 or 3 of the WHO grading scheme is present (44). Fibrosis is associated with an aggressive clinical course, irrespective of the cytogenetics and blast count (45,46). BM biopsy is mandatory for the diagnosis.

2. Pathogenesis of the MDS

2.1 Somatic gene mutations

Recent analysis using next-generation sequencing (NGS) has greatly improved our understanding of the molecular pathogenesis of the MDS. It is estimated that over 90% of patients with MDS will harbor at least one mutation from a set of approximately 40 genes (47–49). However, only 4 to 6 genes are mutated with relatively high frequency while most of them are mutated in a minority of patients. Furthermore, no mutated gene is highly specific for MDS and no single gene is mutated in the majority of cases (47–49). The most frequently mutated genes belong to six major categories: RNA splicing, epigenetics (DNA methylation and histone modifications), transcription factors, signal transduction proteins and components of the cohesion complex. The most common somatic mutations are detailed in Table 7.

Table 7. Most common somatic mutations in patients with MDS

Mutated genes	Frequency in MDS (%)
RNA splicing	
SF3B1	15-30
SRSF2	10-20
U2AF1	<10
ZRSR2	<10
DNA methylation	
TET2	20-30
DNMT3A	~10
IDH1/IDH2	~5
Chromatin modification	
ASXL1	15-20
EZH2	~5
BCOR	<5
Transcription factors	
RUNX1	~10
TP53	10
ETV6	<5
Signal transduction	
NRAS/KRAS	10
CBL	5
JAK2	5
Cohesion complex	
STAG2	5

Several mutations are associated with clinical features and may identify genetic subtypes of MDS with more homogeneous disease phenotypes and prognosis; as for example, *SF3B1* mutation with ring sideroblasts and favorable prognosis (40) and *TP53* mutations with increased blasts, complex karyotype and poor prognosis

(50). Given these associations, somatic mutations carry prognostic information and can be used to assess prognosis of the MDS patients. This topic is discussed in section 3 in detail. Importantly, the genetic variability in MDS reflects the diverse clonal architecture present in these disorders, in which a mutated gene may exist in a small subclone while in other it represents the founding mutation present in every tumor cell (51). Mutational hierarchy analysis has demonstrated that mutations in genes involved in RNA splicing and DNA methylation occur early, that is, they are "founding mutations", whereas driver mutations in genes involved in chromatin modification and signaling often occur later, that is, they are "subclonal mutations" (52,53). Figure 3 shows the relations among founding mutations and subclonal mutations in a clonal evolution from MDS to subsequent AML (54).

Interestingly, three seminal works have identified the presence of somatic mutations tipically present in MDS and AML, in blood samples of adults without myeloid neoplasms, being the most frequently mutated genes *DNMT3A*, *TET2* and *ASXL1* (55–57). This finding has been named as *Clonal hematopoiesis of indeterminate potential* (CHIP) and is present in 10% of individuals older than 65 years with a rising trend with age (56). Notably, the relative risk of developing a hematologic malignancy (MDS or other myeloid or lymphoid neoplasm) is increased in these individuals, but the absolute risk remains very low (0.5–1% per year) (55). Therefore, patients with unexplained cytopenia and somatic mutations in the absence of morphologic or cytogenetic criteria, should not be diagnosed with definitive MDS. Importantly, OS is lower in individuals with CHIP suggesting that factors other than hematological cancers are contributory to death (57,58). In this regard, murine models have provided evidence of an increased cardiovascular mortality secondary to an accelerated atherogenesis driven by inflammasome-mediated endothelial injury, resulting from proinflammatory interactions between endothelium and macrophages derived from circulating clonal monocytes (59). Active research in the this field is on going in order to elucidate the definitive definition of CHIP, the prognostic impact and the best follow-up of CHIP individuals.

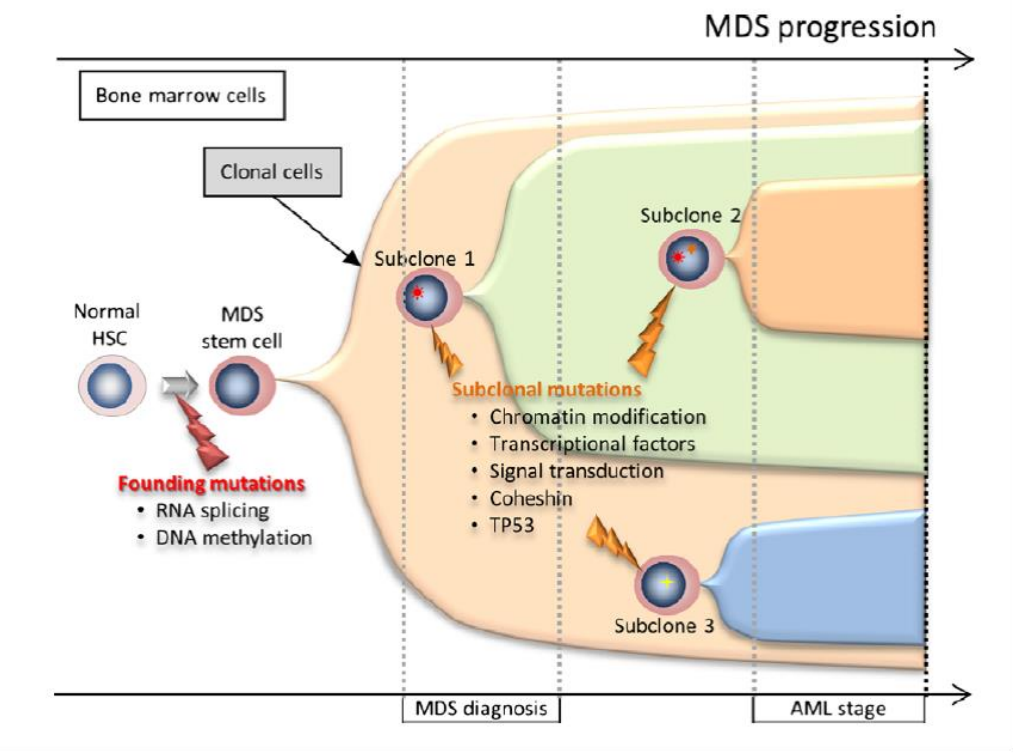


Figure 3. Proposed model of clonal architecture of MDS (Adapted from Harada *et al*, Cancer Science 2015) (54).

2.2 Immune system

As mentioned previously, somatic mutations are critical early drivers of the disorder. However, the factors enabling the emergence, selection, and subsequent leukemic evolution of these clones remain incompletely understood. Recent studies in MDS suggest that chronic activation of the innate immune signaling and its associated inflammatory pathways have been implicated not only in the pathogenesis but also in the evolution to AML (60–62). The main findings that prove the association between the immune system and MDS are detailed below.

2.2.1 MDS and inflammatory and autoimmune disorders

Large-scale epidemiologic studies have shown an increased risk of myeloid diseases in patients affected by chronic infections. Two similar population studies

carried out with data from the Swedish registries and the American Surveillance Epidemiology and End Results-Medicare data base, analyzed the occurrence of infectious diseases in patients with MDS and AML (63,64). Both studies confirmed that history of infection, particularly respiratory, urinary tract and skin infections, were significantly associated with a higher-risk of MDS and AML. The mechanisms for an increased risk for myeloid neoplasms among patients with a previous infection are not clear but several explanations have been hypothesized. First, it could reflect a compromised immune system many years before the MDS diagnosis, making patients more susceptible to transient infections (65,66). Second, persistent activation of the immune system secondary to chronic infections could influence the development of myeloid malignancies by the expansion of clonal myeloid progenitors (67). Lastly, the white cells of the myeloid lineage are components of the innate immune system, the first line of defense against infectious pathogens, and could be susceptible to genetic alterations in myeloblast precursor cells and further clonal expansion (68). Besides infectious diseases, patients with autoimmune disorders (AD) are more prone to develop MDS or AML when compared with matched controls (63,69,70). Conversely, patients with MDS are at higher risk for autoimmune manifestations with a prevalence ranging from 10 to 30% (15,71,72). Vasculitis and rheumatoid arthritis appear to be the most commonly described clinical autoimmune complications, although hypothyroidism, idiopathic immune thrombocytopenia or polymyalgia rheumatica, among others, have been reported as well (15,71,72). Information regarding the onset of the autoimmune disease, the prognostic meaning of these pathologies in the MDS outcomes or their association with the different MDS subtypes or cytogenetics is not conclusive (15,73). Against this background, in the first work that is presented in this thesis, we analyzed the prevalence, clinical characteristics, and impact on the clinical outcome of the presence of AD in a large cohort of patients diagnosed with MDS and chronic myelomonocytic leukemia (CMML), a myelodysplastic/myeloproliferative neoplasm.

Although clinical data strongly suggest a connection between inflammatory and autoimmune diseases and the development of MDS, the role for chronic innate

immune signaling in hematopoietic cells remains to be elucidated. In this regard, the most recent biological insights about the pathophysiological link between the MDS clone and chronic inflammation is outlined below.

2.2.2 Cell-intrinsic dysregulation of innate immune signaling in MDS

Expression of immune-related genes in MDS hematopoietic stem and progenitors' cells (HSPCs)

Overexpression of immune-related genes in HSPCs is reported in >50% of MDS patients (74,75). In MDS HSPCs, Toll-like receptors (TLRs), receptors that recognize pathogens and host cellular by-products critical for antimicrobial host defense and adaptative immune response, are either mutated or overexpressed compared to healthy controls (76,77). Hyperactivation of TLR signaling have been shown to recapitulate MDS phenotype, whereas its suppression has been demonstrated to restore the hematopoietic function of MDS HSPCs in mouse models (77–79). Furthermore, downstream effectors of the innate immune signaling such as MyD88 or receptor-associated kinase-1 and 4 (IRA1 and IRAK4) are also overexpressed or constitutively activated (77–79). Also, the TNF receptor-associated factor 6 (TRAF6), the last driver of TLR signaling and a key effector of the innate immune signaling through the activation of NF-kB pathway, is activated by multiple independent mechanism (Figure 4). In this regard, miR-146 and TRAF-interacting protein with forkhead-associated domain B (TIFAB) haploinsufficiency observed in del(5q), MDS results in TRAF6 overexpression (80,81). Furthermore, loss of miR-145, another del(5q) gene, results in TIRAP repression, a protein that lies on Myd88 pathway (82).

NLRP3 inflammasome and pyroptosis

The inflammasome consist on a family of Nod-like receptors (NLRs) which can lead to inflammatory-mediated cell death via caspase1-dependent IL-1β activation, called pyroptosis (83). Of the NLR family, NLRP3 has been implicated in MDS pyroptosis (84,85). NLRP3 is activated by different damage associated pattern (DAMP) signals, including S100A8 and S100A9 (Figure 4). In addition to activation

by DAMPs, recent studies have shown that inflammasome signaling is associated with common mutations in MDS, including *U2AF1, SRSF2, SF3B1, ASXL1*, and *TET2*, all of which contribute to activation of NLRP3-dependent pyroptosis (84). Importantly, inhibiting the inflammasome restores normal hematopoiesis in mice models. Furthermore, TRAF6 is involved in TLR-mediated activation of the NLRP3 inflammasome in BM macrophages, suggesting that TLR signaling via TRAF6 is also linked to inflammasome activation in MDS (86).

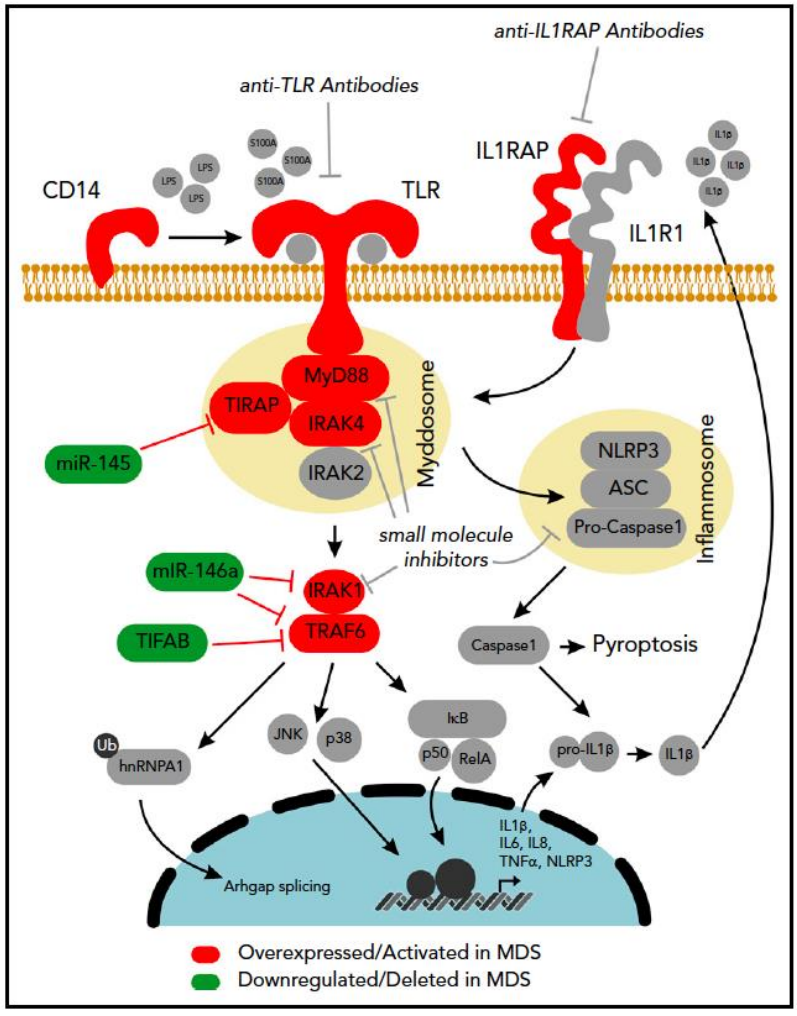


Figure 4. Cell intrinsic dysregulation of innate immune signaling in MDS HSCs (adapted from Varney *et al*, Exp Hematol. 2015) (85).

Innate immune signaling, clonal hematopoiesis (CH) and MDS

Two independent mechanisms have been postulated to explain the role of an altered immune signaling in the pathogenesis of MDS. First, chronic inflammation can induce genomic instability in the HSPCs resulting in CH. Although, CH might not be malignant *per se*, indeed most of the individuals with CHIP will never develop hematopoietic malignancies (57) it might function as a conduit for the development of myeloid neoplasms as a result of a progressive genomic instability and consequent clonal evolution (87,88). On the contrary, the so-called "sterile inflammation", in which the presence of CH drive a chronic activation of the immune signaling that results in a proinflammatory BM microenvironment that favors the development of MDS (89–91).

2.2.3 Cell-extrinsic dysregulation of innate immune signaling in MDS

Immune cells

Myeloid-derived suppressor cells (MDSCs) are absent or rare in healthy hosts but naturally accumulate in situations of trauma and sepsis to temper immune responses (92,93). MDSCs are also observed in the setting of many tumors as key contributors to tumor immune tolerance (92). In the BM of MDS patients, MDSCs are profoundly expanded, particularly in patients with lower-risk diseases, and are responsible of the inflammatory and immunosuppressive effects by causing defects in myeloid and erythroid differentiation and a reduction in T-cell proliferation and functionality. MDSCs are activated by binding of CD33 to S100A9, its specific ligand, that triggers the elaboration of immunosuppressive cytokines IL-10 and transforming growth factor-β, and granzyme-B that directly suppress hematopoiesis (92,94). S100A9 levels are also elevated in MDS BMMCs, supporting not only the increased counts of MDSCs but the increased immunosuppressive cytokines (94). In this regard, S100A9 transgenic mice develop an MDS-like disease. In contrast, blocking S100A9 signaling restored normal hematopoiesis, confirming the implication of MDSCs as initiators of the

MDS. Interestingly, MDSCs in MDS patients do not harbor the same somatic mutations as the MDS clone, indicating that they derive from non-neoplastic HSPC and that probably MDSC activation likely precede the emergence of genetically distinct MDS clones (94).

The role of granulocytes and macrophages is less clear in the MDS pathogenesis. However, recently studies have shown the loss of granulocyte-macrophage progenitors in low-risk patients, probably due to increased apoptosis. Contrary, these progenitors are increased in high-risk MDS due to the expression of antiphagocytic markers in myeloid progenitors such as CD47 (95). Altogether, the loss of MDS myeloid progenitors by apoptosis are, in part, responsible for the cytopenias in low-risk patients, and the up-regulation of the "don't-eat-me" CD47 signal in high-risk is an important transition step leading to progression from lower to higher-risk MDS.

The contribution of the adaptative immune system, specifically T-cell surveillance, has also been well-documented and appears to be regulated in a opposite fashion, from a proinflammatory state in low-risk patients to an anti-inflammatory state in high-risk MDS and AML patients (Figure 5). However, the exact mechanisms responsible of the switch in the cytokine milieu are about to fully elucidated. Low-risk MDS present higher counts of cytotoxic CD8+, helper T (Th17) and natural killer (NK) cells and lower counts of T-regulatory lymphocytes (Tregs) (96–98). Overall, these changes in cell number and functionality cooperate with the release of inflammatory cytokines, such as IL-10, TNFα and TGF-β, and trigger an autoimmune response against hematopoietic cells (clonal and no clonal) that contribute to BM cells apoptosis resulting in peripheral cytopenias (99,100). Contrary, high-risk patients present lower levels and dysfunctionality of CD8+, Th17, NK cells, and increased numbers of Tregs that leads to the acquisition of immune tolerance and increased proliferation rates of the malignant clone with the subsequent acquisition of genetic abnormalities and evolution to AML (97,101,102). Furthermore, negative regulators of T-cell mediate immune responses, PD1/PDL-1(receptor programmed death-1/ programmed death-

1ligand), are found to be overexpressed in CD34+ BM MDS cells supporting the repression of T-cell response hypothesis (103).

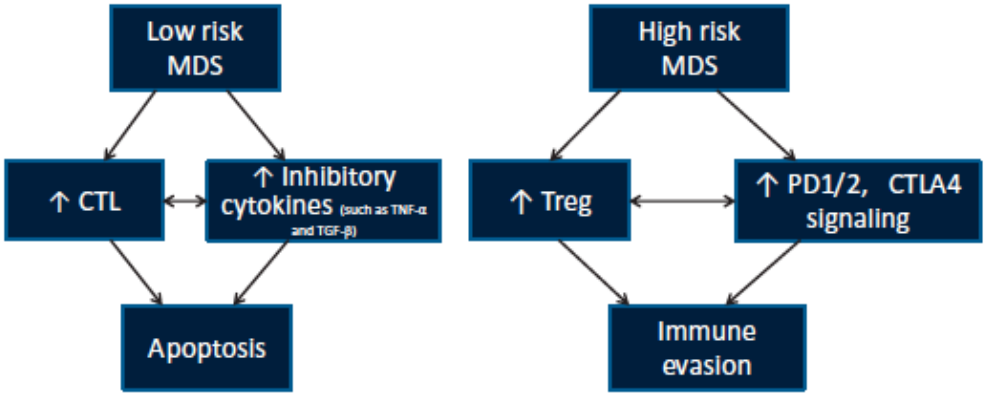


Figure 5. Immunological disturbances in MDS (adapted from Zahr *et al*, Expert Review of Hematology 2016) (104).

Mesenchymal stroma

Multipotent mesenchymal stroma cells (MSCs) are non-hematopoietic stem cells that give rise to all of the BM stroma cells, namely osteoblasts, osteoclasts, fibroblasts, adipocytes or endothelial cells, that shape the BM niche and controls HSC self-renewal and differentiation. Several studies support the hypothesis of the dual role of the BM niche in MDS pathogenesis. In the “niche-driven” model, primary genetic alterations in MSCs drive the proinflammatory microenvironment inducing malignant transformations of the HSC. This was first observed in the pivotal study in which mice bearing mesenchymal deletion of Dicer1, the microRNA-processing endonuclease, or Sbds, Shwachman-Diamond syndrome causing gene, a congenital BM failure syndrome with MDS/AML predisposition, recapitulates MDS phenotype (105). Further studies have shown that increased BM inflammation due to activating mutations in inflammatory pathways, such as

NF-kB pathway and WNT/β-catening signaling, and cellular senescence of the BM-derived stroma cells, resulted in MDS development (106–108). Vice versa, primary (epi)genetic alterations in the hematopoietic stem cells (HSC) have the ability to alter mesenchymal niche components supporting the hypothesis of "niche-facilitated" malignant transformation. Together, the data indicate that inflammation, specifically driven by MSCs in the hematopoietic niche directly or indirectly, leads to attenuation of HSC homeostasis and facilitates neoplastic transformation of the BM.

2.2.4 Immune therapies in MDS

It has been reported 30% of sustained hematologic responses in MDS patients treated with immunosuppressive agents, reinforcing the role of the immune system in the pathogenesis of MDS (109). Furthermore, lenalidomide, an immunomodulatory drug with multiple effects on the immune system, is specifically active in del(5q) MDS (110). The growing knowledge in the innate and inflammatory signals in MDS have provided a strong biological rational for the development of novel immune therapeutic strategies.

Taken together, data strongly point that MDS needs to be considered a disease of a tissue rather than a disease of hematopoietic cells in isolation. Cross-talk between HSPCs and their BM environment likely drives disease initiation and evolution.

2.2.5 Integrated model of the role of inflammation/innate immunity in the pathogenesis of MDS

The MDS HSC might originate from the (epi)genetic changes occurring during aging or generated by genotoxic stress (chemotherapy and/or radiotherapy); or develop after a sustained exposure to inflammatory effectors in the context of an inflammatory condition, such as autoimmune disorders or chronic infections (Figure 6). Regardless of the genesis of the malignant clone, there is an activation of the innate immune signaling pathways resulting in a proinflammatory BM microenvironment with the recruitment of CD8 T, Th17 and NK cells. As a

consequence, BM HSCs increase their cycling rates and express death receptors on their surface. The continuous inflammatory signaling along with expression of death receptors, induce intramedullary apoptosis of probably both HSC, normal and clonal, which result in reduced number of fully differentiated cells and subsequent cytopenias, typically present in low-risk MDS. In addition, as explained above, MDS progenitors present intrinsic defects that favor impaired differentiation contributing to the cytopenias. The persistent BM proinflammatory niche triggers the recruitment of MDSCs and induce profound gene expression changes in the surrounding MSCs. MDSCs exacerbate the defects on differentiation by inducing myeloid skewing and killing erythroid progenitors, and they suppress the autoimmune response by CD8+ T cells and mobilize Tregs, resulting in the switch to an immunotolerant microenvironment. The high proliferation rates induce MDS HSC to accumulate additional genetic alterations that favors resistance to apoptosis and survival advantage, and also contribute to the aberrant proliferation of the clone, resulting in an overpopulation of the BM. The accumulation of genetic abnormalities in the MDS clone along with the ability to scape to the immune system, promotes the progression to AML.

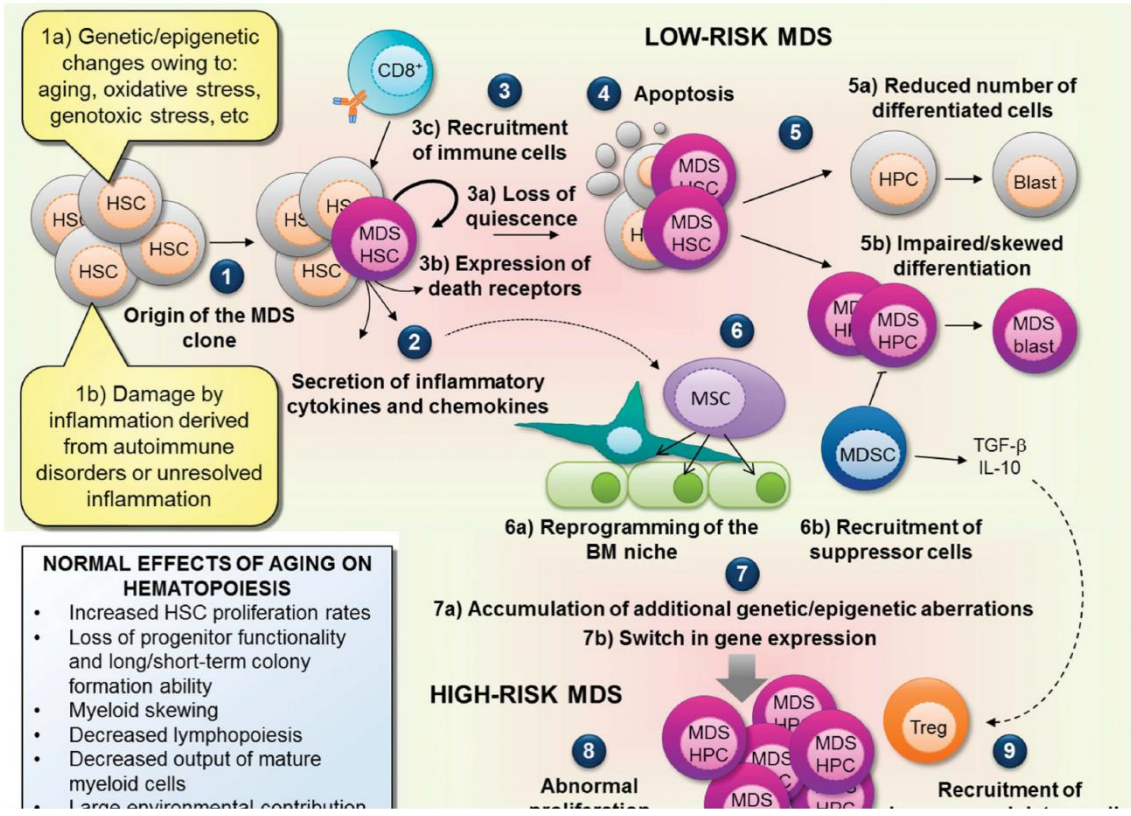


Figure 6. Proposed model for the central role of inflammation/innate immunity in the pathogenesis of MDS (adapted from Gañán-Gómez *et al*, Leukemia 2015) (60).

3. Prognosis assessment of the myelodysplastic syndromes

MDS encompass a wide spectrum of clinical phenotypes that range from largely asymptomatic patients with mild cytopenias and long-life expectancy, to those with severe symptoms due to profound cytopenias and a very poor prognosis. Even patients with similar clinical presentations at diagnosis may evolve differently. This variability is challenging to determine the optimal timing and choice of treatment of MDS patients and how best to counsel them about their expected prognosis. Consensus treatment guidelines for MDS rely on an accurate estimation of risk to determine optimal treatment algorithms, being the therapeutic goals and choices for lower-risk completely different from those for higher-risk patients. Therefore,

accurate determination of prognosis is critical for individualizing risk-adapted therapy for MDS.

3.1 Prognostic scoring systems

In the late 80´s, the first scoring systems were reported all of them based on clinical and laboratory data: The Bornemouth and Goasguen scoring systems included BM blasts and blood counts, while the Spanish contained the age and the Düsseldorf score considered LDH levels. All of them classified patients in different groups with significant differences in survival (21,111,112) Importantly, none of these prognostic scores included chromosomal abnormalities.

3.1.1 International Prognostic scoring system

In 1997 the International Prognostic Scoring System (IPSS) was published and it was a breakthrough prognosis stratification that was used for almost two decades all around the world. It derived by examining over 800 patients who never received disease-modifying agents, and represented a milestone in MDS because for the first time a score recognized the important value of cytogenetic abnormalities in MDS patients (Table 8) (113).

Table 8. International Prognostic Scoring System (113).

Prognostic Variable	0	0.5	1	1.5	2
BM Blasts %	<5	5-10	-	11-20	21-30
Karyotype*	Good	Intermediate	Poor	-	-
Cytopenia/s	0-1	2-3	-	-	-

*Karyotype. Good: Normal, -Y, del(5q), del(20q); Poor: Complex (≥3 abnormalities), abnormalities in chromosome 7; Intermediate: Other single or double abnormalities.

This score divided patients in four prognostic groups with significant differences in OS and risk to AML evolution (Table 9).

Table 9. Risk groups of the IPSS and the predicted median OS and evolution to AML (114).

Risk group	Points	Median OS	25% AML evolution
Low	0	5.7	9.4
Intermediate-1	0.5-1	3.5	3.3
Intermediate-2	1.5-2	1.1	1.1
High	≥2	0.4	0.2

OS: Overall survival; AML: Acute myeloid leukemia.

In addition to the inclusion of the cytogenetic data, the main advantage of the IPSS was its simplicity. Thus, it was the clinical standard for risk assessment in MDS for over 15 years. However, it also presented important limitations. First, it was design and validated to assess the risk only at the moment of the diagnosis. Second, it only included three cytogenetic groups accounting to five specific karyotypic abnormalities, and most of the cytogenetic alterations included in the intermediate group. Third, it only considered the number of cytopenias but not their depth and finally, it outweighed the impact of blasts compared to chromosomal abnormalities (115,116). This leads to an underestimation of risk in many patients, especially in lower-risk MDS. Consequently, further attempts in order to improve the risk assessment were conducted (117,118).

3.1.2 WHO classification-based Prognostic Scoring System

The WHO classification-based Prognostic Scoring System (WPSS), published ten years later, take the advantage of the WHO MDS subtypes instead of the percentage of blasts, while keeping the same cytogenetics groups of the IPSS. The WPSS considers the severity of the anemia, first defined by transfusion

dependency and then in the revised version (WPSS-R) by the hemoglobin thresholds (119,120). This score categorized patients in five risk groups with significant differences in outcomes (Table 10 and 11).

Table 10. WPSS Prognostic score values (119).

Prognostic Variable	0	1	2	3
2001 WHO Subtype	RA, RARS, del(5q)	RCMD, RCMD-RS	RAEB-1	RAEB-2
Karyotype*	Good	Intermediate	Poor	-
Transfusion requirement	No	Regular*	-	-

*Karyotype. Good: Normal, -Y, del(5q), del(20q); Poor: Complex (≥3 abnormalities), abnormalities in chromosome 7; Intermediate: Other single or double abnormalities. RA: Refractory anemia; RARS: Refractory anemia with ring sideroblasts; RCMD: Refractory cytopenia multilineage dysplasia; RCMD-RS: Refractory cytopenia with multilineage dysplasia and ring sideroblasts; RAEB-1: Refractory anemia with excess blasts type 1; RAEB-2: Refractory anemia with excess blasts type 2.

Table 11. Risk groups of the WPSS and the predicted median OS (119).

Risk group	Points	Median OS (months)
Very low	0	103
Low	1	72
Intermediate	2	40
High	3-4	21
Very high	5-6	12

OS: Overall survival.

The main advantage of the WPSS is that it has been validated at times other than diagnosis, making it a dynamic scoring system (121,122). However, it does not address the concerns of the IPSS and the prognostic of low-risk patients remained underestimated.

3.1.3 MD Anderson Lower Risk Prognostic Score

The MD Anderson Lower Risk Prognostic Score (LRPSS) was designed specifically detect patients with lower-risk disease and poor prognosis (118). This score, stratifies lower-risk MDS according to age, blast percentage, cytogenetics and severity of anemia and thrombocytopenia (Table 12).

Table 12. LRPSS Prognostic score values (118).

Prognostic Variable	0	1
Cytogenetics	Unfavorable	-
Age (years)		≥60
Hemoglobin (g/dl)	<10	-
Platelets (x10^9/L)	50-200	<50
BM blasts (%)	≥4	

BM: Bone marrow blasts

Patients could be assigned to one of three categories, being at least one third of the patients classified on the highest-risk category with a median OS similar to that of IPSS intermediate-2 patients, pointing that this group may benefit from early therapeutic intervention.

Table 13. Risk groups of the LRPSS and the predicted median OS (118).

Score	LRPSS Risk group	Median OS (months)
0-2	1	80
3-4	2	27
≥	3	14

OS: Overall survival.

The same group developed a prognostic score which included prior treated patients, secondary MDS and CMML with leukocytosis (123). The MD Anderson Global Prognostic Scoring System (MPSS) considers eight variables (ECOG, age, cytogenetics, hemoglobin, leucocytes, platelets, marrow blasts, and transfusion dependency) to categorize patients in four risk groups. However, the MPSS has not been widely accepted because its complexity, risk overestimation in patients with comorbidities and the simplistic interpretation of the cytogenetics.

3.1.4 Revised International Prognostic Scoring System

The Revised International Prognostic Scoring System (IPSS-R), developed by studying over 7.000 untreated patients with MDS, evaluates the same features as the classic IPSS but in greater detail (Table 15) (124). For the first time, the severity of each cytopenia is considered, the thresholds of blasts percentages are redefined and its prognostic impact is undermined, and which is the most important, the range of cytogenetic groups is increased with the number of explicitly defined karyotype abnormalities more than double.

Table 15. Prognostic score values of the IPSS-R (125).

Prognostic Variable	0	0.5	1	1.5	2	3	4
Karyotype*	Very good	-	Good	-	Intermediate	Poor	Very poor
BM blasts (%)	≤2		>2-<5		5-10	>10	
Hemoglobin (g/dl)	≥10		8-<10		<8		
Platelets ($x10^9$/L)	≥100	50-<100	<50				
Neutrophils ($x10^9$/L)	≥0.8	<0.8					

*Karyotype. Very good: -Y, del(11q); Good: Normal, del(5q), del(21p), del(20q), double including del(5q); Intermediate: del(7q), +8, +19, i(17q) any other single or double independent clones; Poor: -7, inv(3)/(3q)/del(3q), double including -7/del(7q), complex with 3 abnormalities; Very poor: >3 abnormalities.

This results in broader range of risk scores and assignment of patients into one of five groups, incorporating a new risk group, the intermediate-risk (Table 16).

Table 16. Risk groups of the IPSS-R and the predicted median OS (125).

Risk group	Points	Median OS (months)
Very low	≤1.5	8.8
Low	<1.5-3	5.3
Intermediate	>3-4.5	3.0
High	>4.5-6	1.6
Very high	>6	0.8

OS: Overall survival.

Since its publication, IPSS-R has replaced the former IPSS and it has become the gold standard for risk assessment in MDS both in the daily practice and to carry

out clinical trials. Several external validations in different contexts including treated patients, different time other than diagnosis, and in the hematopoietic stem cell transplantation (HSCT) settings have confirmed its high prognostic value (126–128). However, the IPSS-R is not exempt of limitations; it is complex to use, it lacks molecular information, and opens a discussion on the outcomes and management of the intermediate-risk group (129,130). This specific prognostic group, can present a very heterogeneous outcomes; some patients present an indolent disease while others rapidly evolve to AML. The second work conforming this thesis is directed to solve this issue.

3.1.5 Recommendations of the Spanish Group of MDS (GESMD) for the prognostic stratification of MDS

The GESMD has proposed a definition of high-risk MDS (median OS < 30 months) for patients with the following characteristics (131):

1. IPSS and/or WPSS intermediate-2 and high and/or IPSS-R high or very-high
2. IPSS and/or WPSS intermediate-1 and/or IPSS-R intermediate with one or more of the following high-risk factors:
    - -High or very high cytogenetic abnormality in the IPSS-R
    - -Platelets < 30 $x10^9$/L
    - -Neutrophils < 0.5 $x10^9$/L
    - -Grade 2 o 3 BM fibrosis

Whereas, patients without these characteristics are included in the low-risk group. These recommendations are more restrictive; thus, a better characterization of the prognostic risk is presumed. In this regard, a recent paper analyzed the ability of the different newer MDS prognostic indices (IPSS-R, LRPSS, WPSS-R and GESMD) with the aim to identify patients with poor prognoses within the lower-risk categories defined by the IPSS (low and intermediate-1) (132). We found that within the intermediate-1 group, between 17% and 47% of patients were identified as having poor prognoses (defined as those patients with a expected survival of less than 30 months). Accordingly, the IPSS was not sufficiently accurate to

predict the survival of patients in the intermediate-1 group and the use of the newer indices was useful for identification of patients with poor prognosis.

3.2 Prognostic factors in MDS

3.2.1 Prognostic impact of clinical factors

Several factors, other than those included in prognostic scoring systems, have been identified as having prognostic impact in MDS:

- Age; as expected is correlated with OS but not with AML evolution (113,123))
- Comorbidities; higher number of comorbidities are correlated with OS but not with AML evolution (108,133,134)
- High levels of lactate dehydrogenase (LDH) enzyme, β-2 microglobuline and serum erythropoietin (133,135,136)
- High levels of ferritin (>1000 ng/ML) (137)
- Number and severity of cytopenias (12,113,123,125,138)
- Multilineage dysplasia (31,32,139)
- Presence of pseudo-Pelger-Hüet anomaly and mycromegakaryocytes (139)
- Presence of Auer Rods (31–33,101,140)
- Increased blast in peripheral blood and BM (31–33,140)
- Presence of Abnormal Located Immature Precursors (ALIPs) (46)
- BM fibrosis grade 2-3 (141,142)
- Complex karyotype and abnormalities in chromosome 7 ((22)
- Therapy-related MDS, probably influenced by the strong association with karyotypic abnormalities (143)

3.2.2 Prognostic impact of somatic mutations

In the last decade, several studies have demonstrated the prognostic impact of somatic mutations in patients with MDS (47–49). These genetic events are present in nearly every patient with MDS and represent pathophysiologic drivers of disease and evolution indicators, making them a better disease biomarker than clinical

features alone. Two pivotal studies with 439 and 944 patients, respectively, have shown that mutations in *TP53*, *EZH2*, *ETV6*, *RUNX1* and *ASXL1* genes are predictors of poor overall survival in patients with MDS, independently of established risk factors (47,48). In general, a greater number of somatic mutations is associated with shorter OS (49). However, not all genes present equal prognostic significance and in some of them it depends on the clinical context. For example, the splicing factor *SF3B1* is the only recurrently mutated gene associated with favorable prognosis (47,144). However, it loses their beneficial prognostic impact when is present in patients with higher blasts proportion (145). On the other hand, there are several mutated genes that confers adverse prognosis in patients with lower-blast proportion. These genes are *ASXL1, U2AF1* and *SFRSF2*, and lose their unfavorable prognostic significance in patients with higher-risk disease. Other mutated genes as *RUNX1, EZH2* and *TP53* remain adverse across risk groups (145). Interestingly, *TP53* mutations maintain its dismal outcome independently of the presence of complex karyotype (48,50,146). In spite of the enormous progress, mutational status has not yet been incorporated to clinical scoring systems as there is no formal consensus of how best consider them. This can be explained in part because mutations can co-occur in a wide variety of patterns and can be present in either the dominant clone or as a subclone where they might have different impacts. Moreover, the impact of less-frequent mutations is unknown. The molecular-IPSS guidelines are under development and surely will improve how we asses risk prognostication of MDS patients.

3. Recommended prognostic stratification for clinical management

Prognostic assessment is a critical step for the individualization of care of MDS patients as treatment guidelines are risk-adapted. Multiple methods for risk stratification are available, being the IPPS-R currently considered the gold standard (125). Accordingly, patients allocated in the very low and low-risk groups are considered lower-risk MDS, while those in the high and very-high are referred as higher-risk MDS. There are no specific treatment protocols for those patients in

the intermediate-risk group with different strategies recommended (i.e. European Leukemia Net (ELN) guidelines(147), National Comprehensive Cancer Network (NCCN) guidelines (148) and GESMD guidelines (131). Other prognostic factors, especially somatic mutations, can refine estimates of risk. Finally, low or intermediate-risk patients who failed first-line therapy, should be handled as higher-risk.

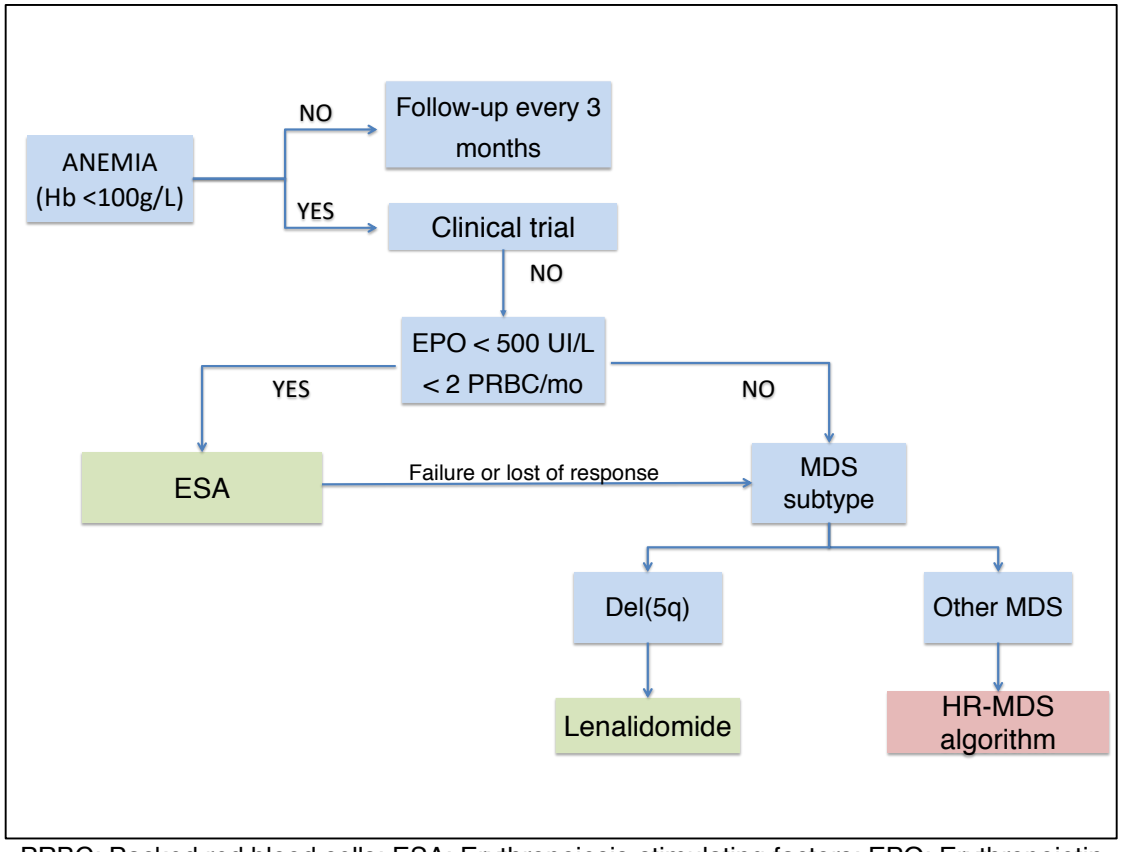
4. Treatment of the MDS patients

Treatment choice of MDS patients is a challenging exercise that relies on patient's age, comorbidities and risk of death and leukemic transformation, according to the IPSS-R. Overall, treatments can be classified in supportive measures (blood transfusions, granulocyte colony-stimulating factor, antibiotics, and iron chelation), non-intensive treatment (erythropoiesis-stimulating factors (ESA)), hypomethylating agents (azacitidine and decitabine) and lenalidomide, and intensive treatment (AML-like chemotherapy and HSCT). In spite of the enormous progress on the biology of the disease, therapeutic options are scarce with only the HSCT as the potentially curative option, but limited to a few patients due to its high treatment related mortality (149). Notably, in the last years, several drugs directed to alleviate cytopenias have shown activity in low-risk MDS patients: Two thrombopoietin (TPO) receptor analogues, Romiplostin a peptide TPO-mimetic administered subcutaneously, and Eltrombopag, an oral nonpeptide TPO-receptor that interacts with the transmembrane domain of the TPO-receptors, resulting in megakaryocyte and platelet development. TPO receptor analogues, have demonstrated improvement in platelet counts and decreased bleeding events in 30% of MDS patients (150,151) and luspatercept (ACE-536), a recombinant fusion protein that promotes late-stage erythroid differentiation by blocking TGFβ superfamily inhibitors of erythropoiesis, especially GDF11. Luspatercept has demonstrated to be effective for the treatment of anemic low-risk MDS patients resistant to ESA, especially in those with ring sideroblasts and/or *SF3B1* mutation (1–6). Based on these results, is expected that luspatercept will be shortly aproved

by the U.S Food and Drugs Administration. Importantly, due to the lack of long-term curative treatments, MDS patients should be included in clinical trials whenever is possible.

4.1.1 Treatment of the low-risk MDS patients

Survival in low-risk patients is expected to be long [OS >30 months] therefore, the goal is to alleviate cytopenias, mainly anemia, in order to improve their quality of life. Treatment of the anemia in lower-risk MDS patients depends on the presence of the deletion of the 5q chromosome. A proposed treatment algorithm is described in Figure 7.

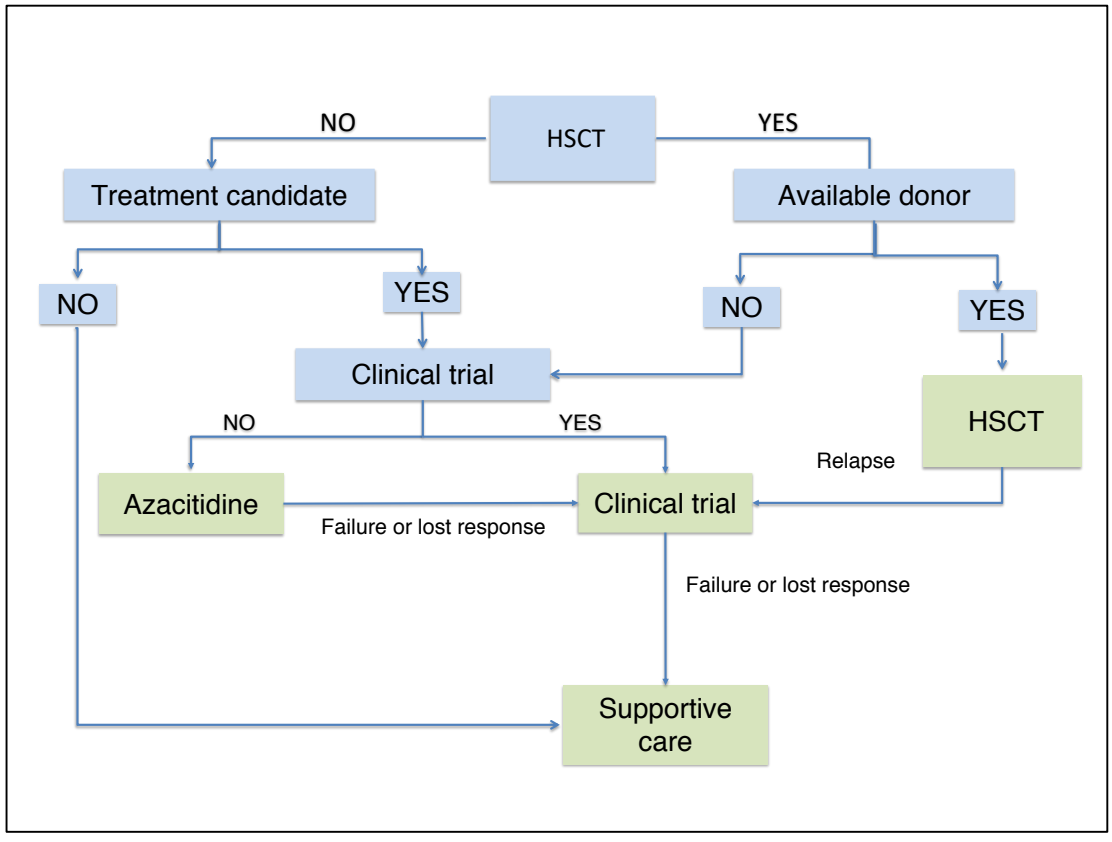


PRBC: Packed red blood cells; ESA: Erythropoiesis-stimulating factors; EPO: Erythropoietin (serum); MDS: Myelodysplastyc syndromes; HR-MDS: High-risk myelodysplastyc syndromes.

Figure 7. Proposed algorithm approach for the treatment of low-risk MDS.

4.1. 2 Treatment of the high-risk MDS patients

Therapeutic strategies in these MDS are focused in overcome the infaust outcomes (OS <30 months and AML transformation in 30% of the patients). Initial approach it depends on whether or not the patient is eligible for HSCT. A proposed treatment algorithm is described in Figure 8.



HSCT: Hematopoietic stem cell transplantation

Figure 8**.** Proposed algorithm approach for the treatment of high-risk MDS.

II. Hypothesis and objectives

Hypothesis

The presence of autoimmune disorders (AD) is common in MDS patients and present prognostic relevance. Moreover, dichotomization of MDS by using the new IPSS-R, identify a high-risk group of patients who are candidate for intensive treatment.

Objectives

In order to evaluate our hypothesis, we developed two studies with the following objectives:

General objective

To improve the strategies for the prognostic evaluation of MDS patients

Specific objectives

- To analyze the prevalence and clinical characteristics of AD in patients with MDS
- To evaluate the prognostic impact of AD in the outcome of MDS patients
- To ascertain which IPSS-R threshold best dichotomizes MDS patients in low vs. high-risk categories

III. Methods and results

The methods and results are presented through the integration of the two published articles:

1. **First work**

Julia Montoro, Laura Gallur, Brayan Merchán, Antonieta Molero, Elisa Roldán Ferrán Martínez-Valle, Guillermo Villacampa, Mayda Navarrete, Margarita, Ortega, Josep Castellví, Silvia Saumell, Sabela Bobillo, Francesc Bosch, David Valcárcel. Autoimmune disorders are common in myelodysplastic syndrome patients and confer an adverse impact on outcomes. Annals of Hematology (2018) 97:1349–1356.

2. **Second work**

Julia Montoro, Helena Pomares, Guillermo Villacampa, Brayan Merchán, Antonieta Molero, Esther Alonso, Laura Gallura, Javier Grau, Olga Salamero, Elisa Roldán, Silvia Saumell, Margarita Ortega, Anna Sureda, Francesc Bosch, Montserrat Arnán and David Valcárcel. Dichotomization of the new revised international prognostic scoring System for a better clinical stratification of patients with myelodysplastic syndromes. Leukemia & Lymphoma (2018)30;1–6.

1. First work

Autoimmune disorders are common in myelodysplastic syndrome patients and confer an adverse impact on outcomes.

The coexistence of AD in patients with MDS has been widely recognized, although with distinct results regarding the prevalence and impact on outcomes.

The aim of this study was to analyze the prevalence, clinical characteristics and outcomes of MDS with AD.

Patients

Patients with diagnosis of MDS and CMML at the Department of Hematology of the University Hospital Vall d'Hebron of Barcelona between January 2011 and October 2016 were consecutively included. Diagnosis of MDS or CMML was performed according to the WHO 2016 criteria and risk stratification was performed following the IPSS-R. All cases were reviewed by three expert cytologists and results were discussed and integrated in the Myeloid Integrated Diagnostic Committee. Transfusion dependency and erythroid response were assessed following the International Working Group 2006 criteria. Clinical characteristics and laboratory values were obtained by electronic medical record review.

Clinical and serological assessment of autoimmune Abnormalities

1. Clinical autoimmune disease

All medical records were reviewed for documented past or active systemic autoimmune disease. Moreover, all patients were interviewed for a past medical history of autoimmune disorder and underwent to a thorough physical examination.

2. Serological autoimmune abnormalities

In addition, serological immune parameters were searched for each patient at the moment of the MDS diagnosis or before any treatment. These tests included:

- Rheumatoid factor, assessed by turbidimetry with an AU5800 analyzer by Beckman Coulter and was considered significant from values above 14 IU/mL
- Antinuclear antibodies (ANAs), detected by indirect immunofluorescence on HEp2 cells (Inova Diagnostics) and considered significant from titer values ≥ 1/320

Patients with clinical or serological suspicion of autoimmune abnormalities were thereafter evaluated by a specialist in autoimmune diseases in pursue of disorder classification. Altogether, MDS and CMML patients were categorized in two groups:

1. MDS with autoimmune disorders (MDS-AD), when they exhibit a clinical autoimmune disease and/or a positive serological test
2. MDS without autoimmune disorders (MDS-noAD), when they were lacking all these findings.

Patients categorized in the very low and low IPSS-R categories were classified as low risk (L-R MDS), whereas those falling in the intermediate, high, and very high IPSS-R subgroup were categorized as intermediate/high risk (I/H-R MDS) patients.

Statistical analysis

Overall survival was defined as the time from diagnosis to death, whereas surviving patients were censored at the last follow-up. Continuous variables were expressed as mean or median, standard deviation and ranges, and categorical variables were expressed as absolute values and percentages. Fisher's exact test for categorical variables and Student's t test or Mann-Whitney test for continuous variables, after checking for normality with the Shapiro-Wilk test, were appropriately used. Survival analysis was calculated using the Kaplan-Meier method and log-rank test was used for statistical comparison. Cox proportional-hazard model was used for multivariable analyses and to obtain HR with CI95%. Statistical analysis was performed by using STATA, version 13.1.

Results

Clinical autoimmune diseases

- Clinical autoimmune diseases were identified in 39 of 142 patients (27.5%) (33 MDS and 6 CMML).
- The most common autoimmune disease was hypothyroidism (8% of patients) followed by rheumatoid arthritis (6.5% of patients).

Abnormal serological immune parameters

- Abnormal serological immune parameters were identified in 38 out of 129 (29.5%) patients; ANAs in 23%, rheumatoid factor in 10%, and positivity for both parameters in 5% patients.
- Among the 39 patients with clinical autoimmune disease, only 20.5% patients presented serological abnormalities. Conversely, 79% with serological abnormalities did not present clinical autoimmune disease.

Prevalence of Myelodysplastic syndromes with autoimmune disorders

- Altogether, and after merging all the clinical and serological parameters for AD, a total of 48% patients were classified as MDS-AD.

Clinical characteristics and outcomes of MD-AD vs. MDS-nonAD

- The presence of AD was significantly associated with female gender and lower hemoglobin value.
- L-R MDS had less autoimmune events (43%) than I/H-R MDS (67%)
- MDS-AD patients showed worst OS than MDS-nonAD (69% vs. 88% at 30 months).
- Only the presence of a clinical immune disorder had an impact on the OS, whereas the isolated presence of immune serological parameters had no impact in OS.
- L-R MDS with AD showed a trend for a worse OS than those lacking AD

- In the multivariate analysis, IPSS-R risk categories (L-R vs. I/H-R) and the presence or absence of AD retained their independent prognostic value

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ORIGINAL ARTICLE

Autoimmune disorders are common in myelodysplastic syndrome patients and confer an adverse impact on outcomes

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Abstract

The coexistence of autoimmune disorders (AD) in patients with myelodysplastic syndrome (MDS) or chronic myelomonocytic leukemia (CMML) has been widely recognized, although with distinct results regarding their prevalence and impact on the outcomes of the underlying hematological process. This study was aimed to analyze the prevalence, clinical characteristics, and outcomes of MDS with AD in a series of 142 patients diagnosed with MDS and CMML. AD was ascertained by both the presence of clinical symptoms or compatible serological tests. In total, 48% patients were diagnosed as having AD, being hypothyroidism the most commonly reported clinical AD (8%) and antinuclear antibodies the most frequent serological parameter identified (23.2%). The presence of AD was associated with female gender, lower hemoglobin levels, and higher IPSS-R. Overall survival for patients with AD was inferior to those with no AD (69 vs. 88% at 30 months; HR 2.75, $P = 0.008$). Notably, clinical but not isolated immune serological parameters had an impact on the outcomes of patients with AD. Finally, in a multivariate analysis, the presence of AD (HR 2.26) along with disease risk categories (very low and low vs. intermediate, high, and very high IPSS-R; HR 4.62) retained their independent prognostic value ($P < 0.001$). In conclusion, AD are prevalent in MDS and CMML patients and have prognostic implications, especially in lower-risk MDS patients.

Keywords Myelodysplastic syndrome · Chronic myelomonocytic leukemia · Autoimmune disorders · Immune dysregulation

Introduction

Myelodysplastic syndromes (MDS) are clonal myeloid neoplasms characterized by ineffective hematopoiesis that usually renders peripheral blood cytopenias, myeloid dysplasia, and an increased risk of transformation to acute myeloid leukemia (AML) [1]. Throughout the past decade, significant efforts have been made to understand the diversity and complexity of the pathophysiology of this disease, leading to the identification of dysfunctional epigenetic mechanisms and genetic defects in numerous pathways. In spite of the enormous progress on the biology of this disease and that some mutations have well-defined clinical and prognostic implications, the whole pathogenic picture remains to be elucidated [2, 3]. Along with these genetic and epigenetic observations, recent studies in MDS and CMML suggest that abnormal activation of the innate immune system and its associated inflammation could be involved not only in the pathogenesis but also in the evolvement to AML [4–7]. Thus, immunodeficiencies like "Monomac syndrome", a sporadic primary immunodeficiency due to *GATA2* mutation characterized by severe monocytopenia and atypical infections, have an increased risk of evolution to MDS or AML [8], and immunodeficient patients due to HIV infection are at higher risk of developing MDS [9].

The relationship between MDS and autoimmune disorders (AD) has been revealed by large-scale epidemiologic studies demonstrating that patients with AD are more prone to

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develop MDS or AML when compared with matched controls [10–12]. Conversely, it has also been shown that patients with MDS or CMML have a prevalence of autoimmune disorders usually around 10 to 30%, being these AD previously reported heterogeneous in their nature and outcome [13–16]. Reinforcing the role of the immune system in the pathogenesis of MDS and CMML, it has been reported that immunosuppression treatments of MDS with antithymocyte globulin and cyclosporine were able to attain a 30% of hematologic responses, particularly in younger patients with hypoplastic bone marrow, presence of trisomy 8, and HLA DR15 [17]. Other immunosuppressive agents like alemtuzumab, a humanized monoclonal antibody that recognizes CD52, have also shown sustained hematologic responses in MDS patients [18].

Against this background, herein we analyzed the prevalence, clinical characteristics, and impact on the clinical outcome of the presence of AD in a large cohort of patients diagnosed with MDS and CMML.

Patients and methods

Patients

Patients with confirmed diagnosis of MDS and CMML at the Department of Hematology of the University Hospital Vall d'Hebron of Barcelona between January 2011 and October 2016 were consecutively included in this study. Diagnosis of MDS or CMML was performed according to the World Health Organization 2016 (WHO 2016) criteria [19] and risk stratification was performed following the Revised International Prognostic Scoring System (IPSS-R) [20]. Transfusion dependency and erythroid response were assessed following the International Working Group 2006 criteria [21]. All cases were reviewed by three expert cytologists (JM, MN, and SS) and results were discussed and integrated in the Myeloid Integrated Diagnostic Committee. When necessary, several bone marrow studies were performed before a formal diagnosis of MDS/CMML was stated. Clinical characteristics and laboratory values were obtained by electronic medical record review.

Clinical and serological assessment of autoimmune abnormalities

All medical records were systematically reviewed for documented past or active systemic autoimmune disease. Moreover, all patients were interviewed for a past medical history of autoimmune disorder and underwent to a thorough physical examination. In addition, serological immune parameters were searched for each patient at the moment of the MDS diagnosis or before any treatment. These tests included rheumatoid factor and antinuclear antibodies (ANAs). Rheumatoid factor was assessed by turbidimetry with an AU5800 analyzer by Beckman Coulter and was considered significant from values above 14 IU/mL, and ANAs were detected by indirect immunofluorescence on HEp2 cells (Inova Diagnostics) and were considered significant from titer values ≥ 1/320 for the purpose of avoiding false positives usually seen in elderly population. Patients with clinical or serological suspicion of autoimmune abnormalities were thereafter evaluated by a specialist in autoimmune diseases (F M-V) in pursue of disorder classification. Altogether, MDS and CMML patients were categorized in two groups: MDS with autoimmune disorders (MDS-AD) when they exhibit a clinical autoimmune disease and/or a positive serological test, and MDS without autoimmune disorders (MDS-noAD) when they were lacking all these findings.

Concerning the disease-risk stratification, patients categorized in the very low and low IPSS-R categories were classified as low risk (L-R MDS), whereas those falling in the intermediate, high, and very high IPSS-R subgroup were categorized as intermediate/high risk (I/H-R MDS) patients. This study was approved by the central ethic committee of the University Hospital Vall d'Hebron, Barcelona, and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Statistical analysis

The main endpoint of this study was to describe and compare the clinical characteristics and outcomes of patients with or without AD. Overall survival (OS) was defined as the time from diagnosis to death, whereas surviving patients were censored at the last follow-up. Continuous variables were expressed as mean or median, standard deviation and ranges, and categorical variables were expressed as absolute values and percentages. Fisher's exact test for categorical variables and Student's *t* test or Mann-Whitney test for continuous variables, after checking for normality with the Shapiro-Wilk test, were appropriately used.

Survival analysis was calculated using the Kaplan-Meier method and log-rank test was used for statistical comparison. Cox proportional-hazard model was used for multivariable analyses and to obtain hazard ratios (HR) with 95% confidence intervals (CI95%). Statistical analysis was performed by using STATA, version 13.1.

Results

Characteristics of the patients

Two hundred and forty-one patients were diagnosed with MDS or CMML during the study period. Among them,

develop MDS or AML when compared with matched controls [10–12]. Conversely, it has also been shown that patients with MDS or CMML have a prevalence of autoimmune disorders usually around 10 to 30%, being these AD previously reported heterogeneous in their nature and outcome [13–16]. Reinforcing the role of the immune system in the pathogenesis of MDS and CMML, it has been reported that immunosuppression treatments of MDS with antithymocyte globulin and cyclosporine were able to attain a 30% of hematologic responses, particularly in younger patients with hypoplastic bone marrow, presence of trisomy 8, and HLA DR15 [17]. Other immunosuppressive agents like alemtuzumab, a humanized monoclonal antibody that recognizes CD52, have also shown sustained hematologic responses in MDS patients [18].

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Results

Characteristics of the patients

Two hundred and forty-one patients were diagnosed with MDS or CMML during the study period. Among them,

clinical and serological information to evaluate the presence of AD was available in 142 patients that represented the final study population. One hundred and twenty-eight were classified as having MDS and 14 were CMML patients. Patients' characteristics are summarized in Table 1. Median age was 76 years (range, 24–95) and 63% were male. According to the IPSS-R, 52 (37%) patients were classified as very low risk; low risk in 63 (44%); intermediate risk in 11 (8%); high risk in 9 (6%); and very high risk in 7 (5%). Thus, 115 (81%) patients were considered L-R MDS, whereas 27 (19%) I/H-R MDS. The median follow-up for survivors was 15.2 months (range 0.1–69.5), and in the last follow-up, 29 patients (21%) had died. Overall survival at 30 months for the whole series was 78% (CI95% 68–86) (Fig. 1a), being of 84% (CI95% 73–91) for L-R MDS and 45% (CI95% 21–68) for I/H-R MDS ($P = 0.009$) (Fig. 1b).

Table 1 Baseline characteristics of the whole series and of the patients with MDS according to the presence or absence of autoimmune disorders (AD)

Characteristics	MDS ($n = 142$)	MDS-AD ($n = 68$, 48%)	MDS-noAD ($n = 74$, 52%)	P
Sex (%)				
Male	89 (63)	35 (51)	54 (73)	*0.01*
Female	53 (37)	33 (49)	20 (27)	
Median age (range), years	76 (24–95)	77 (44–95)	76 (24–90)	NS
WHO 2016 classification (%)				
MDS with unilineage dysplasia	22 (15)	8 (12)	14 (19)	NS
MDS with ring sideroblasts	27 (19)	10 (15)	17 (23)	
MDS with multilineage dysplasia	42 (30)	24 (35)	18 (24)	
MDS with excess blasts-1	18 (13)	9 (13)	9 (12)	
MDS with excess blasts-2	9 (6)	5 (7)	4 (6)	
MDS isolated del(5q)	10 (7)	4 (6)	6 (8)	
Chronic myelomonocytic leukemia-0	10 (7)	5 (7)	5 (7)	
Chronic myelomonocytic leukemia-1	3 (2)	2 (3)	1 (1)	
Chronic myelomonocytic leukemia-2	1 (1)	1 (2)	0 (0)	
Hemoglobin (g/L)	101 (40–147)	97 (40–139)	105 (40–147)	*0.01*
Platelets ($\times 10^9$ cells per L)	173 (2–652)	166 (21–443)	182 (2–652)	NS
Leukocytes ($\times 10^9$ cells per L)	4.9 (1.3–28)	5.2 (1.3–28)	4.8 (1.4–20)	NS
Neutrophils ($\times 10^9$ cells per L)	2.6 (0.2–23)	2.8 (0.2–23)	2.4 (0.3–12)	NS
Bone marrow blasts (%)	3 (0–18)	4 (0–18)	3 (0–14)	NS
Erythropoietin serum, median (U/L)	103 (2–800)	108 (2–800)	99 (3–800)	NS
Transfusion dependency (n, %)	46 (33)	24 (35)	22 (30)	NS
IPSS-R (%)				
Very low	52 (37)	23 (34)	29 (39)	NS
Low	63 (44)	27 (40)	36 (49)	
Intermediate	11 (8)	7 (10)	4 (5)	
High	9 (6)	6 (9)	3 (4)	
Very high	7 (5)	5 (7)	2 (3)	
Grouped IPSS-R (%)				
Low risk	115 (81)	50 (74)	65 (88)	0.03
Intermediate/high risk	27 (19)	18 (26)	9 (12)	
IPSS-R cytogenetic groups (%)				
Very low	13 (9)	4 (6)	9 (13)	NS
Low	103 (72)	46 (68)	57 (77)	
Intermediate	14 (10)	8 (12)	6 (8)	
High	4 (3)	3 (4)	1 (1)	
Very high	8 (6)	7 (10)	1 (1)	
Grouped IPSS-R cytogenetic groups (%)				
Low risk	116 (82)	50 (35%)	66 (46%)	0.01
Intermediate/high risk	26 (18)	18 (13%)	8 (6%)	

Prevalence and characteristics of autoimmune disorders in patients with MDS and CMML

Clinical autoimmune diseases were identified in 39 of 142 patients (27.5%) (33 MDS and 6 CMML) (Table 2). Two of them presented with more than one systemic autoimmune disease: one patient with hypothyroidism and rheumatoid arthritis and the other one with hypothyroidism, rheumatoid arthritis, and ulcerative colitis. The most common autoimmune disease was hypothyroidism (8% of patients) followed by rheumatoid arthritis (6.5% of patients). The diagnosis of autoimmune hypothyroidism was based on the presence of high levels of anti-TPO antibodies, and in the cases that we could not demonstrate the antibodies levels, those patients without previous history of thyroid surgery or radiotherapy, or thyroid toxic medications as lithium, were classified as autoimmune hypothyroidism. Twenty-three (59%), 5 (13%), and 10 (26%) of the clinical autoimmune diseases were diagnosed before, during, or after the MDS diagnosis, respectively. Thirty-three (85%) patients received treatment for the clinical autoimmune disease: steroids in 18 (55%), including seven patients with a combination of steroids and other anti-inflammatory drugs and one patient with splenectomy; replacement therapy (thyroid hormones and B_{12} vitamin), in 11 (33%); nonsteroidal anti-inflammatory drugs in three (9%), and topic treatment in one (3%) patient. Four (10%) patients did not receive any treatment, and in two (5%), this information was not available. Furthermore, abnormal serological immune parameters were identified in 38 out of 129 (29.5%) patients analyzed. The prevalence of positive immune serological tests was as follows: ANAs in 30 out of 129 (23%), rheumatoid factor in 13 out of 126 (10%), and positivity for both parameters in six patients (5%). Of note, among the 39 patients with clinical autoimmune disease, only eight (20.5%) patients presented serological abnormalities. Conversely, 30 out of 38 patients (79%) with serological abnormalities did not present clinical autoimmune disease.

Altogether, and after merging all the clinical and serological parameters for AD, a total of 68 (48%) patients were classified as MDS-AD (60 and 8 patients with MDS and CMML, respectively).

Clinical characteristics and outcome of MDS patients based on the presence of autoimmune disorders

The main clinical, biological, and risk categories between patients classified as having AD or not were compared thereafter (Table 1). The presence of AD was associated with female gender and lower hemoglobin value ($P < 0.01$) (Table 1). In contrast, age, WHO categories, laboratory data, IPSS-R, IPSS-R cytogenetic group, and transfusion dependency did not differ between patients with or without the presence of AD. Moreover, as anemia was significantly more profound in MDS-AD patients, we investigated if this was related to the presence of the AD by analyzing three inflammatory markers: ferritin, erythrocyte sedimentation rate, and C-reactive protein, but we could not find any significant relation (data not shown). Of note, L-R MDS had less autoimmune events (50 out of 115, 43%) than I/H-R MDS (18 out of 27, 67%) ($P = 0.03$).

Survival analysis showed that OS at 30 months was 69% (CI95% 53–80%) and 88% (CI95% 75–95%) for patients with MDS-AD and MDS-noAD, respectively (HR 2.75; CI95% 1.26–6.11; $P = 0.008$) (Fig. 2a). Notably, when considering all the autoimmune manifestations, only the presence of a clinical immune disorder had an impact on the OS (median OS 38.5 months vs. not reached in MDS-AD and MDS-noAD, respectively; HR 3.7; CI95% 1.75–7.87) ($P < 0.0002$) (Fig. 2b), whereas the isolated presence of immune serological parameters had no impact in OS (Fig. 2c). Remarkably, when outcomes were analyzed according to the IPSS-R risk category, L-R MDS with AD showed a trend for a worse OS than those lacking AD (75 vs. 94% at 30 months, $P = 0.06$) (Fig. 3). Only six patients had evolved to AML, a number not sufficient to perform a meaningful AML progression analysis. Finally, in a Cox multivariate analysis, IPSS-R risk categories (L-R vs. I/H-R) and the presence or absence of AD retained their independent prognostic value (risk categories, HR 4.62; CI95% 2.1–10.05; autoimmune abnormalities, HR 2.26; CI95% 1.01–5.07) ($P < 0.001$). Other variables included in the multivariate analysis that did not reach statistical significance were sex, age, WHO classification, IPSS-R, and laboratory data, including hemoglobin, leukocytes, neutrophils, platelets, and blasts.

Discussion

Our study shows that AD are common in patients with MDS and CMML and they are associated with worse prognosis, mainly in lower-risk patients.

The association between MDS and CMML with systemic immune manifestations has been previously recognized with prevalences ranging from 7 to 63% [15, 22–26]. Vasculitis and rheumatoid arthritis appear to be the most commonly described clinical autoimmune complications, although hypothyroidism, idiopathic immune thrombocytopenia, or polymyalgia rheumatica, among others, have been reported as well [14, 23–26]. The observed disparity in both the prevalence and the spectrum of immune diseases among the different studies could be explained by several reasons: the lack of specific clinical manifestations due to the existence

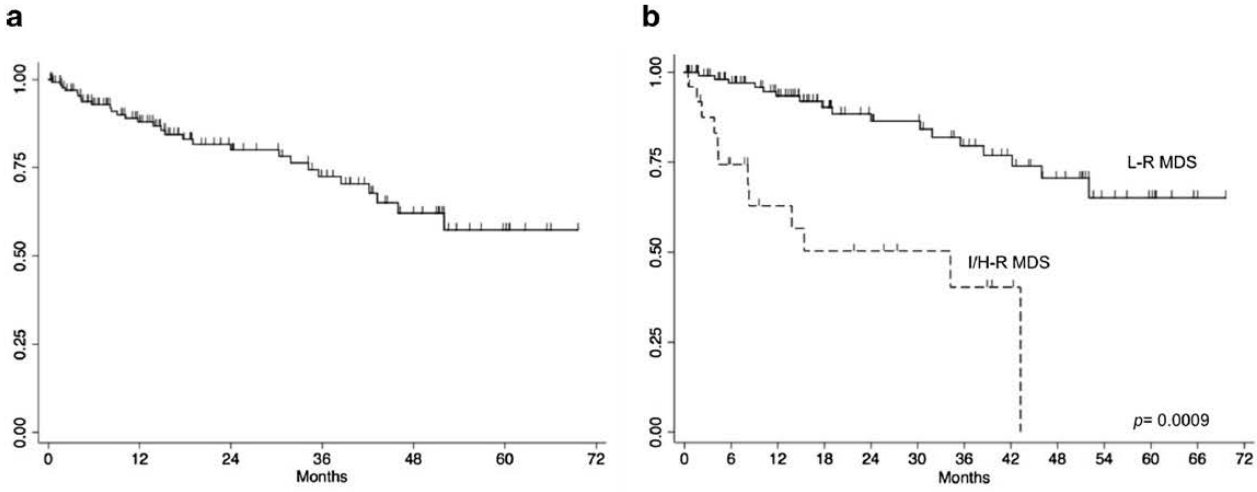

Figure 1 **a** Curve depicting OS of the whole series. **b** OS according to the IPSS-R score. L-R MDS (solid line) disclosed a better OS than I/H-R MDS (dashed line) ($p = 0.009$)

of subtle or atypical symptoms, or the need to perform tissue biopsies to reach the final diagnosis of certain autoimmune diseases. Of note, in this study, we found a high prevalence (48%) of AD in patients with MDS/CMML. The high prevalence of AD in this series could be explained by the scrupulous clinical and serological patient evaluation, although a referral bias or epidemiological reasons could not be ruled out. Considering the whole series of immune events, 29% were based on clinical manifestations, being hypothyroidism the most common clinical disease identified (26%). These findings are in accordance with Komrokji and cols [14], depicting autoimmune hypothyroidism as the most common autoimmune manifestation in around 44% of patients with MDS patients. It should be noted that the main cause of hypothyroidism in the elderly European population is the autoimmune form (Hashimoto thyroiditis), usually found in more than 5% of the general population in epidemiological surveys [27]. Other clinical immune diseases frequently related to MDS and CMML patients as rheumatoid arthritis and polymyalgia rheumatica were also prevalent in our cohort, 21.4 and 14.2%, respectively.

Abnormal serological immune parameters were a frequent finding (29.5%) in our series as well. It is important to highlight, however, that neither ANAs nor rheumatoid factor was helpful for the diagnosis of clinical autoimmune diseases. Thus, only few patients with positive serological test were diagnosed with clinical autoimmune disease and, conversely, most of patients with positive serological

Table 2 Clinical autoimmune diseases identified in patients with MDS and CMML

Autoimmune diseases	$n = 42$*	Prevalence among clinical autoimmune diseases (%)	Prevalence among whole cohort (%)
Hypothyroidism*	11	26.2	8
Rheumatoid arthritis*	9	21.4	6.5
Polymyalgia rheumatica	6	14.2	4
Pernicious anemia	4	9.5	3
Autoimmune vasculitis	3	7.1	2
Inflammatory bowel disease*	2	4.8	1.5
Sjögren syndrome	1	2.4	1
Systemic lupus erythematosus	1	2.4	1
Scleroderma	1	2.4	1
Hemolytic anemia	1	2.4	1
Psoriasis*	1	2.4	1
Not filiated	2	4.8	1.5

*Clinical AD were identified in 39 of 142 patients (27.5%). However, two of them presented with more than one AD: one with hypothyroidism and rheumatoid arthritis, and the other one with hypothyroidism, rheumatoid arthritis, and ulcerative colitis

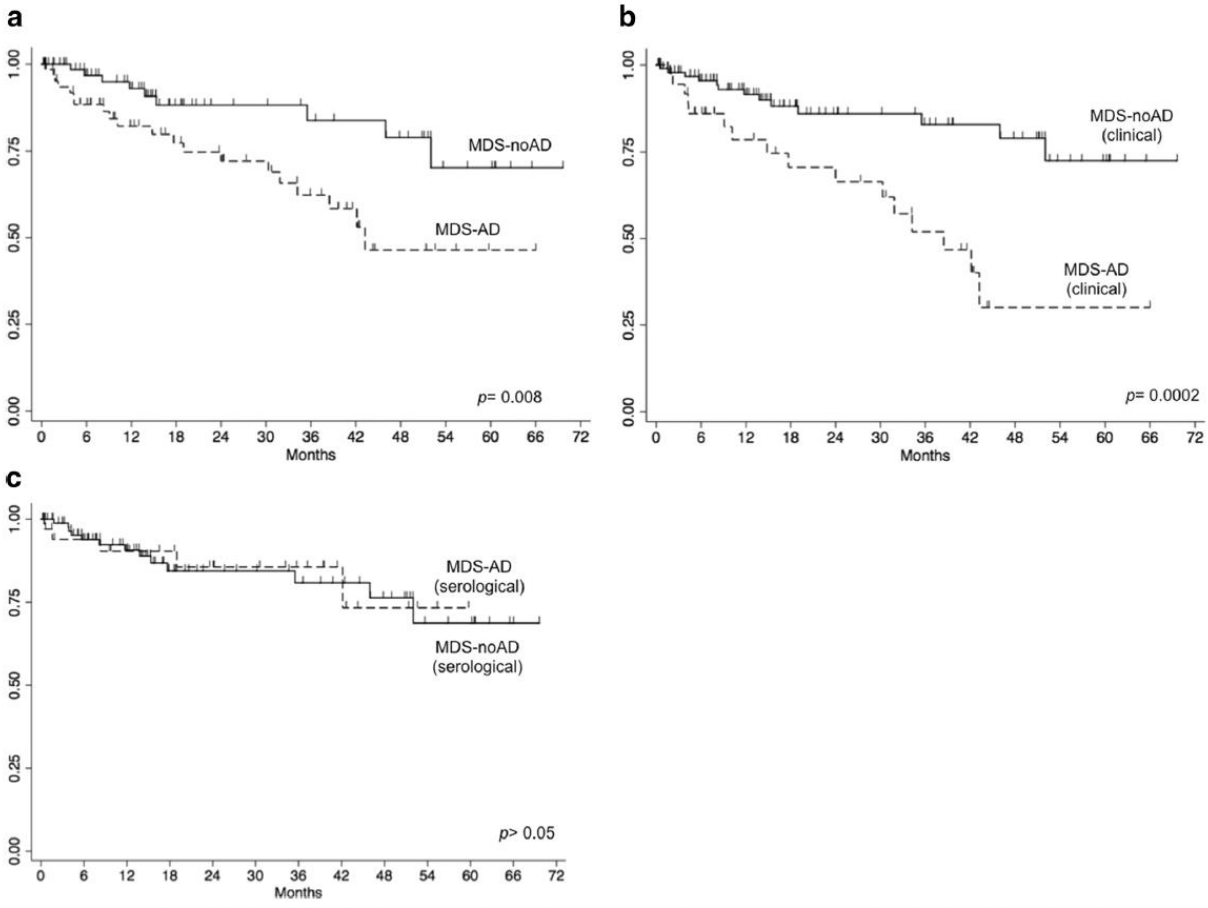

Figure 2 **a** OS of the patients with MDS according to the presence (MDS-AD, dashed line) or absence (MDS-noAD) of autoimmune disorders ($P = 0.008$). **b** Survival of patients with MDS was significantly different when considering solely the presence or absence of clinical autoimmune diseases. **c** In contrast, serological tests for autoimmunity did not discriminate the survival of MDS patients

parameters did not develop an autoimmune systemic disease. This dissimilarity was also noticed by Hamidou and cols, who found that only 3% of MDS patients with vasculitis had positive antineutrophil cytoplasmic antibodies [28]. In our study, ANAs were positive in 23.2% and rheumatoid factor in 10.3% of the patients, figures that were higher than the ones described in the normal population (3 and 5% for ANAs and rheumatoid factor, respectively) [29]. In the light of the above observation, it can be suggested that in MDS patients, autoimmune laboratory results should be interpreted carefully because of their low predictive value, and they should always be evaluated together with well-documented clinical findings in order to diagnose a systemic autoimmune disease. Remarkably, in a new era where immunoregulatory therapies tend to be part of the armamentarium of cancer diseases, awareness of underlying autoimmune complications could be of seminal importance when optimizing the immune modulation of patients with MDS [30, 31].

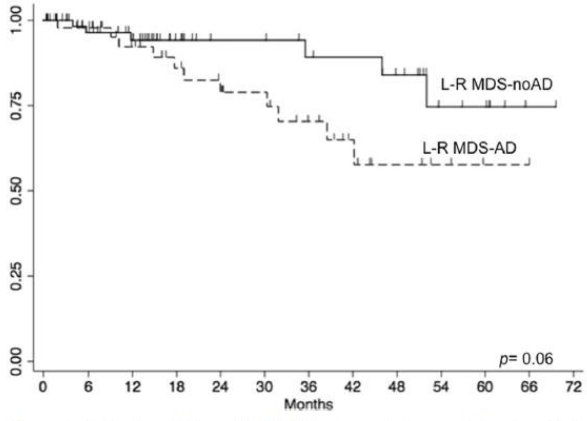

Figure 3 Patients with low-risk MDS having autoimmune disorders (L-R MDS-AD, dashed line) showed a trend for a worse survival than those lacking autoimmune events (L-R MDS-noAD) ($P = 0.06$)

MDS-AD patients were more frequently females, with more severe anemia and higher IPSS-R risk. These results differed from those observed by Komrokji and cols in which MDS patients with AD presented with less transfusion dependence and lower categories of the IPSS-R stratification [14]. Moreover, in the study reported by Menikian and cols, patients with MDS and AD were more frequently male and younger but in agreement with our results, presented higher-risk features [32]. Of note, as it has been reported before [32], most of the clinical autoimmune diseases were diagnosed before the MDS (59%). This sequence suggests that the presence of abnormal inflammation precedes and probably predisposes the appearance of the myeloid neoplasm.

The effect of AD in the outcomes of patients with MDS has also been addressed in several studies. While some studies reported that the presence of AD is not of prognostic relevance [22, 25, 26, 32, 33, 34] or even associated with better outcomes [14], others reported inferior outcomes [15, 33], according to our results. In our study, this negative effect can be explained because MDS-AD patients presented with poorer baseline prognostic features, including lower hemoglobin levels and higher IPSS-R. Furthermore, the association of MDS with AD can mirror the presence of a more profound immune dysregulation, resulting in a more severe bone marrow failure and subsequent disease progression. The heterogeneity of clinical characteristics and prognostic influence between different studies could be explained by relevant differences in the study design, diagnostic criteria for both AD and MDS, and to some extent, differences in management. To minimize the degree of variability in the interpretation of clinical data, in the present study, the diagnosis of MDS and AD were verified by several specialists, this still not entirely prevent for a misdiagnosis or for a patient with atypical or mild manifestations being left out. This issue is in fact the main limitation of our study, and in order to minimize the bias of diagnosis, we included a review from a specialist in autoimmune disorders (F M-V) to confirm all cases of AD, and we are quite sure that all patients reported as MDS-AD had an abnormality in the immune system, but we cannot rule out that a patient with very mild or atypical symptoms could be excluded in the diagnosis procedure.

We think that well-designed prospective studies would be the only way to answer this question. Altogether, we have to admit that it is difficult to correlate any specific clinical feature with the presence of autoimmune diseases in MDS patients, specially taking into account the low number of patients included and the retrospective nature of the studies and also the different criteria used for defining the AD.

In conclusion, AD are common in MDS patients and are associated with worse outcomes, particularly in L-R MDS. Further studies are required to understand the relationship between MDS and AD, especially nowadays that new therapies targeting the immune system will be soon available for patients with MDS.

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Compliance with ethical standards

This study was approved by the central ethic committee of the University Hospital Vall d'Hebron, Barcelona, and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent.

Conflict of interest The authors declare that they have no conflict of interest.

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2. Second work

Dichotomization of the new revised international prognostic scoring System for a better clinical stratification of patients with myelodysplastic syndromes.

The IPSS-R has introduced the intermediate-risk category in which is not clear whether these patients should be managed as low-risk MDS or high-risk.

The aim of this analysis was to ascertain which IPSS-R threshold that better dichotomized MDS patients in low vs. high-risk categories.

Patients

Patients with confirmed MDS at the Department of Hematology of the University Hospital Vall d´Hebron (n= 211) and Institut Català d'Oncologia (n= 153) diagnosed between 2011 and 2016 were included in this study. Diagnosis of MDS was performed in accordance with the 2008 World Health Organization criteria and risk stratification was performed following the IPSS and the IPSS-R. Patients'clinical characteristics and laboratory values were obtained by electronic medical record review. We compared the clinical evolution of the patients of both centers and no significant differences were found. Accordingly, both cohorts were analyzed as a whole.

Statistical analysis

The receiver operating characteristic (ROC) curve and the area under the curve were determined for the IPSS-R score. The Youden's index was used to select the optimum cut-point. Sensitivity, specificity, positive, and negative predictive values were calculated for a range of different IPSS-R cut-points. Continuous variables were expressed as median, standard deviation, and ranges; categorical variables were expressed as absolute values and percentages. Chi-squared test and Student's t-test or Mann-Whitney test were used for categorical and continuous

variables, respectively, as appropriate after checking normality with the Shapiro-Wilk test. Overall survival was defined as the time from diagnosis to death, censoring surviving patients at last follow-up. Last follow-up was calculated since the date of the diagnosis to the last contact or death. Survival analysis was calculated using the Kaplan-Meier method, and log-rank test was used for statistical comparison. Cox proportional-hazard model was used for multivariable analyses and to obtain hazard ratios (HR) with 95% confidence intervals (CI). Statistical analysis was performed by R version 3.3.1.

Results

- An IPSS-R cut-point of 3 was the best to divide patients into low and high-risk subgroups. This cut-point presented 64.1% of sensitivity and 82.6% of specificity.
- According to the cut-point 68% patients were classified as low-risk, whereas 32% patients were allocated in the high-risk subgroup.
- Median OS was 61.3 and 13.9 months in the low and high-risk groups, respectively; The three years cumulative incidence of AML evolution was 5.4% and 38.2% in the low and high-risk groups, respectively.
- Intermediate-risk IPSS-R patients showed an OS closer to the high-risk (16.2 and 13.9 months, respectively), but statistically different. However, it was remarkably distinct to the low-risk group (61.3 months). Similarly, the 3 years cumulative incidence of AML evolution quadruple from low-risk to intermediate (5.4% to 20%), whereas only double when comparing from intermediate to high-risk (20% to 59.6%).

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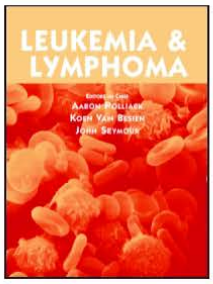

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ORIGINAL ARTICLE – CLINICAL

Dichotomization of the new revised international prognostic scoring system for a better clinical stratification of patients with myelodysplastic syndromes

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ABSTRACT

In clinical practice, patients with myelodysplastic syndromes (MDS) are usually classified in low or high-risk groups to take therapeutic decisions, conservative for low-risk, whereas active for high-risk. Nevertheless, in the Revised International Prognostic Scoring System (IPSS-R) is not well stated which patients are low or high-risk. This study was aimed to ascertain in 364 MDS patients which IPSS-R threshold better dichotomized in low vs. high-risk. The best dichotomization was obtained with an IPSS-R cut-point of 3. Accordingly, 68% patients were classified as low-risk (median OS, 61.3 months) and 32% as high-risk MDS (median OS, 13.9 months) ($p < .001$). Interestingly, the intermediate IPSS-R risk patients presented an OS more related to the high IPSS-R than to the low IPSS-R risk group. In conclusion, an IPSS-R cut-point of 3 led to a meaningful stratification in low and high-risk that can be helpful for the clinical management of MDS patients.

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Myelodysplastic syndromes; revised international prognostic scoring system; international prognostic scoring system; IPSS-R; IPSS

Introduction

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal disorders characterized by impaired hematopoiesis resulting in peripheral blood cytopenias, dysmyelopoiesis, and an increased risk of developing acute myeloid leukemia (AML) [1]. Owing to the fact that MDS are very dissimilar in their genetic characteristics and molecular pathogenesis, the clinical outcomes of patients with MDS are remarkably heterogeneous, with a life expectancy ranging from few months to several years. Therefore, to establish an accurate prognosis evaluation becomes of paramount value for the clinical management of these patients [2,3]. Back in 1997, the International Prognostic Score System (IPSS) delineated four prognostic categories (low, intermediate-1, intermediate-2, and high risk) and became the benchmark for treatment making decisions and stratification in clinical trials [4]. In this scoring system, patients falling into the low and intermediate-1 risk groups were considered as a low-risk category, for which supportive therapy was generally recommended. Conversely, patients included in the intermediate-2 and high-risk groups were merged in the high-risk subgroup for which treatment intervention was usually offered, either with disease-modifying agents (chemotherapy or hypomethylating drugs) and/or allogenic hematopoietic stem cell transplantation (HSCT) [5]. Later in 2012, the Revised International Prognostic Scoring System (IPSS-R) defined five different risk groups: very low, low, intermediate, high, and very high-risk. This new system was rapidly adopted because it allowed a better accuracy in the prognostication of MDS patients. Notably, the newer IPSS-R allocated patients with values ranging from 3.5 to 4.5 points in the intermediate-risk category [6]. Therefore, since the adoption of the IPSS-R it is not clear in clinical practice whether patients allocated in the intermediate-risk category should be managed conservatively like a low-risk category, or considered of high-risk requiring clinical intervention.

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Supplemental data for this article can be accessed here.

The aim of this study was to settle which could be the IPSS-R cut-point that best-divided patients in low and high-risk subgroups and analyzed their clinical characteristics and outcomes. This analysis has potential clinical implications for the daily management of patients diagnosed with MDS and the design of clinical trials.

Patients and methods

Patients

Patients with confirmed MDS at the Department of Hematology of the University Hospital Vall dHebron ($n = 211$) and Institut Català d'Oncologia ($n = 153$) diagnosed between 2011 and 2016 were included in this study. Diagnosis of MDS was performed in accordance with the 2008 World Health Organization criteria [7] and risk stratification was performed following the IPSS and the IPSS-R [4,6]. Patients' clinical characteristics and laboratory values were obtained by electronic medical record review. We compared the clinical evolution of the patients of both centers and no significant differences were found. Accordingly, both cohorts were analyzed as a whole. This study was conducted in accordance with the Declaration of Helsinki.

Statistical analysis

The main endpoint was to determine the IPSS-R cut-point that best-stratified patients into low-risk vs. high-risk and to compare clinical characteristics and outcomes of both risk groups. To obtain such point, overall survival (OS) was evaluated as a dichotomous discrete variable according to a survival of $\geq$24 months. This time point was decided in keeping with the median OS of MDS patients in the IPSS-R classification. Thus, very low, low, and intermediate-risk patients showed OS $\geq$24 months, whereas in high and very high-risk patients it was $<$24 months [6]. This time is also consistent with the median follow-up of our series (27.4 months). The receiver operating characteristic (ROC) curve and the area under the curve (AUC) were determined for the IPSS-R score. The Youden's index, which maximizes the sum of the test sensitivity and specificity, was used to select the optimum cut-point. Additionally, sensitivity, specificity, positive (PPV), and negative (NPV) predictive values were calculated for a range of different IPSS-R cut-points.

Continuous variables were expressed as median, standard deviation, and ranges; categorical variables were expressed as absolute values and percentages. Chi-squared test and Student's *t*-test or Mann–Whitney test were used for categorical and continuous variables, respectively, as appropriate after checking normality with the Shapiro–Wilk test. Overall survival was defined as the time from diagnosis to death, censoring surviving patients at last follow-up. Last follow-up was calculated since the date of the diagnosis to the last contact or death. Survival analysis was calculated using the Kaplan–Meier method, and log-rank test was used for statistical comparison. Cox proportional-hazard model was used for multivariable analyses and to obtain hazard ratios (HR) with 95% confidence intervals (CI). Statistical analysis was performed by R version 3.3.1.

Results

Patients' characteristics

Three hundred and sixty-four patients were diagnosed with MDS during the study period. Median age was 75 years (range 21–95) and 226 patients (62%) were male. The principal features of the patients are summarized in Table 1.

The median follow-up for the whole population was 22.7 months (range 0.1–68) and the median follow-up for survivors was 27.4 months (range 0.1–68). In the last follow-up, 146 (40%) patients have died. The median OS was 40.2 months (CI 95% 32.1–51.7).

An IPSS-R cut-point of 3 is the best to divide patients into low and high-risk subgroups

On the basis of the Youden's index, the optimum IPSS-R cut-point obtained in the ROC curve was 3, giving a 64.1% of sensitivity and 82.6% of specificity. Therefore, the best dichotomization in 2 risk categories, low-risk vs. high-risk, was the division obtained by using an IPSS-R score of 3 points. Furthermore, the AUC was 0.81 (0.75–0.82) showing a good performance of the IPSS-R to predict OS. We then explored the sensitivity, specificity, PPV, NPV, and accuracy of different points of the IPSS-R ranging from 2.5 to 4.5. As expected, we observed that the higher the score in the IPSS-R, the better the specificity and the PPV, but the worse the sensitivity and the NPV (and vice versa) (Supplementary material). Taking into consideration the former results, the point with the best accuracy prediction was 3.

Table 1. Characteristics of the whole series and of the low and high-risk patients according to the cut-point of 3.

	Global cohort $n=364$	Low-risk $n=249$ (68%)	High-risk $n=115$ (32%)	p
Age, years (range)	75 (21–95)	75 (21–95)	73 (39–90)	.09
Gender, male (%)	226 (62)	161 (64.5)	65 (56.5)	.1
Hb g/L (p25/p75)	100 (87–113)	105 (93–118)	89 (77–100)	<.001
Leuc × 109/L (p25/p75)	4.6 (2.9–5.9)	4.9 (3.2–6.2)	4.1 (2.1–5.1)	<.001
ANC × 109/L (p25/p75)	2.5 (1.3–3.3)	2.7 (1.3–3.4)	2 (0.6–2.6)	<.001
Plt × 109/L (p25/p75)	164 (83–230)	186 (104–256)	116 (45–145)	<.001
Blasts BM % (p25/p75)	4 (1–4)	2 (1–2)	8 (4–13)	<.001
WHO 2008 (%)				
RCDU	29 (8)	29 (12)	0 (0)	
RARS	17 (4)	15 (6)	2 (2)	
RCMD	202 (55)	173 (69)	29 (25)	
RAEB-1	47 (13)	10 (4)	37 (32)	<.001
RAEB-2	49 (14)	2 (1)	47 (41)	
MDS del(5q)	18 (5)	18 (7)	0 (0)	
MDS-U	2 (1)	2 (1)	0 (0)	
IPSS-R (%)				
Very Low	108 (30)	108 (29)	0 (0)	
Low	141(39)	141 (39)	0 (0)	<.001
Intermediate	52 (14)	0 (0)	52 (14)	
High	27 (7)	0 (0)	27 (8)	
Very high	36 (10)	0 (0)	36 (10)	
IPSS (%)				
Low	176 (48)	171 (68)	5 (4)	
Intermediate-1	123 (34)	77 (31)	46 (40)	<.001
Intermediate-2	38 (11)	1 (1)	37 (32)	
High	27 (7)	0 (0)	27 (24)	
IPSS-R cytogenetic categories (%)				
Very good	31 (9)	29 (12)	2 (2)	
Good	253 (70)	197 (79)	56 (49)	
Intermediate	41(11)	21 (8)	20 (17)	<.001
Poor	16 (4)	2 (1)	14 (12)	
Very poor	23 (6)	0 (0)	23 (20)	
RBC transfusion dependence (%)	112 (31)	66 (27)	46 (40)	.01
Treatment (%)	207 (57)	142 (57)	91 (79)	<.001
ESAs	121 (58)	104 (42)	17 (15)	<.001
Azacitidine	83 (40)	27 (11)	56 (49)	<.001
Lenalidomide	10 (3)	8 (3)	2 (2)	<.001
HSCT	25 (12)	5 (2)	20 (17)	<.001
Others	26 (13)	18 (7)	8 (7)	<.001

Hb: hemoglobin; Leuc: leucocytes; ANC: absolute neutrophils count; Plt: platelets; BM: bone marrow; RCDU: refractory cytopenia with unilineage dysplasia; RARS: refractory anemia with ring sideroblasts; RCMD: refractory cytopenia with multilineage dysplasia; RAEB-1: refractory anemia with excess blasts type 1; RAEB-2: refractory anemia with excess blasts type 2; MDS-U: MDS unclassified; RBC: red blood cells; ESAs: erythropoiesis-stimulating agents; HSCT: hematopoietic stem cell transplant.

Clinical characteristics and outcomes of the low and high-risk MDS patients according to the cut-point of 3

In agreement with the cut-point of 3, 249 (68%) patients with IPSS-R score ≤ 3 were classified as low-risk MDS, whereas 115 (32%) patients with IPSS-R > 3 were allocated in the high-risk subgroup. Patients' characteristics and treatments of both subgroups are summarized in Table 1.

Median OS was 61.3 months (CI 95% 45.8–NR) in the low-risk and 13.9 months (CI 95% 10.6–18.9) in the high-risk subgroup [HR =3.9 (CI 95% 2.8–5.5); $p = .001$] (Figure 1(a)). Thirty-four (9%) patients evolved to AML, 10 (4%) of the low and 24 (21%) of the high-risk patients and the 3 years cumulative incidence of AML evolution was 5.4% (CI 95% 2.6–11.4) and 38.2% (CI 95% 25.3–54.8) in the low and high-risk groups, respectively [HR =9.5 (CI95% 4.5–20.1); $p < .001$] (Figure 1(b)).

Characteristics and outcomes of the IPSS-R intermediate-risk MDS patients

As the most difficult group of patients to stratify are those in the intermediate-risk group, and to better define their behavior, we focused on these patients to know the characteristics and outcomes. Fifty-two patients (14%) were classified in the intermediate-risk group with a median age of 72 years (43–90) and 32 (62%) were male. Patients' characteristics are summarized in Table 2.

For this subgroup of patients, the median follow-up for survivors was 26.5 months (range 4.9–65.2) and at last follow-up 31 (60%) patients have died. The median OS was 16.2 months (CI 95% 10.6–51.8). Eight

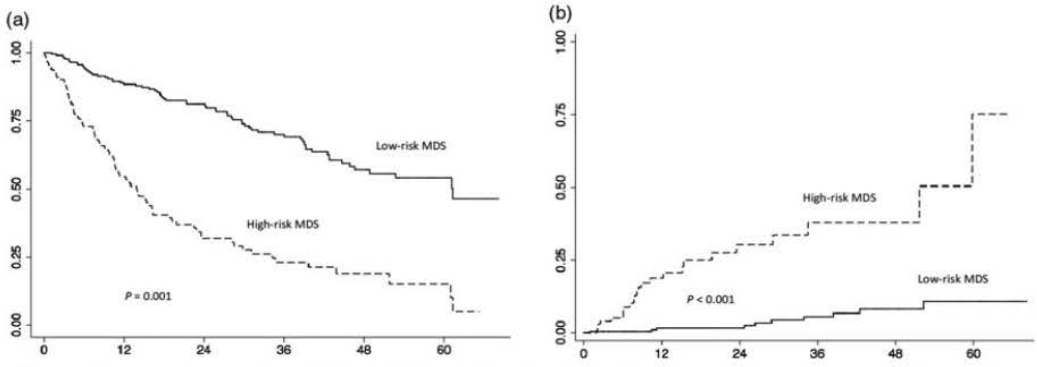

Figure 1. (a) Overall survival of low and high-risk MDS patients by using the IPSS-R score with ≤ 3 points as cut-point. Low-risk MDS (patients with an IPSS-R ≤ 3) showed an OS of 61.3 months (solid line), whereas high-risk MDS (patients with an IPSS-R > 3) showed an OS of 13.9 months (dashed line), $p = .001$. (b) Evolution to AML of low and high-risk MDS patients by using the IPSS-R score with ≤ 3 points as cut-point. Low-risk MDS (patients with an IPSS-R ≤ 3) showed a risk of evolution to AML at 36 months of 5.4% (solid line), whereas high-risk MDS (patients with an IPSS-R > 3) showed an OS of a risk of evolution to AML at 36 months of 38% (dashed line) $p < .001$.

Table 2. Baseline characteristics of intermediate-risk MDS patients.

	Intermediate-risk MDS $n = 52$
Age, years (range)	72 (43–90)
Gender, male (%)	32 (62)
Hb g/L (p25/p75)	95 (40–148)
Leuc × 109/L (p25/p75)	4.2 (1.3–10.5)
ANC × 109/L (p25/p75)	2.2 (0.19–7.2)
Plt × 109/L (p25/p75)	147 (5–419)
Blasts BM% (p25/p75)	5 (0–14)
WHO 2008 (%)	
RARS	2 (4%)
RCMD	22 (42%)
RAEB-1	21 (41%)
RAEB-2	7 (13%)
IPSS-R cytogenetic categories (%)	
Very good	2 (4)
Good	36 (69)
Intermediate	9 (17)
Poor	4 (8)
Very poor	1 (2)
RBC transfusion dependence (%)	18 (35)
Treatment (%)	34 (65)
ESAs	14 (27)
Azacitidine	19 (37)
Lenalidomide	1 (2)
HSCT	10 (19)
Others	3 (6)

Hb: hemoglobin; Leuc: leucocytes; ANC: absolute neutrophils count; Plt: platelets; BM: bone marrow; RARS: refractory anemia with ring sideroblasts; RCMD: refractory cytopenia with multilineage dysplasia; RAEB-1: refractory anemia with excess blasts type 1; RAEB-2: refractory anemia with excess blasts type 2; ESAs: erythropoiesis-stimulating agents; HSCT: hematopoietic stem cell transplant.

(15%) patients evolved to AML and the 3 years cumulative incidence of AML evolution was 20% (CI 95% 9.7–38).

In order to know if the outcomes of the intermediate IPSS-R risk patients were more related to the low IPSS-R risk or to the high IPSS-R MDS, we divided the cohort in low-risk (IPSS-R ≤ 3), intermediate-risk (IPSS-R 3.5–4.5) and high-risk (IPSS-R > 4.5) subgroups. Intermediate-risk patients showed an OS closer to the high-risk, but statistically different (median OS of 16.2 and 9.6 months, respectively; HR =1.9 (CI 95% 1.2–3); $p = .005$); however, it was remarkably distinct to the low-risk group (median OS of 61.3 months; HR =2.8 (CI 95% 1.8–4.4); $p < .001$) (Figure 2). Ten (4%), 8 (15%), and 16 (25%) of the low, intermediate and high-risk patients evolved to AML, respectively, and the 3 years cumulative incidence of AML evolution was 5.4% (CI95% 2.6–11.4), 20% (CI 95% 9.7–36) and 59.6% (CI 95% 37.1–82.9) in the low, intermediate and high-risk patients, respectively; $p = .001$.

Discussion

The results herein presented suggested that an IPSS-R cut-point of 3 could be useful in the clinical setting to stratify MDS patients in low and high-risk subgroups with significant differences in OS and AML evolution. Interestingly, we observed that patients in the intermediate IPSS-R risk group presented an outcome closer to high IPSS-R risk than to low IPSS-R risk MDS, which may be of interest in the decision-making algorithm of these patients.

Myelodysplastic syndromes represent a group of disorders with widely heterogeneous survival and risk to evolution to AML. Accordingly, several prognostic scores have been used, being the IPSS the most commonly applied until 2012, when the revised version (IPSS-R) that includes 5 risk groups was published and became the most common prognostic score used worldwide [4,6]. Nevertheless, after years of applying the IPSS-R, clinicians realized that, as in the classical IPSS, it is relevant to have a dichotomization of this new index because it is crucial to identify which patients are devoted to a more conservative approach

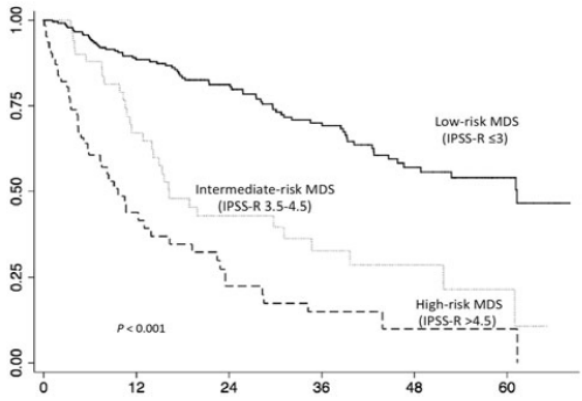

Figure 2. Evolution to AML of low and high-risk MDS patients by using the IPSS-R score with ≤ 3 points as cut-point. Low-risk MDS (patients with an IPSS-R ≤ 3) showed a risk of evolution to AML at 36 months of 5.4% (solid line), whereas high-risk MDS (patients with an IPSS-R > 3) showed an OS of a risk of evolution to AML at 36 months of 38% (dashed line) $p < .001$.

or to a more interventional strategy. This division is extremely important since patients in the low-risk group present longer survival and no treatment has demonstrated to improve their outcomes. Therefore, these patients are not usually considered for intensive therapies, such as allo-HSCT or antileukemic chemotherapy [8,9]. Moreover, hypomethylating agents are not licensed in Europe for low-risk MDS and thereby these patients are only offered supportive care and possibly erythropoiesis-stimulating agents [10,11]. In contrast, high-risk patients with shorter expected survival are candidate to receive a more intensive approach with different options such as hypomethylating agents, chemotherapy, and allo-HSCT, in order to overcome the expected poor prognosis [12–15]. In line with the above, we consider of utmost importance to know, which is the cut-point in the new IPSS-R that best divides patients in low and high-risk subgroups. Moreover, the outcomes of the intermediate-risk group are widely heterogeneous; some patients present an indolent disease, others rapidly evolve to AML. Accordingly, there are no specific therapeutical protocols for these patients with different strategies recommended in the most recently published clinical practice guidelines (i.e. European Leukemia Net and NCCN) [16,17]. Thus, treatment decisions could be extremely difficult.

Because of the retrospective nature of our study, treatment decisions in many patients were based on the former classification of the IPSS; therefore the outcomes could be influenced by treatments decision based on the IPSS. However, despite most of the patients in the intermediate-risk group were treated with disease-modifying agents (75%), the outcomes were poor, supporting that these patients should be considered as high-risk patients. Since this study represents the first focused specifically on the outcomes of the intermediate-risk IPSS-R group, further prospective analyses are recommended in order to validate our results. Furthermore, many clinical trials allow the inclusion of intermediate-risk patients along with high or very high-risk patients to test the efficacy of different drugs. Therefore, it would be important to analyze these different subgroups separately to know if the outcome differs between them. In the meanwhile, a retrospective study analyzing the possible benefit of approved drugs such as hypomethylating agents could help us to understand how to manage these patients.

According to our analysis, the optimal dichotomization in low and high-risk MDS patients is the division obtained by using an IPSS-R score of ≤ 3 points as a cut-point (OS of 61.3 vs. 13.9 months and a 36 months cumulative incidence of AML evolution of 6% and 38% in low and high-risk patients, respectively). Although our data is retrospective in nature, this caveat is somehow overcome owing to the number of patients and the homogeneous management of the patients along the years in both institutions. This cut-point is slightly lower than the obtained by Pfelistöcker *et al.* and colleagues [18] in a subanalysis based on a large cohort of primary untreated MDS patients from the International Working Group for Prognosis in MDS database in which an IPSS-R of 3.5 points was the score suggested to divide in two risk categories with a median OS around 70 and 20 months in low and high-risk patients, figures very similar to our series. Interestingly, both analyses suggest that most of the patients of the IPSS-R intermediate-risk group, two-thirds in Pfelistöcker's study and 100% in ours should be considered as high-risk and therefore deserving an intensive approach. When we compared the outcomes of the intermediate-risk group (IPSS-R 3–4.5) with the low-risk (IPSS-R < 3) and the high-risk (IPSS-R > 4.5), it became evident that the behavior was really close to the high-risk (OS of 16.2 vs. 9.6 months and risk to AML evolution of 20% vs. 60%, for the intermediate and high-risk, respectively) and remarkably different from the low-risk subgroup (OS of 61.1 months and only 6% of risk to AML evolution). These results could support the strategy of adopting a more intense approach for intermediate-risk patients similar to the high-risk groups. In this sense, a recent report of Della Porta *et al*, showed that early

transplant was associated with worse survival in the very low and low IPSS-R risk groups, whereas, in the intermediate, high, and very high-risk groups was associated with an improvement in life expectancy [19].

In conclusion, our data demonstrate that a cut-point of 3 in the IPSS-R is the best for segregating MDS patients in two risk groups (low-risk and high-risk), an observation that may be valuable for the design of clinical trials and for daily management of patients with MDS. Future prospective studies are needed to confirm our previous results.

Potential conflict of interest: Disclosure forms provided by the authors are available with the full text of this article online at https://doi.org/10.1080/10428194.2018.1542151.

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IV. DISCUSSION

The term “myelodysplastic syndromes” includes a group of myeloid diseases with a very heterogeneous clinical presentation and prognosis; from those asymptomatic patients with mild dysplasia, low blast count and long-life expectancy, to those with severe anemia, profound dysplastic features, high blasts count, and dismal outcomes. The fact that the therapeutic approach of these patients is based on the prognostic risk, enhances the importance of a proper prognostic assessment.

There are several approaches to better characterized the prognosis of MDS patients. Commonly, laboratory data such as cytopenias and BM blasts, and chromosomal abnormalities have been the most frequent features employed in prognosis assessment. Furthermore, there have been some attempts with clinical data, such as the presence of comorbidities, that contributes to redefine prognosis risk of MDS. We have attempt to improve the ability of prognosis stratification of MDS patients, trying to analyze both, the classical variables included in the IPSS-R and the possible impact of the presence of autoimmune disorders in patients diagnosed with MDS.

In the first work, we have analyzed the association and the prognostic role between AD and MDS. In the second work, we have analyzed which is the best IPSS-R cut-point that best divided lower and higher-risk MDS. In the next paragraphs, we discuss the results of each work.

First work

The association between MDS and CMML with systemic immune manifestations have been previously recognized with prevalences ranging from 7 to 63% (72,73,154–157). Vasculitis and rheumatoid arthritis appear to be the most commonly described clinical autoimmune complications, although hypothyroidism, idiopathic immune thrombocytopenia, or polymyalgia rheumatica, among others, have been reported as well (15,72,73,156,157). The observed disparity in both the prevalence and the spectrum of immune diseases among the different studies could be explained by several reasons: the lack of specific clinical manifestations due to the existence of subtle or atypical symptoms, or the need to perform tissue

biopsies to reach the final diagnosis of certain autoimmune diseases. Of note, in this study, we found a high prevalence (48%) of AD in patients with MDS/CMML. The high prevalence of AD in this series could be explained by the scrupulous clinical and serological patient evaluation, although a referral bias or epidemiological reasons could not be ruled out. Considering the whole series of immune events, 29% were based on clinical manifestations, being hypothyroidism, the most common clinical disease identified (26%). These findings are in accordance with Komrokji and cols (15), depicting autoimmune hypothyroidism as the most common autoimmune manifestation in around 44% of patients with MDS patients. It should be noted that the main cause of hypothyroidism in the elderly European population is the autoimmune form (Hashimoto thyroiditis), usually found in more than 5% of the general population in epidemiological surveys (158). Other clinical immune diseases frequently related to MDS and CMML patients as rheumatoid arthritis and polymyalgia rheumatica were also prevalent in our cohort, 21.4 and 14.2%, respectively. Abnormal serological immune parameters were a frequent finding (29.5%) in our series as well. It is important to highlight, however, that neither ANAs nor rheumatoid factor was helpful for the diagnosis of clinical autoimmune diseases. Thus, only few patients with positive serological test were diagnosed with clinical autoimmune disease and, conversely, most of patients with positive serological parameters did not develop an autoimmune systemic disease. This dissimilarity was also noticed by Hamidou and cols, who found that only 3% of MDS patients with vasculitis had positive antineutrophil cytoplasmic antibodies (159). In our study, ANAs were positive in 23.2% and rheumatoid factor in 10.3% of the patients, figures that were higher than the ones described in the normal population (3 and 5% for ANAs and rheumatoid factor, respectively). In the light of the above observation, it can be suggested that in MDS patients, autoimmune laboratory results should be interpreted carefully because of their low predictive value, and they should always be evaluated together with well-documented clinical findings in order to diagnose a systemic autoimmune disease. Remarkably, in a new era where immunoregulatory therapies tend to be part of the armamentarium of cancer diseases, awareness of underlying autoimmune complications could be

of seminal importance when optimizing the immune modulation of patients with MDS (160,161). MDS-AD patients were more frequently females, with more severe anemia and higher IPSS-R risk. These results differed from those observed by Komrokji and cols in which MDS patients with AD presented with less transfusion dependence and lower categories of the IPSS-R stratification (15). Moreover, in the study reported by Menikian and cols, patients with MDS and AD were more frequently male and younger but in agreement with our results, presented higher-risk features (162). Of note, as it has been reported before (162), most of the clinical autoimmune diseases were diagnosed before the MDS (59%). This sequence suggests that the exposition of a sustained abnormal activation of the inflammation pathway in the context of the AD, probably enhances the genesis of the MDS HSPC. In this regard, chronic inflammation can induce genomic instability in the normally HSPC resulting in clonal hematopoiesis (60). Although, clonal hematopoiesis might not progress into a myeloid disease, indeed as we stated before, most of the individuals with CHIP will never develop hematopoietic malignancies, it might function as a conduit for the development of MDS as a result of progressive genomic instability in the clonal hematopoietic precursors. In addition, clonal progenitors contribute to the impairment of the already defective immune system by promoting profound changes in the BM microenvironment and immune cells resulting in an immunotolerant microenvironment that favors the immune scape of the clonal hematopoiesis with subsequent acquisition of genomic instability and MDS evolution (60). The effect of AD in the outcomes of patients with MDS has also been addressed in several studies. While some studies reported that the presence of AD is not of prognostic relevance (163,164), or even associated with better outcomes (15), others reported inferior outcomes(154,165), according to our results. In our study, this negative effect can be explained because MDS-AD patients presented with poorer baseline prognostic features, including lower hemoglobin levels and higher IPSS-R. Furthermore, the association of MDS with AD can mirror the presence of a more profound immune dysregulation, resulting in a more severe bone marrow failure and subsequent disease progression. The heterogeneity of clinical characteristics and prognostic

influence between different studies could be explained by relevant differences in the study design, diagnostic criteria for both AD and MDS, and to some extent, differences in management. To minimize the degree of variability in the interpretation of clinical data, in this study, the diagnosis of MDS and AD were verified by several specialists, this still not entirely prevent for a misdiagnosis or for a patient with atypical or mild manifestations being left out. This issue is in fact the main limitation of our study, and in order to minimize the bias of diagnosis, we included a review from a specialist in autoimmune disorders (F M-V) to confirm all cases of AD, and we are quite sure that all patients reported as MDS-AD had an abnormality in the immune system, but we cannot rule out that a patient with very mild or atypical symptoms could be excluded in the diagnosis procedure. We think that well-designed prospective studies would be the only way to answer this question. Altogether, we have to admit that it is difficult to correlate any specific clinical feature with the presence of autoimmune diseases in MDS patients, especially considering the low number of patients included and the retrospective nature of the studies and also the different criteria used for defining the AD. In conclusion, AD are common in MDS patients and are associated with worse outcomes, particularly in L-R MDS.

Further studies are required to understand the relationship between MDS and AD, especially nowadays that new therapies targeting the immune system will be soon available for patients with MDS.

Second work

Prognostic risk assessment represents a cornerstone in the management of MDS patients. Accordingly, several prognostic scores have been used, being the IPSS the most commonly applied until 2012, when the revised version (IPSS-R) that includes 5 risk groups was published and became the most common prognostic score used worldwide (113,125). Nevertheless, after years of applying the IPSS-R, clinicians realized that, as in the classical IPSS, it is relevant to have a dichotomization of this new score because it is crucial to identify which patients are devoted to a more conservative approach or to a more interventional strategy. This

division is extremely important since patients in the low-risk group present longer survival and no treatment has demonstrated to improve their outcomes. Therefore, these patients are not usually considered for intensive therapies, such as HSCT or antileukemic chemotherapy (163,164) .Moreover, hypomethylating agents are not licensed in Europe for low-risk MDS and thereby these patients are only offered supportive care and possibly erythropoiesis-stimulating agents (166,167). In contrast, high-risk patients with shorter expected survival are candidate to receive a more intensive approach with different options such as hypomethylating agents, chemotherapy, and HSCT, in order to overcome the expected poor prognosis (149,168–170). Although there are some efforts trying to incorporate new variables to modify the prognosis stratification such as the presence of point mutations, these techniques are not available in many centers, and thus we sought to improve the utility of IPSS-R which is available in all centers to identify which is the cutoff, which is the cut-point in the new IPSS-R that best divides patients in low and high-risk subgroups. Moreover, the outcomes of the intermediate-risk group are widely heterogeneous; some patients present an indolent disease, others rapidly evolve to AML. Accordingly, there are no specific treatment protocols for these patients with different strategies recommended in the most recently published clinical practice guidelines (i.e. European Leukemia Net and NCCN) (147,148). Thus, treatment decisions could be extremely difficult. Because of the retrospective nature of our study, treatment decisions in many patients were based on the former classification of the IPSS; therefore, the outcomes could be influenced by treatments decision based on the IPSS. However, despite most of the patients in the intermediate-risk group were treated with disease-modifying agents (75%), the outcomes were poor, supporting that these patients should be considered as high-risk patients. Since this study represents the first focused specifically on the outcomes of the intermediate-risk IPSS-R group, further prospective analyses are recommended in order to validate our results. Furthermore, many clinical trials allow the inclusion of intermediate-risk patients along with high or very high-risk patients to test the efficacy of different drugs. Therefore, it would be important to analyze these different subgroups separately to

know if the outcome differs between them. In the meanwhile, a retrospective study analyzing the possible benefit of approved drugs such as hypomethylating agents could help us to understand how to manage these patients. According to our analysis, the optimal dichotomization in low and high-risk MDS patients is the division obtained by using an IPSS-R score of 3 points as a cut-point (OS of 61.3 vs. 13.9 months and 36 months cumulative incidence of AML evolution of 6% and 38% in low and high-risk patients, respectively). Although our data is retrospective in nature, this caveat is somehow overcome owing to the number of patients and the homogeneous management of the patients along the years in both institutions. This cutoff is slightly lower than the obtained by Pfeilstocker et al. and colleagues (129) in a subanalysis based on a large cohort of primary untreated MDS patients from the International Working Group for Prognosis in MDS database in which an IPSS-R of 3.5 points was the score suggested to divide in two risk categories with a median OS around 70 and 20 months in low and high-risk patients, figures very similar to our series. Interestingly, both analyses suggest that most of the patients of the IPSS-R intermediate-risk group, two-thirds in Pfeilstocker's study and 100% in ours should be considered as high-risk and therefore deserving an intensive approach. When we compared the outcomes of the intermediate-risk group (IPSS-R 3–4.5) with the low-risk (IPSS-R < 3) and the high-risk (IPSS-R > 4.5), it became evident that the behavior was really close to the high-risk (OS of 16.2 vs. 9.6 months and risk to AML evolution of 20% vs. 60%, for the intermediate and high-risk, respectively) and remarkably different from the low-risk subgroup (OS of 61.1 months and only 6% of risk to AML evolution). These results could support the strategy of adopting a more intense approach for intermediate-risk patients similar to the high-risk groups. In this sense, a recent report of Della Porta *et al*, showed that early transplant was associated with worse survival in the very low and low IPSS-R risk groups, whereas, in the intermediate, high, and very high-risk groups was associated with an improvement in life expectancy (171). In conclusion, our data demonstrate that a cutoff of 3 in the IPSS-R is the best for segregating MDS patients in two risk groups (low-risk and high-risk), an observation that may be

valuable for the design of clinical trials and for daily management of patients with MDS. Future prospective studies are needed to confirm our previous results.

V. CONCLUSIONS

In the first work that analyzes the association and the prognostic impact between MDS and AD, we showed that:

1. The presence of AD are common in patients with MDS and CMML.
2. The presence of AD confers worse outcomes in MDS patients, particularly in low-risk MDS.

In the second work that analyzes the best IPSS-R cut-point to stratify patients in low vs high-risk, we showed that:

1. An IPSS-R cut-point of 3 could be useful in the clinical setting to stratify MDS patients in low and high-risk subgroups with significant differences in OS and AML evolution.
2. Intermediate IPSS-R risk group presented an outcome closer to high IPSS-R risk than to low IPSS-R risk MDS.

VI. FUTURE DIRECTIONS

Clonal hematopoesis of indeterminate potential or CHIP, the precursor estate of MDS, can alter the immune system and the immune system can promote CHIP. Furthermore, patients with CHIP are more prone to develop hematologic malignancies and cardiovascular events. However, the definitive definition of CHIP is not well established, neither the future consequences of harboring CHIP or the optimal management of these individuals. Therefore, we will establish a Clonal Hematopoiesis Unit (CH Unit) at our Department that will be operative on September 2019. The CH Unit will focus on the detection of CHIP patients and their follow-up. Gathering clinical and mutational data will allow us a better understanding of the biological relationship between CHIP, MDS and immune system.

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