Universitat de Girona

TEMPORAL TRENDS OF INCIDENCE AND SURVIVAL OF MYELOID MALIGNANCIES IN GIRONA: A POPULATION-BASED STUDY DURING THE RECENT FIFTEEN YEARS

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DOCTORAL THESIS

Temporal trends of incidence and survival of myeloid malignancies in Girona: a population-based study during the recent fifteen years.

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Gemma Osca Gelis

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PhD programme in Experimental Sciences and Sustainability

Directed by:

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Thesis delivered to obtain the doctoral degree by the Universitat de Girona

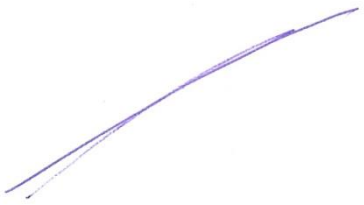
El Dr. Marc Saez Zafra, de la Universitat de Girona i membre del 'Research Group on Statistics, Econometrics and Health' (GRECS) i el Dr. Rafael Marcos Gragera, de la Universitat de Girona i epidemiòleg de la Unitat d'Epidemiologia i Registre de Càncer de Girona (UERCG) de l'Institut Català d'Oncologia (ICO),

Declarem:

Que el treball titulat "**Temporal trends on incidence and survival of myeloid malignancies in Girona: A population-based study during the recent fifteen years**", que presenta **GEMMA OSCA GELIS** per a l'obtenció del Títol de Doctora per la Universitat de Girona (UdG), ha estat realitzat sota la nostra direcció i supervisió durant els últims quatre anys. L'esmentada tesis aporta nous coneixements sobre la incidència i la supervivència poblacional de les neoplàsies mieloides en un àrea ben definida com és la província de Girona. L'epidemiologia d'aquestes patologies es poc coneguda degut principalment als avenços en la seva caracterització i els canvis que ha suposat en la seva classificació.

I, perquè així consti i tingui els efectes oportuns, signem aquest document.

Signatura



Dr. Marc Saez Zafra

Dr. Rafael Marcos Gragera

Girona, 14 d'Abril del 2014.

It always seems impossible until it's done.

Nelson Mandela

To my family and friends

Acknowledgments

Give thanks for a little and you will find a lot.

Hansa proverb

S'acostuma a dir que aquesta part de la tesi és una de les més complicades d'escriure i la veritat és que no t'ho sembla fins que t'hi trobes. Sou tants els que heu estat al meu costat durant aquest llarg camí, que m'és impossible descriure lo afortunada que m'he sentit sempre de tenir-vos prop meu quan us he necessitat. Amb molts de vosaltres hem estat junts des de l'inici, amb altres ens hem anat trobant amb el pas del temps i alguns per desgracia heu anat marxant del meu costat. A tots vosaltres, moltes gràcies.

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List of publications derived from this thesis

There is no substitute for hard work.

Albert Einstein

This thesis is presented in a mixed format. The results section includes the following articles which have been published or sent:

- **Osca-Gelis G**, Puig-Vives M, Saez M, Gallardo D, Lloveras N, Marcos-Gragera R. Population-based incidence of myeloid malignancies: fifteen years of epidemiological data in the province of Girona, Spain. **Haematologica.** 2013 Aug;98(8):e95-7.

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- **Osca-Gelis G**, Puig-Vives M, Saez M, Gallardo D, LLoveras N, Guàrdia R, Marcos-Gragera R. Is the survival of myeloid malignancies really improving? A retrospective 15 years population-based study. Leukemia and Lymphoma. 2014 Aug 18: 1-7.

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- **Osca-Gelis G**, Puig-Vives M, Saez M, Gallardo D, Solé F, Marcos-Gragera R. Incidence and survival of chronic myelomonocytic leukaemia in Girona (Spain): a population-based study, 1993-2007. Leuk Res. 2012 Oct;36(10):1262-6. Erratum in Leuk Res. 2013 Jul;37(7):852.

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  JCR ISI, Hematology: 2nd quartile, position 31 (of 67), Impact Factor (2012): 2.764

Abbreviations, Figures and Tables lists

First learn the meaning of what you say,
and then speak.

Benjamin Franklin

Abbreviations list

-	Monosomy
+	Trisomy
aaDIPSS	Age adjusted Dynamic International Prognostic Scoring System
aCML	Atypical chronic myelogenous leukaemia or atypical chronic myeloid leukaemia
AL	Acute leukaemia
AML	Acute myeloid leukaemia
AMML	Acute myelomonocytic leukaemia
APL	Acute promyelocytic leukaemia
ASR	Standardized incidence rate
ASR_E	European-population standardized incidence rate
ASR_{US}	United States-population 2000 standardized incidence rate
ASR_W	World-population standardized incidence rate
ATRA	All Trans-Retinoic Acid
BCR-ABL1	Fusion gene resulting from t(9;22)(q34;q11)
BM	Bone marrow
CBF	Core-binding factor
CEBPA	CCAAT/enhancer-binding protein alpha
CEL/HES	Chronic eosinophilic leukaemia7hypereosinophilicsyndrome
CML	Chronic myelogenous leukaemia or chronic myeloid leukaemia
CMML	Chronic myelomonocytic leukaemia
CMPDs	Chronic myeloproliferative diseases
CMPDs-u	Chronic myeloprolifeative diseases, unclassifiable
CNL	Chronic neutrophilic leukaemia
CPSS	CMML-specific prognosis scoring system
CR	Crude rate
DCO	Death certificate only

del	Deletion
DIPSS	Dynamic International Prognostic Scoring System
ENCR	European Network for Cancer Registries
ET	Essential thrombocythaemia
FAB	*Grup Franco-Americà-Britànic / Grupo Franco-Americano-Británico* / French-American-British Cooperative Group
FISH	Fluorescense *in situ* hibridation
FLT3	Fetal liver tyrosine kinase 3
GESMD	*Grupo Español de Síndromes Mielodisplásicos*
HAEMACARE	Cancer Registry Based project on Haematological Malignancies
ICD-O	International Classification of Diseases for Oncology
ICUS	Idiopathic cytopenia of uncertain significance
IDESCAT	*Institut d'Estadística de Catalunya*
INE	*Instituto Nacional de Estadística*
inv	Inversion
IPSS	International Prognostic Scoring System
ITD	Internal tandem duplication
IWG-MRT	International Working Group for Myeloproliferative Neoplasms Research and Treatment
JAK2	Janus Kinase 2
JMML	Juvenile myelomonocytic leukaemia
LAM	*Lecuèmia mieloide aguda / Leucemia mieloide aguda*
LMMC	*Leucèmia mielomonocítica crónica / leucemia mielomonocítica crónica*
MDS	Myelodysplastic syndromes
MDS/MPDs	Myelodysplastic/ myeloproliferative diseases
MDS/MPDs-u	Myelodysplastic/ myeloproliferative diseases, unclassifiable
MDS/MPN	Myeodysplastic/myeloproliferative neoplasms
MDS/MPN-u	Myelodysplastic/Myeloproliferative neoplasms, unclassifiable

MDS-u	Myelodysplastic syndromes, unclassifiable
MMs	Myeloid malignancies
MPDs	Myeloproliferative disorders
MPN	Myeloproliferative neoplasms
MPN-u	Myeloproliferative neoplasms, unclassifiable
NMPC	*Neoplàsies mieloproliferatives cròniques / Neoplásias mieloproliferativas crónicas*
NMS	*Neoplàsies mieloides / Neoplásias mieloides*
noc	Not otherwise categorised
nos	Not otherwise specified
NPM1	Nucleophosmin 1
OMS	*Organització Mundial de la Salut / Organización Mundial de la Salud*
OS	Observed survival
p	Chromosomes short arm
PB	Peripheral blood
Ph	Philadelphia chromosome; t(9;22)(q34;q11)
PMF	Primary myelofibrosis
PTK	Protein-tyrosine kinase
PV	Polycythaemia vera
q	Chormosomes long arm
RA	Refractory anaemia
RAEB	Refractory anaemia with excess of blasts
RAEB-T	Refractory anaemia with excess of blasts in transformation
RARS	Refractory anaemia with ringed sideroblasts
RCG	*Registre de Càncer de Girona / Registro de Cáncer de Girona* / Girona Cancer Registry
RCMD	Refractory cytopenia with multilineage dysplasia
RCMD-RS	Refractory cytopenia with multilineage dysplasia and ringed syderoblasts

RCUD	Refractory cytopenia with unilineage dysplasia
RER	Relative excess risk of death
R-IPSS	Revised-IPSS
RS	Relative survival
SCT	Stem cell transplantation
SEHH	*Sociedad Española de Hematología y Hemoterapia*
SM	Systemic mastocytosis
SMD	*Síndromes mielodisplàstiques / Síndromes mielodisplásicos*
SMD/NMPC	*Malalties mielodisplàstiques/mieloproliferatives cròniques / Enfermedades mielodisplásicas/mieloproliferativas crónicas*
t	Translocation
WBC	White blood cells
WHO	World Health Organization
WPSS	WHO classification-based Prognostic Scoring System

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The scariest moment is always just before you start.

Stephen King

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Summary

The harder you work,
the harder it is to surrender.

Vince Lombardi

Resum

Les neoplàsies mielodes (NMs) són un grup heterogeni de malalties hematològiques que presenten diferent incidència, pronòstic i supervivència. Els canvis existents en la seva classificació (Franco-Americana-Britànica (FAB), Organització Mundial de la Salut (OMS) 2001 i OMS 2008) i els pocs estudis de base poblacional publicats disponibles a la literatura, han complicat els anàlisis epidemiològics comparatius d'aquestes malalties a nivell internacional.

És per aquest motiu, que el Registre de Càncer de base poblacional de Girona (RCG) es va plantejar avaluar exhaustivament l'estat actual de les NMs diagnosticades a la nostra província analitzant un període de quinze anys, amb la fi d'incrementar així el nombre de dades existents relacionades amb la incidència i la supervivència d'aquestes patologies poc freqüents.

Entre 1994 i 2008, un total de 1.331 pacients afectats de NMs van ser diagnosticats a la província de Girona: 718 homes i 613 dones amb edats medianes de 72 i 73 anys respectivament. Tenint en compte la classificació de la OMS publicada al 2001, un 24,0% dels casos van ser registrats com leucèmia mieloide aguda (LMA), un 34,4% com síndromes mielodisplàstiques (SMD), un 36,7% presentaven neoplàsies mieloproliferatives cròniques (NMPC) i tan sols un 4,9% dels pacients van ser diagnosticats de síndromes mielodisplàstiques/neoplàsies mieloproliferatives cròniques (SMD/NMPC).

Al comparar les taxes d'incidència del RCG per cadascun dels grans grups de NMs proposats per la classificació de la OMS amb altres estudis internacionals vam veure que, tot i que els resultats eren similars per la LMA, diferien bastant pels grups de SMD, NMPC i SMD/NMPC. Les principals causes atribuïbles a aquestes diferencies podien ser: controvèrsia en els sistemes de classificació, impossibilitat d'un estudi exhaustiu en la majoria dels pacients degut a les comorbiditats associades a la seva avançada edat, increment de pacients asimptomàtics detectats extrahospitalariament i millores en les tècniques diagnòstiques. Analitzant la tendència de la incidència es va apreciar que la LMA era l'únic grup mieloide on la incidència es mantenia estable durant el període d'estudi. Contràriament, tant per SMD com per NMPC i SMD/NMPC, s'observava un augment significatiu de la incidència entre 1994 i 2008. Algunes de les causes que podien haver contribuït en aquest increment eren el refinament de les tècniques moleculars, el descobriment de mutacions com *JAK2* V617F, que faciliten el diagnòstic de patologies com la trombocitèmia essencial, la policitèmia vera i la mielofibrosis idiopàtica i, les millores proposades en les classificacions de les NMs.

En relació a la supervivència d'aquestes patologies, diversos estudis publicats mostren que els Europeus diagnosticats amb NMs tenen una supervivència relativa als 5 anys molt baixa, tot i que és ben conegut que aquesta varia segons els principals grups mieloides proposats per la

classificació de la OMS. A la província de Girona, la supervivència mediana global dels pacients diagnosticats entre 1994 i 2008 va ser de 37 mesos: 6,2 mesos per pacients diagnosticats de LMA, 31,5 mesos per casos amb SMD, 114,7 mesos per pacients amb NMPC i 28,4 mesos per gent amb SMD/NMPC. Donat que el sexe, l'edat dels pacients i l'any de diagnòstic poden influir en el temps de supervivència, al RCG varem analitzar detalladament la supervivència per cadascun d'aquests tres factors pronòstics, essent l'edat el factor més determinant; per tots el grups (LMA, SMD, NMPC i SMD/NMPC) els pacients joves tenien millor pronòstic que aquells d'edat avançada. Estratificant la nostra cohort per edat, va resultar que pacients joves diagnosticats amb SMD o NMPC durant els anys 2004-2008 presentaven millor supervivència en comparació amb aquells diagnosticats durant el període 1994-1998. L'ús individualitzat de les possibles opcions terapèutiques com a conseqüència d'una millor estratificació dels pacients en grups de risc, així com el desenvolupament de noves teràpies dirigides contra les mutacions genètiques recurrents en certes patologies mieloides, va provocar un increment de la supervivència del casos de menor edat. L'Imatinib, fàrmac aprovat per la *Food and Drug Administration* l'any 2001, va suposar una revolució en el tractament de pacients afectes de leucèmia mieloide crònica amb la translocació t(9;22)(q34.1;q11.2). Tot i que a Girona els pacients tractats amb Imatinib presentaven una millor supervivència respecte els que no havien estat tractats, el trasplantament al·logènic de progenitors hematopoètics segueix essent la única teràpia curativa per a pacients diagnosticats de NMs.

Pel que fa a l'increment de supervivència detectat en gent jove amb LMA, tot i no ser significatiu, es va poder relacionar amb una millor estratificació dels pacients en grups de risc basats, no només en alteracions citogenètiques, sinó també amb troballes moleculars com FLT3 i NPM1 que permeten fer una teràpia adaptada al grup de risc.

Degut a que els SMD/NMPC és el grup de NMs menys freqüent, amb una taxa d'incidència que no supera el cas per 100.000 habitants/any, pocs són els estudis publicats que reporten dades epidemiològiques d'aquestes patologies a nivell poblacional, i els existents, descriuen únicament l'entitat més freqüent dins d'aquest grup: la leucèmia mielomonocítica crònica (LMMC). Tot i que aquesta patologia era reconeguda per la classificació de la FAB, no va ser fins la publicació de la classificació de la OMS l'any 2001 que va passar a formar part, juntament amb la leucèmia mielomonocítica juvenil i la leucèmia mieloide crònica atípica, del grup SMD/NPMC, caracteritzat per compartir trets de displàsia i proliferació. Perquè la LMMC és una patologia que afecta a gent d'edat molt avançada i perquè molts casos s'han categoritzat erròniament com a SMD o com a NMPC durant molt de temps, al RCG varem analitzar detalladament l'estat d'aquesta patologia a la província de Girona. Durant el període 1993-2007, es van diagnosticar 61 casos de LMMC (36 homes i 25 dones), la supervivència dels quals variava estadísticament segons l'edat. Pacients de menys de 75 anys tenien una supervivència

mediana de 64 mesos, disminuint fins als 19 mesos en pacients de major edat. Tret del transplantament al·logènic de progenitors hematopoètics, opció terapèutica disponible en un nombre limitat de pacients amb edats inferiors als 65 anys, actualment no existeix cap tractament capaç de curar aquesta malaltia. Degut a les comorbiditats associades a l'edat, pocs són els casos que reben tractament quimioteràpic o teràpia amb agents hipometilants. La majoria dels pacients amb LMMC són tractats amb teràpies de suport per pal·liar la simptomatologia associada a la malaltia.

Resumint, aquesta tesi doctoral agrupa les úniques dades epidemiològiques disponibles de les NMs a la província de Girona, analitza detalladament les tendències de la incidència i supervivència d'aquestes malalties durant un llarg període de temps, i intenta relacionar els possibles canvis amb millores en el mètode de diagnòstic, estratificació dels pacients en grups de risc i tractament. Els nostres resultats poden ser de gran interès a nivell clínic i de salut pública, ja que permeten determinar més acuradament el pronòstic d'aquests pacients, avaluar l'impacte de nous tractaments a nivell poblacional i fomentar la recerca de noves dianes terapèutiques.

Resumen

Las neoplasias mieloides (NMs) son un grupo heterogéneo de enfermedades hematológicas que presentan distinta incidencia, pronóstico y supervivencia. Los cambios existentes en su clasificación (Franco-Americana-Británica (FAB), Organización mundial de la Salud (OMS) 2001 y OMS 2008) y los pocos estudios de base poblacional publicados disponibles en la literatura, han dificultado los análisis epidemiológicos comparativos para estas enfermedades a nivel internacional.

Es por este motivo que el Registro de Cáncer de base poblacional de Girona (RCG) se planteó evaluar exhaustivamente el estado actual de las NMs diagnosticadas en nuestra provincia analizando un período de quince años, con el fin de incrementar de este modo el número de datos existentes relacionados con la incidencia y supervivencia de estas patologías poco frecuentes.

Entre 1994 y 2008, un total de 1.331 pacientes afectados de NMs fueron diagnosticados en la provincia de Girona: 718 hombres y 613 mujeres con edades medianas de 72 y 73 años respectivamente. Teniendo en cuenta la classificación de la OMS publicada en 2001, un 24,0% de los casos fueron registrados como leucemia mieloide aguda (LMA), un 34,4% como síndromes mielodisplásicos (SMD), un 36,7% presentaron neoplasias mieloproliferativas crónicas (NMPC) y solamente un 4,9% de los paciente fueron diagnosticados con síndromes mielodisplásicos/neoplásias mieloproliferativas crónicas (SMD/NMPC).

Al comparar las tasas de incidencia del RCG para cada uno de los grandes grupos de NMs propuestos por la clasificación de la OMS con otros estudios internacionales pudimos ver que, a pesar de que los resultados eran similares para la LMA, diferían notablemente para los grupos de SMD, NMPC y SMD/NMPC. Las principales causas atribuibles a estas diferencias podían ser: controversia en los sistemas de clasificación, imposibilidad de un estudio exhaustivo de los pacientes debido a las comorbilidades asociadas a su avanzada edad, incremento de pacientes asintomáticos diagnosticados extrahospitalariamente y mejoras en las técnicas de diagnóstico. Analizando la tendencia de la incidencia se apreció que la LMA era el único grupo mieloide en el que la incidencia se mantenía estable durante el periodo de estudio. Por lo contrario, tanto para SMD como para NMPC y SMD/NMPC, se observó un incremento significativo de la incidencia entre 1994 y 2008. Algunas de las causas que podían haber contribuido en este incremento eran el perfeccionamiento de las técnicas moleculares, el descubrimiento de mutaciones como *JAK2* V61F, que facilitan el diagnóstico de patologías como la trombocitémia esencial, la policitémia vera y la mielofibrosis idiopática y las mejoras propuestas en la clasificación de las NMs.

En relación a la supervivencia de estas patologías, varios estudios publicados muestran que los Europeos diagnosticados con NMs tiene una supervivencia relativa a los 5 años muy baja, pese a que es bien conocido que ésta varía según los principales grupos mieloides propuestos por la clasificación de la OMS. En la provincia de Girona, la supervivencia mediana global de los pacientes diagnosticados entre 1994 y 2008 fue de 37 meses: 6,2 meses para pacientes diagnosticados con LMA, 31,5 meses para casos con SMD, 114,7 meses para pacientes con NMPC y 28,4 meses para gente con SMD/NMPC. Debido a que sexo, edad de los pacientes y año de diagnóstico pueden influir en el tiempo de supervivencia, el RCG quiso analizar detalladamente la supervivencia para cada uno de estos tres factores pronósticos, siendo la edad el factor más determinante; para todos los grupos (LMA, SMD, NMPC, y SMD/NMPC), los pacientes jóvenes tenían mejor pronóstico que los de avanzada edad. Estratificando nuestra cohorte por edad resultó que pacientes jóvenes diagnosticados con SMD o NMPC durante los años 2004-2008 presentaban mejor supervivencia en comparación con aquellos diagnosticados durante el período 1994-1998. El uso individualizado de las posibles opciones terapéuticas como consecuencia de una mejor estratificación de los pacientes en grupos de riesgo, así como el desarrollo de nuevas terapias dirigidas contra mutaciones genéticas recurrentes en ciertas patologías, provocó un incremento de la supervivencia de los casos de menor edad. El Imatinib, fármaco aprobado por la *Food and Drug Administration* el año 2001, supuso una revolución en el tratamiento de los pacientes con leucemia mieloide crónica con la translocación t(9;22)(q34.1;q11.2). Pese a que en Girona los pacientes tratados con Imatinib presentaban una mejor supervivencia con respecto a los que no se habían tratado, el trasplante alogénico de progenitores hematopoyéticos sigue siendo la única terapia curativa para pacientes con NMs.

En relación al incremento de supervivencia detectado en gente joven con LMA, pese a no ser significativo, se pudo relacionar con una mejor estratificación de los pacientes en grupos de riesgo basados, no sólo en alteraciones citogenéticas, sino también con hallazgos moleculares como FLT3 y NPM1 que permiten hacer una terapia adaptada al grupo de riesgo.

Debido a que los SMD/NMPC son el grupo de NMs menos frecuente, con una tasa de incidencia que no supera el caso por 100.000 habitantes/año, pocos son los estudios publicados que reportan datos epidemiológicos de SMD/NMPC a nivel poblacional, y los existentes, describen únicamente la entidad más frecuente dentro de este grupo: la leucemia mielomonocítica crónica (LMMC). Pese a que esta patología era reconocida por la clasificación de la FAB, no fue hasta la publicación de la clasificación de la OMS en 2001 que pasó a formar parte, juntamente con la leucemia mielomonocítica juvenil y la leucemia mieloide crónica atípica, del grupo SMD/NMPC, caracterizado por compartir rasgos de displasia y proliferación.

Dado que la LMMC es una patología que afecta a gente de edad muy avanzada y que muchos casos se han categorizado erróneamente como SMD o NMPC durante mucho tiempo, los miembros del RCG analizamos detalladamente el estado de esta patología en la provincia de Girona. Durante el período 1993-2007, se diagnosticaron 61 casos de LMMC (36 hombres y 25 mujeres), la supervivencia de los cuales variaba estadísticamente según la edad. Pacientes menores de 75 años tenían una supervivencia mediana de 64 casos, disminuyendo hasta los 19 meses en pacientes de mayor edad. Salvo el trasplante alogénico de progenitores hematopoyéticos, opción terapéutica disponible para un nombre limitado de pacientes con edades inferiores a los 65 años, actualmente no existe ningún tratamiento capaz de curar esta enfermedad. Debido a las comorbilidades asociadas a la edad, pocos son los casos que reciben tratamiento quimioterapéutico o terapia con agentes hipometilantes. La mayoría de los pacientes con LMMC son tratados con terapias de soporte para paliar la sintomatología asociada a su enfermedad.

Resumiendo, esta tesis doctoral agrupa los únicos datos epidemiológicos disponibles de las NMs en la provincia de Girona, analizando detalladamente las tendencias de la incidencia y supervivencia de estas patologías durante un largo periodo de tiempo, e intenta relacionar los posibles cambios con mejoras en el método de diagnóstico, estratificación de los pacientes en grupos de riesgo y tratamiento. Nuestros resultados pueden ser de gran interés a nivel clínico y de salud pública, ya que permiten determinar de manera más precisa el pronóstico de estos pacientes, evaluar el impacto de nuevas terapias a nivel poblacional y fomentar la investigación de nuevas dianas terapéuticas.

Summary

Myeloid malignancies (MMs) are heterogeneous groups of haematological malignancies presenting different incidence, prognosis and survival. Changing classifications (French-American-British (FAB), World Health Organization (WHO) 2001 and WHO 2008) and few population-based published studies available in the literature, complicate international comparative epidemiological studies.

This is the reason why the population-based Girona Cancer Registry (RCG) exhaustively evaluates the actual status of MMs diagnosed in our province analysing a fifteen years period, with the aim to increasing the existing data related with incidence and survival of these infrequent pathologies.

A total of 1,331 patients with MMs were diagnosed in the province of Girona between 1994 and 2008: 718 men and 613 women with median ages of 72 and 73 years respectively. Considering the WHO classification published in 2001, a 24.0% of cases registered were acute myeloid leukaemia (AML), a 34.4% were myelodysplastic syndromes (MDS), a 36.7% presented chronic myeloproliferative diseases (CMPDs) and only a 4.9% of patients were diagnosed with myelodysplastic/myeloproliferative diseases (MDS/MPDs).

Comparing incidence rates for each of main MMs groups proposed by the WHO classification in the province of Girona with other international studies we found that, despite results were similar for AML, were quite different for MDS, CMPDs and MDS/MPDs groups. Principal causes attributable to those differences may be: controversies in classification systems, impossibility to make an exhaustive study of the majority of cases due to the comorbidities associated to the advanced age of patients, increased outpatient setting of asymptomatic patients and improvements in diagnostic techniques. Analysing incidence trend we could appreciate that AML was the only group in which incidence remained stable during the period of study. Contrary, MDS, CMPDs and MDS/MPDs presented a significant increase of incidence since 1994 to 2008. Some reasons that could contributed to that increase were the improvements on molecular diagnostic techniques, the discovery of some mutations such *JAK2* V617F, which led diagnose some pathologies as essential thrombocythaemia, polycythaemia vera and primary myelofibrosis, and meliorations proposed by the MMs classifications.

In relation with survival of these pathologies, some published studies have found that Europeans diagnosed with MMs have poor 5-year relative survival, although it was well known that it depends according to the WHO classification MMs groups. In the province of Girona, the median overall survival for patients diagnosed between 1994 and 2008 were 37 months: 6.2 months for patients diagnosed with AML, 31.5 months for cases with MDS, 114.7 months for patients with CMPDs and 28.4 months for people with MDS/MPDs. Because sex, age of

patients and year of diagnosis could influence survival time, the RCG analysed in detail the survival for each of these prognostic factors, being age the most determinant factor; for all groups (AML, MDS, CMPDs and MDS/MPDs), younger patients had better prognosis with those with advancing age. Stratifying our cohort by age, we found that young patients diagnosed with MDS or MPN during the years 2004-2008 had better survival compared with those diagnosed between 1994 and 1998. The personalised use of the available therapeutic options as a consequence of a better stratification of patients into risk-groups, just like as the development of new targeted therapies against recurrent genetic mutations present in some myeloid malignancies, caused and improvement of survival of young people. Imatinib, drug approved by the Food and Drug Administration in 2001, revolutionized the treatment of patients with chronic myeloid leukaemia with translocation t(9;22)(q34.1;q11.2). Despite patients treated with Imatinib in Girona had a better outcome than those no treated, the allogeneic stem cell transplantation remains the only available curative therapy for patients diagnosed with MMs.

The detected increase for young people with AML, although it was not significant, may be explained by better risk-stratification groups based not only on cytogenetic alterations but also molecular findings such FLT3 or NPM1 that allow a risk-adapted therapy.

Because MDS/MPDs is the less frequent group within MMs, with an incidence rate lower than one case per 100,000 inhabitant/years, there are few reliable population-based published studies reporting epidemiological data on these diseases, and existing ones, are only focused in chronic myelomonocytic leukaemia (LMMC), the most frequent entity within this group. Although this pathology was recognised in the FAB classification, it was not since the publication of the WHO 2001 that it has been categorised, together with juvenile myelomonocytic leukaemia and atypical chronic myeloid leukaemia, in the MDS/MPDs group, characterised by shearing dysplastic and proliferative features. For the reason that CMML is a disease affecting elderly people and because many cases were wrongly registered as MDS or CMPDs during a long period of time, the RCG analysed in detail the status of this pathology in the province of Girona. During the period 1993-2007, 61 cases of CMML (36 men and 25 women) were diagnosed and their survival varied statistically according to age. People aged less than 75 years had a median of survival of 64 months, whereas elderly patients survived a median of 19 months. Actually, haematopoietic stem cell transplantation remains the only curative therapy available for a few number of patients aged less than 65 years. Due to age-related comorbidities, few cases received chemotherapy or therapy with hipomethylating agents. Most of patients with CMML are treated with supportive cares to palliate the disease related symptoms.

Summarizing, this doctoral thesis collect the only available epidemiological data on MMs in the province of Girona, analyze accurately trends of incidence and survival of this pathologies, and try to relate possible changes with improvements in diagnostic methods, better risk-stratification groups and treatment. Our results may be very useful for clinicians and public heath managers in determining the prognosis of patients precisely, evaluating the impact of new treatments within a population and encourage research into new therapeutic targets.

Introduction

Your present circumstances don't determine where you can go;
they merely determine where you start.

Nido Quebein

1. An overview to haematopoiesis

Myeloid malignancies (MMs) are a heterogeneous group of diseases affecting myeloid cell lineages formed during haematopoiesis, in particular through myelopoiesis (Figure 1).

Haematopoiesis is the production, proliferation, differentiation and maturation of blood cells. Bone marrow (BM), peripheral blood (PB), the lymph nodes, the spleen, the liver and the thymus all contribute to this process. In adults, haematopoiesis occurs in BM[1,2].

Under normal conditions, this process begins with a pluripotent stem cell, which is able to proliferate, replicate and differentiate. In response to growth factors, this pluripotent stem cell will differentiate into common myelopoietic or lymphopoietic stem cells which maintain their pluripotent capacity. The lymphopoietic stem cell will proliferate and differentiate into B lymphocytes, T lymphocytes or natural killer cells throughout the lymphopoiesis process. Similarly, myelopoietic stem cells will proliferate and differentiate into granulocytic, monocytic, erythrocytic or megakaryocytic lineages during myelopoiesis; the production, development and maturation of myeloid cells. Once mature, these cells will go into the PB[1-3].

MMs are clonal diseases originating as a consequence of genetic and epigenetic mutations of myelopoietic stem cells or progenitor myeloid cells during myelopoiesis. These alterations perturb key process (such as self-renewal, proliferation and differentiation) and normal myeloid progenitors become pathological cells[3].

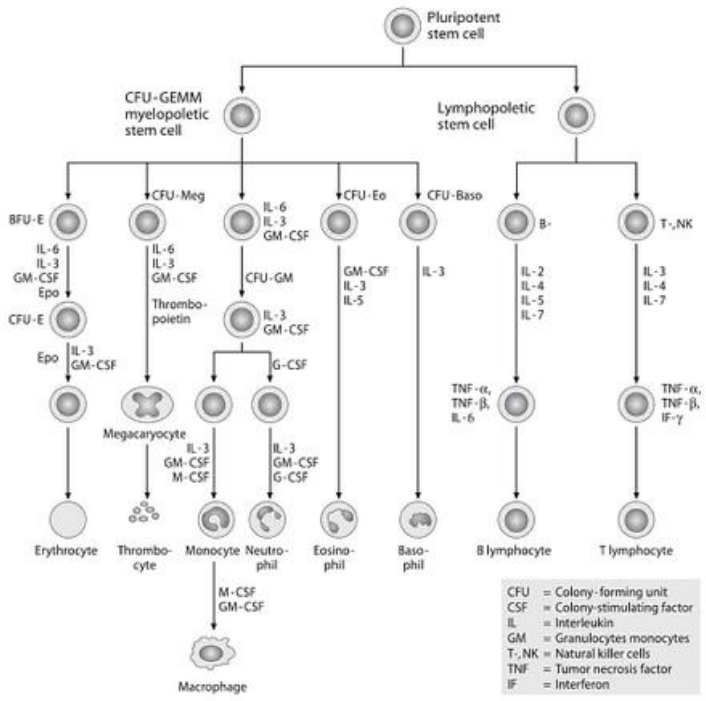


Figure 1. Schematic representation of human haematopoiesis[1].

2. The population-based Girona Cancer Registry

MMs are a heterogeneous group of haematological diseases of myeloid lineage presenting different incidences, prognoses and survival chances, and have few reliable population-based studies available in the literature. The changing definitions and classification systems of MMs and improvements in diagnostic techniques have complicated epidemiological studies and comparative analyses on the incidence and survival of such diseases[4-6].

For all these reasons, and because MMs are not one of the more common cancers within our population, population-based cancer registries have to make an effort to ensure completeness and to guarantee a proper record of these diseases in order to provide factual and precise results.

Since 1994, the population-based Girona Cancer Registry (RCG) has been registering all malignant tumours, *in situ* carcinomas and all cases of cutaneous cancers that are not melanoma in the province of Girona. The RCG is located in the north east of Catalonia, Spain, and covers a population (as of the 2011 census) of 751,806 inhabitants. Information sources for the RCG are regional and taken from community hospitals, haematology and pathology departments, and death certificates. Considering the increasing outpatient diagnosis of some MMs, additional data sources such as results from haematology laboratories where some tests such as flow cytometry, molecular and cytogenetic tests are assessed, along with clinician databases have to be integrated into the RCG to ensure the register's accuracy. Completeness of the RCG is currently 96.3%.

Cases are recorded according to the standards and guidelines for cancer registration in Europe (as proposed by the European Network for Cancer Registries (ENCR)), and are codified using the International Classification of Diseases for Oncology (ICD-O). The second edition of the ICD-O (ICD-O-2) published in 1990, was used by the RCG to register cases diagnosed up until 1997, and the third edition of ICD-O (ICD-O-3) has been used for recording patients diagnosed since 1998 even though it was not printed until 2000[7-9]. Although the myelodysplastic syndromes (MDS) group and some chronic myeloproliferative diseases (CMPDs) such as polycythaemia vera (PV), primary myelofibrosis (PMF), essential thrombocythaemia (ET) and CMPDs unclassifiable (CMPDs-u), were considered neoplasms of uncertain and unknown behaviour in the ICD-O-2, they have in fact been considered to be malignant since the publication of the ICD-O-3.

MM entities were grouped initially using the morphological criteria published by the French-American-British Cooperative Group (FAB) classification, which was first produced in 1976, into three major groups (Table 1)[4,7-10]:

- Acute myeloid leukaemia (AML)

- MDS
- CMPDs

The RCG used this classification system to differentiate the main groups of myeloid disorders until the third edition of the World Health Organization (WHO) classification was published. The foremost problem with the FAB is that there was no relation between the morphology and the codes proposed by the ICD-O-2 used in the registry[9].

In collaboration with the European Association for Haemapathology and the Society for Haemapathology, the WHO published its 2001 classification system in which MMs were reclassified into four major groups (Table 1)[6]:

- AML
- MDS
- CMPDs
- Myelodysplastic/myeloproliferative diseases (MDS/MPDs)

A basic principle is that, while the WHO classification includes many of the criteria of the FAB classification, all other available information (including genetic, immunophenotypic, biological and clinical features) is used to define specific disease entities. The advantage provided by this classification for cancer registries is that WHO and ICD-O-3 merged together cross-referencing with each other, and consequently were able to assign specific codes for distinct morphologies[6-8,11]. Definitions of the major MMs categories and their corresponding entities are detailed in Section 3 of this introduction.

In 2008, the WHO published the second volume of the fourth edition of the WHO series on histological and genetic typing of human tumour classification, namely the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, which incorporated new information that had emerged from basic and clinical investigations[12,13]. Primarily, it included new defining criteria for some diseases as well as some new entities. Some of these are defined by genetic criteria and others by a combination of morphology, immunophenotype and clinical features. This classification system categorized MMs into 5 groups (Table 1):

- AML
- MDS
- Myeloproliferative neoplasms (MPN)
- Myelodysplastic/myeloproliferative neoplasms (MDS/MPN)
- Myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* or *FGFR1*

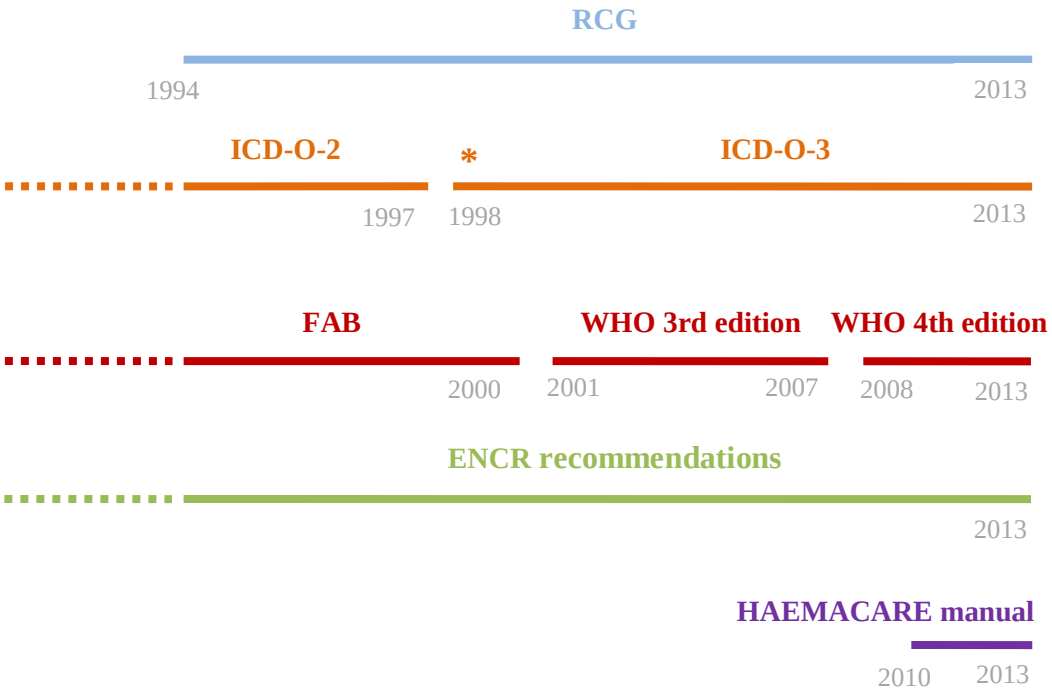
Despite this fourth edition of the WHO series having been available since 2008, the RCG did not use it then and do not use it now. Although the classification of MMs is more accurate as it takes into account numerous criteria to differentiate each entity, the classification lacks a corresponding ICD-O update. Thus, those new diseases defined in the 2008 WHO edition do not have their specific code in the ICD-O-3, and so the RCG still encodes pathologies according to the WHO morphology codes from 2001.

Table 1. Summary of myeloid malignancy groups, according to French-American-British classification (FAB), 2001 World Health Organization (WHO) classification and 2008 World Health Organization (WHO) review[6,13,14].

FAB	WHO 2001	WHO 2008
- Acute myeloid leukaemia	- Acute myeloid leukaemia	- Acute myeloid leukaemia
- Myelodysplastic syndromes	- Myelodysplastic syndromes	- Myelodysplastic syndromes
- Chronic myeloproliferative diseases	- Chronic myeloproliferative diseases	- Myeloproliferative neoplasms
	- Myelodysplastic/ myeloproliferative diseases	- Myelodysplastic/myeloproliferative neoplasms
		- Myeloid and lymphoid neoplasm with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB* or FGFR1

Since 2010, European cancer registries have had a specific Manual for Coding and Reporting Haematological Malignancies which was drafted as part of the HAEMACARE project (Cancer Registry Based project on Haematological Malignancies)[15] . These guidelines were defined by a panel of experts from various European countries who integrated and summarized experiences with existing manuals used in numerous countries to address the problems in the registration and codification of haematological malignancies. This manual is intended to standardize the coding of haematological malignancies in European cancer registries and to make comparative analyses of the incidences and survival rates of these diseases.

Figure 2 summarizes all the classification systems and guidelines for coding and reporting haematological malignancies used by the RCG since its beginnings.



*Despite ICD-O-3 being published in 2001, the GCR used it to register cases diagnosed from 1998 to 2013

RCG (Registre de Càncer de Girona); ICD-O-2 (International Classification of Diseases for Oncology second edition); ICD-O-3 (International Classification of Diseases for Oncology third edition); FAB (French-American-British classification), WHO (World Health Organization classification); ENCR (European Network of Cancer Registries); HAEMACARE (Cancer Registry Based project on Haematological Malignancies).

Figure 2. Recompilation of classification systems and guidelines for coding and reporting in myeloid malignancies used by the Girona Cancer Registry from 1994 to 2013.

3. Myeloid malignancies

3.1. ACUTE MYELOID LEUKAEMIA

3.1.1. Definition and diagnosis

AML is a haematopoietic disorder characterized by a clonal expansion of myeloid blast cells initially in BM, then subsequently in PB, and finally in other tissues. It is a clinically, morphologically and genetically heterogeneous disease. Under normal conditions, the BM produces myeloblasts which, once mature, become granulocytes that are responsible for defending the human body against infections. In AML, myeloblasts are produced in excess and as they are unable to evolve into granulocyte they progressively invade the BM, displacing the normal cells of the blood and other organs (liver, spleen, skin, central nervous system, etc.)[12,13,15,16].

Although risk factors for acquiring AML are not well defined, possible aetiological factors include exposure to ionising radiation, benzene and cytotoxic chemotherapy[17,18].

A BM biopsy is needed to demonstrate any accumulation of blasts resulting from the block in differentiation, characteristic of AML. In the FAB classification a minimum of 30% myeloid blasts in BM used to be required for a diagnosis of AML, whereas the WHO 2001 classification reduced the cut-off point to 20%[10,12].

So therefore, a diagnosis of AML requires[19-22]:

- Clinical data of patients
    - Clinical history of a prior MDS or CMPDs.
    - Knowledge of previous exposure to potentially leukemogenic therapies or agents.
    - History of fatigue, bleeding or recurrent infections with confirmation of an existing PB smears.
- PB and BM samples for evaluating:
    - Morphology
    - Immunophenotype
    - Cytochemistry
    - Cytogenetics
    - Molecular genetics

While morphologic, immunophenotypic and cytochemistry analyses allow us to determine the percentage of blasts and involved cell lineage, cytogenetics and molecular biology tests are

important not only for the sub classification of an AML into a "clinic-pathologic-genetic" entity, but also to determine the prognosis and prediction of treatment response.

3.1.2. Classification

FAB CLASSIFICATION OF AML

For almost 30 years, AML has been registered in keeping with the 1976 FAB classification system. The FAB criteria divides AML into eight subtypes (M0 through to M7) based on cytomorphology and cytochemistry (Table 2). Cytogenetics was only used to characterize any underlying chromosomal abnormalities[14,19-23].

Table 2. French-American-British classification of acute myeloid leukaemia and its associated cytogenetic abnormalities[14,23].

AML entities	Cytogenetic abnormalities
M0: Acute myeloblastic leukaemia, minimally differentiated	
M1: Acute myeloblastic leukaemia, without maturation	
M2: Acute myeloblastic leukaemia, with granulocytic maturation	t(8;21)(q22;q22), t(6;9)
M3: Promyelocytic, or acute promyelocytic leukaemia (APL)	t(15;17)
M4: Acute myelomonocytic leukaemia	inv(16)(p13q22), del (16q)
M4eo: Myelomonocytic together with bone marrow eosinophilia	inv(16), t(16;16)
M5: Acute monoblastic leukaemia (M5a) or acute monocytic leukaemia (M5b)	del(11q), t(9;11); t(11;19)
M6: Acute erythroid leukaemia, including erythroleukaemia (M6a) and very rare pure erythroid leukaemia (M6b)	
M7: Acute megakaryoblastic leukaemia	t(1;2)

AML (acute myeloid entities); t (translocation); q (chromosomes long arm); p (chromosomes short arm); inv (inversion); del (deletion).

The morphologic subtypes of AML also include rare types of leukaemia that are not included in the FAB classification, such as acute basophilic leukaemia, which was proposed as a new subtype, M8, in 1999.

The discovery of some genetic alterations that better predict clinical behaviour and outcome than morphology alone, required genetic data to be incorporated into the classification scheme. The FAB classification, despite making the importance of some cytogenetic alterations, and in

some AML such as acute promyelocytic leukaemia (APL) morphologic alterations predict the cytogenetics, the correlation between morphology and genetics was not always perfect.

After appreciating that AML is a disease that presents two fundamentally different mechanisms of leukemogenesis, a more relevant classification of AML was suggested[14,24,25]. This new classification distinguished:

- AML that evolves from MDS or has similar features to those found in MDS. This group is associated with multilineage dysplasia, poor-risk cytogenetics (loss of genetic material), poor response to therapy and increased incidence with age.

- AML *de novo*. This is related with good-risk cytogenetic abnormalities (specific recurrent chromosomal translocations and inversions), often has a relatively good response to specific treatment and presents good failure-free and overall survival time. The incidence remains constant despite patient age and this type of leukaemia is most likely to be observed in children and young adults. Multilineage dysplasia is not found in this group of AML.

THE WHO 2001 CLASSIFICATION OF AML

The difficulty with the FAB classification system described above was that different subgroups of AML were recognized and classified as unique diseases through the correlation of morphological, genetic and clinical data. In 2001, the WHO, in association with the Society for Hematopathology and the European Association of Hematopathology, published a new classification system which included morphologic findings, genetic, immunophenotypic, biological and clinical features to define specific diseases. This classification identifies four key AML categories and accurately catalogues their corresponding specific entities, including those AML defined by the FAB in the AML not otherwise categorized group (AML noc) (Table 3)[6,10,14].

Table 3. World Health Organization classification (2001) of acute myeloid leukaemia with their corresponding third edition of the International Classification of Diseases for Oncology codes[6,7,10,14].

AML entities	**ICD-O-3 codes**
AML with recurrent cytogenetic abnormalities	
- AML with t(8;21)(q22;q22);(*AML1/ETO*)	9896
- AML with 11q23 (*MLL)* abnormalities	9897
- AML with abnormal bone marrow eosinophils inv(16)(p13;q22) or t(16;16)(p13;q22);(*CBFβ/MYH11*)	9871
- Acute promyelocytic leukaemia (AML with t(15;17)(q22;q11-12) (*PML/RARα*)) and variants	9866
AML with multilineage dysplasia	**9895**
AML and MDS, therapy-related	**9920, 9987**
AML not otherwise categorised	
- AML, minimal differentiated (M0)	9872
- AML, without maturation (M1)	9873
- AML, with maturation (M2)	9874
- Acute myelomonocytic leukaemia (M4)	9867
- Acute monoblastic and monocytic leukaemia (M5)	9891
- Acute erythroid leukaemia (M6)	9840
- Acute megakaryoblastic leukaemia (M7)	9910
- Acute basophilic leukaemia	9870
- Acute panmyelosis with myelofibrosis	9931
- Myeloid sarcoma	9930
AL of ambiguous lineage	**9805**

AML (acute myeloid leukaemia); ICD-O-3 (third edition of the International Classification of Diseases for Oncology); t (translocation); q (chromosomes long arm); p (chromosomes short arm); inv (inversion); MDS (myelodysplastic syndromes); M0-M7 (AML entities present in the French-American-British classification); AL (acute leukaemia.)

This classification of AML follows a hierarchical order[6,15]. According to the ICD-O-3, the diagnosis of this pathology requires the presence of cytogenetic and molecular alterations. If the results of these examinations are not available, we have to evaluate whether multilineage dysplasia is present or if AML can be classified as related to therapy. Only in cases that this information is still not available can the codes proposed by the FAB classification be used to register AML. As a last option, after discarding all previous groups proposed in the WHO classification, AML could be classified as AML not otherwise specified (AML nos), although it is not recommended because this category is very nonspecific.

3.1.2.1. Principal features of AML according to the WHO 2001 classification

1) AML with recurrent cytogenetic abnormalities

This is genetically the best characterized subgroup and consists of AML that present recurrent genetic abnormalities of prognostic significance, mainly balanced translocations and inversions[6,26-29]. The most common aberrations, collectively known as core-binding factor (CBF) abnormalities, are: t(8;21), inv(16) or t(16;16), t(15;17) and various translocations involving the 11q23 breakpoint[28-30]. As was mentioned before, diagnostic karyotyping is one of the most powerful independent prognostic indicators in AML as it is extremely useful in identifying biologically distinct subset of diseases and providing the framework for risk-adapted treatment approaches. This group of AML presents a relatively favourable prognosis and response to appropriate therapy. Overall 5-year relative survival (RS) of AML with recurrent cytogenetic abnormalities is near 55-65%, although it can vary depending on each cytogenetic alteration (Figure 3)[29-31].

AML with t(8;21)(q22;q22);(*AML1/ETO*)

This translocation is one of the most common CBF abnormalities in AML, and found in 5-12% of AML and in one-third of the cases of acute myeloblastic leukaemia with maturation with abnormal karyotype[29,32]. This AML is defined by maturation in neutrophil lineage and it occurs especially in younger patients. It is associated with good response to chemotherapy and high complete remission rate with long-term disease survival after high doses of cytarabine in the consolidation phase (5-year RS of 69%)[27,29,32]. Morphologically, most cases of AML with t(8;21) are correlated with M2 type of the FAB classification[33].

AML with inv(16)(p13q22) or t(16;16)(913;q22);(*CBFβ/MYH11*)

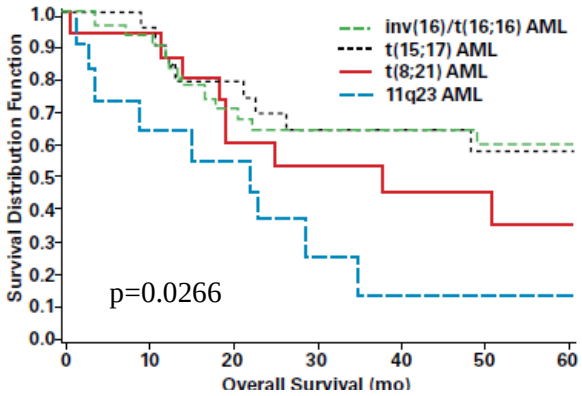
The inv(16)(p13q22) is found in 10-12% of all AML and it is detected especially in younger patients[12]. This AML is related with relatively good prognosis, with a reported 5-year RS of 61%, and higher complete remission when patients are treated with cytarabine in the consolidation phase[27]. Monocytic and granulocytic differentiation and presence of abnormal eosinophils in the BM are characteristic of this AML. The combination of acute myelomonocytic leukaemia (AMML) with abnormal eosinophils is known as AMML Eo[12].

APL (AML with t(15;17)(q22;q12); (*PML/RARα*) and variants)

This AML, which is predominantly found in middle-aged patients, represents 5-8% of all AMLs[12]. It is characterized by a predominance of abnormal promyelocytes, t(15;17), which fuse the *PML* gene to the retinoic acid receptor (*RARα*) gene[32,34,35]. Disease-free survival has significantly increased since the introduction of All Trans-Retinoic Acid (ATRA) as a therapy for AML. ATRA selectively differentiates abnormal promyelocytes from mature granulocytes and the 5-year RS of this disease is almost 65%[36].

AML with 11q23 abnormalities

This type of AML with recurrent cytogenetic abnormalities comprises 5-6% of all cases of AML and although it can occur at any age, it is more common in children[6]. It is associated with monocytic features and the most common 11q23 abnormalities are: t(9;11)(p21;q23) and t(11;19)(q23;p13.3)[32] . Some studies reported the average overall survival is approximately 16-18 months, varying from 1 to 75, and 45% for a 5-year RS[37].



AML (acute myeloid leukaemia); inv (inversion); t (translocation); q (chromosomes long arm).

Figure 3. Overall rate of survival for patients diagnosed with acute myeloid leukaemia with recurrent cytogenetic abnormalities[31].

2) AML with multilineage dysplasia

AML with multilineage dysplasia is an acute leukaemia with more than 20% myeloblasts in BM presenting dysplasia in more than 50% of cells of two or more myeloid lines, generally including megakaryocytes. This type of AML, although it may occurs *de novo*, is mainly associated with MDS-related AML. It is easy to diagnose this group of AML in patients with a history of MDS or MDS/MPDs that has been present for at least of six months before the diagnosis of AML. However, it is more difficult to establish this diagnosis if the patient initially had an acute leukaemia[6,38].

This group of AML is often present in elderly patients which presented several pancytopenia[1]. Chromosomal abnormalities are similar to those found in MDS and often involve a gain or loss of major segments of certain chromosomes such as: -7/del(7q), -5/del(5q), +8, +9, +11, del(11q), del(12p), -18, +19, del(20q) and +21[6,38]. Multilineage dysplasia in AML has an adverse prognosis and low probability of complete remission, with a published 5-year RS of 11% (6-18%)[39].

3) AML and MDS, therapy related

This group of AML emerged as a consequence of cytotoxic chemotherapy and/or radiation therapy exposure. It accounts for 10-20% of all AML and two major groups in relation to the causative agent are[6,40,41]:

- Alkylant agent/radiation. These diseases often develop 5-6 years post-exposure to the mutagenic agent and are mainly related to alterations in chromosomes 5q and/or 7q.
- Topoisomerase II inhibitor. These AML and MDS begin within 2-3 years following the treatment with the topoisomerase II inhibitor and are associated with translocations involving 11q23 (*MLL*) or 21q22 (*RUNX1*).

Although 20-30% of secondary acute leukaemia occurs in the absence of previous chemo-radiotherapy, the majority emerge as a consequence of the treatment for the first cancer such as lymphomas and breast cancer in adults and acute lymphoblastic leukaemia and central nervous system tumours in children[41].

Survival rates for these patients are lower than for those who suffer *de novo* AML, because of the persistence of the primary malignant disease, injury to organs from prior therapy, chronic immunosuppression, damage to marrow stroma and its association with unfavourable cytogenetics[14,37].

[1] **Pancytopenia**: medical condition characterized by a reduction in the number of red and white blood cells, as well as platelets.

4) AML, noc

This category of AML includes those cases which do not fit the criteria for inclusion in one of the previously described groups; those groups being mainly AML as described by the FAB classification[6,19]. Their diagnosis is based on leukemic cell morphological and cytochemical features and their degree of maturation. The main characteristics of each AML grouped in this category are shown in Table 4.

5) Acute leukaemia (AL) of ambiguous lineage

According to the WHO 2001 classification, AL of ambiguous lineage is a group within AML that accounts for acute leukaemia in which[42,43]:

- Morphologic, cytochemical and immunophenotype features of the proliferating blasts lack evidence to classify them as myeloid or lymphoid leukaemia.

  or
- Morphologic and/or immunophenotypic characteristics are both myeloid and lymphoid cells.

  or
- Morphologic and/or immunophenotypic features are both B and T lymphoid lineages (acute biphenotypic leukaemia).

This group of leukaemia is very rare, comprising less than 4% of all cases of acute leukaemia and, although it can occur at any age, it is more frequent in adults than in children[6].

Cytogenetic alterations are present in a high percentage of the cases. Philadelphia (Ph) chromosome is present in a third of the cases, usually with precursor B lymphoid component. Some cases present t(4;11)(q21;q23) or other 11q23 abnormalities. Cases of myeloid/T leukaemia may present complex karyotypes. Prognosis for patients is unfavourable[44].

Table 4. Main characteristics of acute myeloid leukaemia, not otherwise categorised entities according to the 2001 World Health Organization classification: name of each entity, subtype according to the French-American-British classification, genetic alterations, incidence rate and prognosis[6].

WHO 2001	FAB	CHARACTERISTICS	GENETICS	INCIDENCE	PROGNOSIS
Acute myeloblastic leukaemia, minimally differentiated	M0	No evidence of myeloid differentiation	Complex karyotypes, -5/del(5q), -7/del(7q), +8, del(11q)	<5% of all cases of AML. Present mainly in adults IR = 0.1	Lower remission rate and short survival 5-y RS = 17% (4%-37%)
Acute myeloblastic leukaemia, without maturation	M1	High percentage of BM blasts (≥90% of non-erythroid cells) without maturation	No specific recurrent chromosomal abnormalities associated	5-10% of all AML cases. Present in adults (average age of 46 years) IR = 0.1	Poor, particularly in patients with heperleucocytosis
Acute myeloblastic leukaemia, with maturation	M2	Maturation present in ≥10% maturing cells of neutrophil lineage. Monocytes are <20% of BM cells	No specific recurrent chromosomal abnormalities associated	10% of all AML cases. Present at any age, mainly in patients aged >60 years IR = 0.4	Variable 5-y RS = 15% (8%-24%)
Acute myelomonocytic leukaemia	M4	Proliferation (≥20% of BM cells) of both neutrophil and monocyte precursors	Myeloid-associated, non-specific cytogenetic abnormalities such +8	5-10% of all cases of AML. More common in elderly male patients IR = 0.2	Variable 5-y RS = 13% (5%-23%)
Acute monoblastic and monocytic leukaemia		≥80% of leukemic cells are of monocytic lineage	Myeloid-associated, non-specific cytogenetic abnormalities	IR = 0.2	5-y RS = 15% (6%-30%)
- Acute monoblastic leukaemia	M5a	≥80% of monocytic cells are monoblasts		<5% of AML cases, commonly in young patients	Poor
- Acute monocytic leukaemia	M5b	≥80% of monocytic cells are promonocytes	t(8;16)(p11.2;p13.3) is associated with M5b	<5% of AML cases, commonly in adult male patients	Poor

Table 4 continued on the next page.

WHO 2001	FAB	CHARACTERISTICS	GENETICS	INCIDENCE	PROGNOSIS
Acute erythroid leukaemia				IR = 0.1	5-y RS = 5% (0%-24%)
- Erythroleukaemia (erythroid/myeloid)	M6a	≥50% erythroid precursors in the nucleated cell population of BM and ≥20% myeloblasts in the non-erythroid cell population.	No specific recurrent chromosomal abnormalities associated. Complex karyotypes with multilineage structural abnormalities such - 5/del(5q), -7/del(7q) and +8 are common*	<5% of cases of AML, predominantly in adults	Poor
- Pure erythroid leukaemia	M6b	Proliferation of immature cells of the erythroid lineage (>80% of BM cells) with no myeloblastic component.		Extremely rare. Can occur at any age, including childhood.	Poor
Acute megakaryoblastic leukaemia	M7	≥50% of blasts are of the megakaryocyte lineage **	There is no unique chromosomal abnormality	<5% of all AML cases. Can occur at any age.	Poor
Acute basophilic leukaemia		The primary differentiation is to basophils	There is no consistent chromosomal abnormality identified†	Very rare, <1% of all AML	Poor
Acute panmyelosis with myelofibrosis		Is an acute pan myeloid proliferation with accompanying fibrosis of BM	There is no consistent chromosomal abnormality identified††	Very rare, mainly diagnosed in adults. IR = 0.1	Poor
Myeloid sarcoma		Is a tumour mass of myeloblasts or immature myeloid cells occurring in the BM or in extramedullary sites? It may occur concurrently with acute or chronic myeloid leukaemia or other type of MDS or MPD.	-7, +8, MLL-rearrangement, inv(16), +4, -16, 16q-, 5q-, 20q- and +11 are chromosomal aberrations observed in 55% of cases. *NPM1* mutations are present in 16% of cases.	Median age of patients is 56 years and there is a male predominance (1.2:1) IR = 0.01	Prolonged survival if myeloid is an isolated lesion. If a myeloid sarcoma occurs in a setting of MDS, MPN or AML then prognosis is poor.

*WHO 2001 (World Health Organization classification, 2001); FAB (French-American-British classification); BM (bone marrow); AML (acute myeloid leukaemia); IR (World-population-standardized incidence rates (new cases per 100,000 inhabitants/year) reported in Côte d'Or (Burgundy, France)); 5-y RS (relative survival rates at 5 years in % (95% confidence interval) reported by registry of haematological malignancies of Côte d'Or (Burgundy, France)) Genetics: - (monosomy); + (trisomy); del (deletion); t (translocation); inv (inversion); p (chromosomes short arm); q (chromosomes long arm). * Cases with -5/del(5q), -7/del(7q) and/or complex karyotype should be reclassified as AML with multilineage dysplasia if other requirements for that category are satisfied. ** This category excludes cases of AML with myelodysplasia-related changes, AML with t(1;22)(p13;q13), inv(3)(q21;q26.2), t(3;3)(q21;q26.2) and Down syndrome-related cases. †t(6;9)(p23;q34) which was identified as chromosomal aberration is now not considered as specific abnormality because cases are associated with BCR-ABL1 fusion gene. ††No consistent chromosomal abnormalities are defined because it is no sufficient data. Abnormalities in chromosomes 5 and 7 seem to be frequently involved.*

THE WHO 2008 CLASSIFICATION OF AML

The 3rd edition of the WHO classification was written in the era when genetic and molecular abnormalities were incorporated into the diagnostic algorithms of AML. Since its original publication, numerous genetic advances have taken place, which in turn have led to improved diagnostic criteria for AML, especially in the case of the group classified as AML with recurrent cytogenetic abnormalities. That is why the WHO 2008 classification contains the following significant changes to the diagnosis and classification of AML[13,45]:

1. AML with recurrent cytogenetic abnormalities
   a. Although in the WHO 2001 classification, AML with t(8;21)(q22:q22), inv(16)(p13.1q22) or t(16;16)(p13.1;q22), and APL with t(15;17)(q22;q12) are considered acute leukaemia independently of blast count, in the WHO 2008 classification a minimum of 20% of blasts in PB or BM is required for diagnosis of AML with t(9;11)(p22;q23) or other 11q23 abnormalities, as well as other subgroups of AML (except the rare instance of some cases of erythroleukaemia).
   b. In APL with t(15;17)(q22;q12); *PML-RARA*, variant *RARA* translocations involving other partner genes are recognized separately because not all have typical APL features and some do not respond to ATRA.
   c. The category AML with 11q23 (*MLL*) abnormalities, has been redefined in order to focus on AML with t(9;11)(p22;q23); *MLLT3-MLL*. Translocations involving *MLL* and other genes differing from *MLLT3* should be specified in the diagnosis. Moreover, AML with other abnormalities of *MLL* should be not placed in this category.
   d. Three new AML with well defined cytogenetic abnormalities are included in this category: (1) AML with t(6;9)(p23;q34); *DEK-NUP214*, (2) AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2*);* *RPN1-EVL1*; and (3) AML (megakaryoblastic) with t(1;22)(p13;q13); *RBML5-MKL1*.
   e. Two provisional entities are added: AML with mutated *NPM1* and AML with mutated *CEBPA*. Examination of *FLT3* is also recommended in all cases of cytogenetically normal AML, although it is not defined as an independent entity.

2. AML with myelodysplasia-related changes
   a. The name of this group was changed. While in the WHO 2001 it is known as "AML with multilineage dysplasia", in the WHO 2008 it is called "AML with myelodysplasia-related changes".
   b. Cases of AML are assigned to this category if (1) they have a history of a previous MDS of MDS/MPDs that evolved to AML, (2) they have cytogenetic abnormalities related to

MDS, or (3) at least of 50% of dysplastic cells are presented in 2 or more myeloid lineages.

3. Therapy-related myeloid neoplasms. No distinction is made between AML alkylating agent-related and AML topoisomerase II-inhibitor related.

4. AML, not otherwise specified
   a. This group of AML in the WHO 2001 was AML noc and it is renamed as AML nos in the WHO 2008.
   b. Acute erythroid leukaemia and acute megakaryoblastic leukaemia may be reclassified as AML with myelodysplasia-related changes.
   c. Cases previously reported in this category should be reclassified in the appropriate genetic category if they are associated with new cytogenetically defined entities described in 1d. Down syndrome-related cases are also excluded from this category.

5. Myeloid proliferations related to Down syndrome. This is a new category that incorporates Down syndrome-related cases presenting abnormal myelopoiesis as well as MDS and AML. Because MDS and AML related to Down syndrome are biologically identical, they are grouped together in this category.

6. Myeloid sarcoma. This is considered an independent AML category, whereas in WHO 2001 is grouped within AML, noc.

7. Blastic plasmacytoid dendritic cell neoplasms. This is also a new category of AML that includes most cases previously classified as blastic NK-cell lymphoma/leukaemia or agranular $CD4^+$ $CD56^+$ hematodermic neoplasms.

Table 5 shows the categorizing of AML according to the 4th edition of the WHO classification once all the diagnostic improvements have been applied. As mentioned in Section 2 of this introduction, the main problem for the RCG is that, although improvements to the classification of AML were published, these were not able to be applied because cancer registries do not have an updated version of ICD-O and so those new entities emerging since the WHO 2008 classification do not have their specific registration code.

Table 5. Classification of acute myeloid leukaemia according to the 2008 World Health Organization review[12,13]. Entities written in red correspond to the changes made in the WHO 2008 respect of the WHO 2001.

AML entities
AML with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22);*RUNX1-RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16)(p13.1:q22); *CBFβ/MYH11*
- Acute promyelocytic leukaemia with t(15;17)(q22;q21); *PML/RARα*
- AML with t(9;11)(p22;q23); *MLLT3-MLL*
- AML with inv(3)(q21q23.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
- AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
- Provisional entity: AML with mutated *NPM1*
- Provisional entity: AML with mutated *CEBPA*
AML with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Acute myeloid leukaemia, nos
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukaemia
- Acute monoblastic and monocytic leukaemia
- Acute erythroid leukaemia
- Acute megakaryoblastic leukaemia
- Acute basophilic leukaemia
- Acute panmyelosis with myelofibrosis
Myeloid sarcoma
Myeloid proliferations related to Down syndrome
Blastic plasmacytoid dendritic cell neoplasm

AML (acute myeloid leukaemia); t (translocation); q (chromosomes long arm); p (chromosomes short arm); inv (inversion); nos (not otherwise specified).

3.1.3. Coding rules

According to the Manual for Coding and Reporting Haematological Malignancies, AML have to be registered in accordance with the following rules[15]:

- If AML occurs after an MDS, CMPDs or MDS/MPDs, we have to take into account the date of the AML diagnosis:
  - If the diagnosis of AML occurs less than three months after the initial diagnosis of MDS or CMPDs, we should only register the case as AML with the date of incidence being the day of the first hematologic malignancy diagnosis. AML should count as incidence analysis.
  - If the interval between the two diagnoses is greater than 3 months, the AML is considered a transformation and should be coded as a transformation of a previous hematologic malignancy. In this case AML does not have to be considered in the incidence analysis.
- When BM cytology and BM biopsy report different diagnoses, the BM cytology prevails over the BM biopsy when registering the case. The same occurs with immunophenotype and histochemistry, i.e. when these two tests report conflicting results, immunophenotype predominates.
- The cancer registries have to systematically consider the results of cytogenetic and molecular analysis.
- The term sub-acute is now obsolete as we now have to register AML nos.
- Be careful with the term 'secondary' because it is ambiguous; cancer registries have to critically review clinical records to determine the correct definition of this term:
  - AML secondary to previous haematological malignancies (MDS, CMPDs, MDS/MPDs)
  - AML secondary to cytotoxic treatment (chemotherapy, radiotherapy).
- When an AML is diagnosed and no previous MDS or CMPDs is known, although we may suspect it, we have to register the AML as *de novo*.
- Remember that the classification of AML is hierarchical.

3.1.4. Incidence

AML is considered as a disease mainly found in adults, diagnosed in people aged around 65 years. However, it is sometimes seen in children, especially during the first years of life. Some population-based studies report an incidence rate of 3.0-4.0 cases per 100,000 inhabitants/year; although this increases with age or in patients suffering certain chromosomal disorders such as

Down syndrome or Fanconi anaemia. The male to female ratio is 1:1 and AML accounts for 70% of all acute leukaemia[6,12,46].

Table 6 summarizes some incidence results of AML as reported by the few available published studies.

Table 6. Summary of incidence rates of acute myeloid leukaemia available in the literature.

	All AML	**AML with recurrent cytogenetic abnormalities**	**AML with multilineage dysplasia**	**AML/MDS-therapy related**	**AML, noc**	**AML, nos**	**AL of ambiguous lineage**
Maynadié M. et al. Côte d'Or (France)[39] (ASR_W)	2.5	0.4	0.5	0.1	1.2	0.2	
McNally RJ, et al. U.K.[47] (ASR_W)	M: 1.94 F: 1.51						
Ries LAG EM, et al. U.S.[48] (ASR_W)	2.5						
Roda L. et al. French Polynesia[49] (ASR_W)	M: 3.8 F: 1.9						
Broccia G. et al. Sardinia[50] (ASR_W)	M: 2.4 F: 1.7						
Phekoo K.J. et al. South East England[46] (ASR_W)	2.2						
(ASR_E)	3.0						
Bose S. et al. U.K.[51] (ASR_E)	3.2						
Dores G.M. et al. U.S.[52] (ASR_{US})	3.08	0.4	0.24	0.09	1.68		
Sant M. et al. Europe[5] (CR)	3.62	0.14	0.06		3.37		
Visser O. et al. Europe[4] (CR)	3.7				3.4		
Smith A. et al. U.K.[53] (CR)	4.0						

AML (acute myeloid leukaemia); MDS (myelodysplastic syndromes); noc (not otherwise categorised);nos (not otherwise specified); AL (acute leukaemia); ASR_W (World-population standardized incidence rate); ASR_E (European-population standardized incidence rate); ASR_{US} (US-population 2000 standardized incidence rate); CR (crude incidence rate); M (males); F (females). All results of incidence rates are expressed in new cases per 100,000 inhabitants/year with their 95% confidence interval when this is available.

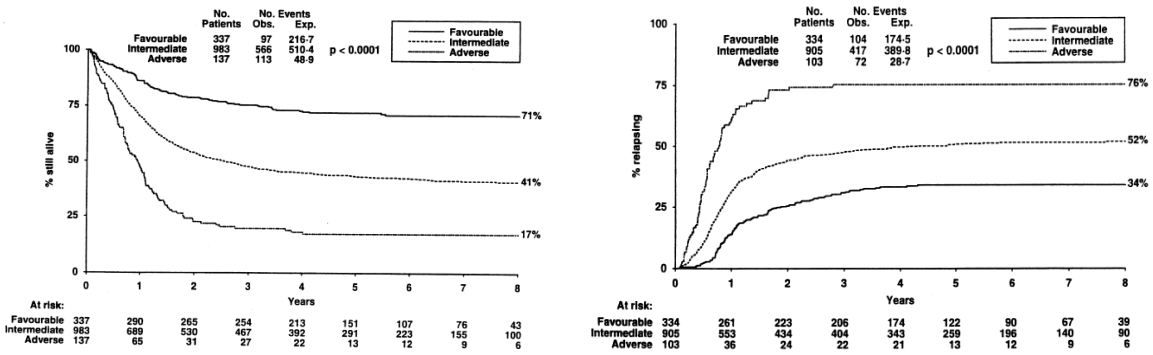
3.1.5. Survival

As previous studies have described, AML is the group with the poorest prognosis within the MM groups, with a 5-year RS of only around 15-18%[4,54]. AML is an aggressive disease that is rapidly fatal without specific therapy. Older patients tend to have a poorer prognostic than younger ones because their tolerance to chemotherapy is low; they exhibit a greater resistance to the primary drug as well as a higher frequency of adverse cytogenetic features[4,6,39,54].

Despite the age of patients at the time of diagnosis, identifying prognostic factors of AML is essential when making therapeutic decisions to determine those patients with a potentially curable disease from those who are incurable. Although some studies have published that cytogenetics is one of the most valuable prognostic factors in AML, the prognostic significance of specific abnormalities is difficult to determine because of the small sample sizes[55-58]. However, the United Kingdom Medical Research Council's Adult and Childhood Leukaemia Working Parties (MRC) have described the importance of diagnostic cytogenetics in AML using data from 1,612 patients[27]. They defined three cytogenetic prognostic groups that predict response to induction therapy, relapse risk and overall survival of AML:

- Favourable prognosis: patients with t(8;21)(q22;q22), t(15;17)(q22;q21) or inv(16)(p13q22).
- Intermediate prognosis: patients with normal karyotype or structural or numerical changes involving trisomy of chromosomes 8, 21 and 22, deletions of 7q and 9q and alterations in 11q23 within others.
- Adverse prognosis: patients with the presence of a complex karyotype, monosomies of chromosome 5 or 7, deletions of 5q or 3q abnormalities.

This cytogenetic stratification system and response to the first course of chemotherapy were the two most important prognostic factors in the MRC AML 10 trial. As shown in Figure 4 (published by Wheatley), statistically significant differences were found when comparing patient survival and relapse according to risk groups detailed before[27].



No. Patients (number of patients); No. Events (number of events); Obs. (observed); Exp. (expected); p (statistically significance); At risk (people at risk).

Figure 4. Survival (A) and relapse (B) of patients diagnosed with AML stratified by prognostic risk groups. Wheatley et al., 1999[29].

Although these risk-stratification groups are currently used, they are now based not only on cytogenetic alterations, but also on molecular findings, which consequently lead to a better stratification of AML patients, especially those with normal karyotype[20]. Established markers testing in AML without chromosomal aberrations are:

- *NPM1*. It is present in approximately 50% of patients with AML without cytogenetic aberrations. Patients with this molecular abnormality and without *FLT3* mutations have a relatively good prognostic, similar to those included in favourable prognosis[59].
- *FLT3* internal tandem duplication (ITD). It is detectable in a third of the cases with normal karyotype. The discovery of this mutation as a single abnormality is related to an unfavourable prognosis. However, if this mutation is identified together with other alterations, such *NPM1* mutation, the negative effect of *FLT3* is reduced[60].

Another molecular marker examined in order to define prognosis of AML patients is *CEBPA*. Although it is not an established marker, mutations in this gene occur in 10% of patients with normal karyotype and it is related to a favourable prognostic[20].

The profound effect of *NPM1*, *FLT3* and *CEBPA* status in AML with normal karyotype suggests the need to assess these mutations in the routine diagnosis of AML. Although the European Leukaemia Net proposes a prognostic system based on four risk groups (favourable, intermediate I, intermediate II and adverse), population-based cancer registries can only make a distinction between three main risk groups: favourable, intermediate and adverse[20].

Information on molecular and cytogenetic tests is not always available for population-based cancer registries, so survival analysis are mainly made using those AML groups proposed by the WHO 2001, and not by risk groups. Thus, the survival results reported by various cancer

registries show that AML with recurrent cytogenetic abnormalities is the group with the longest survival rate, with 5-year RS being nearly 55-65%. In contrast, AML with multilineage dysplasia is the group with the poorest survival rate; a 5-year RS between 4-10% (Table 7)[4,54].

Table 7. Survival of patients diagnosed with acute myeloid leukaemia in different population-based studies and classified according to the 2001 World Health Organization classification.

	All AML	**AML with recurrent cytogenetic abnormalities**	**AML with multilineage dysplasia**	**AML/MDS-therapy related**	**AML, noc**	**AML, nos**	**AL of ambiguous lineage**
Maynadié M. et al. Côte d'Or (France)[39] 5-year RS	19 (15-22)	58 (43-70)	11 (6-18)	2 (0-22)	15 (10-20)	15 (7-26)	
Maynadié M. et al. Europe[54] 5-year RS	17 (16.1-18)	64.1 (57.5-69.9)	4.0 (1.2-9.8)		15.1 (14.2-16.1)		
Visser O. et al. Europe[4] 5-year RS	19				17		
Dores G.M. et al. US[52] 5-year RS	25.4						
Monnereau A. et al. France[61] 5-year NS	M: 17 (16-20) F : 18 (16-21)						
Phekoo K.J. et al. South East England[46] 3-year RS	15 (12-19)						

AML (acute myeloid leukaemia); MDS (myelodysplastic syndromes); noc (not otherwise categorised); nos (not otherwise specified); AL (acute leukaemia); RS (relative survival); NS (net survival); M (males); F (females). Results of survival are expressed in % with their 95% confidence interval when it is available.

3.2. MYELODYSPLASTIC SYNDROMES

3.2.1. Definition and diagnosis

MDS are a heterogeneous group of clonal haematopoietic stem cell diseases characterised by ineffective haematopoiesis in one or more myeloid cell lineages, the presence of cytopenias[2] and morphologic alterations in cells, along with an increased risk of development of an AML[6,62] Neutropenia and/or thrombocytopenia are less frequent and organomegaly[3] is uncommon. Although dysplasia could be accompanied by an increase of myeloblasts in BM, the myeloblasts cannot exceed 20% because this has been the cut-off point for blasts in the diagnosis of AML since the use of the WHO classification[12].

The diagnosis of MDS needs to exclude other pathologies with which share some of the characteristics as well as transient cytopenia and dysplasia. According to the Spanish guidelines (published in 2012) for diagnosis and treatment of MDS and CMML, to diagnose MDS the following has to be considered[62]:

- Clinical data of patients:
    - Presence of anaemia, haemorrhage or infection, physical exploration and size of possible visceromegaly.
    - Exposure to toxic substances, previous chemotherapeutic treatment, immunosupressors, growth factors.
    - Family history of blood diseases and haematological malignancies (AML/MDS)
- PB analysis:
    - Blood count, blood smear and tests to discard other causes of cytopenias.
- BM examination:
    - Cytology of BM for morphologic and genetic analysis. Analysing a minimum of 500-cell differential of all nucleated cells is recommended.
    - Histology of BM if cytology of BM is hypoplastic, presumption of myelofibrosis and for the idiopathic cytopenia of uncertain significance (ICUS).

The specific techniques recommended by the *Grupo Español de Síndromes Mielodisplásicos* /GESMD) and the *Sociedad Española de Hematología y Hemoterapia* (SEHH) to diagnose MDS in special situation included[62]:

- Fluorescence in situ hybridization (FISH) and/or single nucleotide polymorphism/comparative genomic hybridization to diagnose patients when it has not been

[2] **Cytopenia**: reduction in the number of blood cells.
[3] **Organomegaly**: abnormal enlargement of organs.

possible to obtain metaphases, or which are of poor quality or have a normal but less than 20 analysable metaphase karyotype.

- Flow cytometry, useful for detecting phenotypic aberrations that support the diagnosis of MDS and for following the minimal residual disease in patients who could be submitted to allogeneic stem cell transplantation.
- Periodic Acid–Schiff staining in extensions of marrow aspirate to observe the reaction of erythroid lineage.
- Molecular studies:
    - Clonal studies for woman with ICUS.
    - *JAK2* V617F mutation in patients with thrombocytosis.
    - *PDGFRA*, *PDGFRB* and *FGFR1* gene analysis for patients with eosinophilia.
    - *KIT* gene in MDS associated with systemic mastocitosis.
- Myeloid progenitors culture in cases where the study of cytopenias of undetermined significance are required.

3.2.2. Classification

MDS can be classified depending on their aetiology or their morphology. According to their aetiology we can distinguish between:

- Primary MDS or *de novo*. This MDS is diagnosed when the patient has never before received chemotherapy or radiotherapy exposure to treat any other diseases. Possible aetiologies for this type of MDS include benzene exposure, cigarette smoking, exposure to agricultural chemicals or solvents and family history of haematopoietic neoplasms.
- Secondary MDS - exposure to mutagenic agents. This type included patients with MDS who have been treated with chemotherapy or radiotherapy for a previous cancer.

In relation to morphological features, classifications of MDS may change (FAB 1982, WHO 2001 and WHO 2008) but they are principally based on[6,13,63]:

- Percentage of blasts in the BM and PB.
- Degree of dysplasia.
- Presence of ring sideroblasts

FAB CLASSIFICATION OF MDS

In 1982, the FAB group formally introduced the term MDS and proposed detailed guidelines for its diagnosis and classification based on the percentage of blasts in BM and PB, and the lineages affected by the dysplasia. This classification recognized 5 subtypes of MDS: refractory anaemia (RA), refractory anaemia with ringed sideroblasts (RARS), refractory anaemia with excess of blasts (RAEB), refractory anaemia with excess of blasts in transformation (RAEB-T) and chronic myelomonocytic leukaemia (CMML)[64]. Principal morphological characteristics of each subgroup are defined in Table 8.

Table 8. French-American-British classification of myelodysplastic syndromes with their corresponding main characteristics[64]: percentage of blasts in peripheral blood and bone marrow, percentage of monocytes in peripheral blood and percentage of ringed sideroblasts in bone marrow.

MDS subtype	PB blasts (%)	BM blasts (%)	PB monocytes (%)	BM RS (%)
RA	<1	<5	$<1x10^9$/L	<15
RARS	<1	<5 No Auer rods[4]	$<1x10^9$/L	>15
RAEB	<5	5-19 No Auer rods	$<1x10^9$/L	Indifferent
RAEB-T	>5	20-29 No Auer rods	$<1x10^9$/L	Indifferent
CMML	<5	0-20	$>1x10^9$/L	Indifferent

MDS (myelodysplastic syndrome); PB (peripheral blood); BM (bone marrow); RS (ringed sideroblasts); RA (refractory anaemia); RARS (refractory anaemia with ringed sideroblasts); RAEB (refractory anaemia with excess of blasts); RAEB-T (refractory anaemia with excess of blasts in transformation); CMML (chronic myelomonocytic leukaemia).

Although the FAB classification for MDS provided a durable framework into which biological, clinical information and some genetic abnormalities could be integrated, it presented controversies related to specific nomenclature, diagnostic criteria and/or scientific significance.

[4] **Auer rods**: clumps of azurophilic granular material that form elongated needles seen in the cytoplasm of leukemic blasts.

WHO 2001 CLASSIFICATION OF MDS

In the 2001WHO publication, incorporating genetic alterations to morphologic and biological features was the main objective to accurately define MDS[6].

This classification uses many of the concepts and definitions from the FAB classification system, but presents some differences as a consequence of definition refinements:

1) Because the cut-off point of blasts in BM decreased from 30% to 20% for AML diagnosis, patients with MDS can not present more than 20% of blasts. As a consequence, the subgroups RAEB-T in the FAB classification were therefore obsolete.
2) The definition of RA and RARS was refined and refractory cytopenia with multilineage dysplasia (RCMD) became a new subgroup of MDS.
3) RAEB can be classified into two subtypes depending on percentage of blasts in BM and PB: RAEB type 1 and RAEB type 2.
4) MDS with only 5q- aberrations are recognized as a single entity.
5) CMML was eliminated from MDS subgroups and categorized into a newly defined myeloid malignancies group because it shares characteristics of both myeloproliferative and myelodysplasia features.

Once these concepts were applied, the WHO 2001 for MDS distinguished 6 subgroups based on morphologic and genetic characteristics (Table 9). Each of these categories is defined in section 3.2.2.1.

Table 9. Classification of myelodysplastic syndromes, according to the 2001 World Health Organization classification[6].

Myelodysplastic syndromes	**ICD-O-3 codes**
Refractory anaemia	9980
Refractory anaemia with ringed sideroblasts	9982
Refractory cytopenia with multilineage dysplasia	9985
Refractory anaemia with excess of blasts	9983
Myelodysplastic syndrome associated with isolated del(5q) chromosome abnormality (“5q- syndrome”)	9986
Myelodysplastic syndromes, unclassifiable (MDS-u)	9989

3.2.2.1. Principal features of MDS according to the WHO 2001 classification

1) Refractory anaemia

This is an uncommon disease (representing 5-10% of all MDS cases) which affects above all older adults. It is characterized by unilineage dysplasia involving the erythroid series. Although the degree of dysplasia varies, the detection of dyserythropoiesis is essential for its diagnosis. A diagnosis of RA needs to exclude other causes of erythroid abnormalities: drug and toxin exposure, viral illness, immunologic disorders, congenital abnormalities and vitamin deficits. Myeloblasts are <1% in PB and <5% in BM. The erythroid precursors in the BM vary from decreased to markedly increased and dyserythropoiesis varies from slight to moderate. Cytogenetic abnormalities included del(20q), +8 and abnormalities of 5 and/or 7, and despite being useful for RA diagnosis, they are not specific. The average observed survival (OS) of patients with RA is approximately 66 months and close to 6% of them progress to AML[6,65].

2) **Refractory anaemia with ringed sideroblasts**

RARS accounts for 10-12% of all cases of MDS, being more frequent in older patients and in males than in females. Anaemia and dysplasia only in the erythroid lineage are the requisites for its diagnosis, especially with a minimum detection of 15% ringed sideroblasts in a BM smear. Myeloblasts are less than 5% in BM and are absent in the PB. The diagnosis may exclude other causes because anti-tuberculosis treatment and alcoholism can also produce ringed sideroblasts. Although chromosomal aberrations can be present in RARS, they are found in less than 10% of cases. The overall median survival of patients is 6 years and 1-2% of all cases evolve to AML[6,65].

3) Refractory cytopenia with multilineage dysplasia

This is an MDS found in older individuals and represents 24% of MDS. Bi-cytopenia and dysplasia in a minimum of 10% of the cells in two or more of the myeloid lineages are necessary to diagnose it. Myeloblasts are less than 1% in PB and less than 5% in BM. Ringed sideroblasts can be identified, but are less than 15% of nucleated red blood cells. If ringed sideroblasts are more than 15% of erythroid precursors, the case should be classified as refractory cytopenia with multilineage dysplasia and ringed sideroblasts (RCMD-RS).

Clonal chromosomal aberrations find in 50% of cases included +8, -7, del(7q), -5, del(5q), and del(20q), as well as complex karyotypes. The overall median survival of patients with RCMD is approximately 33 months, although it varies depending on the degree of cytopenia and dysplasia. About 11% of patients progress to AML[6,65].

4) Refractory anaemia with excess of blasts

RAEB is an MDS affecting patients over 50 years old and represents nearly 40% of all MDS. It is identifying by the presence of 5-19% of blasts in BM. Two categories of RAEB with different survival and evolution to AML can be differentiated according to the percentage of blasts in PB and BM[6,65]:

- RAEB type 1: 5-9% of blasts in BM and less than 5% of blasts in PB. Approximately 25% of cases progress to AML and median survival is nearly 18 months.
- RAEB type 2: 10-19% of blasts in BM and 5-19% of blasts in PB. A third of patients develop AML and the median survival is 10 months.

Around 30-50% of cases present abnormal cytogenetic such +8, -5, del(5q), -7, del(7q) and del(20q), as well as complex karyotypes. Because of the high percentage of blasts, this group of MDS is the most likely to evolve into AML.

5) Myelodysplastic syndrome associated with isolated del(5q) chromosome abnormality ("5q- syndrome")

The "5q- syndrome" occurs predominantly in middle-aged to older women. This pathology is recognized by an isolated del(5q) involving the bands q31-q33 of chromosome 5 as a single cytogenetic abnormality. The BM is usually hipercellular or normocellular with normal to increased megakaryocytes, and the number of blasts is less than 5%. FISH analysis has confirmed presence of this aberration in differentiating erythroid myeloid and megakaryocytic cells. Patients diagnosed with this pathology have relative good survival (median follow-up 145 months), especially those treated with lenalidomide, an immunomodulatory drug approved by the Food and Drug Administration (FDA) in December 2005 as a first-line therapy for low and intermediate risk MDS with isolated 5q-. Very few cases progress to AML (<20%)[66-68].

6) MDS, unclassifiable

In this category we find all MDS which lack appropriate evidence to classify them into one of the previous groups (RA, RARS, RCMD, RAEB and 5q- syndrome). While the 2001 WHO classification did not describe the morphological criteria to diagnose this category, the 2008 WHO publication does describe three possible situations which would qualify patients for inclusion in this category[6,12,13]:

1. Patients with refractory cytopenia with unilineage dysplasia (RCUD) or RCMD with 1% blasts in PB.
2. Cases of MDS with unilineage dysplasia associated with pancytopenia.
3. Patients with persistent cytopenia(s) with fewer than 1% of blasts in PB and fewer than 5% in BM, unequivocal dysplasia (dyserythropoiesis, dysgranulopoiesis and/or dysmegakaryocytopoiesis) in less than 10% of the cells in one or more myeloid lineage, and which have typical cytogenetic abnormalities of MDS (chromosomal alterations involving chromosomes Y, 5, 20 and/or 7).

The epidemiology of this type of MDS is unknown, and neither is the percentage of patients whose syndrome transforms into AML or the disease survival rate known.

Principal features of MDS syndromes according to the 2001 WHO classification are summarised in Table 10.

Table 10. Summary of principal features of myelodysplastic syndromes, according to the 2001 World Health Organization classification[6].

MDS	Cytopenias	PB blasts (%)	BM blasts (%)	BM RS (%)	Dysplasia
RA	Anaemia	<1	<5	<15	Erythroid
RARS	Anaemia	0	<5	≥15	Erythroid
RCMD	Bicytopenia or pancytopenia	<1	<5	<15	≥2 myeloid cell lines
RAEB-1	Cytopenias	<5	5-9	Indifferent	Unilineage or multilineage
RAEB-2	Cytopenias	5-19	10-19	Indifferent	Unilineage or multilineage
MDS-u	Cytopenias	<1	<5	Indifferent	Unilineage
5q- syndrome	Anaemia	<5	<5	Indifferent	Megakaryocytes with hypolobated nuclei

MDS (myelodysplastic syndrome); PB (peripheral blood); BM (bone marrow); RS (ringed sideroblasts); RA (refractory anaemia); RARS (refractory anaemia with ringed sideroblasts); RCMD (refractory cytopenia with multilineage dysplasia); RAEB (refractory anaemia with excess of blasts); MDS-u (myelodysplastic syndrome, unclassifiable); 5q- syndrome (myelodysplastic syndrome associated with isolated del(5q) chromosome abnormality).

THE WHO 2008 CLASSIFICATION OF MDS

Although the RCG uses the 2001 WHO classification to categorize MDS in concordance with their corresponding ICD-O-3 code, a new revision of MDS classification with several changes was published in 2008[13,45]:

1) 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.

2) RCUD is considered a new category of MDS. Patients who present unilineage dysplasia associated with refractory anaemia (unilineage erythroid dysplasia), refractory neutropenia (unilineage dysgranulopoiesis), or refractory thrombocytopenia (unilineage dysmegakaryocytopoiesis), and who have fewer than 1% of blasts in PB and fewer than 5% in BM have to be classified in this category.

3) All cases presenting multilineage dysplasia are now grouped within the RCMD category, independently of the percentage of ringed sideroblasts (more or less than 15%). RCMD-RS is now incorporated in RCMD.

4) Patients with 2-4% of blasts in PB and less than 5% in BM should be diagnosed as having RAEB type 1 if other clinical and laboratory findings of MDS are present.

5) Refractory cytopenia of childhood has been added as a provisional unit to include children with cytopenia(s) with less than 2% of blasts in PB and less than 5% in BM and evidence of dysplasia in 2 or more lineages. For children with 2-19% of blasts in PB and/or 5-19% in BM, the MDS sub-classification should be made using the same criteria used for adults.

So, after incorporating the new scientific and clinical information to refine diagnostic criteria for MDS diagnosis, the WHO 2008 classification categorizes this myeloid group into the categories shown in Table 11.

Table 11. 2008 World Health Organization classification of myelodysplastic syndromes[12]. Entities written in red correspond to the changes made in the WHO 2008 respect of the WHO 2001.

MDS subtype	Cytopenias	PB blasts (%)	BM blasts (%)	BM RS (%)	Dysplasia
RCUD	Anaemia	<1	<5	<15	Erythroid
RARS	Anaemia	0	<5	≥15	Erythroid
RCMD	Bicytopenia or pancytopenia	<1	<5	<15	≥2 myeloid cell lines
RCMD-RS	Bicytopenia or pancytopenia	<1	<5	≥15	≥2 myeloid cell lines
RAEB-1	Cytopenias	<5	5-9	Indifferent	Unilineage or multilineage
RAEB-2	Cytopenias	5-19	10-19	Indifferent	Unilineage or multilineage
MDS-u	Cytopenias	<1	<5	Indifferent	Unilineage
5q- syndrome	Anaemia	<5	<5	Indifferent	Megakaryocytes with hypolobated nuclei

MDS (Myelodysplastic syndrome); PB (peripheral blood); BM (bone marrow); RS (ringed sideroblasts); RCUD (refractory cytopenia with unilineage dysplasia); RARS (refractory anaemia with ringed sideroblasts); RCMD (refractory cytopenia with multilineage dysplasia); RCMD-RS (refractory cytopenia with multilineage dysplasia and ringed sideroblasts) RAEB (refractory anaemia with excess of blasts); MDS-u (myelodysplastic syndrome, unclassifiable); 5q- syndrome (myelodysplastic syndrome associated with isolated del(5q) chromosome abnormality).

3.2.3. Coding rules

The main coding rules for registration of MDS are those detailed below[15]:

- If RARS is diagnosed, it is important to check whether another tumour is present. If any associated tumour is present, this is what must be recoded and not the RARS because it is considered a reactive process in this context.

- BM aspirate takes precedence over the results of a BM biopsy, except when the aspirate is of poor quality or when marrow aplasia or marrow hyperplasia is present.

- To codify MDS as therapy-related MDS this has to be diagnosed after a cytotoxic treatment such as chemotherapy or radiotherapy. According to the WHO 2001 classification, this MDS should be categorized together with therapy-related AML.

- When a MDS is diagnosed before the ICD-O-3, this case should be registered as ICD-O-2 recommendations, in order to be consistent with other data for the corresponding year. In this situation, MDS should be recoded as benign.

- If MDS transforms into AML, check the percentage of blasts in the BM.

    - $< 20\%$ of blasts in BM: register RAEB
    - $\geq 20\%$: recode AML.
        - In this case it is important to consider the time between the two diagnoses (MDS and AML) as is described in the AML coding rules section.

- RAEB-T (20-30% of blasts in BM) is an obsolete code from the WHO 2001 classification, and is now included in AML group.

- Diagnose of 5q- MDS needs a cytogenetic analysis.

- MDS should be present in the absence of alcoholism, vitamin deficiency and liver failure.

- The percentage of blasts prevails over the percentage of ringed sideroblasts when both are present in a diagnosis.

3.2.4. Incidence

MDS occurs mainly in older adults, with a median age of approximately 70 years, and with a male predominance[69]. Although it was described an incidence rate of 3-5 new cases per 100,000 inhabitants/year, an increase to 20 new cases per 100,000 inhabitants/year was observed in patients over the age of 70[6]. Because MDS were not considered to be malignant in

the ICD-O-2 and the classification systems changed over time, discrepancies in incidence rates were found in published studies (Table 12)[6,9] .

Table 12. Incidence rates of myelodysplastic syndromes classified according the 2001 World Health Organization published in some studies.

	All MDS	RA	RARS	RCMD	RAEB		5q-	MDS-u
					RAEB-1	RAEB-2		
Maynadié M. et al. Côte d'Or (France)[39] (ASR_W)	1.3	0.3	0.4	0.0	0.5		0.0	0.0
Maynadié M. et al. France[70] (ASR_W)	1.7							
McNally RJ, et al. U.K.[47] (ASR_W)	M: 1.71 F: 1.06	M: 0.73 F: 0.54	M: 0.25 F: 0.15		M: 0.46 F: 0.23			
Phekoo K.J. et al. South East England[46] (ASR_W)	2.24	0.85	0.23		0.32			
(ASR_E)	3.47	1.37	0.37		0.5			
Bose S. et al. U.K.[51] (ASR_E)	1.9							
Neukirchen J. et al. Germany[71] (ASR_E)	2.51 (2.16-2.85)	0.30 (0.18-0.42)	0.14 (0.05-0.22)	1.00 (0.78-1.22)	0.25 (0.14-0.36)	0.36 (0.23-0.49)	0.06 (0.01-0.11)	
Ma X. et al. U.S.[72] (ASR_{US})	3.4 (3.3-3.5)							
Visser O. et al. Europe[4] (CR)	1.5							
Sant M. et al. Europe[5] (CR)	1.82 (1.76-1.88)							
Smith A. et al. U.K.[53] (CR)	3.7 (3.4-3.9)							
Iglesias M. et al. Spain[73] (CR)	8.07							

MDS (myelodysplastic syndromes); RA (refractory anaemia); RARS (refractory anaemia with ringed sideroblasts); RCMD (refractory anaemia with multilineage dysplasia); RAEB (refractory anaemia with excess of blasts); RAEB-I (RAEB type I), RAEB-II (RAEB type II), 5q- (MDS associated with isolated del(5q) chromosome abnormality; 5q- syndrome); MDS-u (MDS, unclassifiable); ASR_W (World-population standardized incidence rate); ASR_E (European-population standardized incidence rate); ASR_{US} (US-population 2000 standardized incidence rate); CR (crude incidence rate); M (males); F (females). All results of incidence rates are expressed in new cases per 100,000 inhabitants/year with their 95% confidence interval when this is available.

3.2.5. Survival

MDS are a heterogeneous group of MMs presenting different prognoses, global survival rates and risk of evolution into AML. This, as well as advanced patient age, their comorbidities and associated high morbidity rate and mortality of therapeutic healing potential alternatives, complicate the selection of treatment for an individual patient[6,74,75]. The International Prognostic Scoring System (IPSS), created in 1997, was used to adapt accurate and individualized treatment based on the estimated risk of each patient (Table 13)[6,76]. This scoring system is still used today and differentiates patients with MDS into four risk groups depending on percentage of blasts in bone marrow, karyotype abnormalities and number of cytopenias:

- Low risk group: 0 points of IPSS and median overall survival of 5.7 years.
- Intermediate-1 risk group: 0.5-1 points of IPSS and median overall survival of 3.5 years.
- Intermediate-2 risk group: 1.5-2 points of IPSS and median overall survival of 1.1 years.
- High risk group: 2.5-3.5 points of IPSS and median overall survival of 0.4 years.

Table 13. International Prognostic Scoring System (IPSS)[6,76].

Prognostic variables	**0 points**	**0.5 points**	**1 point**	**1.5 points**	**2 points**
BM blasts (%)	<5	5-10		11-20	21-30
Karyotype	Good: Normal, -Y, del(5q), del(20q) as a single abnormalities	Intermediate: Other single or double abnormalities	Poor: Complex karyotype (≥3 abnormalities) or abnormalities in chromosome 7		
Cytopenias	0-1	2-3			

BM (bone marrow); - (monosomy); del (deletion); q (chromosomes long arm).

Despite the IPSS having been used for many years, this prognostic scoring system has serious weaknesses: the cut-off of percentage of blasts in bone marrow was 30% (FAB classification) instead of 20% (as is described in the WHO classification), unavailability of cytogenetics in over 30% of patients, inadequate relation between cytogenetic abnormalities and risk group stratification (especially for those cases of intermediate risk groups), excessive importance of blasts in association with cytogenetics, as well as lack of identifying other characteristics with an independent prognostic[6,74,75,77]. Taking into account these limitations, a new prognostic index

based on the 2001 WHO classification was proposed in 2007: WHO classification-based prognostic scoring system (WPSS)[78]. Although this new system includes transfusion dependence and recognizes the prognostic value of the 2001 WHO classification to categorize patients into risk groups, its use is not wide spread, probably because no relevant improvement, with respect to the IPSS, has yet been demonstrated.

Over the last few years, newer cytogenetic groupings along with additional variables such haemoglobin value, platelet count, and absolute neutrophil count levels (apart from percentage of blasts) have been identified as prognostic values in MDS. These new prognostic values are compiled in the Revised-IPSS (R-IPSS) published by Greenberg et al. in 2012, and which grouped MDS into five risk factor groups, based on, in particular, cytogenetic abnormalities (Tables 14)[78,79]:

- Very low risk group: 0-1.5 points of R-IPSS. Median OS: 5.4 years.
- Low risk group: >1.5-3 points of R-IPSS. Median OS: 4.8 years.
- Intermediate risk group: >3-4.5 points of R-IPSS. Median OS: 2.7 years.
- High risk group: >4.5-6 points of R-IPSS. Median OS: 1.5 years.
- Very high risk group: >6 points of R-IPSS. Median OS: 0.7 years.

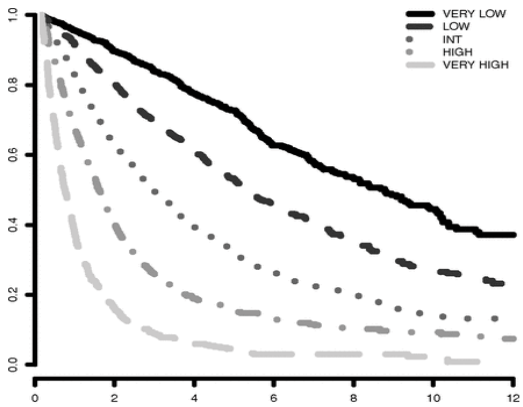
Survival differences between risk groups were well represented in Figure 5.

Table 14. Revised-International Prognostic Scoring System (IPSS-R)[79].

Variables	0 points	0.5 points	1 point	1.5 points	2 points	3 points	4 points
Cytogenetics abnormalities group*	Very low		Low		Intermediate	High	Very high
BM Blasts (%)	0-2		3-4.9		5-10	<10	
Haemoglobin (g/dL)	≥10		8-9.9	<8			
Platelets ($x10^9$/L)	≥100	50-99	<50				
ANC ($x10^9$/L)	≥0.8	<0.8					

BM (bone marrow); ANC (absolute neutrophil count)

*Cytogenetic abnormalities groups: Very Low (-Y, del(11q)); Low (normal karyotype, del(5q), del(12p), del(20q), double including del(5q)); Intermediate (del(7q), +8, +19, i(17q), any other single or double independent clones); High (-7, inv(3)/t(3q), double including -7/del(7q), complex: 3 abnormalities); Very High (Complex karyotype with more than 3 abnormalities.



p<0.001 (statistically significant)

Figure 5. Survival and acute myeloid leukaemia evolution of patients diagnosed with myelodysplastic syndromes, based on Revised-International Prognostic Scoring System (R-IPSS) risk-based categories[79].

As it is difficult for cancer registries to report survival rates of MDS according to risk-stratification groups, and also knowing that MDS are not considered to be malignant in the ICD-O-2, survival rate comparisons are made based on WHO 2001 groupings. Table 15 summarizes survival rates reported in the various works published, and indicates that RA and RARS are the two entities with better survival within the MDS groups, whereas RAEB is the disease with the lowest prognosis, as it is suggested by R-IPSS[6,79].

Table 15. Survival of patients diagnosed with myelodysplastic syndromes according to the 2001 World Health Organization classification.

	All MDS	RA	RARS	RCMD	RAEB		5q-	MDS-u
					RAEB-1	RAEB-2		
Maynadié M. et al. Europe[54] 5-year RS	30.8 (28.8-32.8)	49 (42.7-55.0)	56.1 (47.1-64.2)		11.5 (7.5-16.5)			27.1 (24.7-29.5)
Maynadié M. et al. Côte d'Or[39] (France) 5-year RS	45 (38-52)	57 (39-72)	70 (55-80)	20 (0.5-68)	22 (14-30)		47 (10-77)	31 (5-63)
Monnereau A. et al. France[61] 5-year NS	M: 40 (36-44) F: 51 (47-56)							
Maynadié M. et al. France[70] 5-year OS	23							
Ma X. et al. U.S.[72] 3-year RS	42							
Rollison D.E. et al. U.S.[80] 3-year RS	45 (43-47)							
Phekoo K.J. et al. South East England[46] 3-year RS	45 (41-49)	54 (47-60)	58 (45-69)		21 (13-32)			44 (36-52)

MDS (myelodysplastic syndromes); RA (refractory anaemia); RARS (refractory anaemia with ringed sideroblasts); RCMD (refractory anaemia with multilineage dysplasia); RAEB (refractory anaemia with excess of blasts); RAEB-I (RAEB type I), RAEB-II (RAEB type II), 5q- (MDS associated with isolated del(5q) chromosome abnormality; 5q- syndrome); MDS-u (MDS, unclassifiable); RS (relative survival); NS (net survival); OS (observed survival); M (males); F (females). Results of survival are expressed in % with their 95% confidence interval when this is available.

3.3. MYELOPROLIFERATIVE NEOPLASMS

3.3.1. Diagnosis and treatment

MPN, known as CMPDs in the WHO 2001, are clonal haematopoietic stem cell disorders characterized by the proliferation in BM of one or more myeloid cell lineages, including granulocytes, erythrocytes and megakaryocytes. These diseases present a relatively normal and effective maturation, resulting in an increased number of cells such as granulocytes, red blood cells or platelets in PB. Organomegaly (splenomegaly and hepatomegaly) are commonly found in patients diagnosed with CMPDs. An increase in the percentage of blasts (10-19%) indicates an accelerated stage of the disease or a transformation into an acute stage[6,12].

The diagnosis of CMPDs has been challenging because of similarities among CMPDs and reactive forms of granulocytic, erythroid and/or megakaryocytic hyperplasia characterized by an increased count of mature PB cells. To ensure a diagnosis of CMPDs the following is required[29,81]:

- Clinical data of patient and MPN familial study.
- BM aspirate/biopsy.
- PB tests for evaluating.
- Cytogenetic and molecular analysis.

The WHO diagnostic algorithms for CMPDs are based on clinical, laboratory, morphologic and genetic/molecular findings[29,81-83]. When a CMPDs is suspected, the haemogram test should be correlated with the PB smear and, depending on the suspected disease, additional laboratory studies such as serum erythropoietin, serum lactate dehydrogenase, basic chemical profiles including liver functions, iron studies, and coagulation and platelet function studies may be required to confirm the diagnosis. Cytogenetic and molecular studies based on the *BCR-ABL1* fusion gene and *JAK2* V617F mutation detection are essential to make an accurate diagnosis. Before 2005, CMPDs were distinguished mainly by the presence or absence of the *BCR-ABL1* fusion gene. Since the discovery of the *JAK2* V617F mutation, the diagnosis of CMPDs Ph negative has been simplified. Notwithstanding, cytogenetic and molecular studies may be performed from PB, and a BM study is recommendable for all patients suspected of having a myeloid neoplasm because it is the best material for routine cytogenetic analysis.

3.3.2. Classification

FIRST APPROACH TO MYELOPROLIFERATIVE DISORDERS

William Dameshek (1900-1961), was the first to recognize the 'myeloproliferative disorders (MPDs)' as a distinct clinicopathologic category within myeloid malignancies in 1951[84]. These disorders are mainly characterized by an increased and effective proliferation of one or more myeloid cell lineages in the BM associated to increased PB parameters. Chronic myelogenous leukaemia (CML), PV, ET, PMF and erythroleukaemia (Di Guiglielmo syndrome) are grouped together, based on their clinical and histological similarities. Over the years, erythroleukaemia has been re-classified as acute erythroid leukaemia and moved out of MPN into the AML group.

THE WHO 2001 CLASSIFICATION OF CMPDs

In the 2001 WHO classification, MPDs are assigned the category of myeloid malignancies such as CMPDs, including chronic neutrophilic leukaemia (CNL), chronic eosinophilic leukaemia/hypereosinophilic syndrome (CEL/HES) and CMPDs unclassifiable (CMPDs-u) (Table 16)[6,10]. These diseases are primarily separated according to the presence or absence of the Ph chromosome or the *BCR-ABL1* fusion gene, resulting from t(9;22)(q34;q11). CML is the only entity catalogued as Ph positive, whereas all other so-called *BCR-ABL1* negative. This last group comprises PV, PMF, ET and CMPDs-u cases, along with less frequent cases of pathologies such as CNL, and HES/CEL. The classification of the CMPDs without the *BCR-ABL1* fusion gene requires careful correlation between morphologic finding in BM or PB and the clinical and laboratory results.

Table 16. Chronic myeloproliferative diseases (CMPDs) classification, according to the 2001 World Health Organization guidelines[6].

CMPDs	ICD-O-3 codes
Chronic myelogenous leukaemia	9863, 9875
Chronic neutrophilic leukaemia	9963
Chronic eosinophilic leukaemia and hypereosinophilic syndrome	9964
Polycythaemia vera	9950
Primary myelofibrosis	9961
Essential thrombocythaemia	9962
Chronic myeloproliferative disease, unclassifiable	9960, 9975

3.3.2.1. Principal characteristics of CMPDs according to WHO 2001

1) Chronic myelogenous leukaemia

This is one of the most common entities within CMPDs, with an incidence of 1-1.5 new cases per 100,000 inhabitants/year. Even though it is a disease that can occur at any age, it is more frequent in patients aged 60 years and over and a male predominance has been found[6].

CML is originated in an abnormal pluripotent BM stem cell and it is mainly associated with the Ph chromosome and/or the *BCR-ABL1* fusion gene, found in all myeloid lineages and in some lymphoid cells. This fusion gene, detected in 90-95% of all cases of CML, is the result of a reciprocal translocation between chromosome 9 and chromosome 22 (t(9;22)(q34;q11)). The product of this fusion gene is the BCR-ABL1 tyrosine kinase which remains continuously activated and is thought to be primarily responsible for the pathogenesis of CML (Figure 6)[6].

The natural course of this disease is the progression of an indolent chronic phase (CML-CP) to more aggressive transformed stages: accelerated phase (CML-AP) and blast phase (CML-BP)[85]. Although the median overall survival rate has increased to 5-7 years since the introduction of targeted anti-tyrosine kinase therapy such as Imatinib in 2001, allogeneic stem cell transplantation remains the only curative therapy[85-87].

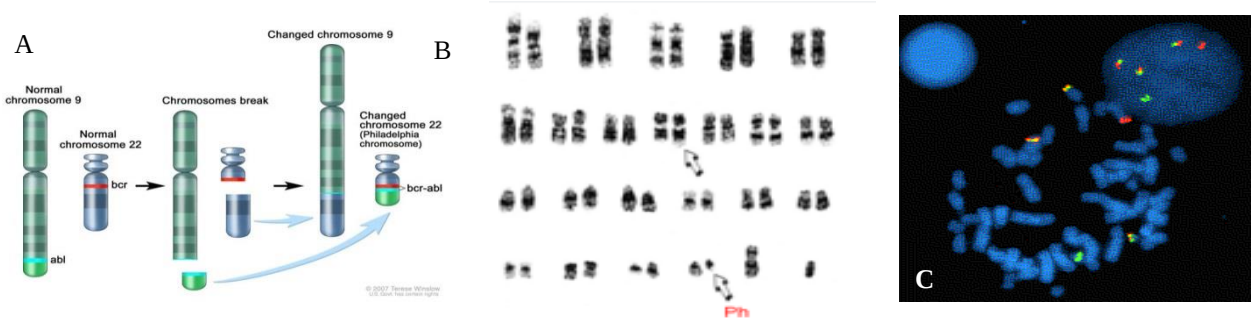


Figure 6. Chromosome Philadelphia positive in chronic myeloid leukaemia. A) Diagram of translocation 9;22; B) Translocation 9;22 present in a karyotype; C) Presence of translocation 9;22 using fluorescence in situ hybridization[88-90].

2) Chronic neutrophilic leukaemia

CNL is a rare CMPD, with less than 100 cases reported (2001 WHO classification) and equally distributed between males and females. Diagnostic criteria for its diagnosis include PB leukocytosis, BM hipercellularity and hepatomegaly. The Ph chromosome is not detected, cytogenetic is principally normal and it is necessary to exclude other MDS, CMPDs and MDS/MPDs for its diagnosis. Patients with CNL can survive from 6 months to more than 20 years[6].

3) Chronic eosinophilic leukaemia and hypereosinophilic syndrome

CEL and HES are CMPDs characterized by an increase of eosinophils in the PB, BM and peripheral tissues.

CEL is defined by an expansion of clonal eosinophils (>1.5x10^9/L) in PB, no presence of the Ph chromosome and less than 20% of blasts in BM. If it is impossible to detect clonal eosinophilia and there is no increase of blasts in BM, clinicians have to diagnosis HES. While these pathologies may be rare, their incidence individually is unknown. HES is more frequent in males than females (male sex ratio 9:1) and even though it can occur at any age, a peak of incidence is found in the fourth decade of life. Even survival rates vary as a 5-year survival rate in 80% of patients with HES was described, whereas splenomegaly and increasing blasts in BM are related to an unfavourable prognostic[6,10].

4) Polycythaemia vera

The increase of red blood cells present in PV is independent of the mechanisms that regulate erythropoiesis. A very useful diagnostic test in PV is to detect the *JAK2* V617F mutation (discovered in 2005) as it is present in more than 95% of all cases[12,91]. Cytogenetic abnormalities such as +8, +9, del(20q), del (13q) and del(1p) are found in only 10-20% of cases. Its incidence varies from 0.02 to 2.8 new cases per 100,000 inhabitants/year and with a male predominance from 1-2:1. The mean age at diagnosis is near 60 years old and it rarely affects patients under 20 years of age. Patient survival varies from few months for those untreated to more than 10 months when they are treated with the therapies currently available. Only 2-3% of patients evolve to AML[6,10,13,91].

5) Primary myelofibrosis

People with PMF present a clonal proliferation of mainly megakaryocyte and granulocyte precursors in the BM with reactive, marrow fibrosis[6,10]. Around 30-40% are asymptomatic at diagnosis time and are discovered by a blood test or a splenomegaly during a routine examination. Its estimated incidence is around 0.5-1.5 new cases per 100,000 inhabitants/year, and occurs, independent of gender, predominantly in patients aged 70 years and over. Although no specific cytogenetic has been identified, del(13q), del(20q) and partial trisomy 1 are the most common recurrent abnormalities and *JAK2* V617F is detected in 50% of ET cases[82]. The median overall survival for these patients is around 5-years post-diagnosis, although it can vary from months to years. Between 5% and 30% of patients develop AML during the course of this disease[6].

6) Essential thrombocythaemia

This clonal CMPD is characterized by an excessive number of platelets produced by mature megakaryocytes. Its incidence is around 0.6-2.5 new cases per 100,0000 inhabitants/year and it is more frequently diagnosed in patients aged between 50-60 years[92,93]. Most cases are asymptomatic so its diagnosis is by routine blood tests, which means this pathology is underreported in cancer registries. An abnormal karyotype is only found in 5-10% of patients and although there is no specific recurrent cytogenetic abnormality, del(13q22), +8 and +9 are the most common alterations in ET. As the *JAK2* V617F mutation is present in around 50-70% of the cases this facilitates its diagnosis. Survival rates reported for patients with ET are more than 10 years and less than 5% of cases evolve into AML or MDS[82].

7) CMPDs, unclassifiable

This group encompasses those patients presenting clinical, laboratory and morphologic features of CMPDs but that can not be included in any of the previous groups. The principal characteristic is that the *BCR-ABL1* fusion gene is not present in these cases. CMPDs-u account for 10-20% of all CMPDs and its incidence depends mainly on the system of classification used to register these cases and on the experience of clinicians in diagnosing CMPDs. Survival is not well documented, and the few published papers report a 5-year RS between 30% and 55%[6,39,54]

- **Mast cell diseases**

Mast cell diseases are clonal haematopoietic disorders characterized by a proliferation of mast cells and their subsequent accumulation in one or more organs. This group of MPN is very heterogeneous, and although approximately 80% of patients have evidence of skin involvement, mastocytosis can also be a highly aggressive neoplasm affecting multiple organs. Mast cell diseases can appear at any age, with cutaneous mastocytosis being more frequent in children and systemic mastocytosis (SM) in young adults. The male to female ratio of SM ranges from 1:1 to 1:3. Survival rates published report a 5-year RS of nearly 80%[6]

Although mast cell diseases were recognized as an independent group of haematological malignancies in the 2001 WHO classification, since the 2008 WHO review they have been considered as one of the MPN entities, so cancer registries have to include it in incidence and survival analysis[13].

THE WHO 2008 CLASSIFICATION OF MPN

The discovery of a mutation in the *JAK2* V617F gene in 2005, at chromosome 9p24, led to an improvement in the diagnosis and pathogenesis of the *BCR-ABL1* negative CMPDs. This mutation is present in more than 90% of patients with PV and in nearly half of the people diagnosed with ET or PMF. As a result of this discovery, the WHO 2001 classification of CMPDs was revised and republished in the WHO 2008. This new version of the now called MPN classification is based on two factors[94]:

- The use of molecular analysis for the diagnosis *BCR-ABL1* negative neoplasms. Almost all MPN are associated with clonal abnormalities involving genes that encode cytoplasmatic or receptor protein-tyrosine kinase (PTK), allowing their identification and classification.
- The use of histological characterization to identify MPN subtypes.

Thus, the major changes proposed by the 4th edition of the WHO classification of MNP in relation to the previous classification schemes are[45]:

1. The nomenclature of this group of MMs has been changed from "myeloproliferative diseases" to "myeloproliferative neoplasms" (MPN) because myeloproliferative is a neoplastic procedure, not a reactive one. Also, the nomenclature of CM has been changed from PMF.
2. Diagnostic algorithms for PV, ET and PMF have changed in order to include information about *JAK2* V617F and other activating mutations. Additional clinical, histological and laboratory information have been included to allow diagnosis and classification of MPN when molecular results are not available.
3. The platelet count threshold for the diagnosis of ET has been decreased from $\geq 600 \times 10^9$/L to $\geq 450 \times 10^9$/L.
4. Some cases previously categorized as CEL may be categorized in a new subgroup of MMs, "myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA*, *PDGFRB*, or *FGFR1*".
5. M, which has many features in common with other MPN entities and is almost always associated with D816V mutation in the *KIT* gene encoding their corresponding receptor PTK, has been included in MPN category.

After applying these criteria in the WHO 2008 changes, MPN are classified as Table 17 shows.

Table 17. Classification of myeloproliferative neoplasms, according to the 2008 World Health Organization review[12]. Entities written in red correspond to the changes made in the WHO 2008 respect of the WHO 2001.

Myeloproliferative neoplasms
Chronic myelogenous leukaemia, *BCR-ABL1* positive
Chronic neutrophilic leukaemia
Polycythaemia vera
Primary myelofibrosis
Essential thrombocythaemia
Chronic eosinophilic leukaemia, not otherwise specified
Mastocytosis
Myeloproliferative neoplasm, unclassifiable

Although the RCG registers cases according to the WHO 2001 and by using the ICD-O-3, mastocytosis are included in the MPN group in incidence and survival analysis, as WHO 2008 recommended, because mastocytosis diseases have their corresponding codes in the ICD-O-3. Despite this group of MMs was called as CMPDs in the WHO 2001 classification, we named it MPN, as the last revision of the WHO classification recommended.

3.3.3. Coding rules

The mainly coding rules for MPN group are[15]:

- CML, PV and PMF can transform into AML. It is important to check the dates of diagnosis in order to codify AML as an incident case or as a transformation (see coding rules of AML).
- PV and ET can evolve into PMF. Also check the diagnosis dates to register PMF as an incident case or as a transformation.
- For PMF diagnosis, the results of a BM biopsy precede a BM aspirate.
- Cytogenetic and molecular analyses are needed for the diagnosis of CML *BCR-ABL1* gene.

3.3.4. Incidence

MPN is the most common incident group of MMs. Its incidence ranges from 6 to 9 new cases per 100,000 inhabitants, according to the 2001 WHO classification[6]. As reported by the few population-based studies available in the literature, incidence rates adjusted using the world population as a standard population are around 3 new cases per 100,000 inhabitants/year, and are between 2 and 5 when crude rate (CR) are published (Table 18). No geographical differences seem to be apparent in CML incidence, but when analyzing MPN Ph negative

divergence is evident[4,95]. Moreover, there has been a significant increase in the incidence of ET since the use of detecting

for the *JAK2* V617F mutation for MPN Ph negative in a routine examination[4,96].

Table 18. Incidence rates of myeloproliferative neoplasms, according to the 2001 World Health Organization classification.

	All MPN	**CML**	**ET**	**PV**	**PMF**	**CEL**	**HES**	**MPN, noc**
Maynadié M. et al. Côte d'Or (France)[39] (ASR_W)	3.2	0.9	1.2	0.6	0.4			0.1
McNally RJ, et al. U.K.[47] (ASR_W)	M: 0.74 F: 0.47	M: 0.33 F: 0.4	M: 0.53 F: 0.35	M: 0.29 F: 0.16				
Girodon F. et al. France[96] (ASR_W)			1.4 (1.2-1.6)	0.6 (0.5-0.7)	0.3 (0.3-0.4)			
Broccia G. et al. Sardinia[50] (ASR_W)	M: 2.4 F: 1.7							
Phekoo K.J. et al. South East England[46] (ASR_W)		0.83	1.13	0.74	0.25			
(ASR_E)		1.09	1.65	1.08	0.37			
Bose S. et al. U.K.[51] (ASR_E)	0.8	1.2	1					
Rollison D.E. et al. U.S.[80] (ASR_{US})	2.01		0.53	0.79	0.25			0.41
Sant M. et al. Europe[5] (CR)	3.34	1.10			1.53			
Visser O. et al. Europe[4] (CR)	3.1	1.2			1.8			0.0
Smith A. et al. U.K.[53] (CR)	5.4 (5.0-5.7)							

MPN (myeloproliferative neoplasms); CML (chronic myeloid leukaemia, BCR-ABL1 positive and not otherwise specified together); ET (essential thrombocythaemia); PV (polycythaemia vera); PMF (primary myelofibrosis); CEL (chronic eosinophilic leukaemia); HES (hipereosinophilic syndrome; MPN noc (MPN not otherwise categorised); ASR_W (World-population standardized incidence rate); ASR_E (European-population standardized incidence rate); ASR_{US}. (US-population 2000 standardized incidence rate); CR (Crude incidence rate); M (males); F (females). All results of incidence rates are expressed in new cases per 100,000 inhabitants/year with their 95% confidence interval when this is available.

3.3.5. Survival

The overall survival of patients with MPN is higher than any other MMs[11]. Some population-based studies reported a 5-year RS of 62-80% of patients, although prognosis varies depending on each entity. If untreated, patients may die within months of their diagnosis. Improvements in diagnostic techniques and the discovery of molecular alterations have led to the development of target therapies for some MPN such Imatinib or *JAK2* V617F inhibitors which, in turn, can increase survival rates[39,54].

There is no overall IPSS for all MPN. However, CML and PMF have their corresponding prognostic scoring systems. In 1984, Sokal et al. created a prognostic index system for patients with CML, which is used even today[97]. It was created by using data for 813 Ph positive patients from six European and American sources and it depends (as reported in their publication) on the following clinical variables and their associated constants:

- Age of patient (years): 0.116(age-43.4)
- Spleen size (cm): 0.0345(spleen-7.51)
- Platelet count ($x10^9$/L): 0.188[(platelet:700)^2-0.563]
- Blood myeloblasts: 0.0887(myeloblasts-2.10)

This scoring system stratifies patients diagnosed with CML into three relative risk groups determined by the sum of previous values:

- Low risk group: <0.8 points of Sokal prognostic index system.
- Intermediate risk group: 0.8-1.2 points of Sokal prognostic index system.
- High risk group: >1.2 points of Sokal prognostic index system.

On behalf of the International Working Group for Myeloproliferative Neoplasms Research and Treatment (IWG-MRT), Francesco Passamonti et al. developed a prognostic score based on a time-dependent risk evaluation for patients diagnosed with PMF: the Dynamic International Prognostic Scoring System (DIPSS)[98]. Previous prognostic scoring systems were based on the evolution risk factors present at the time of diagnosis, whereas DIPSS accounts for modifications of the risk profile after the initial diagnosis. This score was based initially on patient age at diagnosis (<65 years or ≥65 years), white blood cell (WBC) count, haemoglobin, PB blasts and constitutional symptoms such night sweats, pruritus, weight loss and fever. Because the age limit for treating patients with allogeneic SCT is 65 years, Franceseco Passamonti et al. decided to focus DIPSS towards patients younger than 65 years (age-adjusted DIPSS; aaDIPSS), and distinguished 4 risk groups depending on the variables of Table 19:

- Low risk group: 0 points of aaDIPSS. Median survival is not reached.
- Intermediate I risk group: 1-2 points of aaDIPSS. Median survival is near 10 years.

- Intermediate II risk group: 3-4 points of aaDIPSS. Median survival is approximately 5 years.
- High risk group: >4 points of aaDIPSS. Median survival is around 2 years.

Table 19. Age-adjusted Dynamic International Prognostic Scoring System (aaDIPSS) for patients diagnosed with chronic idiopathic myelofibrosis[98].

	Value		
Prognostic variable	0	1	2
WBC count ($x10^9$/L)	≤25	>25	
Haemoglobin (g/dL)	≥10		<10
PB blasts (%)	<1		≥1
Constitutional symptoms (Yes/No)*	No		Yes

WBC (White blood cell); PB (peripheral blood).

** Constitutional symptoms: night sweats, pruritus, weight loss and fever.*

As happens for other MMs groups, cancer registries report survival rates according to the 2001 WHO classification entities, although sometimes they include mast cells in their analysis, as the WHO 2008 recommended. MPN are the group of myeloid diseases with better prognosis, presenting 5-year RS of 62-79%, and ET and PV are the two entities with higher survival within this group (5-year RS around 90%) (Table 20)[4,39].

Table 20. Survival of myeloproliferative neoplasms, according to the 2001 World Health Organization classification.

	All MPN	CML	ET	PV	PMF	CEL	HES	MPN, noc	Mast cell*
Maynadié M. et al. Europe[54] 5-year RS	63.4 (61.8-64.9)	44.9 (42.6-47.3)	89.9 (86.2-92.7)	84.8 (81.5-87.5)	34.6 (27.6-41.6)			55.3 (51.5-58.9)	
Maynadié M. et al. Côte d'Or (France)[39] 5-year RS	79 (74-83)	60 (50-69)	93 (84-97)	97 (77-99)	55 (39-67)			30 (11-52)	
Visser O. et al. Europe[4] 5-year RS	62.0	44							81
Monnereau A. et al. France[61] 5-year NS	M: 70 (67-73) F: 80 (77-82)	M: 49 (44-53) F: 58 (53-65)	M: 79 (76-82) F: 86(84-89)						
Rollison D.E. et al. U.S.[80] 3-year RS	80 (78-82)								
Phekoo K.J. et al. South East England[46] 1-year RS		73 (65-69)	81 (76-85)	80 (73-86)	72 (60-83)				

MPN (myeloproliferative neoplasms); CML (chronic myeloid leukaemia, BCR-ABL1 positive and not otherwise specified together); ET (essential thrombocythaemia); PV (polycythaemia vera); PMF (primary myelofibrosis); CEL (chronic eosinophilic leukaemia); HES (hypereosinophilic syndrome; MPN NOC (MPN not otherwise categorised); RS (relative survival); NS (net survival); M (males); F (females). Results of survival are expressed in % with their 95% confidence interval when it is available.

*Mast cells are grouped in the Myeloproliferative neoplasms group in the 2008 World Health Organization classification review.

3.4. MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

3.4.1. Definition and diagnosis

This category of MMs includes myeloid disorders that present both dysplastic and proliferative features. These characteristics can be found together at the time of initial presentation in some clinical, laboratory and morphologic results[6].

These diseases are identified by hipercellularity of the BM as a consequence of the effective proliferation in one or more myeloid lineages, resulting in increased circulating cells. However, these cells may be morphologically and functionally dysplastic. At same time, the other myeloid lineages may present ineffective proliferation, causing cytopenias. The percentage of blasts in PB and BM is less than 20% and splenomegaly and hepatomegaly are commonly found. Diagnostic criteria for MDS/MPN mainly include[6,62]:

- Clinical data of patients:
    - Absence of a history of a well-defined MPN which develop dysplasia and ineffective hematopoiesis.
    - No existence of secondary causes of monocytosis: neutropenia, chronic and viral infections and autoimmune diseases.
- PB and BM smears:
    - Cytomorphologic analysis: used to evaluate the blast count, persistent PB monocytosis (in CMML) or leukocytosis (in atypical CML (aCML)) and presence of dysplasia.
    - Cytogenetic and molecular tests. Although chromosome abnormalities are found in a few cases, genetic mutations are more common. Even though the *BCR-ABL1* fusion gene has to be negative and *PDGFRA* and *PDGFRB* arrangements do not have to be present, alterations in some genes such as *TET2*, *ASXL1*, *CBL*, *IDH*, *NRAS*, *KRAS*, *RUNX1*, *UTX*, *EZH2*, *DNMT3* and *JAK2* V617F may influence the pathogenesis of the disease.
    - Immunophenotype: this may be useful, especially during the diagnosis of CMML, because the monocytes are characterized by the expression of CD56.

3.4.2. Classification

FAB CLASSIFICATION

This group of MMs emerged since the publication of the WHO 2001 classification, comprising three well-defined entities: CMML, aCML and juvenile myelomonocytic leukaemia (JMML)[6]. Before its publication, the FAB classification only recognized CMML and, although its classification had been

controversial because this pathology shares dysplastic and proliferation characteristics, it was finally categorized as an MDS in 1982 based on its morphologic features[11].

In 1994, FAB distinguished two subtypes within this disease, based on the absolute leukocyte count, each one of which represented nearly 50% of CMML cases[63]:

- Myelodysplastic subtype CMML (MD-CMML): leukocyte count $<13 \times 10^9$/L
- Myeloproliferative subtype CMML (MP-CMML): leukocyte count $\geq 13 \times 10^9$/L

The prognostic value of this distinction was very controversial, and even nowadays it is unknown if the two varieties of CMML represent different clinical-biological entities or whether, to the contrary, they are two different stages of the same disease.

THE 2001 WHO CLASSIFICATION

With the 2001 WHO publication, the controversial classification of CMML was resolved by recognizing this entity as part of the new MMs group called MDS/MPDs, which permits the classification of some entities that have the clinical, laboratory and morphologic features of dysplasia and a proliferation of myeloid cell lineages. This group includes part of the CMML, aCML, JMML and the set of MDS/MPDs unclassifiable (MDS/MPDs-u), which allows other entities with dysplastic and proliferative features that can not be categorized correctly, to be grouped (Table 21)[6].

Moreover, the 2001 WHO differentiated CMML into two subtypes depending on the percentage of blasts in PB and BM: CMML-1 and CMML-2 (see 3.4.2.1).

Table 21. Classification of myelodysplastic /myeloproliferative diseases, according to the 2001 World Health Organization classification[6].

Myelodysplastic / myeloproliferative diseases	**ICD-O-3**
Chronic myelomonocytic leukaemia (CMML) - CMML-1 - CMML-2	9945
Juvenile myelomonocytic leukaemia (JMML)	9946
Atypical chronic myeloid leukaemia (aCML)	9876
Myelodysplastic/myeloproliferative diseases, unclassifiable (MDS/MPDs-u)	9975

3.4.2.1. Principal features of MDS/MPDs according to the 2001 WHO classification.

1) Chronic myelomonocytic leukaemia

CMML is an MDS/MPN characterized by a PB monocytosis. Its incidence varies between 0.3-0.5 new cases per 100,000 inhabitants/year, increasing up to 3 new cases per 100,000 inhabitants/year in the population aged over 60 years. It accounts for 12% of all MDS described in the FAB classification and a male predominance of 1.5:3-1 is found[6,62].

CMML diagnosis requires a persistent PB monocytosis of $>1x10^9$/L, with more than 10% of monocytes of the WBCs. BM is usually hipercellular and may show monocytic or granulocytic hyperplasia. Immunophenotypically, the principal characteristic is an overexpression of CD56, and clonal recurring cytogenetic abnormalities including +8, -7/del(7q) and structural abnormalities of 12p are found[6,62,99].

The WHO classification subdivides CMML into two subcategories depending on the percentage of blasts found in PB and BM:

- CMML-1: <5% in the PB, <10% in the BM.
- CMML-2: 5-19% in PB or 10-19% in BM.

Although survival of patients varies between 1-100 months, most report a median overall survival of 20-40 months. However, some studies described that survival and progression into AML depended on CMML subtypes, because the percentage of blasts is the most important prognostic factor. CMML-1 patients have a median OS of 18-20 months, whereas people with CMML-2 have a survival time of 12-15 months. Progression into AML is more frequent in CMML-2 (20-24%) than in CMML-1 (near 15%) (Figure 7)[100-102].

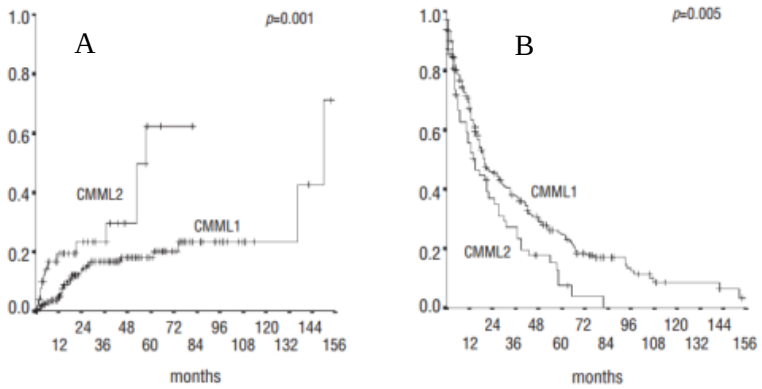


Figure 7. Cumulative risk of AML (A) and survival (B) of patients of the German cohort diagnosed with chronic myelomonocytic leukaemia type 1 vs type 2[102].

2) Juvenile myelomonocytic leukaemia

JMML is an aggressive MDS/MPN manifesting in childhood. Although in the 1980s it was known as juvenile chronic myelogenous leukaemia, the International JMML Working Group, in collaboration with the European Working Group on Myelodysplastic Syndromes in Childhood, renamed it a JMML in the 1990s[103].

While this disease can appear in children aged from 0 to 14 years, the majority of cases are found between birth and 6 years of age; with a median age 2 years. There is a male predominance of 2.5:1. Its incidence is very low, approximately 1.3 new cases per 1,000,000 children aged 0-14 years/year, and represents less than 2-3% of all leukaemia in children and 20-30% of all myelodysplastic and myeloproliferative diseases in younger patients. Diagnosis is based on a persistent monocytosis in PB ($>1x10^9$/L), less than 20% of blasts in PB or BM, absence of the Ph chromosome or the *BCR-ABL1* fusion gene and a minimum of two of the following features: increased haemoglobin F, immature granulocytes in PB, WBC count $>10x10^9$/L, clonal chromosomal abnormalities and granulocytes-monocytes colony stimulating factor hypersensitivity of myeloid progenitors in vitro. Although patient survival varies quite a lot, the overall prognosis is not good. Children with JMML can survive a median of 5 months to more than 4 years, although if untreated, nearly 30% of them have rapid progression and 10-20% evolve into acute leukaemia. The prognosis is better if the disease appears in children aged less than 1 year[6,103].

3) Atypical chronic myeloid leukaemia

This is a rare disease which is predominantly found in patients more than 60 years old with a male predominance from 1:1 to 2.5:1. Its incidence is <2 cases for every 100 cases of CML *BCR-ABL1* positive and it is more aggressive than it. The median survival rate of patients is around 11-25 months and up to 40% develop acute leukaemia within a median time of 18 months after their diagnosis. Principal characteristics of aCML are: leukocytosis, neutrophilia, no basophilia or monocytosis, presence of dysgranulopoiesis often with abnormal chromatin clumping and small megakaryocytes. Despite BM being morphologically similar to CML, the lacks of monocytic differentiation, the presence of dysgranulopoiesis and the cytogenetic and/or molecular genetic studies permit the pathology of this CML to be distinguished[104-106].

4) Myelodysplastic/Myeloproliferative diseases, unclassifiable

This is a group of unknown incidence and prognosis. MDS/MPDs-u is a residual diagnosis reserved for those pathologies that share clinical, laboratory and morphologic features of dysplasia and proliferation and can not be identified as CMML, JMML or aCML. It is important to exclude from this group patients who have a previous history of a well-defined myeloproliferative neoplasm or

those who have received recent cytotoxic or growth factor therapy. The presence of Ph chromosome, del(5q), t(3;3)(q21;q26) or inv(3)(q21q26) are also reasons for exclusion[6].

THE WHO 2008 CLASSIFICATION

The revision of the WHO classification in 2008 renamed this group of MMs as myelodysplastic/myeloproliferative neoplasms (MDS/MPN) and describes some significant and major changes in the diagnosis and their classification (Table 22)[45]:

1. Some cases of CMML with eosinophilia are reclassified to the category "Myeloid/lymphoid neoplasms with eosinophilia and *PDG-FRB* rearrangement".
2. The category aCML has been renamed as atypical CML, *BCR-ABL1*-negative to emphasize that it is not merely a variant of CML, *BCR-ABL1*-postive.
3. RARS-T remains as a provisional entity classified as MDS/MPN, unclassifiable, RARS-T.

Table 22. Classification of myelodysplastic/myeloproliferative neoplasms, according to the 2008 World Health Organization classification review[12]. Entities written in red correspond to the changes made in the WHO 2008 respect of the WHO 2001.

Myelodysplastic/myeloproliferative neoplasms
Chronic myelomonocytic leukaemia
Atypical chronic myeloid leukaemia, *BCR-ABL1* negative
Juvenile myelomonocytic leukaemia
Myelodysplastic/myeloproliferative neoplasms, unclassifiable
*Refractory anaemia with ringed sideroblasts in transformation

**This entity remains as provisional.*

The RCG uses the terminology of the WHO 2008 revision to refer to this group of MMs.

3.4.3. Coding rules

This group of MMs is present in the WHO classification but is not recognized in the ICD-O-3, so cancer registries have to include the following ICD-O-3 codes in this category:

- 9945/3: CMML
- 9946/3: JMML
- 9876/3: aCML
- 9975/3: MDS/MPN-u

CMML can evolve to AML or MDS. If progression occurs in less than three months after the initial diagnosis of CMML, we have to register the AML or MDS with the date of CMML diagnosis, and AML or MDS have to be considered in the incidence analysis. If the diagnosis of AML or MDS occurs after three months of the initial CMML diagnosis, CMML has to be registered as an incident case and AML or MDS will be recorded as a transformation and not account for the incidence[15].

3.4.4. Incidence

MDS/MPN is the less frequent group within MMs. Its incidence varies widely, depending on specific disease. CMML is the most frequent entity, representing approximately 90% of cases diagnosed with MDS/MPN[107]. However, little reliable data on incidence is available in the literature because changing classifications complicate its registration (see 3.4.2 section). The few population-based registries that have "truthful" information on CMML reported an incidence rate of 0.3-0.6 new cases per 100,000 inhabitant/year (Table 23). This incidence varies between less than 1 new case per 100,000 inhabitants/year in younger patients to approximately 3 new cases per 100,000 inhabitants/year in adults over the age of 60 years[108]. Certain incidence rates of aCML and JMML are not available because there are not enough large groups of patients to be validated.

Table 23. Incidence rate of myelodysplastic/myeloproliferative neoplasms, according to the 2001 World Health Organization classification.

	All MDS/MPN	CMML	JMML	aCML
Maynadié M. et al. Côte d'Or (France)[39] (ASR_W)	0.6	0.5	0.0	0.0
McNally RJ, et al. U.K.[47] (ASR_W)		M: 0.27 F: 0.14		
Phekoo K.J. et al. South East England[46] (ASR_W)		0.46		
(ASR_E)		0.28		
Neukirchen J. et al. Germany[71] (ASR_E)		0.41 (0.27-0.54)		
Rollison D.E. et al. U.S.[80] (ASR_{US})		0.37		
Sant M. et al. Europe[5] (CR)	0.35 (0.32-0.37)			
Visser O. et al. Europe[4] (CR)		0.3		
Smith A. et al. U.K.[53] (CR)		0.6		

MDS/MPN (myelodysplastic/myeloproliferative neoplasms); CMML (chronic myelomonocytic leukaemia); JMML (juvenile myelomonocytic leukaemia); aCML (atypical chronic myeloid leukaemia, BCR-ABL1 negative); ASR_W (World-population standardized incidence rate); ASR_E (European-population standardized incidence rate); ASR_{US}. (US-population 2000 standardized incidence rate); CR (Crude incidence rate); M (males); F (females). All results of incidence rates are expressed in new cases per 100,000 inhabitants/year with their 95% confidence interval when it is available.

3.4.5. Survival

Survival of patients diagnosed with MDS/MPN varies markedly depending on the natural course of these diseases, and so can range from months to years[6,53]. It was described that a 5-year RS of patients with MDS/MPN was 23-27%, whereas it was 18-23% for CMML[4,39,53,54]. Due to CMML being the most frequent entity within this group, survival analyses of MDS/MPN are mainly centred on it.

CMML is a disorder with a highly variable prognosis and transformation into AML of between 10-20% at 2 years and 20-40% at 5 years[53,101,102,109]. Therefore, it is necessary to establish an accepted prognostic scoring system which will allow us to predict the individual survival of each patient. Because the IPSS excludes myeloproliferative subtype of CMML and does not identify different risk groups, the GESMD developed a CMML-specific prognostic scoring system (CPSS), which has 4 distinct risk groups for survival and AML evolution depending on FAB and WHO 2001 classifications, transfusion dependence at the time of diagnosis and the presence of cytogenetic alterations (Table 24)[110]:

- Low risk group (score = 0): The average overall survival rate reported was 72 months and probability of AML evolution was 7%.
- Intermediate-1 risk group (score = 1): People survived a median of 31 months and 14% developed AML.
- Intermediate-2 risk group (score = 2-3): The overall survival rate median decreased to 13 months and nearly 40% evolved to AML.
- High risk group (score = 4-5): Patients only survived an average of 5 months and the probability of AML evolution was 73%.

Although this categorization of CMML into risk groups is widely used in clinical practice, cancer registries can only estimate their survival based, when possible, on the subtypes proposed by the WHO classification[6]. However, the percentage of blasts is not recorded for most cases and survival for CMML can only be estimated overall.

Table 24. Chronic myelomonocytic leukaemia-specific prognostic scoring system (CPSS)[110].

Variables	0 point	1 point	2 points
WHO subtype	CMML-1	CMML-2	
FAB subtype	MD-CMML	MP-CMML	
CMML-specific cytogenetic risk stratification (GESMD)	Low	Intermediate	High
Transfusion dependency	No	Yes	

WHO (World Health Organization); FAB (French-American-British Co-operative Leukaemia Group); CMML (chronic myelomonocytic leukaemia); MD (myelodysplastic subtype); MP (myeloprolifertive subtype); GESMD (Grupo Español de Síndromes Mielodisplásicos).

CMML- specific cytogenetic risk stratification (GESMD): Low (normal, -Y); Intermediate (other abnormalities); High (+8, abnormalities in chromosome 7 and complex karyotype (≥3 abnormalities)).

Transfusion dependency was defined as having al least 1 transfusion every 8 weeks over a period of 4 months.

Table 25 shows some reported survival results of MDS/MPN entities according to the 2001 WHO classification.

Table 25. Survival of myelodysplastic/myeloproliferative neoplasms, according to the 2001 World Health Organization classification.

	All MDS/MPN	CMML	JMML	aCML
Maynadié M. et al. Europe[54] 5-year RS	19.6 (16.1-23.4)	19.3 (15.7-23.0)		
Maynadié M. et al. Côte d'Or (France)[39] 5-year RS	34 (24-44)	34 (24-44)		23 (1-62)
Visser O. et al. Europe[4] 5-year RS		18		
Rollison D.E. et al. U.S.[80] 3-year RS		21 (16-26)		
Phekoo K.J. et al. South East England[46] 3-year RS		29 (20-39)		

MDS/MPN (myelodysplastic/myeloproliferative neoplasms); CMML (chronic myelomonocytic leukaemia); JMML (juvenile myelomonocytic leukaemia); aCML (atypical chronic myeloid leukaemia, BCR-ABL1 negative); RS (relative survival). Results of survival are expressed in % with their 95% confidence interval when this is available.

Hypothesis and Objectives

If you don't know where you are going,
you will probably end up somewhere else.

Laurence J. Peter

Hypothesis

Limited population-based data on incidence, prognosis and survival of MMs according to the 2001 WHO classification are available in the literature. Throughout this thesis we formulate the following hypothesis:

1. Incidence of MMs: MMs in particular are not one of the most frequent cancers in our population. According to the 2001 WHO classification, MPN are the highest incident group of MMs and the MDS/MPN group is the less common. An increased incidence trend of MMs over the recent years has been detected, especially for MPN group since the discovery of the *JAK2* V617F mutation.

2. Survival of MMs: Patients diagnosed with MMs have a relative poor prognosis, although it depends on each MMs group and each single entity proposed by the WHO 2001 classification. Age of patients, sex, and period of diagnosis are three prognostic factors that can influence in survival time as well as improvements in diagnostic methods, treatment and better risk-group stratification of patients. The discovery of some targeted therapies such Imatinib for treatment of CML *BCR-ABL* positive may result in an increased overall outcome.

3. Incidence and survival of CMML: Within MMs, CMML is a very rare disease affecting mainly elderly people. Survival is very poor and age of patients may influence on the overall prognosis. No major changes in therapy have been accounted during the last years, so improvement on survival may not be detected.

Objectives

The main objectives of this work are to evaluate incidence and survival rates of MMs overall, for each entity and especially for CMML in the province of Girona, and to associate changes with improvements in diagnostic techniques and therapy.

Specific objectives

1. Incidence of MMs in the province of Girona:

   1.1. Analyse the incidence rate of each MM group and of each MM entity as proposed by the 2001 WHO classification.

   1.2. Evaluate the incidence trend of MMs over fifteen years.

2. Survival of MMs in the province of Girona:

   2.1. Examine the survival of MMs according to the 2001 WHO classification by a) main groups and b) each malignancy individually.

   2.2. Assess possible changes in survival according to gender, age of patients and year of diagnosis, and attempt to relate them to improvements in diagnosis methods, treatment and better risk-group stratification.

   2.3. Evaluate the effect of Imatinib on increasing the prognosis of those patients diagnosed with CML *BCR-ABL1* positive.

3. Incidence and survival of CMML in the province of Girona:

   3.1. Determine the incidence of CMML and analyse variations depending on the age of patients.

   3.2. Estimate survival and evaluate if there are any differences in survival due to the age of patients, gender and year of diagnosis.

   3.3. Attempt to analyse changes in survival with advances in the currently available therapy.

Data and Methods

The value of an idea lies in the using of it.

Thomas A. Edison

1. Incidence of MMs in the province of Girona

Data

Data for incidence analysis were extracted from the population-based RCG, located in the north-east of Catalonia, Spain, and covering a population of 731.864 inhabitants (2008 census). Information sources are regional and taken from community hospitals, haematology and pathology departments, and death certificates. Moreover, the RCG also included molecular, cytogenetic and immunophenotypic results to ensure complete coverage of MMs cases. Cases were registered applying the rules of the ENCR and the Manual for Coding and Reporting Haematological Malignancies[8,15]. Population data used for the statistical analysis were provided by the *Institut d'Estadística de Catalunya* (IDESCAT) and the *Instituto Nacional de Estadística* (INE)[111,112]. We restricted our analysis to incident MMs cases in the province of Girona from 1994 to 2008.

The ICD-O-2 was used by the RCG to diagnose MMs up until 1997 and the ICD-O-3 has been used since 1998. The ICD-O-2 codes were converted into their corresponding ICD-O-3 codes, including MDS, PV and ET as malignant diseases[7,9]. RAEB-T (ICD-O-3 code: 9984/3) was reclassified as AML because the blast percentage cut-off for AML decreased from 30% to 20% according to the 2001 WHO classification. In cases of haematological transformation, only the first tumour was considered for incidence. Therapy related MDS were included in the AML therapy related (ICD-O-3 code: 9920/3) subgroup, as recommended by the WHO. AL of ambiguous lineage (ICD-O-3 code: 9805/3) were grouped with AML nos and cases of CML nos (ICD-O-3 code: 9863/3) were grouped with CML *BCR-ABL1* positive (ICD-O-3 code: 9875/3).

Statistical analysis

The median age (range) was calculated by gender and for each type of MMs. CR and ASR_E per 100,000 inhabitants/year (with their corresponding 95% CI) were performed using the direct method of Epidat software[113]. ASR_W and ASR_{US} were also calculated. Gender ratio was analysed using CR. Age-specific rates for 5-year age groups and time trends related to incidence rates were assessed for the four main myeloid groups. The estimated annual percentage change (EAPC) was calculated with the incidence standardized rates using the Joinpoint log-linear regression model[114].

To predict the number of MMs cases in the Spanish population during 2013, the CR for the Girona province from 1994 to 2008 was used together with the "short-term" projections (predicting 4 years from the last year of observed data) provided by the INE for the period 2011-2021[112]. Firstly, these projections were stratified according to sex and 5-year age groups. Secondly, the number of cases was calculated that would apply if the incidence in Spain were the same as in Girona province by age and sex. Thirdly, the total number of cases for each MMs (and sex) was obtained by adding cases for

each age group. Finally, the overall number of cases was obtained by adding together the results for men and women. The EAPC was not considered due to small number of cases in each stratum.

2. Survival of MMs in the province of Girona

Data

Data used in this study was the same as that used to estimate the incidence of MMs in the province of Girona (n= 1,331) (see data in part 1 of this Section). Of these, 1296 (97.4%) were histological confirmed cases of MMs, 27 (2%) were only diagnosed by death certificate (DCO), 3 (0.2%) were diagnosed by clinical investigation and the diagnosis method was unknown in 5 cases (0.4%). DCO cases were excluded from the survival analysis.

Survival analysis

Survival time was calculated as the difference between the dates of diagnosis and death or, if the patient was alive when the study period ended, then the end of the follow-up study (December 31st, 2012). Vital status was obtained by linking records from the Catalan Registry of Mortality and the National Death Index, and through periodic review of the medical records of patients diagnosed with MMs and whom were not to be found using the previously described information sources.. Cancer-specific mortality was not available.

Survival for patients with AML and MDS therapy related, MDS associated with isolated del(5q), mast cell diseases, other MPN (CEL and HES, and CNL), JMML and aCML was not estimated due to the low number of cases. Median OS was calculated using the Kaplan-Meier method. Relative survival (RS) at 1, 3 and 5 years with their correspoinding 95% CI were obtained using the Pohar-Perme method as the ratio of OS in the study population to the expected survival in the general population of same age, sex and year[115,116]. Survival analysis was calculated overall and according to the WHO's main groups: AML, MDS, MPN and MDS/MPN. RS was also assessed by gender, by three consecutive 5-years period of diagnosis (1994-1998, 1999-2003 and 2004-2008) and by age according to the median age of patients diagnosed in the province of Girona in accordance with their pathology (all MMs: 72 years; AML: 68 years; MDS: 77 years; MPN: 67 years and MDS/MPN: 78 years).

Relative excess risk of death (RER)

To evaluate the importance of age on survival, we stratified our cohort in young and old patients to assess relavite excess risks (RERs) of death adjusted for gender (males/females) and period of diagnosis (1994-1998, 1999-2003, 2004-2008). RERs of death were estimated using a bivariate generalized linear model with a Poisson distribution using 5-year RS for overall MMs, MDS and

MPN groups, and 3-year RS for AML due the low number of patients alive at 5 years after diagnosis. No interactions were found between covariables.

Evaluation of the Imatinib effect on CML survival

To assess the effect of Imatinib on CML patient survival, we selected cases registered as CML *BCR-ABL1* positive [ICD-O-3 code: 9875/3] (n=99) and we checked the treatment received by each patient individually. W also estimated the RERs of death 5-years after diagnosis using a multivariate generalized linear model assuming that the observed number of deaths followed a Poisson distribution. This multivariate analysis included TKI treatment (no/yes), gender (males/females) and age at diagnosis according to the median age of CML diagnosis in the province of Girona (<63 years/≥63 years) as a covariables. Analyses were performed using R 2.14.0 software and statistical significance was determined at p=0.05.

3. Incidence and survival of CMML in the province of Girona

Data

Data for these analyses were also extracted from the population-based RCG. We restricted our statistical analysis to the population diagnosed with CMML and resident in the province of Girona from 1993 to 2007. The ICD-O-2 code for cases registered before 1998 (code: 9868/3) was converted into ICD-O-3 code (code: 9945/3) after consulting the pathological and haematological results of the initial diagnosis.

Incidence analysis

The mean and the standard deviation were calculated overall and by sex and compared using a *t* test. Incidence rates were calculated as the CR, ASR_W using the direct method of Epidat software[113]. The age-specific rate for 5-years overall and by gender was also computed. In order to compare our results with other international studies related to the incidence of CMML, we also calculated ASR_E and ASR_{US}.

Temporal incidence trends in the incidence of CMML were assessed through the estimated annual percentage change (EAPC) and its 95% of CI of the incidence standardized rates using the joinpoint log-linear regression model[114]. The standard parametric Fisher test was used to corroborate whether differences in incidence rates between years were statistically significant[117].

Survival analysis

Follow-up time was calculated as the difference between the date of diagnosis and the date of death, (if the patient had died), or the end of the follow-up study (December 31, 2010) if the patient was

alive when the period of study ended. Vital status of patients was obtained by linking records to the Catalan Registry of Mortality and the National Death Index.

Because we wanted to evaluate and compare survival time of patients with CMML, we calculated overall OS, by sex, by age groups (<75 years and ≥75 years) and by year of diagnosis (1993-1997, 1998-2002, 2003-2007). OS curves were computed using the nonparametric Fleming-Harrington method because risks were not proportional, and G-rho tests were used to compare the curves[118]. Analyses were performed using R software. Statistical significance was determined at $p = 0.05$.

Due to the advanced age of patients and competing causes of mortality other than CMML, RS was estimated with 95% CI. It was computed using WAERS, a web-based application developed by the Catalan Institute of Oncology which uses the Hakulinen method to estimate expected survival[119].

Results

The results you achieve will be in direct proportion to the effort you apply.

Denis Waitley

The results section includes the following articles which have been published or sent:

1. Incidence of MMs in the province of Girona

- **Osca-Gelis G,** Puig-Vives M, Saez M, Gallardo D, Lloveras N, Marcos-Gragera R. Population-based incidence of myeloid malignancies: fifteen years of epidemiological data in the province of Girona, Spain. Haematologica. 2013 Aug;98(8):e95-7.

  doi: 10.3324/haematol.2013.084061. Epub 2013 Jun 28.

2. Survival of MMs in the province of Girona

- **Osca-Gelis G**, Puig-Vives M, Saez M, Gallardo D, LLoveras N, Guàrdia R, Marcos-Gragera R. Is the survival of myeloid malignancies really improving? A retrospective 15 years population-based study. Leukemia and Lymphoma. . 2014 Aug 18: 1-7.

  Epub ahead of print

3. Incidence and survival of CMML in the province of Girona

- **Osca-Gelis G**, Puig-Vives M, Saez M, Gallardo D, Solé F, Marcos-Gragera R. Incidence and survival of chronic myelomonocytic leukaemia in Girona (Spain): a population-based study, 1993-2007. Leuk. Res. 2012 Oct;36(10):1262-6. Erratum in Leuk Res. 2013 Jul;37(7):852.

  doi: 10.1016/j.leukres.2012.06.009. Epub 2012 Jul 9.

MMs are a heterogeneous group of haematological malignancies presenting different rates of incidence, prognosis and survival. A total of 1,331 cases of these diseases were diagnosed in the province of Girona from 1994 to 2008, (718 men (53.9%) and 613 women (46.1%)), with an overall ASR_E of 11.08 new cases per 100,000 inhabitants/year (13.06 in men and 9.56 in women, sex ratio 1.17 and $p<0.005$). Although survival of MMs depends on each main group from the WHO guidelines, the overall 5-year RS for all MMs in the Girona province was 49.7%, with females having a greater survival rate than males and younger patients than older ones. The increasing survival during the whole period of study was only detected for young people.

MPN was the highest incident group among MMs in Girona between 1994 and 2008, with an ASR_E of 4.43 new cases per 100,000 inhabitants/year. A significant male predominance both overall and also in PV entity and an increased incidence trend during the whole period was detected. Patients diagnosed with MPN had the best outcome within MMs (5-year RS of 75.3%; 95% CI: 70.3-80.7), despite survival differing within MPN entities: PV and ET were the two neoplasms with better prognosis, whereas MPN-u was the disease with the poorest outcome. Statistically significant differences were evident when comparing survival by period of diagnosis and age. Younger patients had better outcomes than older ones and increased survival for the period of study was only found in young people. When looking at CML individually, a 5-year RS of 61.2% (95% CI: 51.2-73.1) was found. Despite females having a lower RER compared with males and younger patients having a better prognosis than older ones, differences were not statistically significant. However, when evaluating the effect of Imatinib on survival, we detected that patients treated with TKI had a statistically significant better RER of 0.19 (95% CI: 0.06-0.60) in relation to those untreated.

There were 458 incident cases of MDS, with an overall ASR_E of 3.30 new cases per 100,000 inhabitants/year (4.06 in men and 2.80 in women). The most frequent type within this group was MDS-u and the least frequent were 5q- syndromes. The incidence steadily increased at a statistically significant EAPC of 4.8% (95% CI: 1.8-7.9) over the entire period. Patients diagnosed with MDS had a 5-year RS of 45% (95% CI: 39.3-51.6), with RCMD being the entity with the best outcome within this group and MDS-u and RAEB the two entities with the lowest 5-year RS. Survival of MDS depended on patient age at diagnosis: people younger than 77 years had a significantly better outcome than those over 77 years. Despite an increased survival having been found between 1994 and 2008, it was only detected in young people.

AML was the only MMs group in which the incidence trend over time was stable from 1994 to 2008. The overall ASR_E for AML was 2.91 new cases per 100,000 inhabitants/year (3.48 in men and 2.40 in women), with a statistically significant sex ratio of 1.28. Approximately 54% (171 of

319) of AML cases were AML noc, and within this group the majority of cases were either AMML or AML without maturation. The AML and MDS therapy-related group was the least frequent within the four main AML groups. When analysing survival, AML had the lowest prognosis within the WHO's main MMs groupings, with a 5-year RS of 20.2% (95% CI: 15.8-25.9). The AML with recurrent cytogenetic abnormalities was the subgroup with a better outcome, whereas patients diagnosed with AML nos and AL of ambiguous lineage had the poorest survival within AML. Age clearly influenced survival time, as elderly patients had a poorer prognosis in comparison to younger patients. Although it was not statistically significant, an improvement in the survival of younger patients during the fifteen year study period was detected.

Finally, in the WHO's main grouping of MMs, MDS/MPN had the least incidence with an overall ASR_E of 0.44 cases per 100,000 inhabitants/year (0.59 in men and 0.35 in women). As with the MDS and MPN groups, a significant increase in incidence from 1994 to 2008 was detected. Despite CMML being the most frequent entity within this group, comprising more than 90% of all MDS/MPN cases, it is accepted as a very rare malignancy, with an ASR_E of lower than one case per 100,000 inhabitants/year. Although incidence of CMML was similar by gender for those under 74 years, for older patients the incidence was higher among males than females. The whole 5-year RS for CMML patients was near 30% and differences in survival were only found when comparing patients by age: people under 75 year had a statistically significant better outcome than over 75.

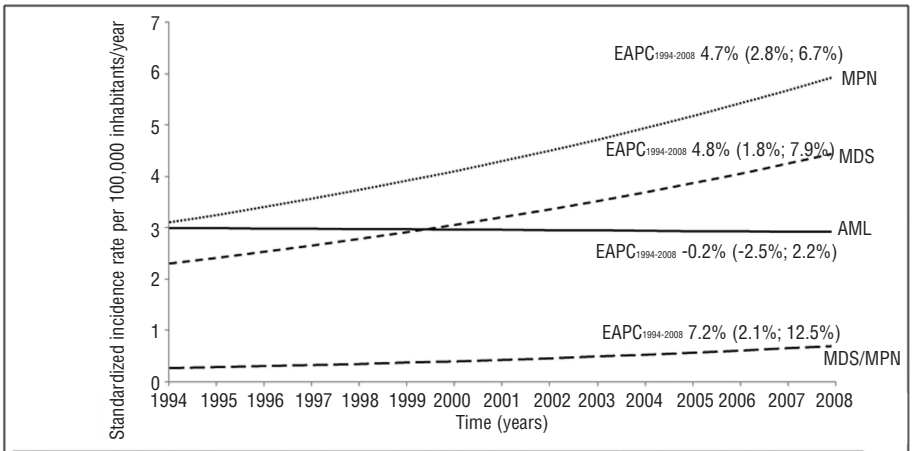
Population-based incidence of myeloid malignancies: fifteen years of epidemiological data in the province of Girona, Spain

Myeloid malignancies (MMs) are a heterogeneous group of hematologic malignancies presenting different incidence, prognosis and survival.[1-3] Changing classifications (FAB 1994, WHO 2001 and WHO 2008) and few available epidemiological data complicate incidence comparisons.[4,5] Taking this into account, the aims of the present study were: a) to calculate the incidence rates and trends of MMs in the Province of Girona, northeastern Spain, between 1994 and 2008 according to the WHO 2001 classification; and b) to predict the number of MMs cases in Spain during 2013. Data were extracted from the population-based Girona Cancer Registry (GCR) located in the north-east of Catalonia, Spain, and covering a population of 731,864 inhabitants (2008 census). Cases were registered according to the rules of the European Network for Cancer Registries and the Manual for Coding and Reporting Haematological Malignancies (HAEMACARE project). To ensure the complete coverage of MMs in the GCR, and especially myeloproliferative neoplasms (MPN) and myelodysplastic syndromes (MDS), a retrospective search was performed. The ICD-O-2 (1990) codes were converted into their corresponding ICD-O-3 (2000) codes, including MDS, poly-

Table 1. Number of cases, median age, incidence rates and sex ratio of myeloid malignancies diagnosed in Girona province (1994-2008) according to the 2001 WHO classification.

Entities	N. of. cases	%	Median age Men	Women	CR	ASRE Men	Women	All	Sex Ratio
Acute myeloid leukemia	319	100	68	68	3.60	3.48	2.40	2.91	1.28*
AML with recurrent cytogenetic abnormalities	35	11.0	52	54	0.40	0.45	0.34	0.39	1.32
AML with t(8;21)(q22;q22)	4	1.3	17	74	0.05	0.08	0.01	0.05	3.50
AML with 11q23 abnormalities	3	0.9	27	57	0.03	0.02	0.05	0.04	0.40
AML with inv(16)(p13;q22) or t(16;16)(p13;q11)	5	1.6	66	57	0.06	0.09	0.03	0.06	4.50
AML with t(15;17)(q22;q11-12)	23	7.2	51	46	0.26	0.27	0.25	0.25	1.08
AML with multilineage dysplasia	46	14.4	71	78	0.52	0.46	0.28	0.36	1.31
AML and MDS therapy related	9	2.8	59	66	0.10	0.07	0.12	0.09	0.50
AML NOC	171	53.6	64	65	1.93	1.90	1.37	1.62	1.25
AML, minimal differentiated	19	6.0	66	62	0.22	0.19	0.22	0.20	0.87
AML, without maturation	37	11.6	62	68	0.42	0.37	0.32	0.34	0.95
AML, with maturation	31	9.7	61	61	0.35	0.44	0.21	0.32	2.09
Acute myelomonocytic leukemia	38	11.9	52	63	0.43	0.52	0.25	0.38	1.97
Acute monoblastic and monocytic leukemia	24	7.5	72	79	0.27	0.22	0.19	0.21	0.86
Acute erythroid leukemia	9	2.8	75	43	0.10	0.07	0.11	0.08	0.82
Acute megakaryoblastic leukemia	3	0.9	75	81	0.03	0.03	0.01	0.02	2.50
Acute basophilic leukemia	0	0.0	–	–	–	–	–	–	–
Acute panmyelosis with myelofibrosis	8	2.5	73	75	0.09	0.07	0.06	0.06	1.00
Myeloid sarcoma	2	0.6	78	37	0.02	0.02	0.02	0.02	1.00
AML NOS and AL of ambiguous lineage	58	18.2	74	80	0.66	0.60	0.30	0.45	1.52
Myelodysplastic syndromes	458	100	77	77	5.18	4.06	2.80	3.30	1.16
Refractory anemia	76	16.6	76	74	0.86	0.58	0.56	0.56	0.96
Refractory anemia with ringed sideroblasts	80	17.5	75	76	0.90	0.79	0.40	0.57	1.41
Refractory anemia with excess of blasts	98	21.4	76	76	1.11	0.78	0.68	0.72	0.92
Refractory cytopenia with multilineage dysplasia	71	15.5	76	78	0.80	0.63	0.46	0.54	1.09
MDS associated with isolated del(5q)	6	1.3	–	68	0.07	–	0.12	0.06	-
MDS unclassifiable	127	27.7	80	81	1.44	1.25	0.60	0.85	1.50**
Myeloproliferative neoplasms	489	100	67	67	5.53	4.93	4.00	4.43	1.10***
Chronic myelogenous leukemia	102	20.9	64	60	1.15	1.13	0.82	0.96	1.32
Polycythemia vera	118	24.1	65	72	1.33	1.46	0.71	1.08	1.62**
Primary myelofibrosis	35	7.2	68	65	0.40	0.36	0.31	0.32	1.19
Essential thrombocythemia	176	36.0	69	65	1.99	1.46	1.77	1.59	0.80
Others	9	1.8	65	55	0.10	0.12	0.06	0.09	2.00
Myeloproliferative neoplasm unclassifiable	42	8.6	74	80	0.48	0.32	0.29	0.30	0.76
Mast cell diseases	7	1.4	56	62	0.08	0.09	0.06	0.08	1.29
Myelodysplastic/myeloproliferative neoplasms	65	100	79	78	0.74	0.59	0.35	0.44	1.33
Chronic myelomonocytic leukemia	61	93.9	79	79	0.69	0.56	0.32	0.41	1.34
Juvenile chronic myelomonocytic leukemia	0	0.0	–	–	–	–	–	–	–
Atypical chronic myeloid leukemia	4	6.2	79	67	0.05	0.03	0.04	0.03	1.00
Myeloid malignancies	1,331		72	73	15.04	13.06	9.56	11.08	1.17***

*N. of cases (number of cases); % (percentage of cases); CR (crude rate per 100,000 inhabitants/year); ASRE (European population standardized incidence rate per 100,000 inhabitants/year); AML (acute myeloid leukemia); AL (acute leukemia); NOC (not otherwise categorized); NOS (not otherwise specified); MDS (myelodysplastic syndromes): * (0.05> P <0.03), ** (0.03> P < 0.01), *** (P < 0.01).*



	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008
AML	2.62	3.25	3.39	2.69	3.07	3.34	2.48	2.82	2.16	3.31	3.35	3.46	1.73	3.40	2.73
MDS	1.48	2.34	2.51	2.09	2.75	4.06	3.03	3.53	3.83	3.84	2.71	4.89	3.00	3.43	4.59
MPN	3.72	3.67	3.06	3.66	4.04	2.97	3.57	3.65	3.33	4.95	5.46	5.81	6.03	5.14	6.06
MDS/MPN	0.09	0.25	0.36	0.34	0.23	0.45	0.28	0.46	0.30	0.85	0.59	0.61	0.64	0.54	0.45

Figure 1. Trends in standardized incidence rate of myeloid malignancy WHO categories in Girona province (1994-2008). This figure shows the modeled standardized incidence rate. Values under the figure represented the real standardized incidence rate by year and myeloid groups.

AML: acute myeloid leukemia; MPN : myeloproliferative neoplasms; MDS/MPN: myelodysplastic/myeloproliferative neoplasms; MDS: myelodysplastic syndromes; EAPC: estimated annual percentage change.

cythemia vera (PV) and essential thrombocythemia (ET) as malignant diseases. Results of crude rate (CR) and European standardized incidence rate (ASRE) were expressed per 100,000 inhabitants/year.

A total of 1,331 cases of MMs were diagnosed in the province of Girona from 1994 to 2008: 718 men (53.9%) and 613 women (46.1%). Acute myeloid leukemia (AML) accounted for 24.0% (319 of 1,331), MDS 34.4% (458 of 1,331), MPN 36.7% (489 of 1,331) including chronic myeloid leukemia (CML), and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) for 4.9% (65 of 1,331).

The MMs CR was 15.04 and ASRE was 11.08 (13.06 in men and 9.56 in women), with a statistically significant male predominance (sex ratio 1.17). Median age was 72 years in men and 73 years in women (Table 1).

Although the highest incidence among MMs groups in the province of Girona was MPN (ASRE = 4.43), diagnosis was often made in outpatient settings and this means these cancers are more likely to be underreported to cancer registries than other cancers. However, our results were similar to those reported in south-east England, although we included mast cell diseases as recommended by the WHO 2008.[5,6] The median age of patients at diagnosis was 67 years and a significant male predominance was found both overall and also in the PV entity (Table 1).

Since ICD-O-3 (International Classification of Diseases for Oncology) was introduced, MDS can now be reported in cancer registries. Due to changes in MMs classification, and the advanced age of patients at diagnosis, these diseases have not been as well documented as other pathologies. The most frequent type within this group was MDS unclassifiable, and the least was 5q- syndrome (Table 1). The incidence of MDS (ASRE = 3.30) was close to that reported in south-east England and Germany.[6,7] The higher incidence of MDS in the province of Girona than in the French study could be due to the fact that the Côte d'Or Department is a hematologic monographic registry. They used positive criteria such as karyotype abnormalities or evolution to a more aggressive type to register cases of refractory anemia in order to avoid the inclusion of false cases.[8] The lower incidence of MDS in the US may be due to the underreporting of cases.[9,10]

The overall ASRE for AML was 2.91, with a statistically male predominance (sex ratio 1.28). The median age of AML patients was 68 years, being lower in AML with recurrent cytogenetic abnormalities and therapy-related AML. Approximately 54% (171 of 319) of cases were AML not otherwise categorized, and within this group, the majority of cases were acute myelomonocytic leukemia and AML without maturation. The AML and MDS therapy-related group was the less frequent within the four main AML groups (Table 1). The incidence rate of AML in the province of Girona was similar to that reported in France, south-east England and Sardinia.[6,8,11] Nevertheless, the proportion of AML with cytogenetic abnormalities in our cohort was lower than in France and the UK.[8,12] This is probably because karyotype tests were made outside the province of Girona and information was not always available for older cases. The most frequent cytogenetic abnormality was the t(15;17), as in France and the UK.[8,12]

Finally, the myeloid group with the lowest incidence was MDS/MPN, with an ASRE of 0.44 (Table 1). The median age at diagnosis was 78 years. Chronic myelomonocytic leukemia (CMML) was the most frequent entity, comprising more than 90% of all MDS/MPN cases. Our results were comparable to others reported in south-east England or Germany.[6,7] Differences were found between our incidence rates and those reported in France, probably because in the province of Girona some cases of CMML were recoded as MDS.[8] The lower incidence in the US could be a consequence of underreported cases.[9,10]

Significant increases in incidence trends were found in the MDS, MPN and MDS/MPN groups (Figure 1), although these could be considered unreliable. The presence of some gene mutations and the improvements in diagnostic techniques could be the reasons for these changes in incidence trends. The discovery of the JAK2 V617F mutation in 2005 could have resulted in an increase in some MPN, such PV, ET, and primary myelofibrosis, which have been identified objectively as pathological since the introduction of molecular biology.[13,14] No increase in incidence of AML was found between 1994-2008, sim-

ilar to results reported from France, the UK and the US.[8,12,15]

According to our predictions, 7,551 new cases of MMs will be diagnosed in Spain for the year 2013, of which 1,772 cases will be AML, 2,647 MDS, 2,744 MPN and 388 MDS/MPN. These results are of interest for clinicians and those involved in public health in order to evaluate the cost of new treatments for these pathologies. Some limitations of this study must be considered. Classification of MMs has changed in recent decades and there could be an underreporting of cases. Also, an increased outpatient diagnosis over time makes it difficult to ensure complete coverage of MMs in cancer registries, especially MDS and MPN.

This study describes in detail the incidence of MMs in a large population-based cohort. MPN were the group with the highest incidence of MMs in the province of Girona and incidence trends increased significantly in MDS, MPN and MDS/MPN. Our results show that recent advances in diagnosis of MMs and an increase in outpatient diagnosis have resulted in changes in incidence rate trends.

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Key words: WHO classification, myeloid malignancies, HAEMACARE project.

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Posted online on August 18, 2014

Abstract

Myeloid malignancies (MMs) are heterogeneous groups of diseases which present different prognoses. Using data from the population-based Girona Cancer Registry, we estimated the relative survival (RS) rates and relative excess risk of death among patients with MMs in the province of Girona between 1994 and 2008. The 5-year RS rate was 49.7%, ranging from 20.2% for acute myeloid leukemia (AML) to 75.3% for myeloproliferative neoplasms (MPN). Marked differences in RS were observed when the age of patients was considered: an increase in RS was mainly found in younger patients with myelodysplastic syndromes and MPN. Furthermore, cases of chronic myeloid leukemia treated with imatinib had a significantly better outcome compared with those that were untreated. Despite the slight improvement in the survival rate of younger patients with AML, RS remained stable for 15 years, as no significant improvements were made in the management of the disease during that period.

Keywords

Relative survival, myeloid malignancies, acute myeloid leukemia, myelodysplastic syndromes, myeloproliferative neoplasms, myelodysplastic/myeloproliferative neoplasms

Osca-Gelis G, Puig-Vives M, Saez M, Gallardo D, Solé F, Marcos-Gragera R. Incidence and survival of chronic myelomonocytic leukaemia in Girona (Spain): a population-based study, 1993-2007. *Leukemia Research*. Vol. 36, issue 10 (2012) : 1262-1266. Erratum in *Leukemia Research*. Vol. 37, issue 7 (2013) : 852

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Abstract

Chronic myelomonocytic leukemia is a very rare blood cancer observed mostly in the elderly. Here we report the incidence trends and survival of patients with chronic myelomonocytic leukemia over a 15-year period (1993–2007). Cases were provided by the population-based Girona Cancer Registry. The crude incidence rate was 0.72/100,000 inhabitants/year. No statistically significant increase in trends was detected over the 15 years. Median overall survival was 28 months although survival markedly decreased with advancing age. The 5-years observed and relative survivals were 20% and 29%, respectively. This is the first population-based study that reports the incidence and survival of chronic myelomonocytic leukemia in Spain

Keywords

Chronic myelomonocytic leukemia; Incidence rate; Relative and observed survival; Population-based cancer registry

Discussion

If you have knowledge,
let others light their candles in it.

Margaret Fuller

1. Incidence of MMs

Limited data on incidence of haematological malignancies of myeloid cell lineage are available in the literature. MMs are not one of the most frequent cancers in our population and changing classifications complicate epidemiological studies and comparative analysis[10,45]. Determining the overall incidence of MMs correctly it is not an easy task for population-based cancer registries and even less so when it comes to reporting incidence rates for each of the MM entities proposed by the 2001 WHO classification. A non-monographic population-based cancer registry codifies not only haematological malignancies, but rather all the pathologies diagnosed in its covered area.

MMs are diseases related with elderly population and their pathology is often not studied exhaustively because their associated comorbidities do not allow for this to happen, making the number of cases with unspecific diagnosis greater than those found in other pathologies. In addition, as diagnostic methods have improved in recent years this has resulted in an increase in outpatient diagnosis, being consequently the completeness of cancer registries not always as comprehensive as we would expect[120]. Furthermore, if we also take into consideration that the molecular biology and cytogenetics laboratories, where some tests are performed, are located outside our coverage area, then we will begin to understand just how laborious it is to register MMs correctly in the province of Girona. This is why the RCG carried out such an exhaustive revision of the actual status of MMs in our province.

As mentioned in the Introduction, because the corresponding ICD-O codes for the revised WHO 2008 classification had not yet been published, the RCG assessed incidence rates are in accordance with the 2001 WHO classification; only adding (as WHO recommended in 2008) mast cell diseases and renaming the MPN and MDS/MPN groups.

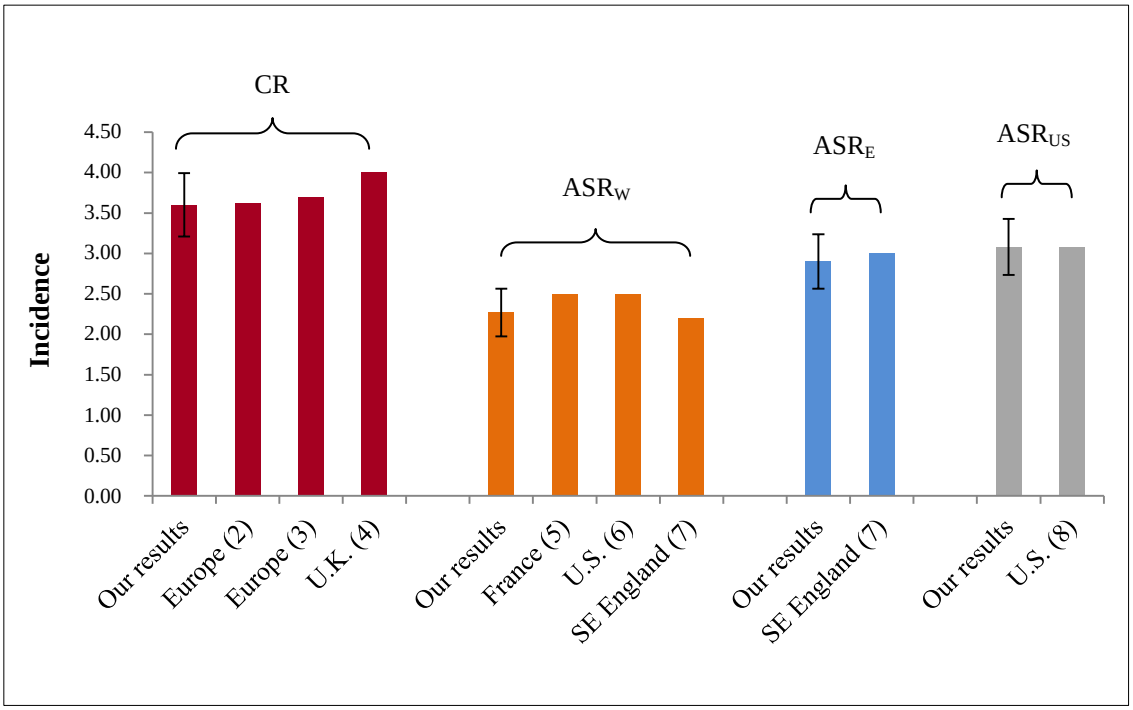
1.1. Analysing the incidence rate of each MM entity as proposed by the 2001 WHO classification

The initial work in this thesis outlines in detail the incidence of MMs in the province of Girona during the period 1994-2008. To ensure the complete coverage of MMs in the RCG, and especially for MPN and MDS, a retrospective search was carried out to include data from clinicians' databases and from haematology laboratories where some test, such as flow cytometry, or molecular and cytogenetic tests, were assessed.

In the province of Girona a total of 1,331 MM cases (718 men and 613 women) were diagnosed over fifteen years. Examining cases using the main MM groups from the WHO, it was found that AML accounted for 24.0% (319 of 1,331 cases), MDS 34.4% (458 cases of the 1,331), MPN 36.7% (489 out of the 1,331) and MDS/MPN for 4.9% (65 of the 1,331).

AML

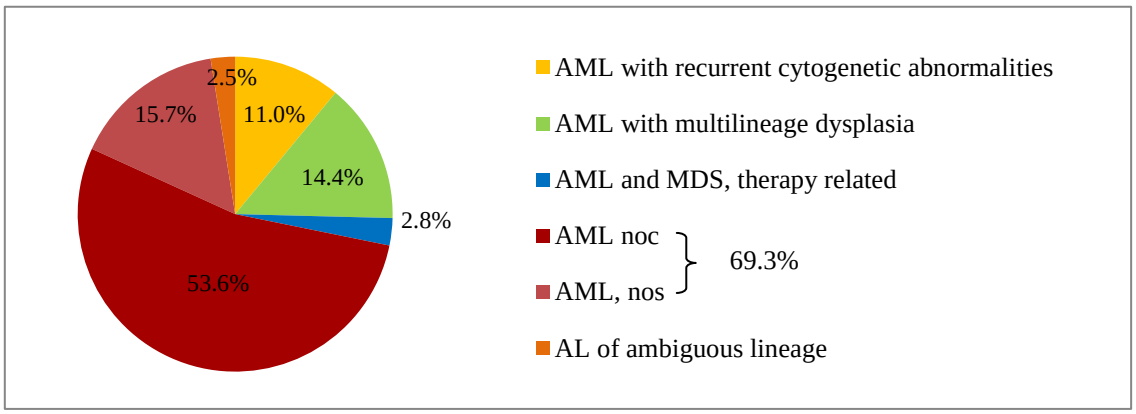
Incidence rates for AML in the Girona province (CR= 3.60, ASR_E= 2.91, ASR_W=2.27 and ASR_{US} = 3.08) were similar to the incidence results reported in France, South East England, the U.K., the U.S.A. and Europe, indicating that recoding system criteria have been stable for many years (Figure 8).



CR (crude rate); ASR_W (World-population standardized incidence rate): ASR_E (European-population standardized incidence rate); ASR_{US}. (US-population 2000 standardized incidence rate): Our results (Girona), Europe (2)[5], Europe (3)[4], UK (4)[53], France (5)[39], U.S. (6)[48], SE England (7)[46], U.S. (8)[52]

Figure 8. Incidence rates for acute myeloid leukaemia reported in some previous epidemiological studies.

The most frequent subgroups of AML in the province of Girona according to the 2001 WHO classification system were AML noc, and AML nos (Figure 9). Because the AML noc group includes almost all the AML entities described by the FAB classification and given that this classification was used in the RCG for many years, it was to be expected that AML noc would be the group comprising most cases. Despite AML classification following a hierarchical order and finding cytogenetic alterations being a priority for proper registration, not all patients were well documented and some had been categorized within nonspecific groups.



AML (acute myeloid leukaemia); MDS (myelodysplastic syndromes); noc (not otherwise categorized); nos (not otherwise specified); AL (acute leukaemia)

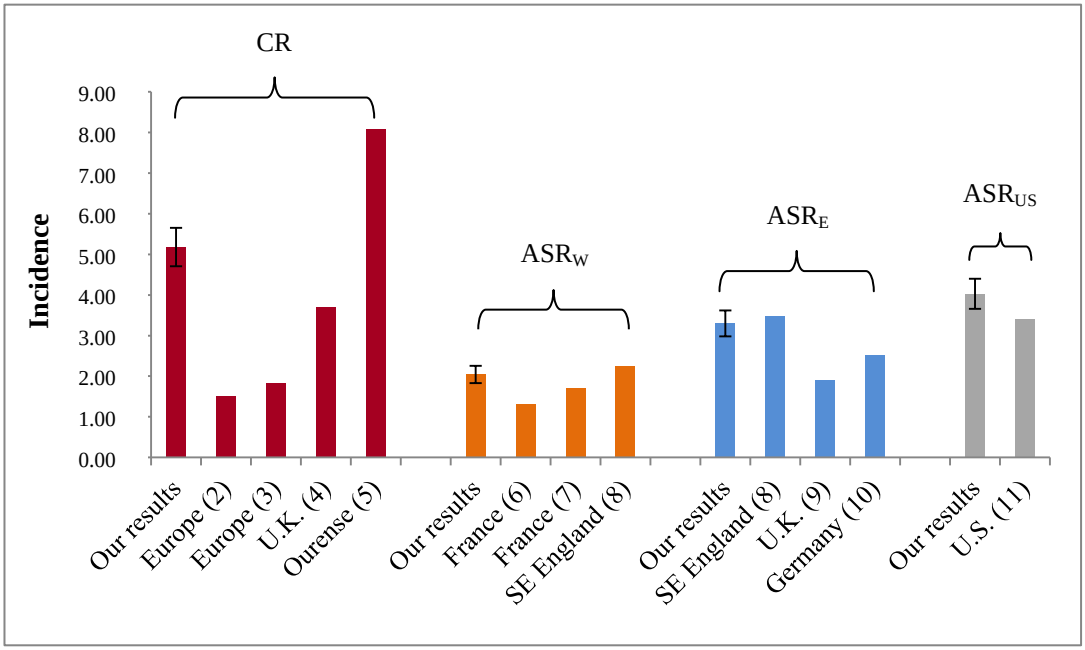
Figure 9. Frequency of acute myeloid leukaemia by main subgroups in the Girona province 1994-2008.

AML with recurrent cytogenetic abnormalities accounted for 11% of all AMLs diagnosed in the Girona province from 1994-2008. This proportion was lower than in France and the UK and likely occurred because of the karyotype tests for patients from Girona mainly being done outside the province and so information for some cases was not always available in the RCG[28,39]. Within this group, AML with t(15;17), also known as APL, was the most frequent entity, in agreement with those result reported by Maynadié et al. and Sanderson et al., being exceptionally associated to breast cancer cases treated with mitoxantrone[28,39,121,122]. Although cases related to a previous breast cancer tend to be classified as therapy-related AML, the classification of AML follows a hierarchical order, so if cases present recurrent cytogenetic alterations they have to be classified within this group despite the patient suffered a previous cancer[15]. Finally, the AML and MDS therapy-related (2.8% of all AML) and AL of ambiguous lineage groups (2.5% of all AML) were those that least numerous in the province of Girona; as it was in France[39].

MDS

The incidence rate of MDS found in the Girona province (CR= 5.18, ASR_E = 3.30, ASR_W = 2.04 and ASR_{US} = 4.03) were very close to those reported in South East England and Germany[28,46,71]. The higher incidence of MDS in Girona than in France could be attributed to the fact that the Registre des Hémopathies Malignes de Côte d'Or is a haematological monographic registry. In order to avoid the inclusion of false cases, they use positive criteria such as karyotypic abnormalities, abnormal progenitor in vitro culture, or the evolution to a more aggressive type of cancer to register cases of RA[39]. Contrary, the lower incidence rates reported in European and

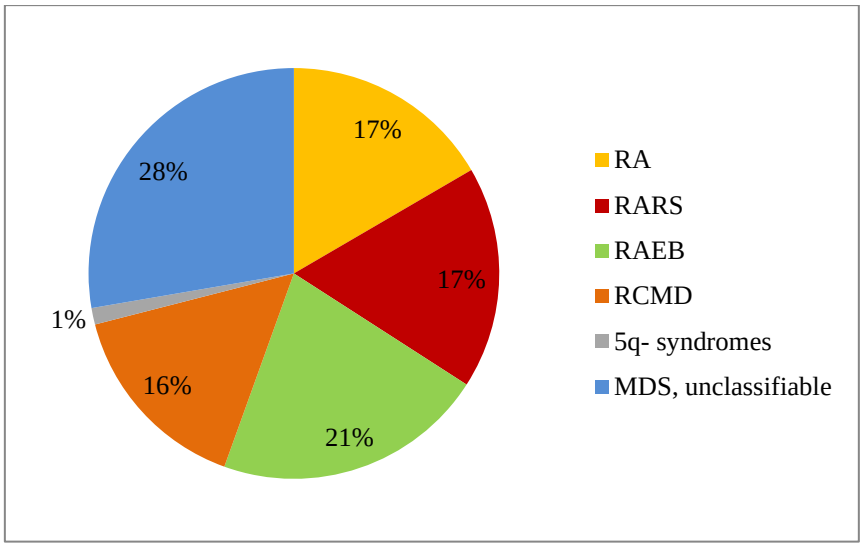
U.S. studies could be a consequence of an underreporting of cases (Figure 10). MDS have been reportable in cancer registries since the ICD-O-3 was introduced[72]. Due to changes in the classification of MMs and the advanced age of patients at diagnosis (median age in Girona = 77 years), the incidence of these diseases has not been as well documented as other pathologies. The RCG recoup some possible MDS which were unable to be not detected using all routine information sources, so this is how we realized the needing to incorporate clinicians' databases (as an additional information source) to ensure the complete registration of all MDS.



CR (crude rate); ASR$_W$ (World-population standardized incidence rate): ASR$_E$ (European-population standardized incidence rate); ASR$_{US}$. (US-population 2000 standardized incidence rate): Our results (Girona), Europe (2)[4], Europe (3)[5], U.K. (4)[53], Ourense (5)[73], France (6)[39], France (7)[70], SE England (8)[46], U.K. (9)[51], Germany(10)[71], U.S. (11)[72].

Figure 10. Incidence rates of myelodysplastic syndromes published in some European and U.S. studies.

Within this group, the most frequent entity in the Girona population was MDS-u (Figure 11). The disproportionate diagnostic activity in our elderly population and the fact that aged patients are not as well catalogued as younger people may have increased the recorded incidences of this entity in our area of study. To the contrary, RAEB was the most frequent MDS in France in the period 1980-2004, RCMD in Germany from 1996 to 2005 and RA in South-East England in the years 1999 and 2000. These results highlight the discrepancies in MDS registration round the world.

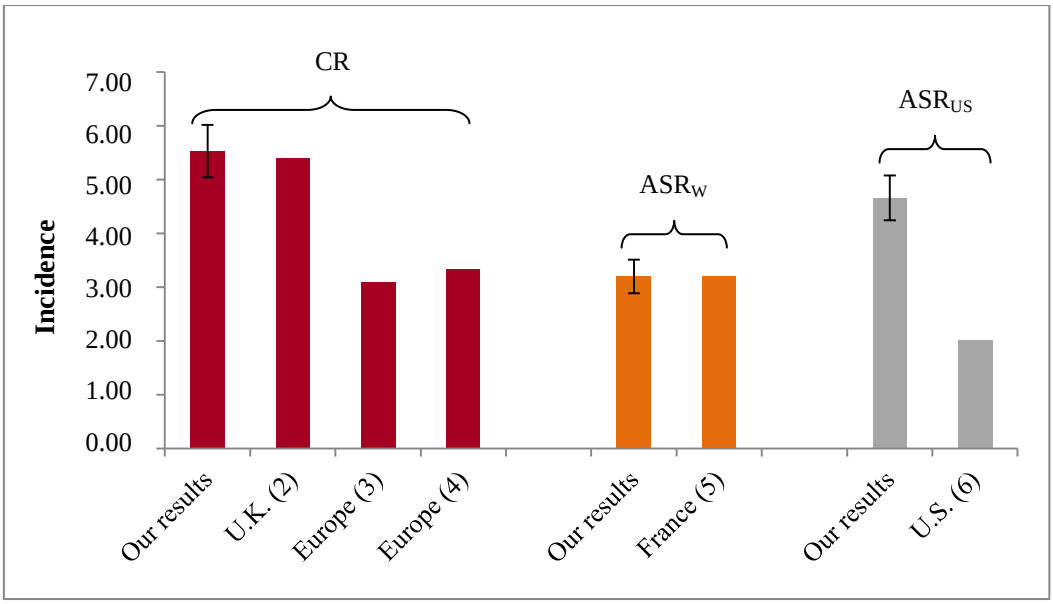


RA (refractory anaemia); RARS (refractory anaemia with ringed sideroblasts); RAEB (refractory anaemia with excess of blasts); RCMD (refractory cytopenia with multilineage dysplasia); MDS (myelodysplastic syndromes)

Figure 11. Frequency of myelodysplastic syndrome entities diagnosed in the province of Girona during 1994-2008.

MPN

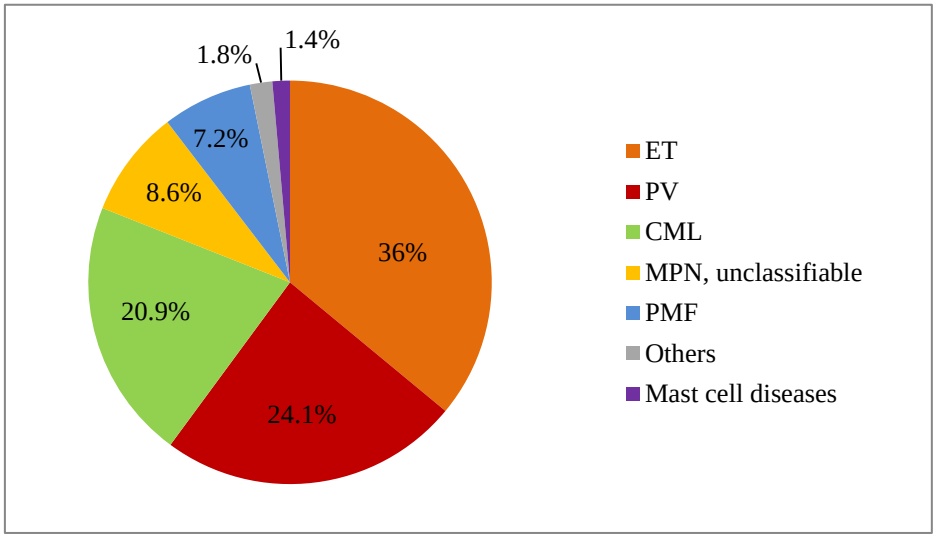
As other studies have demonstrated, MPN was the most frequent group of MMs in the province of Girona. Our incidence rates (CR = 5.56, ASR_W = 3.20, ASR_E = 4.43 and ASR_{US} = 4.66) were similar to the French and U.K. populations, despite the fact that, as recommended by the WHO 2008 review, we included mast cell diseases[39,53]. Meanwhile they were higher than Europe overall and the U.S. (Figure 12)[4,5,39,80]. These differences are mainly due to the underreporting of cases. Cases of MPN without symptoms, which rarely are admitted to hospital, are diagnosed as outpatients. The discovery of the *JAK2* V617F mutation allow the diagnosis of asymptomatic patients with PV, ET and PMF using basically molecular biology tests, which make these cancers more likely than others to be underreported to cancer registries. To ensure the completeness of MPN, the RCG also made a retrospective search of all those cases that may have gone unregistered using only the routine sources of information.



CR (crude rate); ASR$_W$ (World-population standardized incidence rate): ASR$_E$ (European-population standardized incidence rate); ASR$_{US}$. (US-population 2000 standardized incidence rate): Our results (Girona), U.K. (2)[53], Europe (3)[4], Europe (4)[5], France (5)[39], U.S. (6)[80].

Figure 12. Myeloproliferative neoplasm incidence rates as reported in some studies.

Within this group of MMs, PV and ET were the two most frequent entities in the province of Girona, whereas mast cell diseases, CNL and CEL represented less than 2% of MPN (Figure 13). As Titmarsh et al., reported in their meta-analysis comprising populations from Europe, North America and Australasia, ET is the most common MPN, followed by PV and PMF, although they demonstrated the high heterogeneity in the registration of these diseases[123]. Moulard et al., also published data on incidences of 0.68 to 2.6 for PV, 0.38 to 1.7 for ET and 0.1 to 1.00 in the European Union, highlighting the great variety of incidence of these diseases across Europe and also the influence of the *JAK2* V617F mutation discovery in the diagnosis of Ph-negative MPN diseases[95].

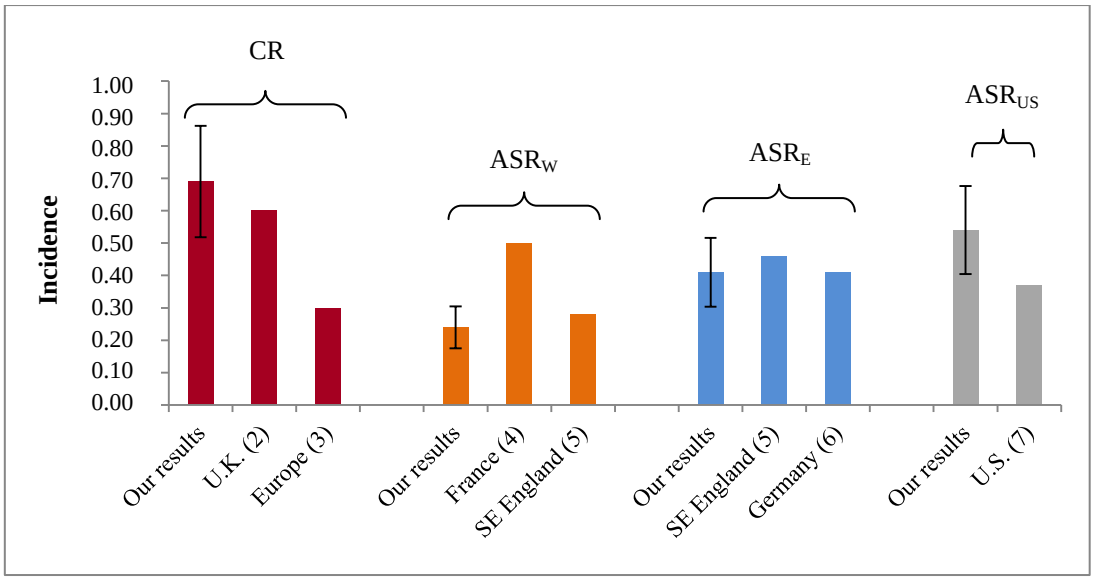


ET (essential thrombocythaemia); PV (polycythaemia vera); CML (chronic myeloid leukaemia); PMF (primary myelofibrosis), Others (chronic eosinophilic leukaemia and hypereosinophilic syndrome)

Figure 13. Frequency of myeloproliferative neoplasm entities diagnosed in the province of Girona during 1994-2008.

MDS/MPN

Finally, the myeloid group with the fewest incidences in the province of Girona was MDS/MPN. Changes in the classification of this group resulted in data collection becoming unreliable and probability data not being properly recoded by population-based cancer registries. Despite CMML was the most frequent entity within this group and comprised more than 90% of all MDS/MPN cases, it is accepted as a very rare malignancy with an incidence of lower than one case per 100,000 inhabitants/year. Our results (CR = 0.69, ASR_E = 0.41, ASR_W = 0.24 and $ASR_{U.S.}$ = 0.54) were comparable to others reported in South East England, the U.K. and Germany. However, differences were found when comparing our incidence with those reported in France, probably because some CMML cases were recoded as MDS in the RCG. The lower incidences in the U.S. and Europe could be a consequence of underreported cases (Figure 14). As discussed in the third paper of this thesis (Section 3 of this part), CMML is an entity whose classification has changed over time, and thus has influenced incidence results.



CR (crude rate); ASR$_W$ (World-population standardized incidence rate): ASR$_E$ (European-population standardized incidence rate); ASR$_{US}$. (US-population 2000 standardized incidence rate): Our results (Girona), U.K. (2)[53], Europe (3)[4], France (4)[39], SE England (5)[46], Germany (6)[71], U.S. (7)[80].

Figure 14. Incidences rates of chronic myelomonocytic leukaemia as reported in some epidemiological studies.

1.2 Evaluate the incidence trend of MMs during fifteen years.

Regarding temporal incidence trend of each MMs group proposed by the 2001 WHO classification we found that incidence of AML remains stable in the province of Girona from 1994 to 2008, which is akin to studies in France, the U.K. and the U.S.[28,39,124]. Although new AML entities have emerged from the successive WHO revision, AML is a malignant entity considered in all MMs classifications. Moreover detection criteria have been stable for many years, so no increasing number of AML cases diagnosed annually in the province of Girona was found[6,7,9,12].

Because patients should be admitted to the hospital for treatment, cancer registries should be able to collect together all the cases devoid of complications, resulting in a completeness of AML.

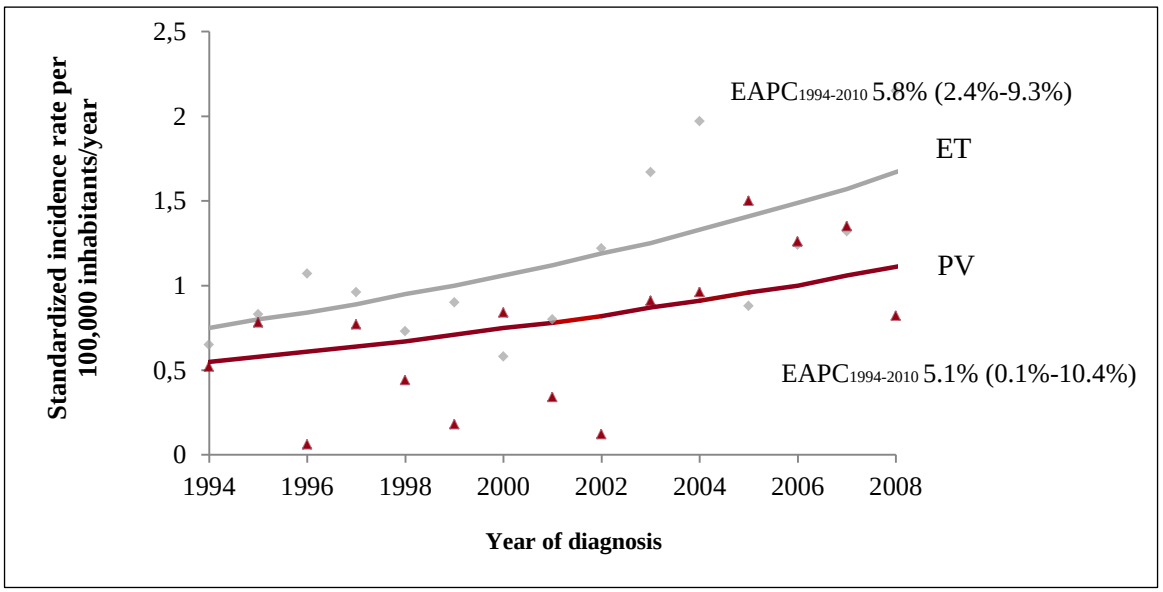
To the contrary, a significant increase in incidence over time in the province of Girona was found concerning the MDS group, and which coincides with that reported in all previous MDS epidemiological studies. Variations on incidence rate could be principally due to:

- Changes in MDS classification[7,9,11]. MDS cases have been recoded as malignant since the publication of the ICD-O-3, whereas before that MDS were considered as being uncertain behaviour pathologies and so cancer registries did not recode them. Thus, this apparent

increase could be a consequence of an over-reporting of cases that had not been registered previously.

- Advances in the diagnostic methods. Despite the fact that MDS is a group of diseases affecting the elderly and that these patients are studied with less accuracy than younger ones, diagnostic improvements have allowed the disease to be detected far more easily, although a specific diagnosis of the disease is not able to be made. In other words, although clinicians are able to detect the MDS, it can not be classified into any of the entities proposed by the WHO classification, and so are registered as MDS-u.
- Improvements in MDS registration. As was mentioned in the introduction, the RCG added clinicians' databases along with the results of cytogenetic and biology molecular tests as information sources to ensure a complete registration of MMs. As these databases include cases diagnosed in more recent years and not for the whole period, this could imply an increase in incidence trend of MDS.

As for MPN incidence trends in Girona province, an annual increase of 4.7% from 1994 to 2008 was found. The discovery of the *JAK2* V617F mutation and improvements in diagnostic techniques would seem to have caused an increase in incidences of MPN, especially in MPN Ph negative such as ET, PV and PMF[45,120,125]. As described by Girodon et al., the observed increase would appear to be a result of a late diagnosis of previous underreported cases, and which, since the introduction of molecular biology as a routine diagnostic method, have been identified objectively as pathological[96]. To verify whether the increased incidence of MPN Ph negative had occurred since 2005, the RCG studied the trend in the incidences of the two more frequent MPN Ph negative entities: ET and PV.



ET (essential thrombocythaemia); PV (polycythemia vera); EAPC (Estimated Annual Percentage of Change).

Figure 15. Temporal incidence trends on essential thrombocythaemia and polycythemia vera diagnosed in the province of Girona from 1994 to 2008.

As Figure 15 shows, incidences of PV and ET increased statistically over time, although significance for PV was borderline. The observed increase could be a consequence of the improvements made when the classification was revised and which ultimately led to MPN being easily categorised[45]. Despite that the *JAK2* V617F mutation can influence on the incidence trend of ET, we could not justify that this increase was in fact due to this mutation detection because the increase of incidence was throughout the whole period, not since 2005.

Lastly, although the group of MDS/MPN was the less frequent in the province of Girona, we could see that the temporal incidence rate increased significantly during the period 1994-2008. Although this group of MMs did not exist before the publication of the WHO 2001 classification, CMML was defined in the FAB classification[11]. Despite this entity sharing myelodysplastic and myeloproliferative features, it was initially catalogued within the MDS group. The main problem was that sometimes it was simply too complicated to diagnose CMML and so it was recorded as either MDS or CML. Since the publication of the 2001 WHO classification, CMML has been codified as an entity of the emergent group called MDS/MPN. With this new group, cancer registries were able to improve their registers. The RCG made a retrospective search to identify possible cases of CMML which might have been recoded as MDS-u or CML nos, and found that very few cases had initially been wrongly recoded. While these changes can result in an increased incidence trend, we decided to consider the rise observed as fictitious, as there were only 65 patients in a fifteen year period and new cases may have significantly influenced the trend.

2. Survival of MMs

One of the uses of population-based cancer registries is to provide survival data of a particular pathology in order to know its prognosis in a concrete population, assess the effects of both available and emerging treatments and, in particular, to inform patients of their survival expectancy.

Along these lines, the second work of this thesis was focused on describing in detail the survival rate of MMs in the province of Girona overall, for each of the groups as proposed by the WHO 2001 classification and for each of pathologies described. In addition, this study also reports on the survival data by sex, age of patients at diagnosis and year of diagnosis, all the while trying to relate possible changes in survival with improvements in the method of diagnosis, treatment and better patient stratification according to risk groups.

There are few published studies reporting MMs survival rates[39,95,123]. As highlighted earlier, changes in the classification of MMs have complicated a proper registration of the different

myeloid pathologies, and this is the preceding step to publishing good survival expectancy data. Consequently, the RCG aimed at increasing published results with this exhaustive study covering all the MMs diagnosed in the province of Girona during the period 1994-2008.

2.1. Examining the MMs survival rates according to WHO classification, by principal groups or by each malignancy individually.

As is well known, MMs are a heterogeneous group of haematological malignancies which present diverse prognoses. Some papers published demonstrate that Europeans with MMs have a poor 5-year RS, despite variations in the WHO groups. AML is the category with the worst scenario, with a 5-year RS of around 15-18%, whereas MPN is the group with the best prognosis of 60-80%[4,39,54,95].

In the Girona province, the median overall survival for all MMs was 37 months: 6.2 months for patients diagnosed with AML, 31.5 months for those with MDS, 114.7 months for people with MPN and 28.4 months for MDS/MPN cases.

AML

Although AML was the group with the worst prognosis within MMs with its 5-year RS of 20.2% in the Girona province, survival differed according to AML subgroups. Patients with AML with recurrent cytogenetic abnormalities had a better survival rate (5-year RS of 60.9%), probably because this group mainly comprises good prognostic cytogenetics such as inv(16), /t(16;16)/del(16q), t(8;21) and t(15;17). This group, which is also known as CBF AML, presents a higher complete remission rate and a lower incidence of relapse, especially in patients receiving high-dose of cytarabine in post-remission treatment. Moreover, because the APL was the most frequent entity within this group, (as mentioned in 1.1 of this section), and patients can be effectively treated with ATRA, the overall result was relatively good[30,126,127].

On the other hand, as found on the Côte d'Or or in other European publications, AML with multilineage dysplasia or AML and MDS-therapy related, were the AML subgroups with the worst survival rates. Some previous studies on the impact of multilineage dysplasia found that the presence of dysgranulopoiesis, bilineage and trilineage dysplasia and dysplastic changes in granulocytes and megakaryocytes were associated with a significant decrease in complete remission, lower disease-free survival and the worse overall outcome[31,38,128,129]. As for the AML and MDS-therapy related group, it was found that these patients presented a more adverse risk to cytogenetic alterations involving chromosomes 5,7, 11, 17 and 19 and to complex karyotypes than patients with AML de novo, making this category more aggressive (Figure 16)[130].

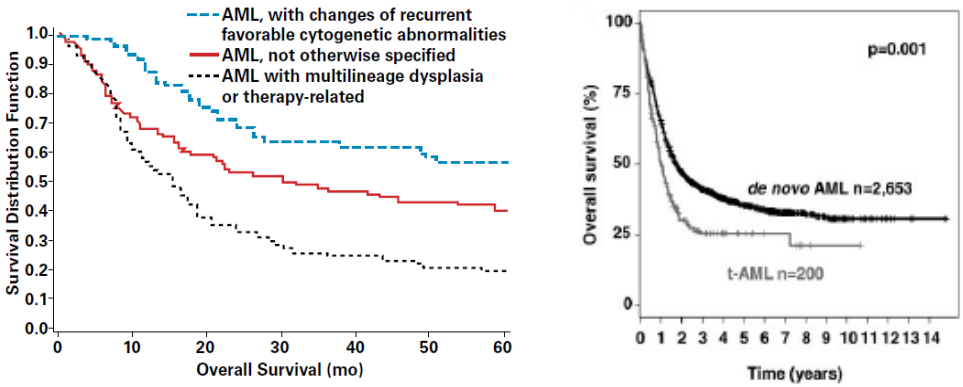
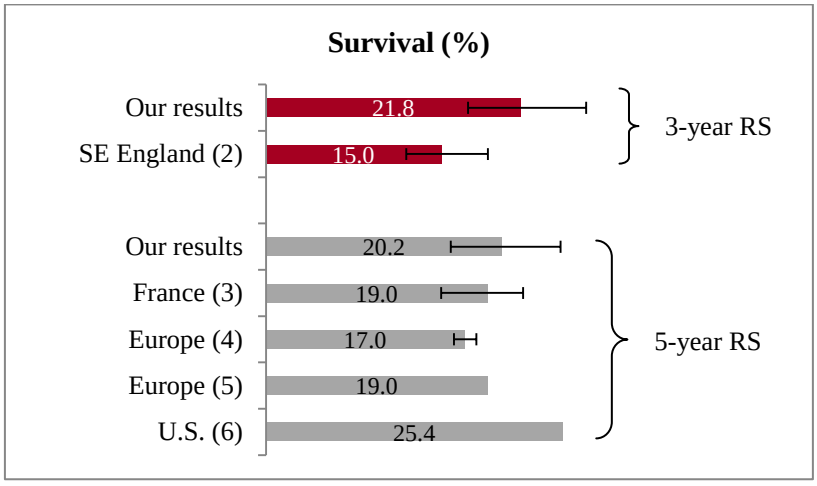


Figure 16. Survival curves as reported by Arber et al and Kayser et al., when comparing the main acute myeloid leukaemia subgroups[31,130].

Comparing the overall survival of AML in Girona, we found that our results were closely related to those reported in other epidemiological studies (Figure 17).

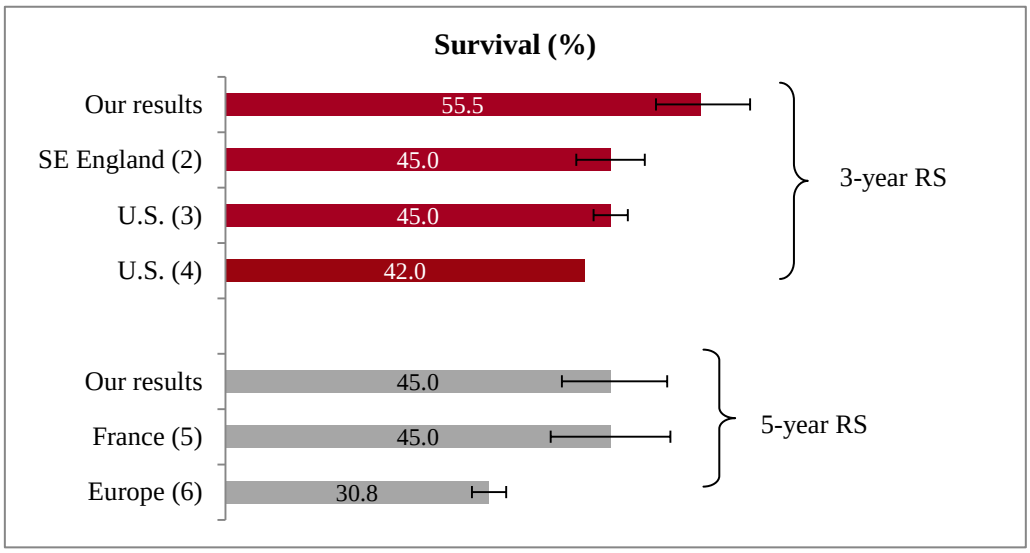


RS (relative survival); Our results(Girona: 1994-2008); SE England: 1999-2000 (2)[46], France: 1980-2004 (3)[39], Europe: 1995-2002 (4)[54], Europe: 1995-2002 (5)[4], U.S.: 2001-2007 (6)[52]

Figure 17. Survival rates of acute myeloid leukaemia patients at 3 and 5 years presented by some epidemiological studies.

MDS

Limited population-based data on survival of MDS are available in the literature. Our results show 3 and 5-year RS of 55.5% and 45%, respectively. This was slightly higher than rates reported in other published papers (Figure 18).



RS (relative survival); Our results(Girona: 1994-2008); SE England: 1999-2000 (2)[46], U.S.: 2001-2004 (3)[80], U.S.: 2001-2002 (4)[72], France: 1980-2004 (5)[39], Europe: 1995-2002 (6)[54]

Figure 18. Population-based data of survival rates of myelodysplastic syndromes.

It is well known that RCMD, RA and RARS are low-risk MDS associated with good survival rates, whereas RAEB and MDS-u have the worse outcomes[62]. Low-risk patients are defined by:

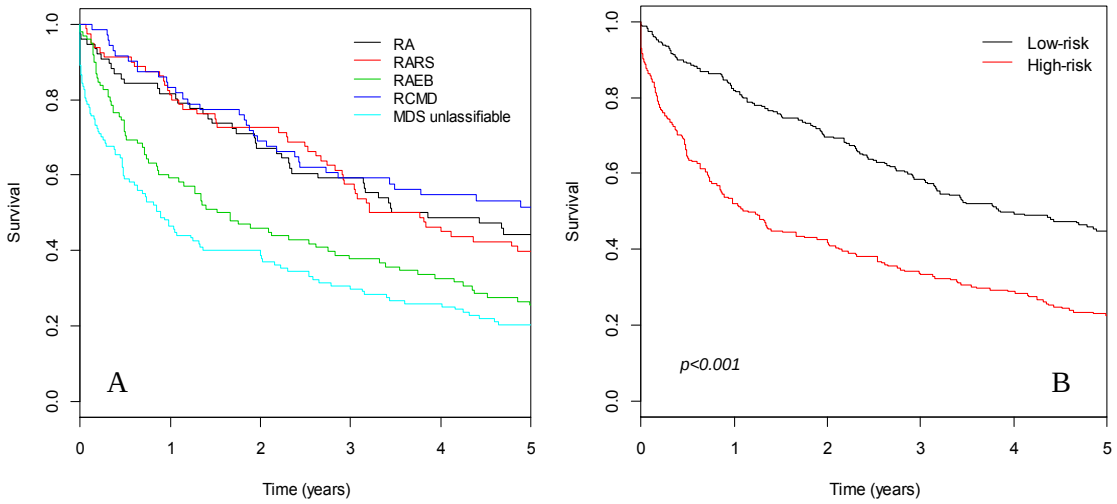
- Low-risk IPSS or low-risk WPSS or very low-risk R-IPSS
- Intermediate-1 IPSS, or intermediate-risk WPSS and R-IPSS without any of the following characteristics:
    - High-risk or very high-risk cytogenetic abnormalities of the R-IPSS.
    - Platelets $<30x10^9$/L.
    - Neutrophils $<0.5\ x10^9$/L.
    - Presence of thick and diffused myelofibrosis (grade 2-3).

Whereas, high-risk patients are those characterized by:

- Intermediate-2 or high-risk IPSS, and/or high or very high-risk WPSS and/or R-IPSS.
- Intermediate-1 IPSS, and/or high or very high-risk WPSS or R-IPSS presenting one of the next qualities:
    - High or very high-risk cytogenetic abnormalities of the R-IPSS.
    - Platelets $<30x10^9$/L.
    - Neutrophils $<0.5\ x10^9$/L.
    - Presence of thick and diffused myelofibrosis (grade 2-3).

Although the RCG was not able to stratify MDS through important risk assessment data such as IPSS, we were able to broadly stratify MDS according to their histology in two risk groups: low

and high. As depicted in Figure 19A, it was found that RA, RARS and RCMD could be categorized as low-risk MDS, whereas RAEB and MDS-u corresponded to high-risk groups after excluding 5q- patients due to low number of cases. By comparing these two groups (Figure 19B) we found that, while low-risk patients had a median OS of 46 months, those in the high-risk groups only survived 14 months ($p<0.001$), as the literature verified.



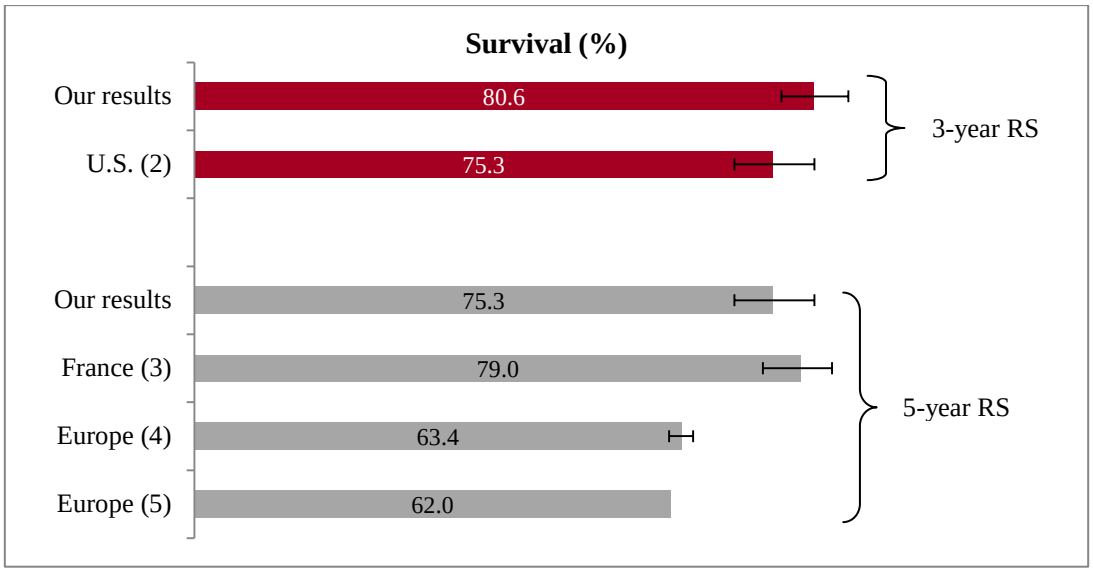
RA (refractory anaemia); RARS (refractory anaemia with ringed sideroblasts); RAEB (refractory anaemia with excess of blasts); RCMD (refractory cytopenia with multilineage dysplasia); MDS (myelodysplastic syndromes)

Figure 19. Myelodysplastic syndrome survival in the province of Girona during the period 1994-2008. A) by each entity individually and B) by grouping entities in risk groups.

The high percentages of blasts in PB and BM in patients with RAEB, along with the increase in age of patients with MDS-u remain two of the possible explanations in comprehending the poor survival rates of the high-risk group[4,39,62]. Thus, depending on the frequency of RAEB and MDS-u in a defined population, the overall survival of MDS could vary, which in turn, led us to rationalize the difference between MDS survival rates across Europe.

MPN

With the exception of those diagnosed with CML, few data on the survival of patients with MPN have been published[4,39,54,62,80]. Within MMs, MPN patients had the best outcome with a 5-year RS in the Girona province of 75.3%. These results are comparable to those reported in the U.S. and France[39,80]. However, survival in Girona was better than in Europe overall (Figure 20)[4,54]. Differences could be explained by the fact that Imatinib was not introduced throughout Europe at the same time, so this could well be why the overall survival rate of the MPN group was lower than expected.



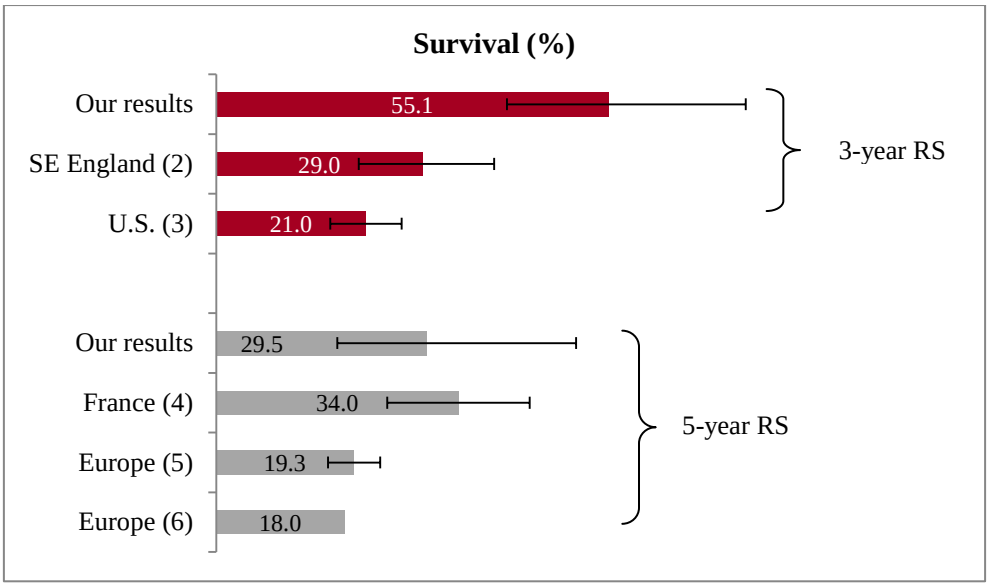
RS (relative survival); Our results(Girona: 1994-2008); U.S.: 2001-2004 (2)[80], France: 1980-2004 (3)[39], Europe: 1995-2002 (4)[54], Europe: 1995-2002 (5)[4]

Figure 20. Published myeloproliferative neoplasm survival rates.

Despite the good prognosis of MPN, survival does differ greatly between MPN entities. PV and ET were the two neoplasms with better survival in Girona, with a 5-year RS of 91.9% and 85.2% respectively, as was found in other clinical and population-based data[54,80,92,98,131]. On the other hand, MPN-u was the entity with the lowest survival, which is being mainly related with oldest patients. Although CML Ph-positive has been correctly defined since the description of the Ph chromosome in the 1970s, several differences were found in the rates of survival across Europe. This was probably due to the inclusion of different CML as CMML in the EUROCARE 4 series which serves to decrease the overall survival rate of this group[54].

MDS/MPN

As mentioned in 1.1 of this section, the MDS/MPN group is the least frequent of MMs and CMML represents over 90% of the cases in this category, so few of the available population-based survival studies have focused on it[4,39]. As CMML is a disease which presents a worse prognosis than MDS this justifies isolating CMML from MDS. The 5-year RS of CMML was lower than in France but higher than in other European works (Figure 21)[4,39,54]. Differences in survival may basically be attributed to the incompleteness of case registration and the frequencies of each CMML subtype (Section 3 of discussion).



RS (relative survival); Our results(Girona: 1994-2008);SE England: 1999-2000 (2)[80], U.S.: 2001-2004 (3)[80], France: 1980-2004 (4)[39], Europe: 1995-2002 (5)[54], Europe. 1995-2002 (6)[4]

Figure 21. Chronic myelomonocytic leukaemia population-based survival rates.

2.2. Assessing possible changes in survival according to gender, patient age and year of diagnosis and try to relate it to improvements in therapy and better risk stratification.

As mentioned in some previous papers related to the survival rate of MMs, gender, patients' age and year of diagnosis are three of the major factors in any disease prognostic. This is the main reason why the RCG wanted to evaluate how these variables influenced the survival of MMs overall as well as each of the main groups proposed by the WHO classification.

For the overall MMs diagnosed in the province of Girona during 1994-2008 according to gender, age and year of diagnosis, the rate of survival was better in women, young patients and those diagnosed during 2004-2008, despite differences were not always significant.

It is well known that women have a better overall outcome than men, but the reasons for the differences in survival rates between the sexes are not yet well understood. While variances in incidence by gender were well described by risk factors, disparities in survival remain unknown. In 2011, Cook et al., published a paper in which general cancer survival differences according to sex were associated to the risk factors and incidence of the pathology. Thus, the increased risk of developing the disease and a poorer prognosis for males (in relation to females) were related to hormone, body mass index, viral infections, carcinogenic susceptibility and health care access and utilization[132]. However, we do not know with any certainty whether these factors directly influence the survival disparities related to gender found in MMs. The most scientific approximation that relates a poorer prognosis in males with MMs, particularly with MDS, was

recently published by Radivoyevitch and Sauntharajah[133]. They suggest that discrepancies may be due to a faster cellular and molecular aging in men as was measured by telomere length or DNA methylation profiles.

In relation to the age of patients at diagnosis, it has been well documented that older patients had poorer rates of survival than younger patients[47,108,134]. In general terms, the comorbidities associated with more elderly patients, the inability to undergo intensive chemotherapy or have a transplant make the overall survival rate of these patients very low.

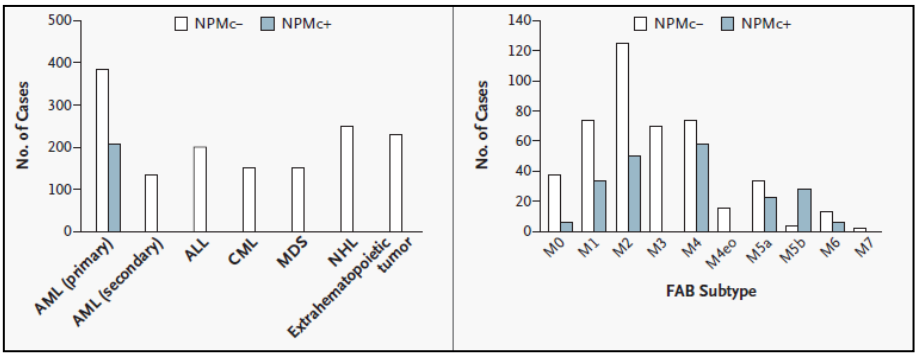
The increased survival rate found during the period of study, especially for younger patients, could be related to improvements in therapy and better stratification of patients in risk-groups, as detailed below for each of the MM groups.

AML

Age of patients at diagnosis was the principal factor to influence survival. The increased survival observed overall and particularly in young patients during the period 1994-2008 may not be related to the development of new drugs used to treat AML. ATRA, used to treat APL, was already in use before 1994, so we could not assess changes in survival related to its effectiveness. In 1981 Breitman TR et al., speculated that ATRA may have therapeutic value in the treatment of APL because it appeared to induce granulocytic differentiation of leukemic promyelocytes in short-term culture of human APL cells[135]. Treatment with ATRA alone for patients newly diagnosed with APL was proposed for first time in 1988 by Huang et al., and for patients in relapse in 1990 by Castaigne et al.[136,137]. Several years later, because secondary resistance to ATRA had been observed, Fenaux et al. (1993), reported improvements in event-free survival in newly diagnosed APL patients when combining ATRA with anthracycline and Arabinofuranosyl Cytidine (Ara-C) chemotherapy. Since that publication, numerous studies have reported the improvement in survival of patients diagnosed with APL and treated with ATRA.

Thus, this increased survival could be a consequence of better risk-stratification groups based, not only on cytogenetic alterations, but also molecular findings such as FLT3 and NPM1 which allow for risk-adapted therapy. NPM1 is a nucleocytoplasmic protein that continuously shuttles between nucleus and cytoplasm and regulates the ARF-p53 tumour suppressor pathway. The *NPM1* gene is involved in 50% to 60% of leukaemia and lymphoma associated chromosome translocations that result in fusion proteins retaining the amino terminus of NPM1, one of these translocations being the NPM1-myeloid leukaemia factor 1 (NPM1-MLF1)[138,139]. Despite the *NPM1* gene itself not seeming to have transformation potential, it is able to bring about the oncogenic potential of the fused protein. Falini et al., identified a large subgroup of patients diagnosed with primary AML with normal karyotype who had cytoplasmatic NPM1 in

leukemic blasts and the mutated *NPM1* gene. This pattern was found in almost all the AML subtypes described by the FAB classification, although it was more frequent in monocytic AML (Figure 22).



No. of cases (number of cases); NPMC- (negative for cytoplasmatic nucleophosmin); NPMC+ (positive for cytoplasmatic nucleophosmin); AML (acute myeloid leukaemia); ALL (acute lymphoid leukaemia); CML (chronic myeloid leukaemia); MDS (myelodysplastic syndromes); NHL (non-Hodgkin lymphoma); FAB (French-American-British classification)

Figure22. Frequency of cytoplasmatic NPM1 in leukemic blasts of patients diagnosed with acute myeloid leukaemia as reported by Falini et al. A) Percentage of NPM1 in some haematological malignancies, and B) according to FAB subtypes of acute myeloid leukaemia[59].

Moreover they found that *FLT3*-ITD mutation was twice as frequent compared with negative cases for cytoplasmatic NPM1. The *FLT3* gene encodes a receptor tyrosine kinase that regulates haematopoiesis. It is mutated in a third of AMLs, either by ITD or by point mutations usually involving the kinase domain[140]. The *FLT3*-ITD mutation is powerful adverse prognostic indicator for relapse in AML, as described by Gale et al. (Figure 23)[60].

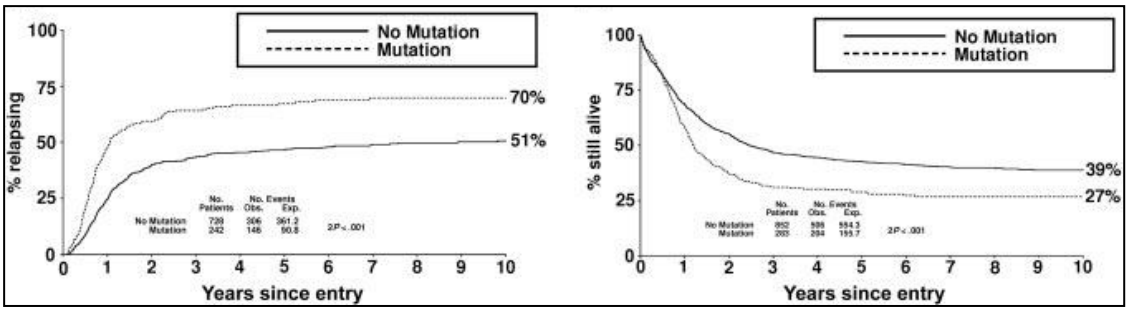


Figure 23. Overall survival and relapse risk (in percentage) of patients diagnosed with AML depending on the presence or absence of the *FLT3*-ITD mutation. Data from a clinical study published by Gale et al. in 2005[60].

Although Falini et al., stated that *NPM1* appears to be an independent predictor for responsiveness to induction treatment, Döhner et al. found a significant interaction between *NPM1* and *FLT3*-ITD, and 63% of those patients with *NPM1* mutated/No mutated *FLT3*-ITD reached complete remission after intensive therapy (Figure 24)[139].

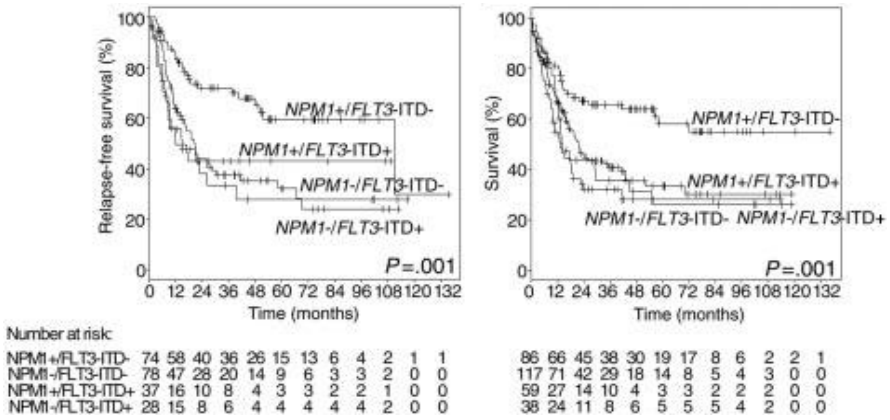


Figure 24. Survival and relapse-free survival (in percentage) of patients, depending on *NPM1* and *FLT3*-ITD status, diagnosed with acute myeloid leukaemia. Data published by Döhner et al**.** in 2005[139]**.**

The identification of *NPM1* mutation had a significant effect on the WHO classification. In the 2008 WHO review, molecular findings, such as cytogenetic abnormalities, were considered as prognostic factors, and AML with mutated *NPM1* was considered a provisional entity of the recurrent genetic abnormality AML subgroup[12]. In contrast, despite the high frequency of *FLT3* mutations and their impact on the prognosis of AML, this does not define any specific entity that coexists in many AML subtypes. The major problem of the population-based cancer registries, as is mentioned in the Introduction and the Discussion, is firstly that the corresponding ICD-O of the 2008 WHO classification is not yet available, and secondly that molecular and cytogenetic results are not always available, so we cannot register some AMLs in their specific corresponding group. Thus, although an increase in overall survival is appreciated, it cannot be precisely related to the cytogenetic and molecular findings that have enabled the better risk-stratification groups of patients.

Another reason that can lead to increased survival is allogeneic haematopoietic SCT, the principal antileukaemic treatment to prevent relapse in younger patients. In 2003, Suciu et al., published a study analysing the benefits of allogeneic (donor-receptor) compared with autologous (recipient only) SCT in the treatment of patients younger than 46 years and with their AML in its first complete remission[141]. They found that allogeneic haematopoietic SCT had a significantly better outcome than autologous SCT, especially for those patients with high or very high risk cytogenetics, and that disease free survival was higher in those patients who had a donor rather than those included in the no donor group. Burnett et al., also reported the importance of transplants in young and standard-risk patients in order to avoid relapse after chemotherapy, but they also highlighted that there were several reasons why these may not translate into improvement in overall survival for patients with AML[142]. This is basically

because, despite some technical improvements in allogeneic haematopoietic SCT, evitable procedural mortality still remains. In regards to our results, and related to Brunett el al., no statistically significant improvements in survival were found between 1994 and 2008. Nor were any differences in survival according to gender found independently of patients' age, as suggested by other studies.

MDS

With MDS, it was found that older patients had a RER of 1.97. Survival of patients younger than 77 (median age of diagnosis) increased, independent of sex, from 1994 to 2008. This fact could be related with the use of demethylating drugs such as azacitidine and decitabine, which are associated with a prognosis improvement in patients diagnosed mainly with high-risk MDS. Azacitidine, used in clinical trials since 1984, was approved by the FDA in 2004 for treatment of symptomatic MDS[143]. One of the trials with azacitidine in patients with MDS published by Silverman et al., demonstrated that azacitidine decreased the risk of transformation into AML and extended survival of patients aged more than 65 years diagnosed with high-risk MDS (including RAEB, RAEB-T), and also in patients with intermediate-2 and high-risk MDS of the IPSS[144]. Azacitidine would seem to alter the natural history of MDS, modulating the behaviour or the MDS clone without the need to eradicate it. Moreover, they also described that azacitidine delays the onset of red blood cells and platelet transfusion in patients who were transfusion-independent at the start of the study. Decitabine also inhibits further DNA methylation. In a phase II study of decitabine in patients with MDS, Wijermans et al., found an overall response rate of 42-45%, including complete remissions in 20-28% of patients[145,146]. This good response from MDS patients treated with decitabine was also confirmed in a phase III study. When comparing best supportive care and historical intensive chemotherapy, Kantarjian et al., found that decitabine produced the best response and complete remission in all IPSS groups, and all the decitabine treated patients became transfusion-independent. Moreover, decitabine also decreased the risk of transformation into AML (Figure 25)[147].

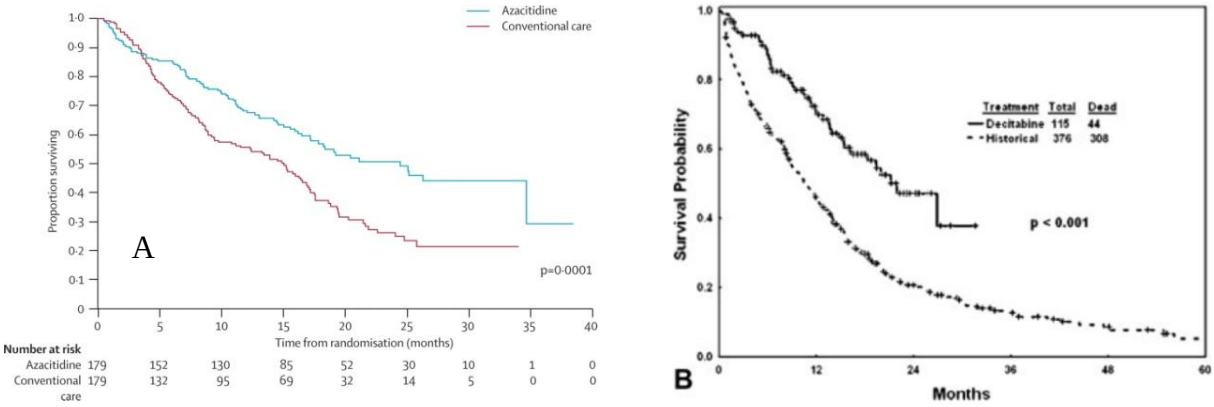


Figure 25. Increased survival of patients diagnosed with myelodysplastic syndromes treated with hypomethylating agents. A) Azacitidine vs. conditional care[148] and B) Decitabine vs. historical chemotherapy[149]. Data published by Fenaux et al. and Kantarjian et al.

Thanks to the widespread use of reduced-intensity conditions with haematopoietic SCT, advances made in the approach to allogeneic transplants for high-risk patients aged 50-70 may also be related to increased survival (mainly in the case of young patients) during the period of study. As allogeneic haematopoietic SCT is the only curative option for patients diagnosed with MDS, it is therefore limited to patients younger than the 50 to 55 year old group and who have no significant dysfunction of any other major organ. The nonmyeloablative or reduced-intensity conditioning regimens could be carried out in patients with MDS of whom conventional allogeneic haematopoietic SCT was considered inappropriate[150]. Because the median age of diagnosed MDS in Girona was 77 years old, there is a high percentage of cases considered as young that could in fact benefit from reduced-intensity conditions with haematopoietic SCT and increase overall survival.

Moreover, progression support therapies for patients not receiving intensive treatment, such as the availability of better antibiotics or antifungal drugs, may also have a positive effect on survival. Although PB transfusion is actually a very safe therapy, there is the possibility of disease transmission between donor and receptor and/or secondary effects. Immune or no immune adverse transfusion reactions can appear immediately or 24 hours after post-transfusion. Providing appropriate antibiotic and/or antifungal prophylaxis could prevent patient fatality and in fact increase their overall survival[62].

Even though we did not find any differences in survival by sex when analysing the overall MDS group, they were present when we stratified our sample according to age of patients. While young women had a statistically significant RER of 0.56 over men, this difference disappeared when contrasting by gender in the older age group. Although we suspect that young women might be benefitting from a protective effect from hormones, we were not able to corroborate

this assumption statistically due to the low number of women aged 50 years or less; the approximate age when hormonal changes in women, due to menopause, start to appear.

MPN

With regards to the whole MPN in the province of Girona, we found that age and year of diagnosis were the most crucial prognostic factors for survival time. Patients older than 67 years had a RER of 2.53 compared with those younger ones. The increased survival throughout the whole period was found only for young patients and could be related to both the use of TKI treatment for CML and improvements in MPN Ph-negative diagnoses and management. As CML is later explained independently in Section 2.3, we will now focus on MPN-Ph negative.

Since the utilization of molecular techniques in the routine diagnosis and the discovery of the *JAK2* V617F mutation, MPN Ph-negative cases have been much more objective and better categorized. This improvement in detection method has led to patients being diagnosed much earlier on, and consequently they receive treatment, if needed, rapidly.

Until the discovery of *JAK2* V617F in 2005, several papers on the survival of these MPN, (also known as non-CML MPN), were written. The latest one available was published by Price et al., and accurately described survival rates in MPN Ph-negative by using data from a nation-wide US population. Apart from analysing by subtype, they also assessed survival by age, and found (consistent with other studies) that older patients diagnosed with ET and PV had a much poorer prognosis than younger ones. Survival of PMF, however, was similar for patients included in the 64-75 years, 75-84 years and 85+ year age groups[151].

As our period of study ends in 2008, we were not able to analyze the effect of the *JAK2* V617F inhibitors on survival of MPN Ph-negative diagnosed in the province of Girona, however, several paper presented preliminary results from clinical trials with *JAK2* V617F inhibitors, in particular in the case of PMF. While patients with PV and ET are usually treated with cytoreductive agents, apart from hydroxyurea, there are few drugs available for the treatment of PMF. In this disease, therapy is mainly palliative and oriented to alleviating the symptoms caused by splenomegaly or cytopenias. Because there are no approved drugs for PMF treatment, most *JAK2* V617F inhibitor studies are focused on precisely on this. The principal benefits of these inhibitors observed (summarized in Table 26) were a reduction in splenomegaly, elimination of debilitating disease-related symptoms and weight gain.

Table 26. Preliminary results of clinical studies with *JAK2* V617F inhibitors in chronic idiopathic myelofibrosis, polycythemia vera and essential thromobythemia.

Study	Therapy	N	Responses	Toxicity
PMF				
Santos et al.[152]	CEP-701 Phase I	22	Reduction in spleen size and improvement in cytopenias in 27% of cases	GI toxicity, cytopenias
Hexner et al.[153]	CEP-701 Phase I	26	Reduction in splenomegaly (median 5.8 cm)	GI toxicity, cytopenias
Verstovsek et al.[154]	INCB018424 Phases I/II	153	Reduction in spleen size in 44% of cases, response rate in 52% of patients, transfusion independency in 14% of cases and improvement in exercise ability, systemic symptoms, decrease in cytokines in majority of patients	Thrombocytopenia, anaemia
Verstovsek et al.[155]	SB1518 Phase I	43	Reduction in spleen size in 28% of cases	GI toxicity, thrombocytopenia
Seymour et al.[156]	SB1518 Phase I	20	Reduction in spleen size in 11/13 evaluable patients, transfusion independency in 2/9 cases	GI toxicity
Pardanani et al.[157]	TG101348 Phases I/II	59	Reduction in splenomegaly in 49% of cases, improvement in systemic symptoms, leukocytosis, thrombocytosis and improvement in BM fibrosis in selected cases	Anaemia, thrombocytopenia and GI toxicity
Shah et al.[158]	XL019 Phase I	30	Improvement in splenomegaly, systemic symptoms, anaemia and leukocytosis	Neurological toxicity (study closed)
PV and ET				
Moliterno et al.[159]	CEP-701 Phase II	39	Reduction in spleen size in 83% of patients, reduction in phlebotomy requirements in 60% of cases	GI toxicity, thrombotic episodes
Verstovsek et al.[160]	INCB018424 Phase II	73	PV→ 97% of response; 45% of complete response and 52% of partial response ET → 90% or response; 13% of complete response and 77% of partial response	Anaemia, thrombocytopenia

N (number of patients); PMF (primary myelofibrosis); GI (gastrointestinal); PV (polycythemia vera); ET (essential thrombocythaemia).

MDS/MPN

Due to the low number of cases, a multivariate analysis was not able to be done. Reported data on survival of this MM group is well documented in Section 3.2 of this discussion.

2.3 Evaluating the effect of Imatinib on improving the prognosis of patients with CML and *BCR-ABL1* positive.

During the course of our study one of the most significant improvements found to be effective in the treatment of MMs was Imatinib, a selective inhibitor of BCR-ABL1 tyrosine kinase activity. Although hematopoietic SCT is the currently available curative therapy for CML, Imatinib is now considered the gold standard therapy for CML treatment as it induces complete cytogenetic and major molecular responses together with long-term disease-free survival[161]. Before its availability, interferon alpha plus low-dose cytarabine was considered standard therapy for patients with CML who were not planning to undergo allogeneic haematopoietic SCT. However in 2001 after an initial phase I dose-escalation study of Imatinib, which included 83 patients with chronic-phase CML, Imatinib was accepted by the FDA as the first-line therapy for CML Ph-positive cases and from the beginning of 2002 was applied across Europe[4,54,162]. A phase II study involving 532 patients with late chronic-phase CML who had had an unsatisfactory response to interferon alpha and an International Randomized Study of Interferon and STI571 (a multicenter, open label, phase III study which included 1,106 patients diagnosed with CML between 2000 and 2001) also clearly demonstrated the benefits of Imatinib[163,164].

Although population-based observational studies do have significant limitations that must be considered when evaluating treatment benefits (i.e. no detailed information on comorbidity or performance status nor a specific treatment plan, coupled with the identification of comparative benefit being prone to multiple biases), they are indeed necessary and complementary to randomized controlled trials (the gold standard studies for evaluating efficacy of new therapies) to ensure that results translate into benefits for the general population. This is why the RCG attempts to evaluate the effects of Imatinib in terms of improving the prognosis of patients with CML in the province of Girona.

This is the reason why the RCG attempts to evaluate its effect on improving the prognosis of patients with CML in the province of Girona.

The overall 5-year RS for patients with CML was 61.2% (Figure 26A). After reviewing all the medical records for each patient and individually verifying whether they were/are being treated with TKI or not, we found the 5-year RS was close to 80% for those who received treatment, whereas it was 43.5% for those who were not treated (Figure 26B).

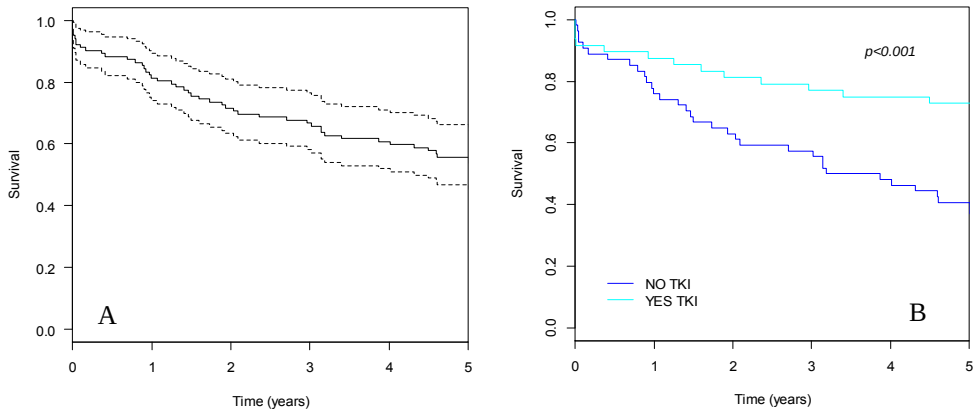


Figure 26. Survival curves of patients diagnosed with chronic myeloid leukaemia in the province of Girona during the period 1994-2008. A) Overall survival with a 95% confidence interval, and B) Comparing patients who received tyrosine kinase inhibitor (TKI) treatment vs. patients not treated.

Thus, our results confirmed that since the implementation of Imatinib as the gold standard treatment for patients with CML Ph-positive, the patient survival rates has greatly increased, with an RER of 0.19 with respect to those not treated with Imatinib and, independent of age or gender.

3. Incidence and survival of CMML

The last study of this thesis consists of a detailed determining of the incidence and survival of CMML in the province of Girona.

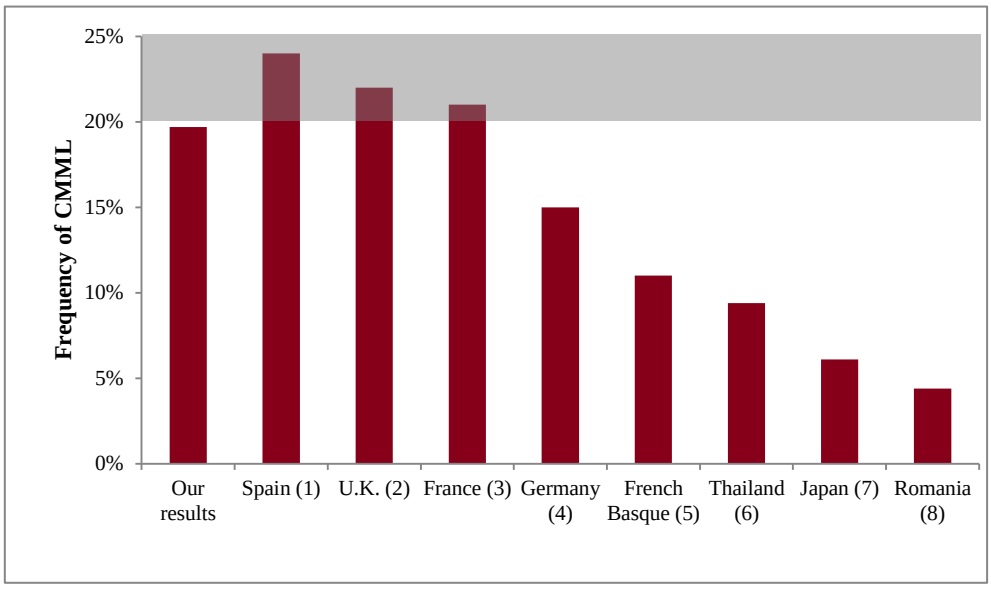
CMML is a rare haematological malignancy characterized by shearing myelodysplastic and myeloproliferative features at the time of diagnosis. Its classification within MMs was controversial for many years and some cases were wrongly registered as MDS-u and others as CML nos[102].

Even though the FAB classification categorized it within the MDS group as an independent entity, it was not until the publication of the 2001 WHO classification that CMML was reclassified within a new emergent group called MDS/MPN, of which it is now the most frequent entity[11]. As there are few population-based studies reporting the incidence and survival rates of this disease, the RCG decided to analyse the current situation of this pathology in the province of Girona.

3.1. Determining the incidence of CMML in the province of Girona during the period 1993-2007.

As previous studies reported, CMML is an infrequent myeloid disease associated with elderly people. For cancer registries it was not easy to classify all the cases of CMML correctly, because its classification was under discussion until the appearance of the 2001 WHO document. To report a "true" incidence rate of this malignancy, the RCG initially had to review some cases reported as nonspecific MMs, such as MDS and CML, to verify their diagnoses. Finally, we confirmed that 61 cases of CMML were diagnosed during the period 1993-2007 in the province of Girona. These cases accounted for 1.5% of all haematological malignancies diagnosed in the province of Girona over the same time period and 6.5% of all MMs according to the 2001 WHO classification.

According to the FAB classification, CMML was described as accounting for approximately 20-25% of MDS cases[100]. The frequency of CMML within MDS in Girona was higher than that in Romanian, Chinese, Thai and French Basque study group samples, indicating the difficulty of properly registering CMML in cancer registries. Having said this, it should be noted that the results were similar and in line with those from the UK and French population studies (Figure 27).



CMML (chronicc myelomonocytic leukaemia); Our results (Girona); Spain (1)[165], U.K. (2)[166], France (3)[70], Germany (4)[167], French Basque (5)[168], Thailand (6)[169], Japan (7)[170],Romania (8)[171].

Figure 27. Frequency of chronic myelomonocytic leukaemia according to the FAB classification as reported in a number of epidemiological studies. In grey is marked the percentage range to be achieved by chronic myelomonocytic leukaemia within myelodysplastic group according to the FAB classification.

The incidence of CMML described in the literature is lower than one case per 100,000 inhabitants/year. CR in Girona was 0.72 of new cases per 100,000 inhabitants/year, with an annual average of 4 new cases per year. This result was similar to those reported in the South-East England, German and U.K. studies[46,47,71]

Compared to other myeloid diseases such as AML, CMML is a haematological malignant diagnosed and managed sometimes outside hospitals, and although independent laboratories reported cases to local cancer registries, the completeness of case reporting is not always well documented. The cases of CMML that are always registered in population-based registries are those of patients who are hospitalized.

In terms of the age of the patient, we found that more than 70% of patients were over 75 years old at the time of diagnosis, with the median age being 79 years old. Only 26.2% of cases were under 75. By analysing the age-specific rate we found that the under 74 year incidence level was very low and alike by gender, but for older age groups the incidence level increased up to 16 new cases per 100,000 inhabitants/year for men and 8 new cases per 100.000 inhabitants/year for women. Although this male predominance, differences were not statistically significant.

The incidence rate according to CMML subtypes (CMML-1 and CMML-2) was not able to be estimated because the RCG did not collect clinical data, for all patients, on the percentage of blasts at diagnosis. We only had this information in the cases where the clinician specified the percentage of blasts in BM and PB or specified the subtype.

3.2. Estimating survival and evaluating if there are differences in survival between the age of patients, the gender or the year of diagnosis.

In this work, we also reported the survival of CMML overall, by gender, age and period of diagnosis. The 5-year OS and RS for all the CMML cases diagnosed in Girona between 1993 and 2007 were 20% and 29%, respectively. After comparing our results with other international studies we found that there were differences between our results and those of other countries. The RS of CMML in the province of Girona was higher than the RS reported for South-East England and U.S. populations, whereas it was lower than the RS for the French group[39,46,80]. Some of the possible causes to explain those differences could be:

- Low number of CMML cases. When survival is assessed for infrequent diseases in a population, few variations in the number of cases can influence in the survival analysis.
- Age of patients. As will be discussed below, the age of patients is an important prognostic factor in any disease. Elderly patients have associated comorbidities and therefore they do not receive such aggressive treatments as young people do and as a

consequence present the poorest prognosis. The number of patients of advanced age in a specific study group can also vary survival rates.

- Percentage of blasts in PB and BM at the time of diagnosis. Depending on the percentage of blasts in PB and BM, patients with CMML are categorised into 2 subtypes: CMML-1 and CMML-2 (see Introduction of this thesis). This value is not always available for population-based registries, so cancer registries mainly reported the incidence rate of CMML overall, rather than by subtypes. Knowing that CMML-2 has a poorer prognosis than CMML-1 it is logical to expect better or worse survival depending on the frequency of each subtype in the population of study.
- Percentage of progression to AML. Because of the high percentage of blasts in PB and BM in patients with CMML-2, there is a 18% (15%-30%) possibility of transformation into AML between 1 and 125 months after the initial diagnosis of CMML. This evolution causes survival rates to decrease by 14 months or less. In the Girona group, the percentage of AML transformation was lower than that described in the literature[13,102].

When analysing survival by gender, we found that men diagnosed with CMML in Girona had a lower survival rate than women, although differences between the two were not statistically significant. However, statistically significant differences in survival were found when comparing patients by age group and time of diagnosis. As explained previously, elderly patients have extremely poor prognosis compared to younger patients because aged patients could not be treated with intensive chemotherapy and allogeneic haematopoietic SCT transplantation is not an available therapeutic option for them[13,103]. In the Girona sample, the median OS for patients younger than 75 years old was 64 months, whereas for patients aged over 75 years the median OS was only 19 months. These results are similar to earlier published papers reporting survival of CMML by age[172]. Lastly, the increased survival in the period of study could be mainly related to better disease diagnosis and improvements in therapeutic options.

3.3. Attempting to analyze changes in survival with advances in treatment.

In point of fact, allogeneic haematopoietic SCT remains the only curative therapy for CMML, but it is a possible option in only a minority of patients aged below 65 years. Because these patients are included in the clinical trials of MDS patients, few studies report specifically on transplant survival of this disease. One of the latest papers published by Cheng et al., in 2012 summarized the status of haematopoietic stem cell transplantation in patients with CMML by compiling eight available studies on this area[173]. Table 27 compiles the main characteristics of these clinical trials.

Table 17. Summary of clinical trial information available on allogeneic haematopoietic stem cell transplantation for patients diagnosed with chronic myelomonocytic leukaemia.

Information of study	Patient characteristics	Disease burden transplant	Outcomes
Eissa et al. Fred Hutchinson Cancer Research Center, 1986-2008[174]	N= 85 Age= 51 (1-69) Time diag->tx = Unknown	CMML-1 = 57 CMML-2 = 26	10 years RFS = 40% NRM = 35% RR = 25%
Krishnamurthy et al. Kings College, UK, 1998-2007[175]	N= 18 Age= 54 (38-66) Time diag->tx = 17 months	Blasts <5% = 8 5-9% = 9 10-19% = 0 >20% = 1	3 years RFS <5% blasts = 47% >5% blasts = 20% OS= 31% RR = 47%
Ocheni et al. Hamburg, Germany, 2003-2007[176]	N= 12 Age= 56 (38-67) Time diag->tx = 7 months	CMML-1 = 7 CMML-2 = 3 Unknown = 2	2 years RFS = 67% OS = 75% RR = 17%
Laport et al. Standford and Fred Hutchinson Cancer Research Center, 1998-2004[177]	N= 7 Age= 58 (39-62) Time diag->tx = 10 months	Unknown = 7	3 years RFS = 43% OS = 43% RR = 57%
Elliot et al. Mayo clinic, 1992-2004[178]	N= 17 Age= 50 (20-60) Time diag->tx = 7 months	Unknown	3 years RFS = 18% OS = 18% RR = 41% (6 months)
Kerbauy et al. Fred Hutchinson Cancer Research Center[179]	N= 43 Age= 48 (1-66) Time diag->tx = 8 months	CMML-1 = 32 CMML-2 = 11	4 years RFS = 41% NRM = 35% RR = 25%
Mittal et al. MD Anderson, 1991-2001[180]	N= 8 Age= 51 (20-64) Time diag->tx = Unknown	Not available	3 years RFS = 25% OS = 37.5% RR = 62.5%
Kroger et al., EBMT, Germany, 1988-2000[181]	N= 50 Age= 44 (19-61) Time diag->tx = 9 months	Blasts ≤5% = 28 6-10% = 10 11-20% = 6 Unknown = 5	5 years RFS = 18% OS = 21% RR = 49%

EBMT (eight bone marrow transplantation institutions); N (number of cases); Time diag->tx (median time for diagnosis to transplant); CMML (chronic myelomonocytic leukaemia); RFS (relapse-free survival); NRM (non-relapse mortality); OS (observed survival); RR (relapse rate).

Despite the poor outcome of CMML patients, these studies seemed to prove the curative potential of a transplant. However, the insufficient data available on transplants for this disease along with the complexity of this process, do not allow us to determine with any certainty if there is in fact improved survival.

Other available therapeutic options include: chemotherapy, hypomethylating agents, transfusion support and a combination of new agents. Despite chemotherapy not being able to cure patients

in most cases, it does lead to being able to temporarily control splenomegaly and hyperleukocytosis. The most common drugs used at low doses for CMML treatment are: hydroxyurea, oral etoposide or cytarabine[182-185]. Wattel et al., published in 1996 the results of a randomized study which demonstrated that the hydroxyurea was the best chemotherapeutic option for treating patients with CMML[182]. When hydroxyurea competes with oral etoposide, hydroxyurea presented the best response in less time and with a better overall survival. Although cytarabine has been widely used in the treatment of CMML, its benefits are uncertain because it is not specifically used and because the number of patients in the available series is very low.

As aberrant methylation was described as also being present in CMML, the FDA finally approved azacitidine and decitabine for its treatment[186]. There are several published works reporting that azacitidine produces good responses in patients with CMML. An abstract of the principal results found in some these studies is shown in Table 28.

Table 28. Summary of principal characteristics from the available clinical studies on patients with chronic myelomonocytic leukaemia treated with azacitidine.

Study	N of patients	Median age	ORR	CR	Median OS (months)
Costa et al.[187]	38	70.5	39	11	12
Thorpe et al.[188]	10	69	60	40	20
Fianchi et al.[189]	31	69	51	42	37
Wong et al.[190]	11	65	55	36	17.3
Adès et al.[191]	76	70	43	17	29

N (number of patients); ORR (overall response rate); CR (complete remission including marrow CR); OS (overall survival).

There are also several clinical trials demonstrating the benefeits of decitabine in patients with CMML, although these are less frequent. Wijermans et al., reported its effectiveness in the treatment of CMML using data on three multicenter phase 2 studies and a multicenter phase 3 study[192]. Results showed that the decitabine produced an overall response rate (ORR) of 25% (14% complete remission and 11% partial remission), hematologic improvement in 11% of patients and disease stability in 39% of cases.

Another therapeutic option for CMML patients that has been published recently is the use of combined agents. As demonstrated in the results of a phase 2 trial of combination therapy using thalidomide, arsenic trioxide, dexamethasone and ascorbic acid (TADA scheme), published in 2012, TADA appears to be relatively well tolerated and allows for clinical responses in patients with MDS/MPN[193]. Finally, patients with symptomatic anaemia may benefit from red cell transfusion[74].

Thus, all the therapeutic options described above may result in increased overall survival of patients diagnosed with CMML during the period of study. Summarizing all this information in cancer registries is a meticulous task that requires the availability of staff with expertise in this area. This is why population-based cancer studies focus their survival studies on well-defined prognostic factors such as age, sex and even the diagnostic period.

Estimating the survival of CMML according to treatment would be easy if there was a widespread and well stipulated therapeutic option for all individuals suffering from this pathology. Because nowadays therapies applied are more individualized and addressed to the patients' individual needs, all we can do in the RCG is to make assumptions about which therapies can cause changes in survival basing our hypothesis on results previously published by experts in this pathology.

So, although CMML is a pathology associated with a poor prognosis, the increased survival rates found in the province of Girona according to year of diagnosis could be due mainly to improvements in transplantation and to the use of hypomethylating agents and chemotherapy[179].

Conclusions

There is no real ending.
It's just the place where you stop the story.

Frank Herbert

1) Incidence of MMs in the province of Girona

- MPN was the group with the highest incidence in the province of Girona during the period 1994-2008, followed by the MDS, AML and MDS/MPN groups.
- Incidence trends since 1994 to 2008 increased significantly in MDS, MPN and MDS/MPN, whereas remain stable for AML.
- Recent advances in diagnostic methods of MMs, such as *JAK2* V617F mutation detection, and an increase in outpatient settings have resulted in changes in incidence rates and trends.

2) Survival of MMs in the province of Girona

- MPN was the group with highest survival in the province of Girona during 1994-2008, whereas AML presented the poorest outcome.
- Age of patients at diagnosis determine survival of both overall and also in each of MMs groups proposed by the WHO 2001 classification.
- The increased survival in young patients diagnosed with AML and MDS was found to be possibly related to better risk-risk stratification groups.
- The use of Imatinib as the gold standard therapy for CML treatment was reaffirmed as it resulted in an overall increased survival for patients diagnosed with this disease.

3) Incidence and survival of CMML

- CMML is a rare disease with an incidence rate lower than one new case per 100,000 inhabitants/year, and affecting predominantly males.
- Survival depends on gender and the age of patients at the time of diagnosis. Men had lower survival rates than women, and people aged more than 75 years had poorer outcome than those aged less than 75 years.
- No improvements in therapy were made since 1994 to 2008, and despite new drugs having been approved as treatment for CMML, potential curative therapy is only available to a limited number of patients.

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Never memorize something that you can look up.

Albert Einstein

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