REGISTRY STUDIES ON MYELODYSPLASTIC SYNDROME AND SECONDARY ACUTE MYELOID LEUKEMIA
European and Swedish perspectives

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Cover illustration: Stairs of emotions; *Men känslotrappan, alltså ibland är man, ser man horisonten här uppe då, blå och fin här. Här har du botten… här vill man ju kanske inte leva, en symbolbild hur man vandrar upp och ner för den här trappan va och jag är jätteglad att det finns en ledstång, för det första kan det dämpa fallet, man kan hålla sig i en ledstång så man inte slår i så hårt va, man kanske inte ... ja skadar sig, rent ut sagt. Och samtidigt så kan man då liksom ha hjälp med att ta sig upp. För så är det ju det går ju upp och ner. ”*

Courtesy of Berit Söderberg

For my family; Per, Kristina and Katarina

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ABSTRACT

The aims were (I) to describe a European lower risk MDS population and the use of erythropoietin stimulation agents (ESA), (II) to describe the AML population in Sweden 1997-2006 with emphasis on secondary AML (s-AML) and therapy-related AML (t-AML), (III) to investigate the use and effect of allogeneic hematopoietic stem cell transplantation (HSCT) in the AML population in Sweden 1997-2013, and (IV) to merge patients from the Swedish AML Registry 2009-13 with patients from the Swedish MDS Registry 2009-14 in order to describe the patients with s-AML after MDS from time of MDS diagnosis and time of AML diagnosis. **Patients, methods and results:** (I) ESA treatment were given to 45.6% patients with lower risk MDS, median duration 27.5 months. A propensity model, comparing ESA-treated and untreated was used. Median time to first post-ESA treatment transfusion was 6.1 months in patients transfused before ESA treatment compared to 23.3 months in non-transfused patients (p<0,0001), showing that ESAs can significantly delay the onset of a regular transfusion need in patients with lower-risk MDS. (II) Of 3,363 AML patients with induction therapy, 73.6% were de novo AML, 18.7% had antecedent hematological disease (AHD-AML), and 7.7% had t-AML. S-AML-patients were older compared to de novo AML and had higher cytogenetic risk scores. Multivariate analysis showed that AHD-AML and t-AML were independent risk factors for inferior survival in the younger age groups. (III) Of 3337 intensively treated patients, 21% underwent HSCT at any stage of the disease. Five-year survival without and with allogeneic HSCT were 0% vs 50% for MPN-AML, 3% vs 39% for MDS-AML, 8% vs. 48% for t-AML and 24% vs. 57% for *de novo* AML-patients. Presence of any chronic graft versus host disease (cGvHD) compared to no cGvHD and a GvHD grade 1 or lower was significantly associated to better survival in a multivariable analysis. Allogeneic HSC is the only option for cure in S-AML. (IV)We found 257 patients with sufficient information from both AML and MDS registries for further examination. 72.2% had high risk cytogenetics and 66.8%, had performance status 0-1 at AML diagnosis. Median time from MDS diagnosis to AML diagnosis was 10.8 months. Median survival time for S-AML was 4.93 months. Allogeneic HSCT improves survival significantly in the younger age groups.

Keywords: Myelodysplastic syndromes, secondary acute myeloid leukemia, erythropoietin stimulating agents

SAMMANFATTNING PÅ SVENSKA

Denna avhandling baserar sig på en europeisk prospektiv registerstudie av lågrisk myelodysplastisk syndrom (MDS) (I), samt tre studier från de svenska leukemi-och MDS-registren (II-IV).

MDS och akut myeloisk leukemi (AML) är närbesläktade sjukdomar. Båda är maligna sjukdomar som uppstår i och påverkar benmärgen och produktionen av röda och vita blodkroppar samt blodplättar (trombocyter).

Vid lägre risk MDS är det viktigaste är att behandla konsekvenserna av låga blodvärden, så som anemi, leukopeni och trombocytopeni. Högre risk MDS har en större benägenhet att gå över i akut myeloisk leukemi, och här syftar behandlingen till att få kontroll på, och eventuellt försöka behandla bort sjukdomen helt.

MDS kan – i likhet med myeloproliferativa sjukdomar – utvecklas till en sekundär akut myeloisk leukemi. (s-AML). Andra orsaker till sekundär AML är tidigare cytostatika eller strålbehandling, terapirelaterad AML (t-AML), där benmärgens stamceller har tagit skada av tidigare behandling.

Arbete (I) är från en stor europeisk prospektiv registerstudie som samlar in patienter med lågrisk MDS från små och större sjukhus i 17 länder. Vi valde att i en kohort om drygt 1800 patienter studera effekten av erytropoietin-stimulerande medel (ESA) hos patienter med lågrisk MDS. Patienter med hemoglobin <10 g/dL eller transfusionsbehov som antingen har fått behandling med ESA eller inte, beroende på lokala riktlinjer blev jämfört i en propensity-modell. Strikta kriterier för respons blev definierat, och man kunde visa att patienter med ESA-behandling har signifikant längre tid till första blodtransfusion jämfört med patienter som fick blodtransfusion innan ESA (23,3 vs 6,1 månader, p=0,0001). Patienter med respons hade en signifikant bättre överlevnad jämfört med patienter utan svar på ESA (HR 0,65, 95% CI 0.45–0,893, $P = 0,018$). Det var ingen signifikant skillnad mellan ESA- behandlade och icke-behandlade med avseende på utveckling till AML, och en icke-signifikant trend mot bättre överlevnad.

I (II) är alla patienter från det svenska akut-leukemiregistret under perioden 1997–2006 undersökt, där totalt 3,363 vuxna patienter fick induktionsterapi (intensiv behandling) med syfte att uppnå remission. Merparten (73,6%) hade de novo AML (AML utan tidigare sjukdom), Tidigare hematologisk sjukdom (AHD-AML) som MDS eller

Myeloproliferativ sjukdom (MPN) fanns hos 18,7% och 7,7% hade terapirelaterad AML (t-AML). Patienter med sekundär-AML var signifikant äldre än de novo AML-patienterna och fler hade en sämre cytogenetisk riskprofil. Det var fler män i AHD-AML gruppen, och fler kvinnor i t-AML-gruppen. AHD-AML och t-AML var oberoende riskfaktorer för sämre överlevnad hos patienter <80 år.

I (III) har man bedömd effekten av allogen stamcellstransplantation (HSCT) hos patienter med sekundär AML jämfört med de novo AML. Alla patienter i AML-registret under perioden 1997–2013 som fick induktionsterapi, totalt 3330 patienter blev undersökt. Allogen HSCT i första remission blev genomgått av 17% av patienterna med de novo AML, 12% av patienter med AHD-AML och 14% av patienter med t-AML. Fem års överlevnad var 0% vs 50% for MPN-AML med och utan allogen HSCT, respektive 3% vs 39% for MDS-AML, 8% vs. 48% for t-AML och 24% vs. 57% for *de novo* AML-patienter. Slutsatsen blir att allogen HSCT är den enda möjligheten för bot vid S-AML.

I (IV) är information från svenska MDS-registret sammanfogat med AML-registret 2009–14 för att bedöma utvecklingen från MDS till S-AML. I AML-registret var 335 av 2181 (15,3%) patienter registrerade med MDS som tidigare sjukdom. Efter validering och komplettering av journaler hittade vi 257 patienter med tillräcklig information från MDS- och AML-diagnos. Vid MDS-diagnos hade 13,5% låg risk MDS risk, 72,2% hög risk MDS och 14,5% hade MDS-MPN. Cytogenetik saknandes i 34,6% av fallen vid MDS-diagnos, av de resterande var 14,4% låg risk (VRL/LR), 18,2% Intermediär risk and 32,7% hög risk (HR/VHR). Vid AML-diagnos saknades cytogenetik i 60,3% av fallen. Av de resterande hade 0% lågrisk, 20,2% intermediärrisk och 19,5% högrisk.

Mer än 2/3 av patienterna var uppegående och aktiva (WHO-performance status 0–1) vid tidpunkten för AML-diagnos, trots en medianöverlevnad på endast 4,9 månader. Allogen HSCT förbättrade överlevnaden betydligt hos patienter <70 år.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Garelius** HK, Johnston WT, Smith AG, Park S, de Swart L, Fenaux P, Symeonidis A, Sanz G, Čermák J, Stauder R, Malcovati L, Mittelman M, van de Loosdrecht AA, van Marrewijk CJ, Bowen D, Crouch S, de Witte TJ, Hellström-Lindberg E.

   Erythropoiesis-stimulating agents significantly delay the onset of a regular transfusion need in nontransfused patients with lower-risk myelodysplastic syndrome.

   J Intern Med. 2017 Mar; 281(3):284–299. doi: 10.1111/joim.12579.

II. Hulegårdh E, Nilsson C, Lazarevic V, **Garelius H**, Antunovic P, Rangert Derolf Å, Möllgård L, Uggla B, Wennström L, Wahlin A, Höglund M, Juliusson G, Stockelberg D, Lehmann S.

   Characterization and prognostic features of secondary acute myeloid leukemia in a population-based setting: a report from the Swedish Acute Leukemia Registry.

   Am J Hematol. 2015 Mar;90(3):208-14. doi: 10.1002/ajh.23908.

III. Nilsson C, Hulegårdh E, Lazarevic V, **Garelius HK**, Remberger M, Möllgård L, Stockelberg D, Lehmann S.

   The effect of allogeneic bone marrow transplantation in first remission in patients with secondary acute myeloid leukemia in the population-based Swedish AML Registry 1997-2013

   Manuscript.

IV. **Garelius HK**, Genell A, Nilsson C, Hulegårdh, Ejerblad E, Nilsson L, Lehmann S, Stockelberg D, Hellström-Lindberg E and Möllgård L.

   Acute myeloid leukemia secondary to myelodysplasia. Results from the Swedish AML and MDS Registries 2009-14.

   Manuscript

CONTENT

ABBREVIATIONS

aGvHD	acute Graft versus Host Disease
AHD	Antecedent hematological disease
allogeneic HCT	allogeneic hematopoietic stem cell transplantation
AML	Acute myeloid leukemia
AML-registry	The Swedish INCA Registry for AML
ANC	absolute neutrophil count
APL	acute promyelocyte leukemia
ATG	Anti thymocyte globuline
ATRA	All-trans retinoic acid
CCR	conventional care regimens
cGvHD	chronic Graft versus Host Disease
CPRT	conventional post remission therapy
CR	complete remission
CR1	complete remission after the first chemotherapy cycle
de novo AML	AML without previous hematological disease
ESA	Erythropoietin stimulating agents
EUMDS	European Myelodysplastic Syndromes (MDS) Registry
FAB- classification	French American British Classification
FDA	Food and Drug Administration
GvHD	Graft versus Host Disease
Hb	Hemoglobin
HI	Hematological improvement
HLA-DR15	Human leukocyte antigen DR 15
HMA	hypomethylating agents
HSCT	hematopoietic stem cell transplantation
IC	Intensive or Induction chemotherapy
INCA	Information network for cancer diagnosis in Sweden
int-1	intermediate risk 1 in IPSS
int-2	Intermediate 2 in IPSS

IPSS	International Prognostic Scoring System
LDAC	low-dose cytarabine
MDS	Myelodysplastic Syndrome
MDS-registry	The Swedish INCA Registry for MDS
MFC	Multiparameter Flow cytometry
MPN	Myeloproliferative neoplasms
MPO	myeloperoxidase
MRD	Minimal or measurable residual disease
NGS	Next Generation Sequencing
NRM	non-relapse mortality
PML-RARA	promyelocytic leukemia/retinoic acid receptor alpha
R-IPSS	Revised International Prognostic Scoring System
RBC	red blood cells
RQ-PCR	real-time quantitative polymerase chain reaction
s-AML	Secondary acute myeloid leukemia
s-epo	serum-erythropoietin
SALR	Swedish Acute Leukemia Registry
t-AML	Therapy-related AML
Transfusions	in this context, Erythrocyte transfusions
WBC	white blood cells

DEFINITIONS IN SHORT

Myelodysplastic syndrome (MDS)	A group of clonal hematopoietic diseases characterized by immature hematopoiesis. Typically, one or more of the cell lines in bone marrow is affected with low blood cell counts. It can also present itself with immature blasts up to 19%. There is an increased risk of progression to AML.
Acute myeloid leukemia (AML)	A malignant clonal disease in the bone marrow with >20% blasts affecting a myeloid cell line.
Secondary acute myeloid leukemia (s-AML)	Acute myeloid leukemia in patients with former malignant hematopoietic disease such as MDS or myeloproliferative neoplasia (MPN), or patients who have been treated with irradiation of chemotherapeutic agents

1 INTRODUCTION

This thesis is based on 4 registry studies. The first (I) is a large European study from the European Network on myelodysplastic syndromes (MDS) (EUMDS) with patients from 17 countries (1).

The three last papers are based on the Swedish Acute Leukemia Registry (SALR)(2) and the Swedish Information Network for Cancer (INCA) (3)Acute Myeloid Leukemia (AML) - and myelodysplastic syndromes (MDS)-registries(4).

1.1 MYELODYSPLASTIC SYNDROMES:

Myelodysplastic syndromes (MDS) comprise a heterogeneous group of myeloid neoplasms defined by peripheral cytopenia, bone marrow (BM) failure, with more than 10% dysplasia in one or more myeloid cell lines (5-7) and genetic instability with increased risk to transform to secondary acute myeloid leukemia (AML) (8).

The bone marrow percentage of myeloblasts is restricted to 0-19%. The hematopoiesis is ineffective with increased apoptosis. Karyotyping is essential in order to diagnose MDS correctly (6). With conventional chromosome analysis, cytogenetic changes can be seen in approximately 55% of the cases (9, 10), but with more sophisticated diagnostics such as Next Generation Sequencing (NGS), gene mutations can be found in up to 90% of the cases(11, 12).
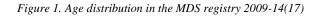
The myelodysplastic syndromes as a group of diseases can overlap between AML, aplastic anemia, and myeloproliferative neoplasms (MPN), and it can sometimes be difficult to distinguish which diagnosis that is most correct. For patients with low risk MDS, it is recommended to have two separate bone marrow samples with an interval of 3 months in order to be certain of the diagnosis. The cytopenias (hemoglobin (Hb) <10g/dL, platelets <100 x10^9/L and absolute neutrophil count (ANC) 1.8 x10^9/L) should be persistent in > 4 months to fulfill the diagnostic criteria (8). For patients with an elevated blast count, it is also recommended to take two separate bone marrow samples, but with a shorter interval in case the disease progresses to AML.

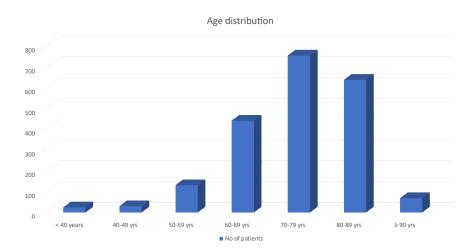The development of MDS is slower than in AML, especially in the lower risk groups. The challenge here is to treat the effects of cytopenias, such as anemia, thrombocytopenia and neutropenia. With high risk MDS, the aim is a more curative treatment including allogeneic hematopoietic stem cell transplantation (HSCT) in order to eradicate the malignant clone or at least improve the levels of cytopenias (13).

EPIDEMIOLOGY

In Sweden, about 350 new patients are diagnosed with MDS each year, representing a crude incidence of 4 per 100 000 inhabitants, comparable to other registries (14, 15). A study from Düsseldorf reports an incidence of 4,15 per 100 000 inhabitants(14), and from USA, the incidence was 3.3 per 100 000 in 2001-2003, increasing to 4.9 per 100 000 for the years 2007-2011, probably due to increased awareness of the disease more than an actual increase (15). There is a risk of underdiagnosing MDS, as especially the lower risk MDS diagnosis may be difficult (15, 16).

The male/female ratio in the Swedish MDS-registry 2009-14 is 59/41. Age distribution in MDS (fig. 1) in the MDS registry 2009-14 (17). The median age is 75 years, 77 years for women and 75 years for men.

Figure 1. Age distribution in the MDS registry 2009-14(17)

ETIOLOGY

The etiology in MDS is in most cases unknown. Former exposure to benzene, smoking and agricultural chemicals (18) can predispose for MDS. Rare cases of inherited or de novo germline mutations are now easier to diagnose with new methods such as deep sequencing (19), and specific mutations have been identified that are associated with MDS (TET2, SF3B1, ASXL1, SRSF2, DNMT3A, and RUNX1 and ASXL1)(11).
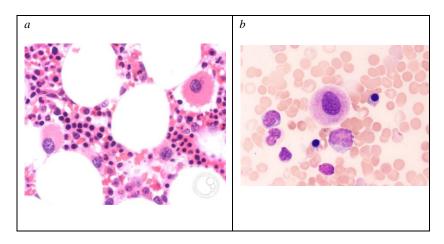
1.1.1 DIAGNOSTICS MDS

The diagnosis of MDS is based on several different diagnostic procedures: A careful clinical assessment is always important. The age, general health, performance status (WHO Performance status (20) or ECOG(21)) and assessing comorbidities are important when deciding what kind of treatment this particular patient is going to receive.

MORPHOLOGY: BONE MARROW SMEAR AND BONE MARROW BIOPSY

The morphological examination of peripheral blood and bone marrow is a prerequisite for establishing MDS (5, 6, 22)(Fig. 2a and b). It is important that the quality of the smears and biopsy are good (6). The major morphological finding in MDS is dysplasia that should be found in >10 per cent of the cells, present in one or more of the hematopoietic cell lines. Both bone marrow biopsy and smear should be done to diagnose a patient properly. Bone marrow biopsy is necessary to evaluate the cellularity in the bone marrow and the amount of fibrosis. Bone marrow smears are better in distinguishing the morphology of the cells.

Figure 2(a) MDS with isolated del (5q) chromosome abnormality. Bone marrow biopsy specimen (H&E stain.) From ASH image bank. Author: James W. Vardiman ID 1446(b) MDS. (b) Bone marrow aspirate smear (May Grünwald -Giemsa stain) with dysplastic megakaryocyte. Courtesy of Bone Marrow Laboratory, Section of Clinical Chemistry, Sahlgrenska University Hospital

CYTOGENETICS

About-50-70% of the MDS cases have chromosome aberrations (11, 23), and some chromosome changes define special entities of MDS, such as del5(q)(22). A proper karyotyping is necessary in order to classify and risk score a patient with MDS (6). G-banding to visualize the chromosomes is the traditional way (24). It is reliable but is time-consuming in culture and also requires special visual skills to identify changes. Fluorescence *in situ* hybridization (FISH)(24) is used to detect specific areas on the chromosome.

As we learn more about both the AML and MDS diseases, we are beginning to see that there can be genetic lesions in families that predispose to AML or MDS (25).

NEXT GENERATION SEQUENCING

Next generation sequencing (NGS) or deep sequencing is a method that is becoming increasingly more used. It is a method that enables amplification of genes, so that mutations can be detected on a very low level. (11, 12). Whole Genome Sequencing is now commercially available as methods for investigating the whole human genome (26). This has made it possible to be more accurate in our risk assessment.

With ordinary cytogenetic methods, about 50-70% of MDS patients have cytogenetic changes at diagnosis (27). With NGS, 80-90% of the patients have mutations (11). In the next few years, we will probably see proposals on new risk assessments for both AML and MDS which incorporates mutations found by these new methods (28) .

1.1.2 MDS CLASSIFICATION

In the first AML classification paper in 1976 (29), a preleukemia variant is mentioned, but it was in 1982 that the first classification of MDS came(5). This was a classification based mostly on morphological and cytochemical methods (Table 1).

Table 1. FAB classification

Low risk	RA Refractory anemia	<5 % blasts
	RARS Refractory anemia with ring sideroblasts	>15% Ring sideroblasts
	CMML Chronic myelomonocytic leukemia	<20% blasts
High risk	RAEB Refractory anemia with excess of blasts	5-20% blasts
	RAEB Refractory anemia with excess of blasts	20-30% blasts

This was a huge leap into trying to systematize a heterogeneous group of conditions that up to then had been poorly defined. In the beginning, it was not clear whether this should be classified as malignant diseases or not, which is reflected in our coding system ICD-10(30) as it is classified as neoplasms of uncertain or unknown behavior. The first WHO classification was presented in 2001, (7) (Table 2) now with more extensive diagnostic methods than morphology and cytochemistry, with revisions in 2008(22) (Table 3) and 2016 (6).

Table 2. WHO classification of MDS 2001(31) compared to the FAB classification

FAB 1982	WHO 2001
Refractory anemia (RA)	RA Refractory cytopenias with multilinear dysplasia (RCMD) MDS associated with isolated del(5q)
Refractory anemia with ring sideroblasts (RARS)	Refractory anemia with ring sideroblasts (RARS)
Refractory anemia with excess of blasts (RAEB)	RAEB 1
	RAEB 2
Chronic myelomonocytic leukemia (CMML)	Mixed MDS/MPN
Refractory anemia with excess of blasts in transformation (RAEB-t)	AML

Table 3. WHO classification of MDS 2008 (22)

MDS	Refractory cytopenia with unilinear dysplasia:	Refractory anemia (RA) Refractory neutropenia (RN), Refractory thrombocytopenia (RT)
	Refractory anemia with ringed sideroblasts	RARS
	Refractory cytopenia with multilinear dysplasia:	RCMD
	MDS associated with isolated del(5q)	MDS del(5q)
	Refractory anemia with excess of blasts –1	RAEB-1 5-10% blasts
	Refractory anemia with excess of blasts -2	RAEB-2 10-20% blasts
	MSD- unclassifiable	MDS-U
MDS/MPN	CMML	Peripheral monocytosis >1 x 10 9/L, BCR-ABL neg., < 20% blasts CMML 1:< 10% blasts in BM and <5% blasts in peripheral blood CMML 2:10-19% blasts in bone marrow and/or 5-19% peripheral blasts
	Atypical CML, BCR-ABL neg.	

	Juvenile myelomonocytic leukemia JMML	
	MDS/MPN unclassifiable	
	RARS associated with marked thrombocytosis RARS-T	

In 2016, the latest classification of both MDS and AML was presented (6). For both diseases, this classification adds some more specific entities thanks to the new diagnostic methods now available. The 2016 classification will not be presented in detail, as it is the WHO 2008 classification that is relevant for these studies.

1.1.3 PROGNOSTIC SCORING SYSTEMS AND RISK ASSESSMENT IN MDS

In 1997, the first risk score system for MDS, International prognostic scoring system (IPSS) was introduced (32). Patients were divided in risk groups depending on blast counts, karyotype and degree of cytopenias (Table 4). The patients were divided into 4 groups, Low, Intermediate-1(int-1), Intermediate-2 (int-2) and High risk (32). Low and Int-1 were grouped as low risk and Int-2 and High risk grouped as high risk. Since then, other risk score methods have emerged, such as WHO classification-based prognostic scoring system for myelodysplasia (WPSS) (33) which uses the WHO classification (2001)(7) in the scoring system, as well as transfusion need.

In the revised international prognostic scoring system (R-IPSS) (table 5) (34), hemoglobin value is used as a pseudomarker for transfusion need. It also includes absolute neutrophil count (ANC), platelets and cytogenetic changes that are a bit more refined as compared to IPSS. The blast count is also more refined than in the IPSS score (see table 4 and 5). R-IPSS and WPSS have been compared in a Dutch (35) and a Swedish study, (36) and R-IPSS come out as more predictable. A proper risk classification is a part of the decision-making with regards to treatment (13, 37). In order to do a risk classification, it is necessary to do a proper diagnostic work-up, including counting blasts down to 2 per cent, and cytogenetics. It has been shown that patients without a thorough diagnostic work-up, the survival of the patients is poorer (38), possibly indicating that the patients that we choose not to diagnose properly, are more often elderly and have other diseases.

Currently, there are several groups (39, 40) working on establishing a new prognostic scoring system that also include mutations, where the Swedish MDS Biobank is a part of the patient pool that is the basis of the studies in one of the groups (Jädersten M, personal information).

Table 4. International prognostic scoring system (IPSS)(32)

Score	0	0.5	1	1.5
% BM blasts	< 5%	5-10%	-	11-19%
Karyotype	good	INT	Poor	-
Cytopenia	0-1	2-3	-	-

Karyotype: good=normal, -Y, del(5q), del(20q), poor=complex (≥abnormalities) or chromosome 7 anomalies; Intermediate = other abnormalities

Cytopenias: Hb <10g/dl, Absolute neutrophil count (ANC) <1.8x10^9/L, Platelets <100x10^9/L

Risk group	**Score value**	**Median survival (years)**
Low risk:	0	5.7
Intermediate 1	0.5-1	3.5
Intermediate 2	1.5-2.0	1.2
High risk:	≥ 2.5	0.4

Table 5. Revised international prognostic scoring system (R-IPSS), including prognostic variables(34)

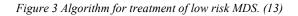| Prognostic variable	0	0.5	1	1.5	2	3	4
Cytogenetics	Very good		Good		Intermediate	Poor	Very poor
BM blasts, %	≤ 2		>2-<5		5-10	>10	
Hemoglobin	≥10		8-<10	<8			
Platelets	≥ 100	50-<100	<50				
ANC	≥ 0.8	≤0.8					

Cytogenetics: Very good: -Y, del(11q), Good: normal, del(5q), del(20q), del(12p), double incl. de(5q) Intermediate: del(7q), +8, +19, i(17q), or any other single or double independent clones. Anomalies. Poor: -7, inv (3)/t(3q)/del(3q), double including -7/del(7q), complex (3 abnormalities) very poor: (>3 abnormalities)
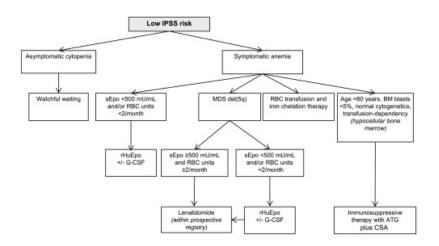
Risk group	Score value	Median survival
Very low	≤1,5	8.8
Low	2-3	5.3
Intermediate 1	3,5 – 4,5	3.0
High	5-6	1.6
Very high	>6	0.8

1.1.4   MDS TREATMENT

TREATMENT LOWER RISK MDS

Treatment of lower-risk MDS is highly dependent on age and symptoms. Older patients who are not candidates for potentially curative treatment with allogeneic stem cell transplantation are mainly treated based on symptoms, and asymptomatic patients with R-IPSS low or very low risk MDS can live many years after diagnosis (34), and watchful waiting can be recommended for some patients in these groups. However, it is important to carefully evaluate symptoms of anemia that sometimes can be missed by the physician and may lead to reduced quality of life. Several studies have shown a clear association between Hb level and quality of life (QoL) in MDS (41-43). (Fig.3)

Figure 3 Algorithm for treatment of low risk MDS. (13)

Therapeutic algorithm for adult patients with primary MDS and low IPSS score. BM, bone marrow; sEpo, serum erythropoietin.

SUPPORTIVE CARE:

The onset of anemia in low risk MDS is often slow but gradually signs of fatigue develops. Depending on age and heart condition, palpitations, angina pectoris and shortness of breath can be found. Elevating the Hb level can alleviate these symptoms, either by transfusions or with erythropoietin (42).

For patients with low risk MDS and a need for treatment due to low blood counts, the aim of the treatment is to alleviate the problems associated with anemia, thrombocytopenia and leukopenia. (see Fig. 3).

TRANSFUSION THERAPY FOR ANEMIA:

When ESA (alone or in combination with G-CSF) no longer have effect, most patients are confined to transfusion therapy. In a study by the NMDS group(42) we showed that a Hb elevated to 120g/L increased QoL, irrespective of whether Hb was increased by transfusions or darbopoietin. Moreover, the rate of transfusions did not increase once the higher Hb level was reached. The level at which transfusion is necessary varies. The Nordic Guidelines recommend individual transfusions triggers and targets(44). Younger persons can manage with Hb levels down to 70g/L, but most often, 80 g/L is chosen as an arbitrary threshold for transfusions for patients <60 years, 90g/L to patients up to 80 years. Often the patient experience and can tell when a transfusion is necessary. Comorbidities as angina, reduced lung functions, makes it necessary to increase the threshold for transfusions. In everyday practice, we accept an Hb level that is lower. Our ESA study (1) also showed that the trigger level for transfusions in Europe varies from >100g/L in Sweden and The Netherlands and <80g/L in Poland and Romania indicating that access to erythrocyte transfusions can vary within the countries.

IRON CHELATION:

With regular transfusions, the risk of iron overload is imminent. Iron chelation is widely accepted for patients with thalassemia(45, 46), and is recommended in many of the care programs for low risk MDS (13, 44, 47, 48). One study from France (45) showed prolonged survival in patients treated with chelation compared to transfused patients with MDS without chelation, but no prospective study with MDS and chelation has been done. The use of iron chelators in MDS is not always sufficient (49). This can be due to side effects among the most commonly used iron chelators om the market. There are 3 iron chelators available: Deferoxamine, which can only be given as an iv. infusion or a sc injection (44), deferiprone which has the risk of neutropenia as side effect (50), and deferasirox (46), with risk of liver or kidney damage and nausea as a bothersome side effects. There are also studies that have shown that careful chelation before allogeneic HSCT improves the survival (51). It is generally recommended to start chelation in MDS patients that have received >20 units of red blood cells (RBC) or when the ferritin levels increases >1000μg/L(44, 52).

NEUTROPENIA AND INFECTIONS IN MDS

Proper treatment of infections is important in patients with low white blood cell count (WBC). A Cochrane review recommends prophylactic antibiotics to neutropenic patients (53). Although prophylactic antibiotics is not recommended in our care program (44), it is recommended to start antibiotics as soon as possible when there are signs of infection. Prophylactic agents against candida (fluconazole) and herpes infections (acyclovir) can also be given. G-CSF can be considered as prophylaxis for severely neutropenic patients with recurring serious infections or during infectious episodes. Published data are limited. It may be considered during azacitidine treatment. Long-acting G-CSF has not been evaluated in MDS and cannot be recommended.(44)

ESA AND G-CSF

Low hemoglobin counts can be treated with erythropoietin stimulating factors (ESA) (54) (55, 56), and combining them with granulocyte-stimulating factors (G-CSF) can have a synergistic effect (57, 58). In 2003 the Nordic MDS group proposed a model for deciding which patients to treat with ESA based on s-erythropoietin (s-epo) and transfusion need (Table 6) (59). Basically, it says that the chances of responding to ESA is better if the patient has a low transfusion need and a low s-epo (< 500 U/L). The model has been validated several times. Park et al. conducted a study in 2010 showing that patients with a low transfusion need, s-epo below 100U/L and HB>90 had a better response to ESA. Patients with RCMD-RS and shorter time between diagnosis and ESA start had longer ESA responses(60).

A Canadian group emphasizes the importance of starting ESA at a lower EPO level (below 100 U/L, and have added low risk criteria in their algorithm for staring ESA(61), and treatment with ESA is now established as being important in low risk MDS in order to postpone transfusion need. (13). In a study that compared an ESA treated cohort from Sweden with a cohort from Pavia that did not receive ESA could show that an increased survival was seen in the ESA group (improved overall survival (hazard ratio, 0.61; 95% CI, 0.44 to 0.83; P = .002). No impact on transformation to AML was seen (62).

Table 6. Decision model for the use of epo:

Transfusion need	Point	S-epo	Point
<2 unit's RBC/month		<50 U/l	
≥2 units RBC/month	1	≥500 U/l	1
Predicted response:	0 point 74 %	1 point 23%	2 points 7%

IMMUNOSUPPRESSIVE TREATMENT:

Hypoplastic MDS and aplastic anemia can sometimes be difficult to differ from each other. The hypoplastic MDS is characterized by pancytopenia and low bone marrow cellularity. Patients with hypoplastic MDS can respond to Anti thymocyte globuline (ATG)(44, 63), similar to what is seen in aplastic anemia, especially in patients with the HLA phenotype HLA DR15.

SPECIFIC TREATMENT FOR CERTAIN SUBGROUPS

Lenalidomide: Patients with a 5q deletion is defined as a special entity in MDS ((19, 22), typically with anemia and thrombocytosis. The patients respond to ESA, but the effect is not long lasting. Lenalidomide has been shown to efficiently treat anemia in this condition (64, 65) Lenalidomide can also alleviate anemia in a low risk MDS population refractory to ESA without del 5q (66). Patients with TP53 mutation has an increased risk of transformation to AML (67). Lenalidomide is recommended in Europe within the MDS Post-Authorization Safety Study(PASS) (68) and is approved by FDA in the USA(69).

Luspatercept: Refractory anemia with ring sideroblasts (RARS) (≥15% erythroblasts with at least 5 siderotic granules covering at least a third of the circumference of the nucleus) (70) has been defined as a specific entity since the first classification (5, 22) of low risk MDS. RARS is characterized clinically by anemia as the cardinal symptom. The patients have response to ESA but often a very short response. There is a strong association with spliceosome mutations (such as SF3B1) and ring sideroblast anemia (12).
Phase II studies have shown (71) that luspatercept can reverse the anemia in low risk MDS especially in the group of patients with the SF3B1 mutation. The mechanism of action is different from ESA. There is an ongoing phase 3 study investigating the effect of luspatercept on patients with ring sideroblasts and hopefully luspatercept can be an alternative to ESA in postponing the transfusion need in the low risk MDS patients. It is not yet recommended by EMA.
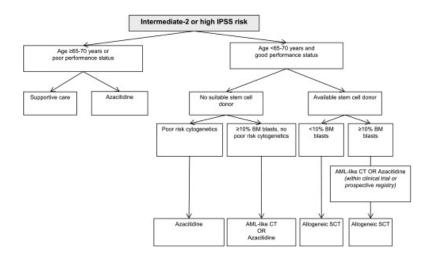
TREATMENT HIGHER RISK MDS

For patients with high risk MDS, the treatment aim is to remove or reduce the malignant clone. The only curable way to do this is through allogeneic HSCT (13, 44). If the blast count is >10%, it is generally recommended to reduce the malignant clone before transplantation (13). Pretreatment is either with induction treatment similar to the induction treatment in AML (44), or by using hypomethylating agents (HMA) such as azacitidine (72) or decitabine (73). The non-relapse mortality (NRM) after allogeneic HSCT is 36%, varying from 32% with reduced intensity conditioning (RIC) and 44% with myeloablative treatment (MAC); (HR, 0.84; P = 0.05) and long- term survival is 31%(74). Patients with MDS tend to have longer time to regenerate the bone marrow after induction, thus rendering them more prone to complications such as infections.

More and more, induction therapy is reserved for the younger and fit patients, whereas HMA is a better treatment option for elderly patients (13, 44). For patients where allogeneic HSCT is not an option, HMA is a good alternative. The overall survival with azacitidine were 24.5 months compared to 15 months with conventional care regimens (best supportive care only, low-dose cytarabine (LDAC), or intensive chemotherapy (IC)) in a phase III study in patients with higher risk MDS or AML up to 30% blasts (72). A metaanalysis has shown that the results with azacitidine is better than with decitabine (73). In the Nordic countries, the recommendation is to use azacitidine before decitabine (44) We do not yet have any good treatment options after HMA failure, but studies with new agents such as guadecitabine are trying to address this difficult issue (75).

PALLIATIVE CARE/SUPPORTIVE CARE

When HMA no longer are working, or the patient is considered too frail for treatment, supportive care is necessary. The aim of this treatment is to keep the patient healthy enough to avoid in-patient care. Erythrocyte transfusions, antibiotics when necessary or platelet transfusions when bleeding can be good alternatives. Hydroxyurea can be a good option in more proliferative patients.

Figure 4 Therapeutic algorithm for adult patients with primary MDS and Intermediate-2 or high IPSS score(13)
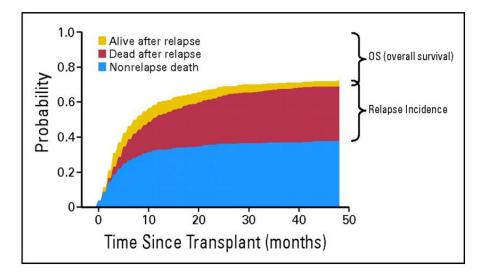

Therapeutic algorithm for adult patients with primary MDS and intermediate-2 or high IPSS score. CT, chemotherapy.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) FOR THE TREATMENT OF MDS

 Younger (<70 years) and fit patients with high risk MDS or lower risk MDS that are transfusion-dependent or suffers from chronic cytopenia can be treated with allogeneic HSCT (13) (44) (76). The risks with allogeneic HSCT is risk of death in relapse which increases when using non-myeloablative treatment for HSCT (74)(fig. 5). On the other hand, the risk of non–relapse mortality or mortality due to side effects of treatment increases when using stronger or myeloablative treatment. The general recommendation is to use a reduced intensity treatment (13) for patients with MDS preferably with a combination of treosulphan and fludarabine (77). The risk of relapse also increases with increasing risk score (78), making it important to transplant before the disease progresses.

Figure 5 MDS patients: Stacked cumulative incidence curves from a competing risk model evaluating the proportion of patients in a particular state with respect to the presence or absence of relapse, as a function of time after transplant. OS, overall survival. (74)

TREATMENT INTERMEDIATE RISK MDS

With R-IPSS, an intermediate group of patients emerges. It is up to the clinician to decide whether the patient should receive treatment more in analogy with the higher risk patients with a lower risk patient. Careful monitoring is necessary to follow the patient and see how the disease develops.

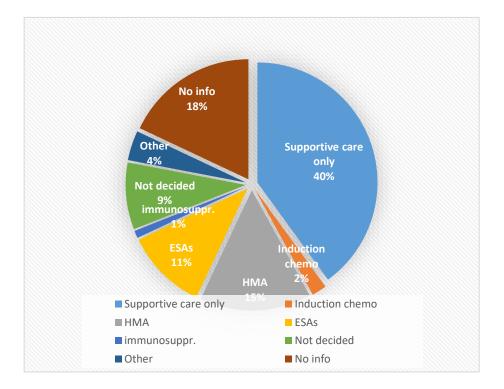*Figure 6 Treatment decision at diagnosis all MDS categories (From MDS report 2009-13)(79)*

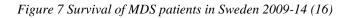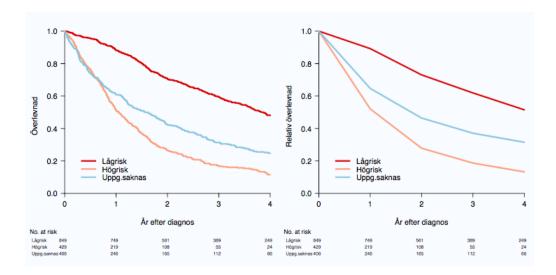Figure 6 shows the clinician's treatment decisions in The Swedish MDS registry at time of registration.

SURVIVAL IN MDS

The prognosis varies for low and high risk MDS. Both survival and risk of progression to AML differ significantly. The relative 2–year survival for low risk and high risk MDS are 77 and 29 per cent, respectively.(16) (fig.7).

Figure 7 Survival of MDS patients in Sweden 2009-14 (16)

1.2 ACUTE MYELOID LEUKEMIA

Acute myeloid leukemia (AML) is defined as a hematopoietic myeloid stem cell disorder with more than 20% blasts in the bone marrow or peripheral blood (29). As we now learn that this disease is dynamic and changeable, the definition changes as well: "A complex, dynamic disease, characterized by multiple somatically acquired driver mutations, coexisting competing clones, and disease evolution over time" (9).
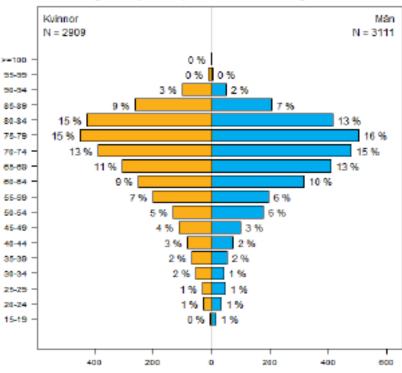
Acute myeloid leukemia (AML) is a heterogeneous clonal disorder of hematopoietic progenitor cells and the most common malignant myeloid disorder in adults (80). The bone marrow is often hypercellular and dominated by one or more malignant blast clones which destroy the environment of the normal hematopoietic cells. Cytogenetic changes that can be seen in more than 50% of the cases with AML, and specific mutations have been shown to be important in the risk assessment of AML (81).

The important challenge in AML is to eradicate the malignant cells, thus allowing the normal hematopoiesis to regenerate in the bone marrow. It is often a rapidly developing disease, necessitating treatment as soon as possible.

EPIDEMIOLOGY

In Sweden, the median age for AML is 71 years, 71 for men and 72 years for women (82). There are slightly more men than women that are diagnosed with AML. The incidence in Sweden is relatively stable, approximately 3.5 per 100 000 per year (82), comparable to the incidence in the US of 4.2 per100 000 inhabitants per year (83). In a large study from Europe, the incidence of AML was estimated to 3.7 per 100 000 inhabitants.(84) (Fig. 8).

Figure 8 Age and gender distribution of AML in the Swedish AML registry 1997-2014 (82)

ETIOLOGY

The causes of AML are not well known. Age is a risk factor, as well as some genetic disorders, such as Downs syndrome (85). Exposure to smoking, benzene, herbicides and former treatment with radiation or chemotherapy such as alkylating agents increases the risk of AML. Most cases of AML appear *de novo*, without any previous cause (85). Approximately 25 per cent of AML cases are secondary either to previous hematological disease such as MDS or MPN, or to chemotherapy or radiation (2, 86).

1.2.1   AML DIAGNOSTICS

The diagnosis of AML is based on several different diagnostic procedures: Clinical assessment is essential to determine what kind of treatment that is best suited for the patient.

The malignant clonal nature of the blasts is determined by morphology, cytochemistry or by using Multiparametric flow cytometry (MFC) (22). Cytogenetic methods such as chromosome analysis(10, 87), Fluorescence in situ hybridization (FISH)(24) and mutational analyses against specific mutations that are associated with AML (e.g. CEBPA, NPM1, FLT3-ITD)(22) are used. As in MDS, certain cytogenetic aberrations and mutations are risk defining in AML(9).

Next Generation Sequencing (NGS) (88) is a relatively new method that enables amplification of genes, so that mutations can be detected on a very low level. It is now available in all university hospitals in Sweden and will be important in future classification of acute leukemia.

MORPHOLOGY

The morphological examination of peripheral blood and bone marrow is essential in AML diagnosis (Fig. 9) with the exception of Myelosarcoma (22). Twenty per cent blasts is a prerequisite for the AML diagnosis, except AML with t(8,21)(q22;q22.1), AML with inv(16(p13.1q22) or t(16;16)(p13.1;q22) and Acute promyelocyte leukemia (APL) with PML-RARA t(15;17)(q22;q12)(19). It is possible to diagnose a patient with AML solely based on the peripheral blood count if the blast count is >20%. Cytochemical staining such as myeloperoxidase (MPO) are used in recognizing the myeloid lineage of cells, but it does not exclude myeloid lineage, because early monoblasts and myeloblasts can lack MPO.

Figure 9 AML with t(8;21)(q22;q22); RUNX1-RUNX1T1) bone marrow smear with May Grünwald Giemsa stain Description: Centrosomes are evidence of myeloid differentiation. Copyright © 2018 American Society of Hematology. ID 2597
From ASH image bank. Author: Peter Maslak

More sophisticated methods are needed to make the diagnosis as precise as possible. The new methods are necessary in providing information for risk assessment both for MDS and AML. (22).

MULTIPARAMETER FLOW CYTOMETRY (MFC) AND MINIMAL OR MEASURABLE RESIDUAL DISEASE (MRD)

Multiparameter Flow cytometry (MFC) or immunophenotyping with flow cytometry or immunohistochemistry on trephine biopsy is a way of identifying a malignant clone in the bone marrow or blood (22)(Fig.10). With this method, the blast amount can be better assessed than by morphology alone. By using a set of predefined antibodies, it is possible to identify malignant clones in bone marrow or blood in 85-90% of AML patients (89).

Immunophenotyping is also used for identifying a malignant clone that can be followed by measurable residual disease (MRD) after treatment as a method for evaluating the effect of treatment, especially important in AML (90).
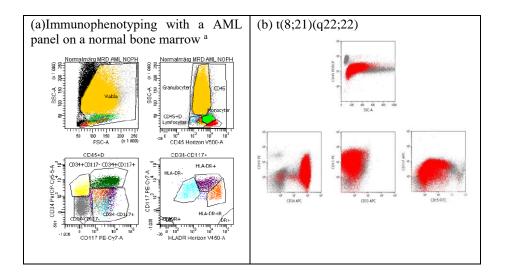
Measurable (minimal) residual disease (MRD) can be defined as detectable leukemia in blood or bone marrow in a patient that otherwise fulfills the criteria for complete remission. Detecting MRD with MFC or molecular genetic methods indicates an increased risk of relapse (91) and is important in assessing risks in AML patients (89, 92).

By using a set of antibodies, specific clones of malignant cells can be identified. These cells can be recognized either because they have 1) AML defining changes (Leukemia-Associated ImmunoPhenotypes (LAIP) where the phenotype is specific for AML (93), or 2) a phenotype that can be classified as Different-from-Normal (DfN)(9).

Other ways of defining MRD is by using molecular methods to identify specific mutations that have been found earlier at diagnosis (9, 19). RT-qPCR for t(15;17)(q24;q21); *PML-RARA* has been used to monitor high risk APL (94). Other molecular markers that are suitable for MRD monitoring are t(8;21)(q22;q22); *RUNX1-RUNX1T1*, inv16(p13q22)/t(16;16)(p12;q22); *CBFB-MYH11*, t(9;11)(p21;q23); *KMT2A-MLLT3* (*MLL-AF9*) (95)and NPM1 mutations (96).

Analyzing measurable residual disease (MRD) is recommended in all patients that are being evaluated for allogeneic HSCT(97) (94).

(a)Immunophenotyping with a AML panel on a normal bone marrow [a]	(b) t(8;21)(q22;22)

CYTOGENETICS

Cytogenetics is important in the risk assessment of AML. About 55% of all AML cases have cytogenetic changes (87). In the first FAB-classification (table 7,) morphology was the important defining feature. Now, the classification uses specific genetic changes, such as t(8;21) (Fig.11A and B) or t(15;17) in APL to classify AML more specifically, see table 8).

Figure 11 Illustration of different cytogenetic methods

(a)Chromosome analysis showing a translocation 8,21 in AML [a]	(b)Fish showing t(8;21)

Courtesy of cytogenetic lab, Section of Clinical Chemistry, Sahlgrenska university hospital

The number of chromosomes should if possible be counted in at least 20 cells in metaphasis and as many as possible should be karyotyped (Fig 11a). FISH for t(15;17)(q24;q21) and RT-PCR for PML-RARA must be done when acute promyelocyte leukemia is suspected (Fig.11b).

MUTATIONS AND NEXT GENERATION SEQUENCING (NGS)

In a study of 200 cases of de novo AML 23 genes were found to be commonly mutated, and another 237 were mutated in 2 or more cases (98). This confirms the fact that AML is genetically a heterogeneous disease and so far, just a fraction of these mutations have clinical relevance. Mutations in NMP1, FLT3-ITD and CEBPA are risk-defining mutations used in clinical routine in AML and should be analyzed both at diagnosis and when possible as MRD markers (9, 94). Other mutations that seems to be important for AML prognosis are TP53, ASXL1, DNMT3A and RUNX1 (99, 100). In a near future several other mutations will probably be used in clinical routine both for prognostic decisions and hopefully for coming new targeted therapies.

Mutations in NMP1, FLT3-ITD and CEBPA are risk-defining mutations used in clinical routine in AML and should be analyzed both at diagnosis and when possible as MRD marker(9, 94).

Next generation sequencing is now available at all university hospital lab in Sweden. With this method, mutations can be seen in almost 90% of the AML cases (88). In a near future several of these mutations will probably be used in clinical routine both for prognostic decisions and hopefully for coming new targeted therapies. In Sweden, a defined panel of 54 known mutations in AML can be detected using a predefined kit from Illumina (101). Many of the molecular analyses we use today can probably be replaced by NGS methods(9). Both in MDS and AML, groups are working to incorporate the new knowledge about mutations in the risk assessment models (6, 11).

1.2.2 CLASSIFICATION AML

The first proper classification on AML came in 1976 by a group of French, American and British (FAB) hematopathologists (29). This classification was based on cytomorphology and a few cytochemical methods. The theory is that a hematopoietic stem cell in the bone marrow differentiates to mature myeloid cells and a when a malignant clone occurs, the maturation to normal hematopoietic cells is abrupted and immature blasts occur in the peripheral blood. The FAB-classification identified nine different variants in the development of acute myeloid leukemia. (Table 7) The classification of AML has become more sophisticated over the years as new diagnostic methods has been introduced, making the classification more accurate, but also more complicated. Specific cytogenetic changes and specific mutations have been included as separate entities. New classifications of AML and MDS came in 2001(7) and 2008 (22) (table 8). The latest update of the WHO classification was published 2016 (6). In the papers from this thesis, the WHO classification from 2008 were used.

Table 7. FAB-classification from 1976(29)

FAB subtype	Name	Adult AML patients (%)
M0	Undifferentiated acute myeloblastic leukemia	5%
M1	Acute myeloblastic leukemia with minimal maturation	15%
M2	Acute myeloblastic leukemia with maturation	25%
M3	Acute promyelocytic leukemia	10%
M4	Acute myelomonocytic leukemia	20%
M4eos	Acute myelomonocytic leukemia with eosinophilia	5%
M5	Acute monocytic leukemia	10%
M6	Acute erythroid leukemia	5%
M7	Acute megakaryocytic leukemia	5%

Table 8. The WHO Classification of AML (2008)(22)

Acute myeloid leukemia (AML) with recurrent genetic abnormalities*	AML with t(8;21)8q22;q22);RUNX1-RUNX1T1
	AML with inversion(16)(p13.1q22) or t(16,16)(p13;q22);CBFB-MYH11
	Acute promyelocytic leukemia with t(15;17)(q22;q12);PML-RARA
	AML with t(9;11) (p22;q23); MLLT3-MLL
	AML with t(6;9) (p23;q24); DEK-NUP214
	AML with inv(3) (q21q26.2) ort(3;3)(q21;q26.2);RPN1-EVI1
	AML (megakaryoblastic) with 1(1;22)(p13;q13;RBM15-MKL1
AML with gene mutations	FLT3-ITD
	CEBPA
	NPM1
	KIT
	MLL
Acute myeloid leukemia with myelodysplasia-related changes	>20% blasts in blood or BM, previous history of MDS or MDS/MPN, or multilineage dysplasia
	Absence of prior cytotoxic treatment for an unrelated disease and recurrent cytogenetic abnormalities as described above*
Therapy-related myeloid neoplasms	Includes T-MDS, T-MPN, T-AML

Acute myeloid leukemia, not otherwise specified	AML with minimal differentiation
	AML without maturation
	AML with maturation
	Acute myelomonocytic leukemia
	Acute monoblastic and monocytic leukemia
	Acute erythroid leukemia
	Acute megakaryoblastic leukemia
	Acute basophilic leukemia
	Acute panmyelosis with myelofibrosis
Myeloid sarcoma	
Myeloid proliferations related to Down's syndrome	
Blastic plasmacytoid dendritic cell neoplasm	
Acute leukemia of ambiguous lineage	

1.2.3 AML RISK ASSESSMENT

Risk assessment is important in deciding which therapy should be chosen for the individual patient. It is also important to assess factors that are not associated with leukemia such as age, general health and comorbidities in order to judge if the patient can tolerate induction chemotherapy.

One of the most important therapy decisions in AML treatment is if an allogeneic stem cell transplantation should be performed in first remission. This decision is based on risk factors associated with the AML disease e.g. mutational status and cytogenetic changes.

Secondary AML is not mentioned as a separate risk factor, but it is known that it affects the prognosis in younger patients (2). Table 9 illustrates which cytogenetic changes and mutations that are regarded as risk factors in the Swedish AML guidelines, Patients with intermediate or high risk will be candidates for an allogeneic stem cell transplantation if they are considered fit for the treatment depending on comorbidities and age. The European Leukemia Net (ELN) has also proposed a risk assessment model (Table 10).

Table 9. Risk assessment in the Swedish AML guidelines based on cytogenetic changes and mutations (102)

Risk category	Genetic abnormality
Low risk	APL with t(15:17)/q22:q21), t/inv(16)(p13q22), t(8;21) if not CD56+/c-kit+. NPM1pos if FLT3 neg. Double mutated CEBPA with a normal karyotype
Intermediate risk	Normal karyotype without FLT3-ITD, mutated NPM1 or double mutated CEBPA. • Normal karyotype and both NPM1-pos and FLT3-ITD-pos. Neither low or high risk, including t(9;1)
High risk	FLT3-ITD pos., 5q-/-5/-7, t(11q23) except t(9;11), t(6;9), t/inv(3)(q21q26) or t(3;3)(q21;q26), complex with >3 deviations, KMT2A-rearrangement.

Table10 shows the risk stratification proposed by the ELN group(81), adding mutations such as RUNX1-RUNX1T1, mutated RUNX1, mutated ASXL1, mutated TP53 into the risk categories.

Table 10. 2017 ELN risk stratification by genetics (9)

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated NPM1 without *FLT3*-ITD [ow]
	Biallelic mutated *CEBPA*
Intermediate:	Mutated *NPM1* and *FLT3*-ITD[high]
	Wild-type *NPM1* without *FLT3* -ITD or with *FLT3*-ITD [ow] (without adverse-risk genetic lesions
	T(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;))(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL-1*
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM(EVI1)*
	-5 or del(5q);-7;-17/abn(17p)
	Complex karyotype, monosomal karyotype
	Wild-type *NPM1* and *FLT3*-ITD[high]
	Mutated *RUNX1,* Mutated *ASXL1*or Mutated *TP53*

1.2.4   AML TREATMENT

In most cases, the AML treatment should be initiated as soon as possible after diagnosis. The age, general health, performance status (WHO Performance status (20) or ECOG(21)) and assessing comorbidities are important when deciding what kind of treatment this particular patient is going to receive. It is also important to know something about the patient's former health, such as former exposure to chemotherapy or irradiation or antecedent hematological disease such as MDS or myeloproliferative neoplasms (MPN) (103).

This means that the first treatment given is based upon the clinical assessment, morphological diagnosis, immunophenotyping, and a limited genetic assessment. The full risk assessment will take place later when all genetic factors have been analyzed. These results will form the basis for coming treatment decisions including allogeneic stem cell transplantation.

INDUCTION THERAPY

AML is a life-threatening disease, often with a relatively short disease history. Untreated, the survival is short (104). In the Swedish national care program(94), the ambition is to decide and start treatment within 6 days from when the suspicion towards leukemia is raised, and this is done in 71% of the cases in Sweden(82).

The first goal in AML treatment is to achieve a complete remission (CR). CR is defined as < 5% blasts in the bone marrow, presence of regenerating cells, no Auer rods, absence of extramedullary leukemia, no peripheral blasts, ANC > 1 x 10^9/L, platelets >100 x 10^9/L and no need for erythrocyte transfusions (81, 105). The most efficient way to achieve a CR is by *intensive chemotherapy*(106) in patients fit for treatment. According to the last report from the Swedish AML-registry, 58% of the patients started intensive chemotherapy. Median age of these were 64 years, and 76 % achieved a CR (89 % up to age 60 years, 63 % between 70-79 years)(82). An earlier study from the Swedish AML registry has shown that survival in patients aged 70-79 increased were higher in regions where more patients were offered induction therapy compared to palliative treatment only(106). These data emphasize the importance of proper induction chemotherapy up to the age of <80 years if the patient is fit. The induction regimen in Sweden consists of daunorubicine 60 mg/m^2 daily for 3 days combined with intermediate dose of cytarabine 1g/m^2 twice daily for 5 days (94). In clinical trials using daunorubicine doses up to 90 mg/m^2 the effect has been similar to the standard dose 60 mg/m^2(107). The international standard is daunorubicine 60mg/m^2 for 3 days, combined with cytarabine100-200 mg as a continuous infusion (9). Risk assessment based on the results from cytogenetic examination, mutational analysis and ideally NGS should be done before start of the second course of intensive chemotherapy. The second chemotherapy course is equivalent to the first, followed by a third course with only 2+5 days of treatment, and then, finally the fourth course with only intermediate dose of cytarabine. If the patients fail to respond to the first induction treatment changing of the chemotherapeutic agents can be tried in order to achieve remission e.g. combinations including fludarabine, idarubicine, etoposide or amsacrine in combination with cytarabine. In APL, the standard intensive induction therapy has been replaced by a combination with an anthracycline, all-trans retinoic acid (ATRA) and arsenic trioxide(108).

HYPOMETHYLATING AGENTS

For patients unfit to manage induction therapy, hypomethylating agents (HMA) such as azacitidine (AZA) or decitabine is approved by EMA (112, 113). This can be a good option for elderly patients (114) and patients not fit for induction therapy.

ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

For AML-patients, allogeneic HSCT should be performed in patients with high or intermediate risk up to the age of around 70 years if there is no significant comorbidity (109). It is not clear whether a myeloablative conditioning regimen should be preferred to a reduced intensity regimen (110). In general, for fit patients <60 years, a myeloablative regimen is recommended (109). For patients >60 years, a reduced conditioning (RIC) regimen is preferred in order to reduce toxicity (9). In the Swedish AML-registry, allogeneic HSCT have been reported for 24 % of the patients up to 70 years (37 % up to 50 years)(9, 81, 82, 111).

MAINTENANCE

The combination of Interleukin-2 and histamine as maintenance treatment after induction and consolidation treatment resulted in an increased leukemia free survival in a phase III randomized trial (115). Subgroup analysis have shown that the positive effect with IL-2 and histamine is mainly in leukemia with a monocytic differentiation (116). Patients with myelomonocytic or monocytic leukemia in complete remission can be treated with this as maintenance after induction and consolidation.

NEW DRUGS

New drugs used for patients with special mutations are being introduced in the treatment. The FLT3-inhibitor Midostaurin can be given as part of induction and consolidation to patients <60 years (117) followed by 1 year of maintenance. Midostaurin was approved by EMA in 2017(118).

Gemtuzumab ozogamicin (Mylotarg®), is an antibody chemically linked to calicheamicin, a specific compound that recognizes and binds specifically to the CD33 protein. It is effective in CD33 positive AML especially the Core Binding Factor (CBF) subgroups t(8;21) and inv(16) (119), but has been associated with toxic effects and increased death rates when given in doses 6mg/m2, but with better overall survival together with standard induction therapy when given in doses 3 mg/m2 for patients with favorable or intermediate risk profile(120). It is not yet recommended in standard therapy by the Swedish authorities.

There are ongoing studies on more specific and potent FLT3-inhibitors like qutuizartinib and crenolanib (which even inhibits KIT and PDGFRA) and gilteritinib (which even inhibits ASXL1)(120).

SUPPORTIVE CARE

Supportive care during induction chemotherapy is vital for managing the problems that inevitably come. Liberal use of broad spectrum antibiotics in neutropenic phase, antimycotic and antiviral treatment is part of supportive care, as well as total parenteral nutrition, transfusions of red blood cells and platelets, and access to intensive care when needed. The AML treatment has up to now not changed fundamentally during the last 30 years but still the survival have improved for every 5-year period partly due to more effective supportive care (82).
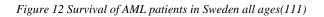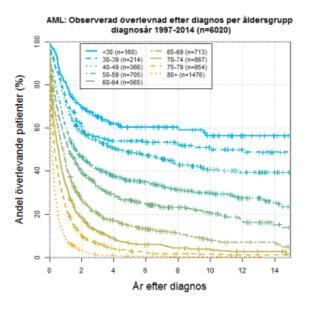
PALLIATIVE CARE

As we can see from the survival curves below (Fig.12 and 13), AML is still a disease with a dismal prognosis, especially for patients >70 years of age(121). Many patients can live a relatively good life with proper palliative care. The symptoms can be alleviated by reducing the tumor burden if the leukemia is very proliferative by using hydroxyurea (122) or to use low-dose cytarabine. In the palliative setting red blood cell transfusions can improve the quality of life and be useful for the patients (94). Platelet transfusions on the other hand should be administered more cautiously because the risk of immunization is greater with the risk of losing the effect when it is needed. The recommendation is therefore to only give platelet transfusions in case of active bleeding (94).

The aim in this situation is to provide the patient with treatment that enables them to stay at home for as long as possible, and to alleviate symptoms such as fatigue and fever. For most patients with AML, pain is not a major problem (4).

1.2.5 AML PROGNOSIS AND SURVIVAL

There are marked differences in survival for AML patients depending on age, see fig. 12. Fig.13 shows that the observed survival for all patients still are low, but patients diagnosed during 2007-2014 have an improved chance of survival compared to patients diagnosed during 1997-2006 (p<0.001)

Figure 12 Survival of AML patients in Sweden all ages(111)

Figure 13 Observed survival for patients with AML diagnosis 1997-2006 and 2007-14(111)

1.3   SECONDARY AML

AML that is a result of a progression from either MDS or Myeloproliferative neoplasia (MPN) or caused by previous radiotherapy or chemotherapy is called secondary AML (2). Secondary AML is defined as AML either in patients with a previous (> 3 months) antecedent hematological disease (AHD) in the myeloid cell lines such as either MDS or my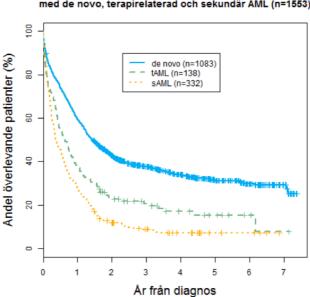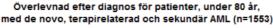eloproliferative neoplasms (MPN), or a therapy-related AML (t-AML) in patients that have either been exposed to radiotherapy or chemotherapy earlier in life (2). In the most recent update of the WHO-classification, these are named "Therapy-related myeloid malignancies, regardless if they are therapy-related MDS or AML(19). Treatment of s-AML is principally not different from *de novo* AML, but as the patients often are older and the prognosis is poor, less patients receive induction therapy (2). Figure 14 shows that the survival for S-AML and t-AML are poorer than for *de novo* AML.

Figure 14 Total survival for patients < 80 years with de novo AML, therapy-related AML and secondary AML. From the Swedish AML-registry 2007-2011. (123)

2 AIMS

The first paper (I) in my thesis is based on a large European prospective longitudinal observational study enrolling lower risk MDS patients from 17 European countries from both university hospitals and smaller regional hospitals. The aim of this study was to describe the usage and clinical impact of erythropoiesis-stimulating agents (ESAs) in 1696 patients enrolled between 2008 and 2014.

Paper II-IV describes Patients with secondary AML in three different ways:

In paper II, the whole acute leukemia population from the Swedish Acute Leukemia Registry (SALR) during the period 1997-2006 is described and characterized comparing secondary AML (s-AML) with de novo AML with regards to gender, age, cytogenetic risk and survival. Paper III is also from the Swedish Acute Leukemia registry (SALR) 1997-2013 and aims to investigate patients with secondary AML that undergo allogeneic hematopoietic stem cell transplantation (HSCT) compared to those treated with intensive chemotherapy (IC) only. In this study, only patients receiving intensive chemotherapy were included.

Paper IV have merged patients with information of former MDS from the AML registry with patients from the MDS registry 2009-14 in order to describe the development of the disease with regards to age, gender and transfusion need with information from both MDS diagnosis and AML diagnosis, and to assess how different factors impact survival.

3 PATIENTS AND METHODS

3.1.1 PATIENTS

Paper I: Of 1680 patients with lower risk MDS, ESA treatment was administered to 773 patients (45.6%), median duration of 27.5 months, range 0–77 months. Outcomes were assessed in 897 patients (484 ESA treated and 413 untreated).

Paper II: S-AML was divided into three group; patients with s- AML after MDS or MPN (called antecedent hematological disease, AHD-AML), or therapy-related AML (t-AML) where AML is secondary to previous chemotherapy or radiation. The study comprised 3,363 adult patients that had received induction therapy with the intention to achieve remission where 2,474 (73.6%) were de novo AML, 630 (18.7%) AHD-AML, and 259 (7.7%) t-AML.

Paper III: All patients from the AML-registry 1997-2013 that received intensive treatment (non-APL), 3337 of 5873 patients. Of these, 707 (21%) underwent HSCT at any stage of the disease, whereof 576 (22%) with de novo AML, 74 (17%) with AHD-AML and 57 (20%) with t-AML.

Paper IV: All patients registered with MDS as antecedent disease in the AML-registry 2009-14 were examined. These patients were merged with all MDS-patients from the MDS-registry 2009-14. In all patients registered in the AML-registry, but without information in the MDS-registry, missing data was completed by reading electronic journals where that was possible from Nov 2016 to November 2017.

3.1.2 ABOUT THE REGISTRIES

The European Myelodysplastic Syndrome (MDS) Registry (EUMDS) is an initiative of the Leukaemia Net MDS Work Package (www.leukemia-net.org). It is a prospective observational study aiming to collect information on newly diagnosed patients with Low or Intermediate-1 Score according to the International Prognostic Scoring System (ref. http://www.eumds.org/) It was started in 2008 and has until now included patients from 17 European countries. It includes patients from all kinds of hospitals seeking to be truly population based instead of only from university hospitals.

The Swedish Cancer Registry (SCR) started in 1958(124). It is mandatory to register all malignant diseases into this, giving close to a 100% coverage of all malignant diseases in Sweden. Both diagnosing doctors in pathology and clinicians have an obligation to register in the SCR. The SCR include data for age, gender, domicile, hospital, clinical and morphological diagnosis, stadium of the cancer, and time of diagnosis (125).

The Swedish Acute Leukemia Registry (SALR) started in 1997(126). The coverage here has been more than 95% over the years, providing a reliable source of population based research.(106) (126, 127). In 2007, it was fully digitalized, and separated into *The Swedish AML-registry* (including Acute Promyelocyte Leukemia (APL))(128) and *The Swedish Acute Lymphatic Leukemia* (ALL)-Registry (129). It provides more specific diagnoses and risk factors, including cytogenetic and mutational examinations, and treatment choice.

The *Swedish MDS-registry* started in 2009 (17). The coverage is >95%. All these registries are administered and maintained by the Regional Cancer Centers in Sweden, the AML-registry being located to Lund, Skåne, and the MDS –registry to Uppsala.

3.1.3 STATISTICS:

In paper I, the effects of ESAs on outcomes were assessed using proportional hazards models weighting observations by propensity to receive ESA treatment within a subset of anemic patients with or without a regular transfusion need.

In paper II-IV, continuous variables were compared using the Mann–Whitney U-test and the Pearson's chi-squared test for categorical data. Median follow-up time was calculated with the Reverse Kaplan-Meier method. Survival was estimated using the Kaplan–Meier method and compared through the log-rank test. The Cox proportional hazards model was used for multivariable analyses of survival. Propensity score matching analysis was performed using the R MatchIt package (130) with nearest neighbor matching and a caliper of 0.25 on continuous variables and exact match on categorical variables. Cumulative incidences of NRM and relapse were calculated considering competing risks using the R cmprsk package(131, 132). Two-sided P-values with a significance level of 0.05 were used in all analyses. The software used were SPSS (version 22 and 24) and R (version ver. 2.15.1and 3.3.3)(133). In paper IV, the date of diagnosis refers to the AML diagnosis secondary to the previous MDS diagnosis. Patients were censored at the end of follow-up in the study or loss to follow-up.

4 RESULTS

4.1.1 PAPER I:

ESA treatment (median duration of 27.5 months, range 0–77 months) was administered to 773 patients (45.6%). Outcomes were assessed in 897 patients (484 ESA treated and 413 untreated). ESA treatment was associated with a non-significant survival benefit (HR 0.82, 95% CI 0.65–1.04, $P = 0.09$); this benefit was larger among patients without prior transfusions ($P = 0.07$). Among 539 patients for whom response to ESA treatment could be defined, median time to first post-ESA treatment transfusion was 6.1 months (IQR 4.3–15.9 months) in those transfused before ESA treatment compared to 23.3 months (IQR 7.0–47.8 months) in patients without prior transfusions (HR 2.4, 95% CI 1.7–3.3, $P < 0.0001$) Responding patients had a longer time to first post-ESA transfusion compared to non-responders (Fig.15a). Pretransfused patients had a shorter time to post ESA-transfusions, both responders and non-responders. (Fig 15b). Responding patients had a better prognosis in terms of a lower risk of death (HR 0.65, 95% CI 0.45–0.893, $P = 0.018$). There was no significant effect on the risk of progression to acute myeloid leukemia (HR 0.71, 95% CI 0.39–1.29, $P = 0.27$).

Figure 15(a) Comparison of time to first post ESA treatment transfusion between ESA treated patients who did or did not respond to ESA (a) Time to first ESA treatment was significantly improved amongst patients responding to ESA treatment compared to those not responding (HR 0.43, 95% CI: 0.32-0.57, P <0,0001)
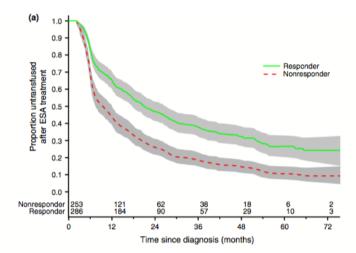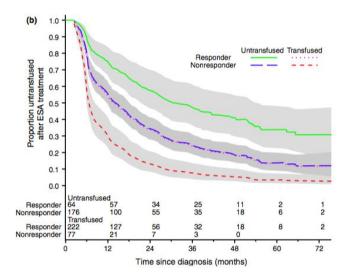
Figure 15(b) The response effect on time to first post-ESA transfusion was evident when stratified by pre-ESA transfusion experience (solid line vs. long-dashed line for untransfused patients and short-dashed line vs. dotted line for transfused patients)

4.1.2 Paper II:

This paper describes the secondary AML population (therapy-related, MDS-AML and MPN-AML) in comparison to de novo AML. S-AML was significantly different from de novo AML with regards to age (higher age) gender (more men in the AHD-AML group, more women in the t-AML group) and cytogenetic risk (higher risk).

Figure 16 Overall survival (OS). (A) OS irrespective of treatment or age (B) OS in patients given intensive treatment irrespective of age (C) OS in patients <65 years with intensive treatment (D-F) OS according to cytogenetics irrespective of treatment (2)

In total, 3,363 patients diagnosed with AML between 1997 and 2006 were included. Of these, 2,474 (73.6%) were classified as de novo AML, 630 (18.7%) as AHD-AML, and 259 (7.7%) as t-AML, resulting in 889 (26.4%) cases of secondary AML. (fig.16A, Overall Survival depending on de novo or secondary AML).

Intensive induction chemotherapy (IC) with the intent to obtain a complete remission (CR) was given to 1,967 (58%) patients. IC was less commonly given to t-AML and AHD-AML patients compared to de novo AML (P = 0.018 and P < 0.001, respectively); this was found in younger as well as in older patients (Fig 16B). In patients < 65 years, IC was considerably more common and was given in 94% of the patients with de novo AML, in 69% of AHD-AML, and in 82% of t-AML patients (fig 16 c). Fig 16D-F shows survival according to cytogenetic risk.

In patients who received IC, CR rates were significantly lower in both types of secondary AML with 39% CR in AHD-AML and 54% in t-AML compared to 72% in de novo AML (P < 0.001 for both comparisons). Decreased CR rates in secondary AML were seen independently of cytogenetic risk group. Interestingly, in patients with secondary AML who received IC, CR rates were similar in younger and older. This in contrast to de novo AML where CR rates were substantially higher in younger patients (P < 0.001).

Seventeen per cent (434 patients) with de novo AML, 12% (54 patients) with AHD-AML and 14% (40 patients) with t-AML underwent allogeneic HSCT in first remission. No patients with MDS-AML or MPN-AML treated with IC only survived more than 4 years, while 4-year survival in allogeneic HSCT treated was 48% and 44%, respectively. Patients with intermediate or high risk < 65 years with CR more than 3 months had a greater advantage with transplantation compared to de novo AML. Patients that received allogeneic HSCT had significantly better survival compared to those receiving IC (7-year survival 43% compared to 8% p <0,001). A multivariate analysis showed that AHD-AML and t-AML were independent risk factors for inferior survival in the younger age groups, but not significant in patients in the age above 80 years. Patients with s-AML had a worse prognosis compared to de novo AML with intensive treatment, but palliative treatment had an even worse prognosis.

4.1.3 PAPER III

Study population

The study population included all 5873 adult patients diagnosed with AML during the 17-year study period from 1997-2013. Of 3337 intensively treated patients (non-APL), 707 (21%) underwent HSCT at any stage of the disease. Of patients with de novo AML 576 (22%) underwent a HSCT, 74 (17%) of AHD-AML and 57 (20%) of t-AML, respectively. Of transplanted s-AML patients, 100 (76%) were transplanted in first remission (CR1); 55 (74%) in AHD-AML and 45 (79%) in t-AML (Fig.17). The rest of the HSCT patients were transplanted in refractory or relapsed status or in later CRs. The proportion of patients that entered CR1 and that underwent HSCT in CR1 was similar between *de novo* AML, AHD-AML and t-AML with 23%, 28% and 27%, respectively.

Figure 17 Proportion of patients reaching CR and proportion of patients undergoing HSCT within the groups CR or no CR. (3)

There were more patients with higher risk cytogenetics in the secondary AML groups where the adverse risk group constituted 36% of the transplanted *de novo* AML patients, 50% of AHD-AML and 50% of t-AML patients, respectively.

For donor type, conditioning, stem cell source, female donor to male recipient, EBMT score or time from CR1 to HSCT, there was no significant difference between AHD-AML and t-AML.

Survival in transplanted and non-transplanted secondary AML patients

For AHD-AML patients not HSCT-transplanted, 5-year survival was 0% compared to 50% for MPN-AML patients that did undergo HSCT; respective 5-year survival for MDS-AML patients were 3% compared to 39%. Corresponding 5-year survival were 8% vs. 48% for t-AML patients and 24% vs. 57%, for *de novo* AML. For patients reaching a CR1, 5-year survival was, 39%, 45%, 54% and 61% for MPN-AML, MDS-AML, t-AML and de novo AML, respectively in patients undergoing HSCT compared to 0%, 7%, 16%, and 34% for those that did not undergo HSCT.

In order to allow for a more accurate comparison between transplanted and non-transplanted patients and to compare the impact of transplantation between s-AML and de novo AML, we selected patients ≤ 65 years that had been in CR1 for at least 3 months, excluding patients with favorable karyotype (Fig. 18).

Figure 18 Overall survival after CR1 in patients treated either with HCT (line) or conventional post remission therapy (dotted line) in de novo AML (blue) and s-AML patients (red). Patients with CR1 shorter than 90 days, age above 65 years or patients with a favorable karyotype are excluded from the analysis

In this analysis, secondary AML patients had a similar benefit from HSCT compared to patients with de novo AML and the projected 5-year survival was 63% in *de novo* AML with HSCT and 44% with conventional post remission therapy (CPRT) compared to 47% and 20%, respectively, in secondary AML. Additional independent factors impacting on survival in s-AML were cytogenetic risk, but not the type of secondary AML or age and for *de novo* AML.

Prognostic factors for outcome after HSCT in secondary AML
Survival was favorably associated with peripheral stem cells rather than bone marrow as graft source, a mild cGvHD versus no cGvHD and aGvHD of grade 0-1 rather than above 1. There was no difference in outcome with regard to gender or age of the patients, the type of secondary AML, cytogenetic risk, donor age, early or late period (1997-2004 versus 2005-2013), HCT-CI score, a myeloablative or a non-myeloablative conditioning, CMV reactivation or with female donor and male recipient.
In a multivariable analysis, presence of any cGvHD compared to no cGvHD and a GvHD grade 1 or lower remained significantly associated to better survival.

4.1.4 PAPER IV:

In the AML-Registry 2009-14, 335 of 2181 patients were registered with MDS as antecedent hematological disease. By merging all patients from the AML-Registry between 2009-14 (2181 patients), together with all patients from the MDS-Registry (2102 patients) during the same period, we found 169 patients registered in the AML-registry only and 166 patients with information from both registries.

After completion of missing data, 257 patients had sufficient information from both registries for further examination. Thirty-eight patients were classified as therapy-related MDS due to former treatment with either chemotherapy or irradiation, 2 patients had a wrong diagnosis (1 MPN and 1 hyperesosinophilic syndrome) and 38 patients did not have sufficient information from the time of MDS diagnosis.

At MDS-diagnosis, 13.5% were defined as low risk, 72.2% were high risk and 14.5% had MDS/MPN disease. Cytogenetics were missing in 34.6% at MDS diagnosis, the rest had the following R-IPSS score: 14.4% Low risk (VRL/LR), 18.2% Intermediate and 32.7% high risk (HR/VHR).

The coverage of MDS cases as compared to the Cancer registry was 95% for the period 2009-14 (17). The coverage for the AML- Registry were 92.4 per cent for the same period (82).

The Male/Female ratio was 62/38%. Median age at MDS diagnosis was 72 (range 24-91) and median age at AML diagnosis was 74 years (range 24-91).

According to the cytogenetic risk according to Grimwade (102, 134) and Lazarevic (102) found at AML diagnosis, there were 19.1% with high risk, 20.6% with intermediate risk, no patients with cytogenetic low risk, and 60.3% of the patients did not have any cytogenetics taken at time of AML diagnosis.

Of those with information available, 51.5% were transfusion dependent with regard to erythrocytes, while 5.5% received platelet transfusions at time of MDS diagnosis.

WHO performance status(20) were recorded at time of AML diagnosis. A majority of the patients; 66.8%, had performance status 0-1, 14.8% had performance status 2 and 14.9% performance status 3-4 at AML diagnosis.

Eighty-six patients (33.5%) were diagnosed with AML with dysplasia-related changes, and a large proportion of the patients ended up with

more general diagnoses such as Acute myeloid leukemia, not otherwise specified (n=66 pts, 25.7%) or Acute undifferentiated leukemia (n=9).

Treatment

The median time from MDS diagnosis to AML diagnosis was 10.8 months for all patients. The median time from MDS to AML for patients treated with HMA was 13.3 months, intensive chemotherapy (IC) 11.5 months and supportive care 11.2 months, for ESA 7.2 months and other 8.6 month. There were no significant differences between these groups. Regardless of the treatment choice at MDS diagnosis, 12.0% was offered HMA at AML diagnosis with a median observed survival at 7.6 months, 40.5% IC with a median observed survival at 11.6 months, 46.7 % palliative care (PC) with a median observed survival at 2.65 months and 2 patients had no decision made. Complete remission after treatment for AML was achieved in 19.8 % of the cases.

One patient received an allogeneic HSCT after MDS diagnosis and developed AML after HSCT. Twenty-nine patients were transplanted after AML –diagnosis, in total 11.7% of the population.

Survival

The median survival time for the whole population with MDS-AML is 4.93 months (CI 3.77- 6.6) (fig.19a). Figure 19b shows survival from the time of AML diagnosis in relation to R-IPSS at MDS diagnosis and by age at AML-diagnosis (fig.19 c). Treatment category at MDS diagnosis (fig. 19d) show no significant differences. Treatment category at AML diagnosis (fig. 19e) shows that patients receiving either IC or HMA have a significantly better survival compared to patients with palliative care only, but there is no significant difference between HMA and IC. WHO performance status at AML diagnosis (fig. 19f): The median survival is significantly better with WHO PS 1 compared to median survival in WHO PS 2-4, although the median survival is less than a year in all groups. Remission status after AML treatment (fig.19g): If a patient achieves a complete remission after induction therapy for AML, there is a significantly better survival compared to patients that do not achieve CR and patients without any registration. Transplantation status (fig. 19h): Median survival for transplanted patients were 17.65 (CI 12.67 - NA) months compared to 6.27 (CI 4.93 - 8.43) months for patients not transplanted.

Figure 19 a-d a: Overall observed survival in patients with MDS-AML 2009-14, b;
Survival by R-IPSS category at MDS diagnosis, c: Survival by age, d: Survival by
treatment category at MDS diagnosis

Figure 19e-h: Survival by treatment category at AML diagnosis, f: Survival by WHO performance status, g: Survival by remission status: CR No, CR no info, CR yes, h: Survival by transplantation status Allogeneic HSCT No, No info or Yes

There was no significant difference in survival in regards to treatment received at time of MDS diagnosis from a proportional hazard regression model. A proportional hazard regression model showed no significant differences in this material between HMA and induction therapy, but both have a significantly better survival than palliative care.

5 DISCUSSION

5.1.1 PAPER I

The aim of this study was to analyze treatment patterns of ESAs, as well as their effects on long- term outcome in a large prospectively observational cohort of patients with lower-risk MDS. The higher median age of patients in the EUMDS registry (74.4 years) compared to other registries in which a majority of patients came from university hospitals (e.g. Düsseldorf,72 years; Pavia,65.3 years (135, 136)) may be due to the wider recruitment. It may also be a reflection of an ageing population given the more recent establishment of the EUMDS registry. Our results revealed marked variations in ESA use across Europe. Most, but not all countries follow guidelines as recently proposed by the European LeukemiaNet (13). However, in some countries, transfusion need is a prerequisite for treatment initiation, an approach that is not supported by the findings of this analysis. Furthermore, there were marked variations in pre-ESA treatment Hb levels between the countries, with Sweden and the Netherlands starting ESAs at higher Hb levels than for example Portugal, Poland and Romania, where patients were usually transfusion-dependent before the start of treatment. Despite treatment recommendation in most care programs (13, 137, 138), this study shows that less than half of the MDS population receives ESAs at any time-point. This seems to be due both to national financial and legal restrictions and to treatment traditions that do not follow European guidelines.

It is important to note that a significant proportion of transfusion-dependent patients, 28%, achieved both transfusion independency and a clear increase in Hb levels in response to treatment. The median treatment duration of 27.5 months indicates response duration of around 2 years, in line with previous reports (62).

Serum EPO is used as a predictor of response to ESA treatment(59). Amongst the patients with available serum EPO measurements in this study, only a few patients had serum EPO levels above 200 U/ L, which is in accordance with previous findings.

5.1.2 PAPER II

This study gives the first detailed description of AHD-AML and t- AML in a large population-based AML cohort.

The proportion of patients with secondary AML found in our study (26.4%) was higher compared to most previous studies (139, 140). CR rates were significantly lower in both types of secondary AML regardless of age, performance status, and cytogenetic risk. CR rates in our study differ somewhat to what was reported in a larger t-AML German study(141), where CR rates were 67% in de novo AML and 63% in t-AML compared to 72 and 54%, respectively, in our study. However, the German study was not population-based and the cohort was significantly younger and included fewer patients with high-risk cytogenetics.

Survival was poorer for both AHD-AML and t-AML compared to de novo AML regardless of cytogenetic risk group. This difference was more pronounced in the younger age group. Although secondary AML is less common among younger AML patients, the fact that secondary AML has such a strong and independent impact on survival in the younger age groups is of major clinical importance. In contrast, in elderly patients, information about secondary AML does not contribute to the prognostic assessment.

The data in this study are based on information retrieved from the routine diagnostic procedures performed at the time of the diagnosis (between1997 and 2006) and additional material for further molecular testing was not possible to obtain. Thus, good covering of mutational data on NPM1, FLT3, and CEBPA as well as more recently discovered recurrent mutations in AML is lacking in this cohort but important for future studies.

The poor survival in secondary AML is in part due to the difficulty to obtain a CR. However, remission duration is short and the survival analysis from the time of CR shows that the poor outcome remains after CR regardless of cytogenetic risk. Primary treatment resistance seems to be the major reason for the poor outcome of secondary AML. As secondary AML is associated to known poor prognosis (86, 141, 142), multivariable models are essential. A recent population-based Danish study on secondary AML failed to show that secondary AML was an independent risk factor (86). In contrast to the Danish study, in our study

both t-AML and AHD- AML seems to be factors that independently and strongly predict a poor outcome in AML.

In addition to treatment outcome, several baseline characteristics differed between the of secondary AML and de novo AML. A significant female predominance was found in t-AML, which is likely due to the fact that breast cancer, the most common female cancer preceding t-AML, has good long-term survival

The median latency period between MDS and AML was 1 year, indicating that most MDS patients who progress to AML do so within a short time frame. Median latency times between MPN and AML were between 7 and 8 years, whereas the median latency between the malignancy and t-AML was slightly longer, 5.8 years (142-144). Median latency between a non-malignant disease and t-AML is seldom reported but was shown to be 14.3 years in our cohort. Almost half of t-AML patients showed high- risk cytogenetics, which is similar to the literature (86, 141, 142). However, previous data on cytogenetics in AHD-AML are very limited and our study shows a considerably higher proportion of high-risk cytogenetics compared with the previously largest population-based study (86).

5.1.3 PAPER III

In this study, we aimed to define the role of allogeneic hematopoietic stem cell transplantation (HSCT) in patients with s-AML in a large population-based cohort, representing a real-life setting. Including all Swedish patients diagnosed during a 17-year period, we were able to show that HSCT constitutes the only realistic curable treatment alternative in AML patients with an antecedent hematological disorder. This conclusion is based on a 5-year survival rate of 0% and 3% respectively for all MPN-AML and MDS-AML that did not undergo HSCT compared to 50% and 39%, respectively, for those who did. For t-AML patients, the chance to survive without HSCT is slightly higher (8%) but still, the chance of cure is low compared to t-AML undergoing HSCT (48%) and *de novo* AML not undergoing HSCT (24%). A matched analysis similar to what previously have been used to estimate the role of HSCT in retrospective cohorts (145) were done in order to better define comparable groups. These analyses showed that the improvement in outcome after HSCT compared to conventional post-remission therapy remains, both in multivariate analysis and matching models. The survival benefit of HSCT as post-remission treatment in CR1 was significant in non-favorable risk s-AML patients who had been in a first CR for at least 90 days. The improvement was similar compared to patients with *de novo* AML, but at survival levels of approx. 20% points lower in both transplanted and non-transplanted patients. In a multivariate analysis, HSCT was significant both in s-AML and non-favorable *de novo* AML with a HR of 0.45 and 0.61, respectively. In the matched analysis of s-AML, both OS and DFS were significantly better in the transplanted group with a five-year OS difference of 48% vs. 20%.

Somewhat surprisingly, the only significant factors that predicted better survival after HSCT in s-AML were the presence of cGvHD and absence of severe aGvHD. No patient- or AML-related factors such as cytogenetics and age were significant in uni- or multivariable analyses. This points to transplantation-related factors as key elements in survival of transplanted s-AML patients.

5.1.4 PAPER IV

In this registry study, we identified 257 patients with AML secondary to MDS. We have analyzed characteristics at MDS diagnosis and AML diagnosis and tried to evaluate how these factors impact on outcome. The classification of AML in this population is in many cases uncertain, only reflecting that the patients may be in the end-stage of their disease, the diagnosis of acute leukemia is only implying a progression of the disease in a patient that will be treated palliative.

The majority of this population have died. It is interesting, and also in accordance with what we see as clinicians, that even patients with WHO performance status 0-1 have a median survival of only 8.1 months in this study. In our experience, it is relatively often that an AML patient can be relatively healthy up to a short time before death.

A relatively high proportion of these patients receive induction treatment (40.5 %) in contrast to only 12.1% for hypomethylating agents (HMA). One explanation is that 29 percent (69 pts) of the patients were treated with HMA already at time of MDS diagnosis and may have lost the effect on HMA. Ten patients continued HMA after the AML diagnosis was established. It is not unusual to see that the MDS disease often progress quickly after HMA failure (146), which is in accordance to our findings.

In a multivariate analysis, we found that performance status 0-1 and allogeneic HSCT was significantly associated with better survival. As we have shown in our previous study (3), the only way of long time survival is through an allogeneic HSCT.

The major strengths of the Swedish blood cancer registries are the population-based setting and the relatively high coverage, which mean we can draw some important conclusions from this material in general. It would have strengthened this material if we had been able to do Next Generation Sequencing (NGS) on material from the patients from both MDS and AML diagnosis which could have provided us with valuable information in the risk evaluation of the patients (11, 88, 147). Biobanking of AML and MDS patients have started at a later point, and we have therefore not included results from this such as Next Generation Sequencing (NGS) in to this project.

There are other weaknesses to this study. In this dismal population, it may not come as a surprise that a large part of the patients has not done the basic diagnostics, such as cytogenetics, neither at the time of MDS

diagnosis or (even fewer) at time of AML diagnosis. Many patients have been diagnosed with AML, not otherwise specified, also an indication that the importance of thorough diagnostic is low. The reason may be that the patients are old, this condition is secondary to another serious condition, and the consequences of a thorough diagnostic procedure may not be large.

6 CONCLUSION

I: An important conclusion of this large observational registry study is that the response rate to ESAs as well as the capacity of these agents to significantly delay the onset of a regular transfusion need is most pronounced in transfusion-naïve patients, and patients with a transfusion requirement of less than 2 units per month, thus corroborating the findings from a small retrospective study by the French GFM group (60). Hence, we propose that ESAs should be recommended as first-line treatment in low-risk MDS patients with symptomatic anemia before the onset of a regular transfusion need.

II-IV: We found that secondary AML has a considerable impact in younger patients with a worse survival compared to *de novo* AML in contrast to a lack of independent prognostic impact in elderly patients. Secondary AML is a broad term where t-AML and the different types of AML with antecedent hematological disorders are addressed in different ways. The two major subtypes of secondary AML display important differences compared with de novo AML when it comes to age, gender, and cytogenetics in a population-based cohort, and importantly, each of t-AML and AHD-AML confer a poor prognosis independently of other risk factors. However, the prognostic impact of secondary AML is highly significant for younger patients, whereas it does not add prognostic information in elderly AML patients. Nevertheless, despite poor outcome in AHD-AML and t-AML, intensive treatment remains the chance to cure and long-term survival. The results in paper III and IV confirm this clearly, the only option for cure with s-AML is by allogeneic HSCT.

7 FUTURE PERSPECTIVES

Low risk MDS is a malignant disease, often chronic in its character. The aim of the treatment is to prolong survival without too much morbidity. The EUMDS Registry show us that too few anemic patients with lower-risk MDS actually receive treatment with ESA. This study implies that we should start ESA as soon as that the patients go below Hb 100 g/L. We know that long-time treatment with transfusions can be very debilitating for the patients, and that postponing transfusion start for these patients can postpone the iron overload effects and, thus, hopefully, also improve quality of life and survival. We are currently using the EUMDS registry to investigate HRQoL in the low risk patients with regards to ESA use. We will also repeat the ESA analysis in an extended cohort of patients from 2008-17, where the preliminary results indicate long lasting effect of ESA.

Our three studies of Secondary AML have showed that this is a condition with dismal results, often considered being the end-stage of a former malignant disease such as MDS or MPN. We have also showed that these patients can respond to intensive chemotherapy and allogeneic HSCT. Hopefully, our three studies are small contributions in this field, indicating the need for intensive chemotherapy and allogeneic HSCT in the patients that can tolerate this treatment.

Currently, we are working on establishing a link between the Acute Leukemia -biobank and the MDS-biobank and the registries, thus further enhancing research. As the diagnostics with NGS is improving, this will eventually also be included in the registries. It is unique to have these population-based registries with information from both university hospitals and smaller hospitals, securing a true population-based basis for research and reports (38, 102, 121, 148).

New methods such as NGS can be helpful in determining the prognosis of secondary AML (149), and determine whether the patients have s-AML or T-AML. Certain mutations such as TP 53 have especially dismal outcome even after an allogeneic HSCT(150). In the future, our aim is to match our registries with biobanks, making these even more valuable in this research field.

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