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PREDICTORS OF PROGNOSIS IN ACUTE MYELOID LEUKEMIA

A CLINICAL AND EPIDEMIOLOGICAL STUDY

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To my family and
in memory of my father

ABSTRACT

AML is a malignant disorder characterized by clonal expansion of immature myeloid hematopoietic stem cells, myeloblasts, in bone marrow, blood and/or other tissue. Despite advances in treatment the majority of patients eventually die from this aggressive disease.

We conducted a study including 9,729 AML patients diagnosed in Sweden 1973-2005 to define survival patterns over time. One-year relative survival ratios (RSRs) improved in all age groups. Improvement in 5-year RSRs was restricted to patients <80 years. The 5-year RSRs in the last calendar period were 0.65, 0.58, 0.36, 0.15, 0.05, and 0.01 for the age groups 0-18, 19-40, 41-60, 61-70, 71-80, and 80+ years, respectively. Intensification of induction and consolidation treatment, an increasing rate of allografted patients, a continuous improvement in supportive care measures, and a more precise risk stratification of patients are probably the most important factors contributing to the improvement. We also assessed the impact of socioeconomic status (SES) on survival in 9,165 patients with AML. Overall, higher white-collar workers had lower mortality compared to other SES groups (p=0.005). In AML patients, a consistently higher overall mortality was observed in blue-collar workers compared to higher white-collar workers in the last three calendar periods (hazard ratio [HR]=1.26; 95% confidence interval (CI) 1.05-1.51; HR=1.23; 1.05-1.45; HR=1.28; 1.04-1.57, respectively). Differences in comorbidities, management, and life-style factors are likely to explain these findings.

We determined expression patterns of CD33 and CD15 in leukemic blasts from 129 patients with AML using flow cytometry (FC) and a standard panel of triple antibody combinations. Five patterns, corresponding to the consecutive stages of myeloid differentiation, were identified [I:CD33-/CD15- (n=18), II: CD33+/CD15- (n=43), III: CD33++/CD15 heterogeneous (n=10), IV: CD33+/CD15+ (n=50), V: CD33-/CD15+ (n=8)]. Patients with pattern II had the highest relapse rate and shortest median overall survival (OS; 8 months), but they were also the oldest (median age 72 years) and had a high frequency of unfavorable cytogenetics. Pattern V patients had a short OS (median 14 months) even though they were the youngest (median age 50 years) and had high remission rate. Age (p=0.004), cytogenetics (p=0.011), CD15 expression (p=0.031), and the immunophenotypic classification (p=0.024) were all independent significant predictors for OS.

The presence of minimal residual disease (MRD) in AML patients in complete remission (CR) is a predictor of poor prognosis. We determined MRD status by FC in 45 AML patients ≤60 years old in first CR. MRD was determined after induction (MRD1; n=43) and/or at the end of post-remission chemotherapy (MRD2; n=31). Patients with detectable MRD at either time-point who underwent allogeneic or autologous stem cell transplantation (SCT) had significantly better 5-year relapse-free survival than patients not transplanted (MRD1: 83%, 54%, and 8%, respectively, p<0.0001; MRD2: 80%, 53%, and 0%, respectively, p=0.003).

We identified 11,039 patients with myeloproliferative neoplasms (MPNs) from the Swedish Cancer Registry and major hematology units. Through record-linkage with the Cancer Registry patients who developed AML (n=271) and myelodysplastic syndromes (MDS; n=21) were identified. For each patient with a subsequent AML/MDS diagnosis (cases) two matched patients without AML/MDS (controls) were identified. After exclusions the final study population consisted of 162 cases (153 AML, 9 MDS) and 242 controls. 25% of patients with AML/MDS development were never exposed to cytotoxic agents. Compared to no hydroxyurea (HU) exposure the odds ratios (with 95% CIs) for 1-499 g, 500-999 g, >1000 g of HU were 1.22 (0.61-2.45), 1.41 (0.58-3.40), and 1.35 (0.55-3.32), respectively for AML/MDS development (not significant). In contrast, MPN patients who received radioactive phosphorus (P^{32}) >1000 MBq and alkylating agents >1 g had a 4.60-fold (2.15-9.85; p<0.0001) and 3.39-fold (1.08-10.59; p=0.036) increased risk of AML/MDS, respectively. Lower exposures to P^{32} and alkylators were not associated with a significantly increased risk of AML/MDS.

LIST OF PUBLICATIONS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I. **Rangert Derolf Å**, Kristinsson SY, Andersson T M-L, Landgren O, Dickman PW, Björkholm M. Improved patient survival for acute myeloid leukemia: a population-based study of 9729 patients diagnosed in Sweden between 1973 and 2005. Blood 2009;113(16):3666-72.

II. Kristinsson SY, **Rangert Derolf Å**, Edgren G, Dickman PW, Björkholm M. Socioeconomic differences in patient survival are increasing for acute myeloid leukemia and multiple myeloma in Sweden. J Clin Oncol 2009;27(12):2073-80.

III. **Rangert Derolf Å**, Björklund E, Mazur J, Björkholm M, Porwit A. Expression patterns of CD33 and CD15 predict outcome in patients with acute myeloid leukemia. Leuk Lymphoma 2008; 49(7):1279-91.

IV. Laane E, **Rangert Derolf Å**, Björklund E, Mazur J, Everaus H, Söderhäll S, Björkholm M, Porwit-MacDonald A. The effect of allogeneic stem cell transplantation on outcome in younger acute myeloid leukemia patients with minimal residual disease detected by flow cytometry at the end of post-remission chemotherapy. Haematologica 2006; 91(6):833-36.

V. Björkholm M, **Rangert Derolf Å**, Hultcrantz M, Kristinsson SY, Ekstrand C, Andreasson B, Birgegård G, Linder O, Malm C, Markevärn B, Nilsson L, Samuelsson J, Granath F, Landgren O. Treatment related risk factors for transformation to acute myeloid leukemia and myelodysplastic syndromes in chronic myeloproliferative neoplasms – a population-based nested case-control study. Manuscript to be submitted.

CONTENTS

LIST OF ABBREVIATIONS

Allo-SCT	Allogeneic stem cell transplantation
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
ATRA	All-*trans* retinoic acid
Auto-SCT	Autologous stem cell transplantation
CD	Cluster of differentiation
CEBPA	CCAT/enhancer binding protein alpha
CI	Confidence interval
CR	Complete remission
DA	Daunorubicin and cytosine arabinoside
EBMT	European group for blood and marrow transplantation
ET	Essential thrombocythemia
FAB	French-American-British
FC	Flow cytometry
FCM	Flow cytometer
FLT3	FMS-like tyrosine kinase 3
G-CSF	Granulocyte colony stimulating factor
GM-CSF	Granulocyte macrophage colony stimulating factor
GVHD	Graft-versus-Host Disease
HR	Hazard ratio
HU	Hydroxyurea
ICD	International Classification of Diseases
ICE	Idarubicin, cytosine arabinoside, and etoposide
ITD	Internal tandem duplication
JAK2	Janus kinase 2
LGMS	Leukemia Group of Middle Sweden
MDS	Myelodysplastic syndrome
MM	Multiple myeloma
MoAb	Monoclonal antibodies
MPN	Myeloproliferative neoplasm
MRD	Minimal residual disease
NPM1	Nucleophosmin 1
OR	Odds ratio
OS	Overall survival
P^{32}	Radioactive phosphorus
PCR	Polymerase chain reaction
PMF	Primary myelofibrosis
PV	Polycythemia vera
RFS	Relapse free survival
RSR	Relative survival ratio
SCT	Stem cell transplantation
SES	Socioeconomic status
SIR	Standardized incidence rate
SNOMED	Systemized Nomenclature of Medicine-Clinical Terms
TRM	Treatment related mortality
WHO	World Health Organization

1 INTRODUCTION

1.1 HISTORY

In 1845 Rudolf Virchow (Figure 1) described a disease characterized by an enlarged spleen and excess of white blood cells. He named it ”leukemia” which is derived from Greek meaning ”white blood”.[1-3] At this time there had been other descriptions of patients with similar findings and it was generally considered that the white color of the blood was caused by the vessels being invaded by pus. John Hughes Bennett argued that this was the case and wrote a paper entitled “Two cases of disease and enlargement of the spleen in which death takes place from the presence of purulent matter in the blood”.[1] Alfred Donné, on the other hand, described several cases with a great excess of white blood cells in his book of 1844 and wrote that “Blood of such patients contains so many white blood cells that at first glance I thought they contained purulent matter. In fact, I believe that the excess of white blood cells is due to an arrest of maturation of blood.”[1] However, Donné’s work was not acknowledged. Virchow continued his investigations and broke with the conventional wisdom and eventually recognized leukemia as an autonomous disease.

Nikolaus Friedrich was first to describe a case of acute leukemia in 1857. The clinical course differed from the two forms of indolent leukemia, splenic and lymphatic, described by Virchow.[2] At this time the function of the bone marrow was considered to be mechanical, to protect the blood vessels. Therefore it was not examined routinely at autopsy. In 1870 Ernst Neumann discovered changes in the bone marrow in a patient with leukemia and thus introduced the term “myelogenous” leukemia. Another important contribution to the understanding of the leukemias was made by Friedrich Mosler in 1876, when he became the first physician to collect biopsy material from the bone marrow by sternal puncture. When Virchow developed his theories about leukemia, he believed that the white (or rather colorless) blood cells seen in leukemia were immature red cells. Paul Erlich was able to characterize the white blood cells further with his staining techniques and classified them into the lymphatic and myeloid systems in 1892.[2]

Figure 1. Rudolf Virchow (1821-1902)

Despite early work of describing and classifying acute leukemias, it was not until 1976 that a uniform classification system was generally accepted. In 1976 the French-American-British (FAB) co-operative group published “Proposals for the classification of the acute leukaemias”[4] in which they classified acute leukemias based on morphological characteristics of the leukemic blast in association with cytochemical reactivity patterns. Six main types of acute myeloid leukemia (AML) were defined according to the direction of differentiation along

one or more cell lineages and the degree of maturation. Modifications were made in 1985.[5] The FAB classification was in use until 2001 when the World Health Organization (WHO) introduced a new classification in order to highlight the biologic and prognostic relevance of the cytogenetic abnormalities.[6] It categorizes AML based on genetic findings, relation to cytotoxic therapy, and presence of myelodysplasia-related changes.[7] Cases that do not fulfill criteria for inclusion in one of these groups are assigned to the group of "acute myeloid leukemia, not otherwise specified" and classified according to the major lineages involved and the degree of maturation. The WHO classification was revised in 2008[8-9] and is described in Table 1.

Tabell 1. Classification of acute myeloid leukemia according to the World Health Organization[8]

Acute myeloid leukemia with recurrent genetic abnormalities
t(8;21)(q22;q22); *RUNX1-RUNX1T1**
inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11**
t(15;17)(q22;q12); *PML-RARA** (acute promyelocytic leukemia)
t(9;11)(p22;q23); *MLLT3-MLL*
t(6;9)(p23;q34); *DEK-NUP214*
inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*
t(1;22)(p13;q13)*; RBM15-MKL1* (acute megakaryoblastic leukemia)
Provisional entity: acute myeloid leukemia with mutated *NPM1*
Provisional entity: acute myeloid leukemia with mutated *CEBPA*
Acute myeloid leukemia with myelodysplasia-related changes
Therapy-related myeloid neoplasms
Myeloid sarcoma
Acute myeloid leukemia, not otherwise specified**
Acute myeloid leukemia with minimal differentiation
Acute myeloid leukemia without maturation
Acute myeloid leukemia with maturation
Acute myelomonocytic leukemia
Acute monoblastic and monocytic leukemia
Acute erythroid leukemia
Acute megakaryoblastic leukemia
Acute basophilic leukemia
Acute panmyelosis with myelofibrosis
Acute leukemia of ambiguous lineage
Acute undifferentiated leukemia
Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*
Mixed phenotype acute leukemia with t(v;11q23); *MLL*-rearranged
Mixed phenotype acute leukemia, B/myeloid
Mixed phenotype acute leukemia, T/myeloid
Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma

*The diagnosis of AML is established without regard to blast cell count

**The category of AML not otherwise specified encompasses those cases that do not fulfill criteria for inclusion in one of the above described groups

1.2 ACUTE MYELOID LEUKEMIA

1.2.1 Definition

AML is a malignant disorder characterized by clonal expansion of immature myeloid hematopoietic stem cells, myeloblasts, in bone marrow, blood and/or other tissue. The diagnosis is typically established when at least 20% of nucleated cells in a bone marrow sample are myeloblasts (Figure 2). In AML with some specific genetic abnormalities the diagnosis is established irrespective of the blast cell count. Immunophenotypic analysis by flow cytometry (FC) has a central role in distinguishing between minimally differentiated AML and acute lymphoblastic leukemia (ALL). Mixed phenotype acute leukemia can contain separate blast populations of different lineages or one population with characteristics of different lineages. FC analysis is especially important when establishing the diagnosis in this leukemia subset.[8]

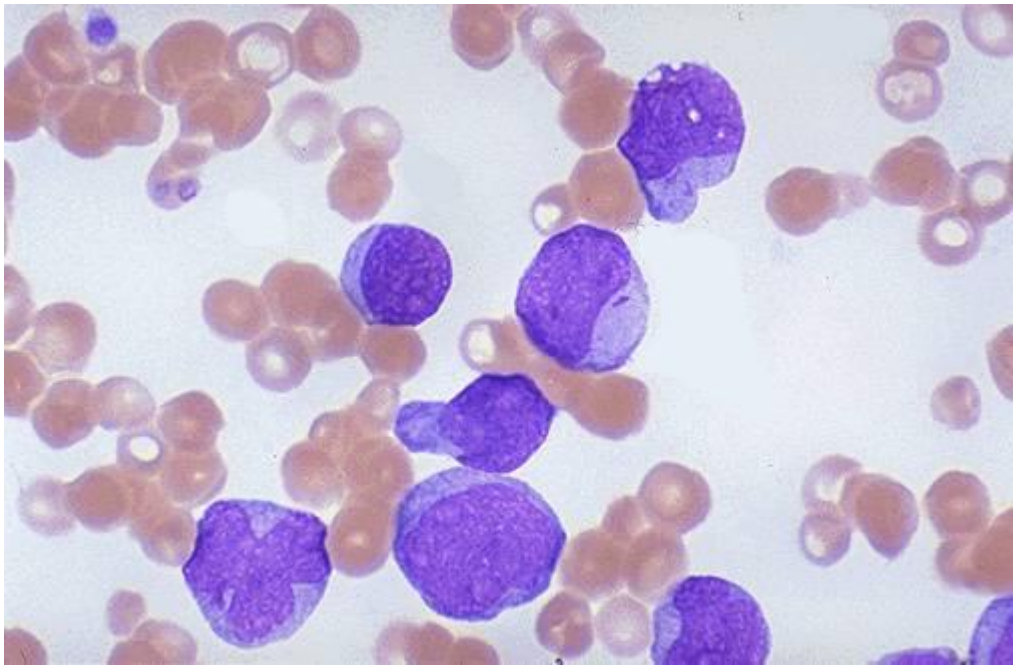

Figure 2. A bone marrow smear from a patient with acute myeloid leukemia showing a predominance of myeloblasts and presence of Auer rods

1.2.2 Epidemiology

Approximately 400 patients are diagnosed with AML in Sweden every year. This corresponds to an annual incidence of 3-4/100,000 inhabitants, which is similar to what is seen in other Western countries.[10-12] AML is diagnosed in all ages, but the incidence increases with increasing age and the median age at diagnoses is just below 70 years. Males are slightly more affected than females.[10-12]

1.2.3 Etiology and pathogenesis

The etiology of AML is unknown in most patients. However, some have a preceding diagnosis of another hematologic disease such as a myelodysplastic syndrome (MDS) or a myeloproliferative neoplasm (MPN). Another well established risk factor is

exposure to chemotherapeutic agents, especially alkylating agents and topoisomerase II inhibitors.[13] Exposure to high doses of ionizing irradiation and chronic benzene exposure are other accepted risk factors.[8, 14] Cigarette smoking has been reported to increase the risk for AML by 20-100%.[15-17]

Development of AML in a patient is considered to be a process of multiple genetic changes in a hematopoietic stem cell. Mutations can either affect cell proliferation or cell survival (class I mutations) or they can affect differentiation and maturation of the hematopoietic cells (class II mutations). The presence of one of these mutation classes is not enough to induce leukemia, but when the two types are combined the disease can develop.[13, 18-19]

1.2.4 Clinical signs and symptoms

AML is an aggressive disease where the symptoms typically appear rapidly. The expansion of myeloid blasts in the bone marrow leads to impairment of normal hematopoiesis. Thus, the patients often present with symptoms of anemia, bleeding, and infections.[14] Skeletal pain is experienced by some patients and is directly related to the expansion of myeloblasts in the bone marrow. In addition, leukemic infiltration may produce organ specific symptoms. Hyperleukocytosis can occur and is associated with organ malperfusion and failure.[20-21] These patients are also at risk for cerebral and other hemorraghes.[21-22] Acute promyelocytic leukemia (APL; AML with t(15;17)(q22;q12); *PML-RARA*) differs clinically from other AML subtypes in that patients carry a high risk of bleeding and thromboembolic events, even in the absence of leukocytosis, before and during early treatment.[23-25]

1.2.5 Treatment

Intensive chemotherapy and achievement of complete remission (CR) is necessary for long-term survival in AML. Complete remission (CR) is defined as <5% leukemic blasts in the bone marrow in combination with a neutrophil count of $>1.0x10^9$/L and a platelet count of $>100x10^9$/L.[26] Curative treatment was not available until the late 1960's, when the use of daunorubicin and cytosine arabinoside (DA) was introduced.[27-28] These drugs combined made it possible to induce CR in AML patients. In a small subpopulation, there was even a potential of long-term survival. Improvement in supportive care over the years has enabled intensification of treatment. Several antileukemic drugs have been introduced since then, but no other drug combination has been convincingly shown to be better.[14, 29-33] Thus, DA remains the cornerstone of AML treatment[34], though addition of etoposide[35] and the substitution of daunorubicin for idarubicin or mitoxantrone may improve overall survival in certain groups of patients.[36-38] In recent publications it is suggested that dose intensification of daunorubicin in both younger and older patients is tolerable and leads to superior survival.[39-40] The addition of granulocyte or granulocyte-macrophage colony-stimulating factor (G-CSF; GM-CSF) has been postulated to make leukemic blast more sensitive to chemotherapy. While patients belonging to the intermediate cytogenetic risk group may benefit from the addition of G-CSF[41], no survival advantages have been shown regarding the addition of GM-CSF.[42-43]

The concept of consolidation treatment, including high doses of cytosine arabinoside, has been developed to prevent relapse of the disease.[44-46] As a result, once the patient has achieved CR, consolidation treatment (usually 3-4 courses) is given. In addition, allogeneic stem cell transplantation (allo-SCT) was introduced as a clinical option in the mid 1970's and reduces the risk of relapse and improves survival in selected patient groups.[47-51] Autologous SCT (auto-SCT) has been a part of AML treatment since the 1980's but does not seem to significantly improve survival compared to conventional consolidation treatment.[50, 52-53] An important difference between the two types of SCT is that the allo-SCT takes advantage of the donor's immune system and there is clearly a graft-versus-leukemia effect. The drawback of the allo-SCT is the graft-versus-host disease (GVHD) which causes morbidity and mortality in a certain number of patients.[54] Allografted patients are also susceptible to bacterial, viral, and fungal infections several months after the transplantation has been performed, which is a major cause of therapy related mortality (TRM) in this group of patients.[55]

APL is treated differently from all other subtypes of AML. The vitamin A derivative, all-*trans* retinoic acid (ATRA), introduced in the early 1990's, has the ability to induce differentiation of leukemic promyelocytes in patients with APL and can induce CR as a single drug. In combination with an anthracycline-based chemotherapy the results are further improved.[23, 56] In addition, arsenic trioxide has the ability to induce CR in patients with refractory and relapsed APL and is successfully used in combination with chemotherapy in this group of patients.[23, 56]

When DA was first introduced, TRM was substantial because of a high incidence of severe infectious complications.[57] The introduction of empirical broad-spectrum antibiotics, antifungal and antiviral therapies, which are especially important in the allo-SCT setting, has reduced infection related mortality.[58-62] Altogether, in modern AML treatment, short-term mortality resulting from infectious complications has been reported to be as low as 4%.[63] In addition, patients treated with intensive chemotherapy and/or allo-SCT are often dependent on transfusion of erythrocytes and platelets for survival. Before platelet transfusions were made possible in the early 1960's, many patients had fatal hemmorrhage.[3, 64] Another important accomplishment in transfusion medicine is the testing for infectious diseases, especially in preventing transmission of

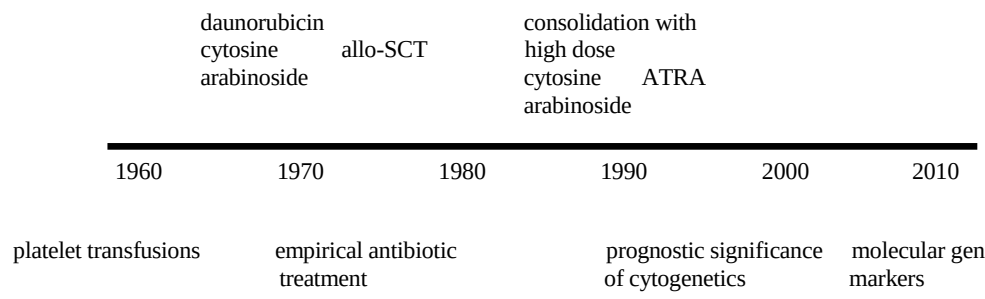

Figure 3. Time-line showing important achievements in the management of acute myeloid leukemia

hepatitis and HIV.[64-65] In the allo-SCT setting, refinement of conditioning regimens and the use of larger amounts of stem cells have also contributed to lower TRM.[59, 61] In a recent report from the Swedish Acute Leukemia Registry the overall allo-SCT TRM in patients treated 1997-2006 was estimated to be 14-19% in patients younger than 60 years of age.[66] Risk stratification based on cytogenetic analyses[67-69], and in recent years, molecular genetic analyses[70-73] has significantly contributed to an improved identification of patients who benefit from allo-SCT.

In all, survival of patients with AML is depending on successful induction treatment in combination with consolidation treatment and, in an increasing number of patients, allo-SCT. The importance of high quality supportive care during the entire treatment period cannot be overestimated.

1.2.6 Prognosis and prognostic factors

Despite advances in antileukemic treatment and supportive care the prognosis in AML remains rather poor. It is estimated that only 30-40% of younger patients and 10-20% of older patients survive five years or more after diagnosis.[74-75] However, most survival data arrive from clinical trials that are associated with a various degree of patient selection and elderly/ frail patients are often not included.[76-77] Population-based data are scarce, though reports from England and USA suggest that survival has improved over the years.[12, 78]

AML is a heterogeneous disease where outcome depends on many factors and treatment, including SCT, has to be tailored for each patient depending on the individual prognostic factors. The prognostic factors can be divided into three main categories 1) patient-related prognostic factors, 2) leukemia-related prognostic factors, and 3) response to treatment.

Patient-related prognostic factors

Old age is a well established independent predictor of poor prognosis in AML patients.[51, 77, 79] AML in the elderly is also often associated with drug resistance and unfavorable cytogenetics.[79-80] Despite the general poor prognosis in elderly, some elderly patients seem to benefit from intensive chemotherapy and although few, long-term survivors exist.[81-82] Poor performance status and comorbidities are more common in elderly patients and often present in the same patient. However, they are also independently associated with increased induction treatment mortality.[22, 83-84] For a number of cancers, most pronounced in cancer of breast, large bowel, bladder, and uterus, low socioeconomic status (SES) is associated with both increased risk and poorer outcome.[85] Shorter survival among young AML patients within lower SES groups has been reported[86] but there are few studies on the impact of SES in AML and an association between SES and outcome in AML has not been well established.[87-88]

Leukemia-related prognostic factors

Chromosome abnormalities are detected in approximately 55% of adult AML patients.[89-90] In addition, more than 50% of patients with a normal karyotype have somatically acquired mutations in the nucleophosmin 1 (*NPM1*)[70], FMS-like tyrosine kinase 3 (*FLT3*)[71], and/or CCAT/enhancer binding protein alpha (*CEBPA*) genes.[72-73, 91]

Thus, about 75% of all AML patients can be genetically characterized. The information on cytogenetic and molecular genetic abnormalities is used to determine what risk category the patient belongs to (Table 2).

Table 2. Risk groups depending on cytogenetic and molecular genetic abnormalities[34]

Favorable	APL with t(15;17)(q22;q12): *PML-RARA* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11** t(8;21)(q22;q22); *RUNX1-RUNX1T1** Mutated *NPM1* without *FLT3-ITD* (normal karyotype) Mutated *CEBPA* (normal karyotype)
Intermediate	Mutated *NPM1* and *FLT3-ITD* (normal karyotype) Wild type *NPM1* and *FLT3-ITD* (normal karyotype) Wild type *NPM1* without *FLT3-ITD* (normal karyotype) t(9;11)(p22;q23); *MLLT3-MLL* Cytogenetic abnormality not classified as favorable or unfavorable
Unfavorable	inv(3)(q21q26.2) or t(3;3)(q21q26.2); *RPN1-EVI1* t(6;9)(p23;q34); *DEK-NUP214* t(v:11)(v;q23); *MLL* rearranged. del(5q) or -5 -7 abnl(17p) complex karyotype**

* Exception: not favorable if *kit*-mutation of codon 816 is present
** Three or more chromosome abnormalities in the absence of one of the WHO designated recurring translocations or inversions, i.e. t(15;17), t(8;21), inv(16) el. t(16;16), t(9;11), t(v;11)(v;q23), t(6;9), inv(3)/t(3;3)

Therapy-related AML (secondary to treatment with chemotherapy and/or radiation) and AML secondary to a preceding hematological malignancy such as MDS or MPN are associated with lower rates of CR and a higher risk of relapse if CR is achieved.[14] Also within this group of patients cytogenetic findings can be used for risk stratified therapy.[92]

Patients with hyperleukocytosis at diagnosis have higher short-term mortality, mainly due to cerebral hemorrhage and/or respiratory failure, but once in CR their risk of relapse is probably not increased.[21-22]

The use of immunophenotypic markers or patterns as prediction of prognosis in AML has not been widely accepted due to diverging results from the published studies.[93]

Response to treatment

It is well accepted that a patient with a slow response to treatment, i.e. needing two or more cycles of chemotherapy to achieve CR, has a higher risk of relapse and shorter survival.[94] Results from several studies indicate that early evaluation of the bone marrow can be used to predict prognosis. The German cooperative AML-group has shown that the presence of more than 10% blast cells one week after end of induction chemotherapy is associated with lower rates of remission, shorter relapse-free and overall survival.[95-96] In addition, early clearance of peripheral blasts measured by flow cytometry has been reported to be associated with the achievement of CR.[97]

Minimal residual disease (MRD) in AML is defined as remaining leukemic cells, usually detected by molecular genetic or flow cytometry methods, in a patient in morphological CR. The presence of MRD is clearly associated with a higher relapse rate and shorter survival.[98-99] For details see section 1.3.2.

1.2.7 Risk-adapted therapy

As previously mentioned, allo-SCT has the ability to prevent relapse in AML. The advantageous effect of allo-SCT is not only due to the high dose chemotherapy given as conditioning before the transplant. Rather, the graft-versus-leukemia effect is considered to be at least as important.[54] Allo-SCT with reduced intensity conditioning is an alternative for the older patients and the impact on outcome is currently evaluated in on-going studies.

Despite improvements in supportive care, TRM in allografted patients remains substantial. As a consequence, relapse-free survival may be improved, while no improvement regarding overall survival is seen. This challenging issue has led to the concept of risk-adapted therapy, i.e. patients with a low risk of relapse probably do not benefit from allo-SCT while patients with a high risk of relapse may.[47-49] The prognostic factors discussed above are used to select the strategy for the patients on an individual basis. Thus, patients allocated to the favorable risk group are generally not considered for allo-SCT, while patients with intermediate or high risk features are likely to benefit from allo-SCT.[34] In addition, the risk associated with the transplant itself as assessed by age, comorbidity and other transplant related risk factors needs to be taken into account when making individual clinical decisions.[48, 100-101]

1.3 FLOW CYTOMETRY IN ACUTE MYELOID LEUKEMIA

Flow cytometry (FC) is a laser-based technology which most widely used clinical application is to classify hematopoietic cells by cell surface immunofluorescence. The flow cytometer (FCM) scans single particles or cells as they flow in a liquid medium past an excitation light source. Analysis is based on size and granularity and whether the cells are carrying fluorescent molecules either in the form of antibodies or dies. Photo detectors convert the signals to electric impulses, which are processed by a computer. Fluorescence occurs when a fluorescent molecule absorbs light at one wavelength, reaches an excited state and then returns to the ground state, emitting a light at a different (longer) wavelength.[102-103]

Immunophenotypic characterization of blast cells by FC is used to distinguish between ALL and AML[104] and is also of great importance when establishing the diagnosis of mixed phenotype acute leukemia.[8]

Leukemic blasts in AML express normal myeloid differentiation antigens.[104] In addition, they often display leukemia-associated immunophenotypes (LAIPs; Figure 4), which are immunophenotypes that differ from their normal counterparts or is uncommon in regenerating bone marrow. LAIPs are usually divided into four groups based on the type of aberrant findings: 1) cross-lineage infidelity (i.e. lymphoid antigens expressed on myeloid blast cells), 2) asynchronous antigen expression (expression of a combination of myeloid-associated antigens which is not found in the

normal myeloid differentiation such as the co-expression of CD34 and CD15 or absence of CD13 in CD33 positive cells), 3) antigen over-expression (abnormally high expression of normal myeloid antigens), and 4) lack of antigen expression (absence of myeloid-specific antigens on myeloid blasts).[105-106] The leukemic clone may express more than one LAIP.

LAIPs may reflect underlying molecular abnormalities.[107-108] Specific immunophenotypic features have been found in most of the cytogenetically defined AML subsets.[108] Also, patients with defined molecular genetic markers often present with an associated immunphenotype.[19, 109-110] Defining LAIP at diagnosis enables monitoring of MRD and certain immunophenotypic profiles have been proposed to be of prognostic relevance.

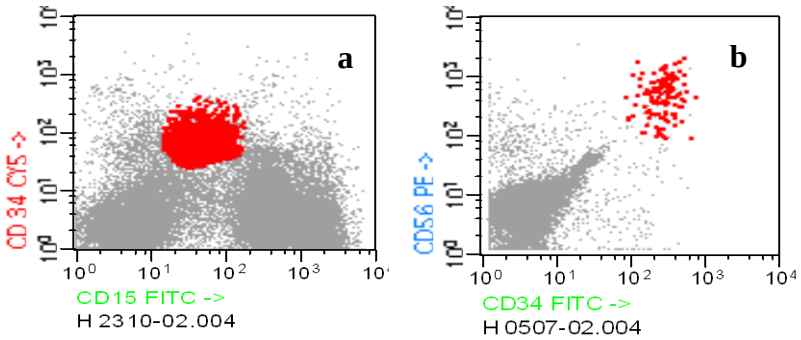

Figure 4. Examples of leukemia-associated aberrant phenotypes in acute myeloid leukemia
a: Co-expression of CD34 and CD15 as an example of asynchronous antigen expression and,
b:Co-expression of CD56 and CD34 exemplifies cross-lineage infidelity

1.3.1 Immunophenotype in relation to prognosis

There are a number of reports on prognosis in relation to expression of individual antigens and combinations of antigens in AML. Some investigators have found an association between CD34 positivity and shorter survival.[111-112] However, other authors could not relate CD34 expression to outcome.[113-115] The current opinion is that CD34 expression can be associated with both favorable and adverse cytogenetics and thus, CD34 *per se* cannot be used to predict prognosis in AML.[93] CD56 expression has been associated with shorter survival in AML with t(8;21).[116] Some[117-118], but not all[119], studies showed similar results in other categories of AML. CD7 is the most common lymphoid marker observed in AML. Some authors have demonstrated shorter survival in patients with CD7+ AML, but it has not been confirmed by others (reviewed in[93]). CD15 expression has been associated with a higher CR rate after standard induction chemotherapy [120-122] and with longer survival.[123]

Combinations of antigens have been used with the aim to construct prognostic scores and new immunophenotypic classifications of AML. Casanovas *et al.*[124] formed a new classification based on seven antigens. In their study, overall survival was shorter in the group of patients having leukemic blasts expressing pan-myeloid markers and CD7. Another prognostic score was created by Legrand *et al.*[125], who demonstrated that no single antigen was of prognostic value, but that co-expression of pan-myeloid markers was associated with a better prognosis. Repp *et al.*[126] reported that certain

individual antigens (such as CD9, CD13, CD33, and CD34) were related to a worse prognosis and if combined their prognostic discriminatory capacity improved. Recently, it was suggested that the combination of CD33 and CD34 expression can be used for predicting prognosis in patients older than 60 years.[127] Thus, despite many efforts to define the prognostic value of immunophenotype in AML, this issue remains controversial.

1.3.2 Minimal residual disease detected by flow cytometry

MRD is defined as persistence of very low levels of leukemic cells in a bone marrow with morphological CR. The two most commonly used methods for investigating MRD are based on the detection, with high sensitivity, of either molecular or immunophenotypic markers expressed by the leukemic clone. The polymerase chain reaction (PCR) technique has a very high sensitivity (one target cell per 10^4-10^6 nucleated bone marrow cells) but the method is limited to the fraction of AML patients with specific genetic lesions.[128-129] FC allows a sensitivity of one leukemic cell per 10^3-10^4 normal bone marrow cells and can be used in more than 90% of AML patients.[130-131] In MRD studies, follow-up samples can be acquired using a so-called live gate procedure when only cells with lineage associated markers are saved (CD19 in B-ALL, CD7 in T-ALL and myeloid marker of choice in AML). These cells are then screened for the possible persistence of residual cells with the same LAIPs as those identified at diagnosis.[102, 132] The FC technique is under continuous development and five to eight color FCMs are becoming available, which will probably increase the sensitivity.[133] When molecular genetic techniques and FC are used to determine MRD in the same patient the concordance rate is high.[134]

When FC is used to monitor MRD in an AML patient, the LAIP of the patient's leukemic blasts has to be characterized at diagnosis. The most common measure points thereafter are at first CR and at the end of consolidation treatment. It is evident that the presence of MRD at these time-points is strongly associated with an increased risk of relapse [98-99, 106, 130-131, 135-136] and shorter overall survival. Some authors report a stronger association between risk of relapse and MRD-positivity post consolidation than after induction treatment.[99, 130-131] However, it has also been suggested that MRD measurement at day 16 from start of induction treatment can be of prognostic relevance.[137] The presence of MRD seems to be associated with high risk karyotypes, but the prognostic significance probably remains within each karyotypic risk group.[98-99, 134]

There is no consensus on how to use the MRD information in the clinical setting. The proposed use has been to select patients for SCT, though there are few reports in the literature regarding the benefit of SCT in MRD-positive patients. Auto-SCT does not seem to reduce the risk of relapse in MRD-positive patients[138], while there is some evidence that allo-SCT may improve relapse-free survival in these patients.[135-136] APL patients, on the other hand, are monitored regarding *PML-RARA* with PCR analysis and treatment is started if a molecular relapse is detected.[23, 139] The use of FC to detect early relapse and start treatment before a clinical relapse is overt in non-APL AML has not been extensively investigated.

1.4 MYELOPROLIFERATIVE NEOPLASMS

1.4.1 Definition

Myeloproliferative neoplasms (MPNs) including polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF) are stem cell-derived clonal diseases characterized by proliferation of one or more of the myeloid lineages, i.e. granulocytic, erythroid and megakaryocytic.[140] Initially, MPNs are characterized by a hypercellular bone marrow with an effective hematopoieses but the disease can progress into a stage with marrow failure due to myelofibrosis, ineffective hematopoieses or transformation into an acute blast phase.[141-143] The diagnostic criteria for PV, ET, and PMF according to the WHO classification of 2008 are given in Table 3.[8] Chronic myelogenous leukemia is genetically characterized by the *BCR-ABL1* fusion gene and considered a specific entity among the MPNs[8] and will not be discussed further in this thesis.

Table 3. Diagnostic criteria for polycythemia vera, essential thrombocythemia, and primary myelofibrosis as defined by the World Health Organization classification of 2008[8]
Polycythemia vera: Diagnosis requires the presence of both major criteria and one minor criterion or the presence of the first major criterion together with two minor criteria
Major criteria
1. Hemoglobin >18.5 g/dL in men, >16.5 g/dL in women
2. Presence of *JAK2* mutation
Minor criteria
1. Bone marrow biopsy showing hypercellularity for age with trilineage growth with prominent erythroid, granulocytic and megakaryocytic proliferation
2. Serum erythropoietin level below the reference range for normal
3. Endogenous erythroid colony formation in vitro
Essential thrombocythemia: Diagnosis requires meeting all four criteria
1. Sustained platelet count $\geq 450 \times 10^9$/L
2. Bone marrow biopsy showing proliferation mainly of the megakaryocytic lineage with increased numbers of enlarged, mature megakaryocytes. No significant increase or left-shift of neutrophil granulopoiesis or erythropoieses
3. Not meeting WHO criteria for polycythemia vera, primary myelofibrosis, *BCR-ABL1* positive chronic myelogenous leukemia, myelodysplastic syndrome, or other myeloid neoplasm
4. Demonstration of *JAK2* mutation or other clonal marker, or in the absence of *JAK2*, no evidence for reactive thrombocytosis
Primary myelofibrosis: Diagnosis requires meeting all 3 major and 2 minor criteria
Major criteria
1. Presence of megakaryocyte proliferation and atypia, usually accompanied by reticulin and/or collagen fibrosis, or in the absence of significant reticulin fibrosis, the megakaryocyte changes must be accompanied by an increased bone marrow cellularity characterized by granulocytic proliferation and often decreased erythropoiesis
2. Not meeting WHO criteria for polycythemia vera, *BCR-ABL1* positive chronic myelogenous leukemia, myelodysplastic syndrome, or other myeloid neoplasm
3. Demonstration of *JAK2* mutation or other clonal marker, or in the absence of *JAK2*, no evidence that the bone marrow fibrosis or other changes are secondary to infection, chronic inflammatory condition, lymphoid neoplasm, metastatic malignancy, or toxic myelopathy
Minor criteria
1. Leukoerythroblastosis
2. Increase in serum lactate dehydrogenase level
3. Anemia
4. Splenomegaly

1.4.2 Epidemiology

MPNs are mainly disorders of the middle ages. The median age at diagnosis ranges between 60-70 years depending on MPN subtype, where ET is more common than PV and PMF in younger individuals.[144-146] Females are more commonly diagnosed with ET[146-147] while men are more frequent among PMF patients.[146, 148] Some authors report a higher incidence of PV in men[149-150], but these findings have not been confirmed by others.[145-146] The annual incidence of all subtypes combined is estimated to be 6-10/100,000 inhabitants.[8, 144]

1.4.3 Etiology and pathogenesis

The underlying cause is unknown in most cases. A genetic predisposition has been reported in some families[151-152] and relatives of MPN patients seem to have a significantly increased risk of MPN.[146] The Janus kinase 2 (*JAK2*) mutation is present in 95% of PV patients and in approximately 50% of ET and PMF patients[153] and gives a proliferative advantage of hematopoietic precursor cells.[154] The mutation occurs at a primitive stem cell level and is chronologically an early event. However, there is evidence to suggest that the *JAK2* mutation may not be the initial clonogenic event in PV or other MPNs and its presence may not be mandatory for endogenous colony formation.[153]

1.4.4 Clinical signs and symptoms

Approximately half of all MPN patients are reported to be asymptomatic at diagnosis.[140, 147] The disease is often indolent and the morbidity related to MPN is mainly related to venous or arterial thrombosis.[155-156] Hemorrhage may also occur, especially in ET patients.[157] Some patients experience pruritus (typically aquagenic and mainly in PV), erythromelalgia or other symptoms of acral ischemia. Splenomegaly is common and is often due to extramedullary hematopoiesis. In addition, some patients with PMF experience fatigue, weight loss, night sweats, and low-grade fever.

1.4.5 Treatment

The treatment in PV and ET is focused on reducing the risk of thromboembolic events and bleeding. Low-dose aspirin has been shown to reduce the risk of both arterial and venous thrombosis[158] and is recommended to almost all patients. Patients with PV are treated with phlebotomy to reduce the hematocrit below 0.45. In addition, patients who are considered at risk for thromboembolic events (i.e. patients above the age of 60 years, with platelet counts $>1500x10^9$/L, and/or with a thromboembolic history) also receive cytoreductive treatment.[159] Cytoreduction can be accomplished by several different approaches. Hydroxyurea (HU) is the treatment of choice in patients above the age of 60. In the elderly patient, treatment with radioactive phosphorus (P^{32}) and alkylating agents is also an option. In younger patients, on the other hand, treatment with interferon is recommended as first line therapy. In addition, ET patients may be treated with anagrelide. Some PMF patients with anemia may be successfully treated with erythropoietin.[160] HU can be used to reduce spleen size and general symptoms.[159]

1.4.6 Prognosis

The prognosis differs substantially between the subsets of MPNs. Life expectancy of patients with ET[150, 161] has been reported to be similar to that of the general population while in PV patients life expectancy has been observed to be reduced.[150, 162] PMF clearly has the worst prognosis with an average survival of less than five years.[143, 148, 162] However, there is a well recognized risk of transformation to AML or MDS in a subset of patients in all three MPN subtypes. The risk has been reported to be highest in PMF followed by PV and ET (8-20%, 5-10%, and 2-5% at 5-15 years, respectively).[148, 161-167] Patients diagnosed with AML secondary to MPN have a very poor prognosis with a median survival of a few months.[143, 163] In addition, patients with PV and ET have a risk of progression to PMF.[141, 161] Risk factors at diagnosis of PV and ET that could be used to predict leukemic transformation are lacking. However, it is established that the risk of transformation is increased in patients treated with P^{32} or alkylating agents.[165, 168-169] The issue of the leukemogenic potential of HU, on the other hand, remains controversial.[170] In fact, AML/MDS incidence rates of 10-14%[171-172] with HU used alone and 30% when preceded by busulphan treatment have been reported.[172] Others have found no increased risk for transformation in patients treated with HU.[161, 167, 170, 173] The diverging results from the studies referred to above are likely explained by the fact that most of them are single institution studies including small numbers of patients with transformation to AML/MDS and, in many cases, a median follow-up time of less than ten years. The launching of large randomized trials addressing the issue of the risk for treatment related AML/MDS transformation has been hampered by the relative rarity of MPNs, late appearing transformation events in a long-term disease course and reluctance to randomize patients to potentially leukemogenic therapies.

1.5 SWEDISH POPULATION REGISTRIES

Sweden has a long history of population registries, the first was introduced as early as 1686 for military purposes, with the first report of survival in 1746. The personal identification code system for all Swedish citizens was established in 1947. Information regarding patients diagnosed with a malignant disorder is reported to the Swedish Cancer Registry which was established in 1958. Every physician and pathologist/cytologist is obliged by law to report each occurrence of cancer to the registry. The registry contains information on diagnosis, sex, date of birth, date of diagnosis, and hospital where the diagnosis was made.[10, 174] In a recent validation study focusing on lymphoproliferative malignancies diagnosed 1964-2003 the completeness and overall diagnostic accuracy of the registry was found to be >90-95%.[175] In addition, there is the Swedish Adult Acute Leukemia Registry founded in 1997 by the Swedish Society of Hematology. This registry contains clinical information such as comorbidity, the patient's cytogenetic risk group, applied treatment, and results of treatment.[81] Allo- and auto-SCTs performed in Sweden are also reported to the European Group for Blood and Marrow Transplantation (EBMT) registry, which was established in 1974. For each person the date and cause of death is registered in the national Cause of Death Registry. Statistics Sweden performed mandatory censuses every fifth year between 1960 and 1990 collecting information on individuals' occupational status, income,

housing etc. This information is gathered in the Swedish National Census Database.[176] All these registries and the possibility of linkage between registries using the personal identification code system provide an excellent platform for performing epidemiological research.

2 AIMS

Overall aim
To improve management of patients with AML by identifying factors associated with risk of disease and outcome

Specific aims
To define outcome of patients with AML in Sweden over a long time period in relation to age, gender, year of diagnosis, and region of diagnosis and to relate the survival patterns to prevailing management strategies

To estimate the potential effect of socioeconomic status on survival in AML, using multiple myeloma as an indolent disease comparator

To seek for an association between the leukemic cell immunophenotype and outcome in AML

To evaluate the prognostic significance of minimal residual disease after induction and consolidation treatment with a special focus on the role of stem cell transplantation in younger adult AML patients

To define treatment related risk factors for transformation to AML in patients with myeloproliferative neoplasms

3 EPIDEMIOLOGICAL STUDIES ON SURVIVAL IN ACUTE MYELOID LEUKEMIA (I, II)

3.1 PATIENTS AND METHODS

In studies I and II we included information on all AML patients reported to the Swedish Cancer Registry from 1973 to 2005 using the International Classification of Diseases version 7 (ICD-7). The diagnoses coded as 2050 (acute myeloid leukemia), 2059 (acute myelomonocytic leukemia/acute myeloid leukemia non-specified), 2060 (acute monocytic leukemia), and 2069 (acute monocytic leukemia non-specified) were included. Using Systemized Nomenclature of Medicine-Clinical Terms (SNOMED) codes (introduced in 1993) we were able to define patients with acute promyelocytic leukemia (APL; SNOMED code 98663) diagnosed 1993-2005. Analysis of patients with a preceding myelodysplastic syndrome (MDS) was restricted to the same time period due to the fact that MDS was not reported to the Swedish Cancer Registry until the early 1990's. Patients with APL or prior MDS were also included in the analysis of the whole AML cohort. Patients with a preceding cancer diagnosis including a hematological malignancy were included in study I but excluded in study II. Additionally, in study II we included patients with multiple myeloma (MM) diagnosed from 1973 to 2005. Based on previous findings regarding the effect of hospital-type at diagnosis and gender on survival in MM[177], we were inclined to perform a study to assess the impact of socioeconomic status (SES) on survival in AML and MM. We chose to investigate these two hematological malignancies due to their different clinical characteristics. While AML is an aggressive malignancy which requires immediate management and is potentially curable[14], MM is in most cases an indolent lymphoproliferative disorder with little or no prospect of cure.[178] We used occupational status as a proxy for SES, gathered from the Swedish National Census Databases[176], established from mandatory censuses conducted in 1960, 1970, 1980, and 1990. Seven SES groups were determined: higher white-collar worker, lower white-collar worker, self-employed, farmer, blue-collar worker, retired, and unknown. Information from the Cancer Registry included date of birth, sex, date of diagnosis, region, and hospital where the diagnosis was established. Date of death was obtained from the Cause of Death Registry. Information on the number of SCTs performed on AML patients during this time period was obtained from the EBMT registry (I).

3.1.1 Statistical methods

Relative survival ratios (RSRs) were computed as measures of AML survival (I).[179-180] An important advantage of using relative survival is that it does not rely on the accurate classification of cause of death. Instead, it provides a measure of total excess mortality associated with a diagnosis of AML irrespective of whether the excess mortality is directly or indirectly due to the cancer. The RSR is defined as the observed survival in the patient group divided by the expected survival of a comparable group from the general population, which is assumed to be free from the cancer in question. One-year, five-year, and ten-year RSRs can be interpreted as the proportion of AML patients who

survived their malignancy at one, five, and ten years, respectively. Expected survival was estimated using the Ederer II method[181] from Swedish population life-tables stratified by age, gender, and calendar period. One-, five-, and ten-year RSRs were calculated for four calendar periods: 1973-1980, 1981-1988, 1989-1996, and 1997-2005 and six age categories: 0-18, 19-40, 41-60, 61-70, 71-80, and older than 80 years. In addition, patients diagnosed with APL or prior MDS were studied separately in two calendar periods (1993-1999 and 2000-2005). For APL patients, three-year RSRs were used as outcome variable due to reduced observation time. Poisson regression was used to model excess mortality.[182] The estimates of this model are interpreted as excess mortality ratios. As an example, an excess mortality ratio of 1.5 for males/females indicates that males experience 50% higher excess mortality than females.

We estimated survival in relation to SES using the Kaplan-Meier method (II).[183] Secondly, the relative risk of death (any cause and cause-specific) in relation to SES and calendar period was estimated using Cox's proportional hazards regression. We conducted both univariate and multivariate analysis, adjusted for sex, area of residence at diagnosis, age at diagnosis (≤54, 55-64, 65-72, 73-78, 79-83, or ≥84 years), and calendar period at diagnosis (1973-1979, 1980-1989, 1990-1999, and 2000-2005). To investigate whether mortality in relation to SES had changed over time, we also conducted analyses stratified by calendar period.

3.2 RESULTS AND DISCUSSION

3.2.1 Survival in acute myeloid leukemia patients (I)

A total of 9,729 AML patients (4,954 males and 4,775 females; median age 69 years) were included in the study. Forty percent of the patients were diagnosed in the Leukemia Group of Middle Sweden's regions (LGMS; Stockholm-Gotland, Uppsala, and Örebro regions). A total of 949 SCTs were reported to the EBMT register during the study period, 626 allo- and 323 auto-SCT. More than half of the SCTs were carried out during the last calendar period with allo-SCT dominating.

We observed significant improvements in survival during the 33 year study period. One-year RSRs improved significantly for all age categories (Table 4) while

Table 4. One-year relative survival ratios with 95% confidence intervals in acute myeloid leukemia patients stratified by age category and calendar period

	Calendar period			
Age category (years)	**1973-1980 (95% CI)**	**1981-1988 (95% CI)**	**1989-1996 (95% CI)**	**1997-2005 (95% CI)**
0-18	0.40 (0.30,0.49)	0.62 (0.51,0.70)	0.73 (0.63,0.81)	0.81 (0.73,0.86)
19-40	0.37 (0.31,0.44)	0.61 (0.54,0.68)	0.71 (0.64,0.77)	0.74 (0.67,0.80)
41-60	0.31 (0.27,0.36)	0.44 (0.40,0.49)	0.61 (0.57,0.66)	0.61 (0.56,0.64)
61-70	0.19 (0.16,0.23)	0.32 (0.28,0.36)	0.46 (0.41,0.50)	0.48 (0.44,0.52)
71-80	0.12 (0.08,0.14)	0.15 (0.13,0.18)	0.26 (0.23,0.29)	0.28 (0.25,0.30)
81+	0.09 (0.05,0.13)	0.05 (0.03,0.09)	0.13 (0.10,0.17)	0.16 (0.13,0.19)

five-year RSRs improved for all but patients older than 80 years of age (Table 5). As expected, improvement in survival was most pronounced in younger patients. However, even among patients up to 80 years of age diagnosed in the last calendar period a fraction of long-term survivors was observed. Ten-year RSRs differed only little from five-year RSRs, suggesting that most patients who survive for five years are cured from the disease.

Table 5. Five-year relative survival ratios with 95% confidence intervals in acute myeloid leukemia patients stratified by age category and calendar period

	Calendar period			
Age category (years)	**1973-1980 (95% CI)**	**1981-1988 (95% CI)**	**1989-1996 (95% CI)**	**1997-2005 (95% CI)**
0-18	0.17 (0.10,0.25)	0.31 (0.22,0.41)	0.53 (0.42,0.62)	0.65 (0.56,0.73)
19-40	0.09 (0.06,0.14)	0.21 (0.15,0.27)	0.38 (0.32,0.45)	0.58 (0.51,0.65)
41-60	0.06 (0.04,0.09)	0.12 (0.09,0.16)	0.24 (0.21,0.29)	0.36 (0.32,0.41)
61-70	0.04 (0.02,0.06)	0.07 (0.05,0.09)	0.14 (0.11,0.17)	0.15 (0.12,0.18)
71-80	0.03 (0.01,0.05)	0.02 (0.01,0.04)	0.06 (0.04,0.08)	0.05 (0.04,0.07)
81+	0.03 (0.01,0.09)	0.00 (0.00,0.00)	0.01 (0.004,0.04)	0.01 (0.001,0.04)

Males had a 5% higher mortality compared to females during the first five years after diagnosis (p=0.032; Table 6), which is consistent with a previous report of female predominance among long-term survivors.[74] Overall, patients resident in LGMS regions had a 6% lower mortality during the first five years after diagnosis (p=0.006; Table 6), mainly confined to differences observed in the first calendar period. There was no significant difference in excess mortality ratios between patients diagnosed at university compared to non-university hospitals.

One hundred and eleven patients with APL were diagnosed between 1993 and 2005, constituting 2.5% of all AML cases during this period. This reflects the lower incidence of this AML subtype in Scandinavia compared to that seen in Southern Europe, Latin America, and Asia.[184] Patients diagnosed with APL were younger (median age 54 years; 56 and 47 years in the two calendar periods under study, respectively) than those diagnosed with other subtypes of AML, which is consistent with the published literature.[184] The fact that APL is mainly a disease of younger patients may contribute to the differences in incidence of this disease between Scandinavia, where the population is older, and for example Latin America with a younger population. Overall three-year RSR in APL was 0.61, being 0.53 (95% CI: 0.38;0.66) in patients diagnosed in 1993-1999 and 0.69 (95% CI: 0.55;0.79) in patients diagnosed 2000-2005. Forty-eight (43%) of the APL patients had deceased at follow-up. One-month mortality rate was 23%; 27% in 1993-1999 and 18% in 2000-2005. Bearing the limited number of APL patients in mind, short-term mortality appears lower in the later calendar period. An improved immediate management of this patient

group including the introduction of all-*trans* retinoic acid (ATRA) has likely contributed to this finding.[56, 185]

There were 219 AML patients (median age 72 years) with a preceding MDS diagnosis. Mortality of patients with prior MDS was 51% higher than in patients with *de novo* AML when adjusted for age (Table 6) and RSRs in this group of patients did not improve during the second calendar period.

Table 6. Excess mortality ratios and 95% confidence intervals during the first five years after acute myeloid leukemia diagnosis by calendar period, sex, region of residency, age at diagnosis, and prior myelodysplastic syndrome

	Excess mortality ratio	CI	p
Calendar period of AML diagnosis			
1973-1980	1.00 (reference)		
1981-1988	0.77	0.73, 0.83	<0.001
1989-1996	0.50	0.47, 0.54	<0.001
1997-2005	0.44	0.42, 0.47	<0.001
Sex			
Male	1.00 (reference)		
Female	0.95	0.91, 0.996	0.032
Region of residency			
LGMS	0.94	0.90,0.98	0.006
Other	1.00 (reference)		
Age at AML diagnosis, years			
0-18	1.00 (reference)		
19-40	1.26	1.08, 1.46	0.003
41-60	1.80	1.57, 2.07	<0.001
61-70	2.72	2.38, 3.11	<0.001
71-80	4.71	4.12, 5.37	<0.001
Older than 80	7.62	6.64, 8.74	<0.001
Prior MDS*			
No	1.00 (reference)		
Yes	1.51	1.31, 1.74	<0.001

All variables are simultaneously adjusted for all other variables in the table excluding prior MDS diagnosis

*Analyzed on patients diagnosed 1993-2005 only, adjusted for all other variables in the table

Most probably, a combination of factors related to diagnostics and treatment have contributed to the observed improvements in survival of AML patients. Improvement in one-year RSRs is likely to reflect higher CR rates due to more effective induction treatment and better management of short term toxicity. The introduction of consolidation treatment[186-187] has lowered the relapse rate and contributes in part to the improvement in five-and ten-year RSRs. Especially important was the introduction of high-dose cytosine arabinoside consolidation treatment in the early 1990's.[45-46] In addition, allo-SCT reduces the risk of relapse in certain high and intermediate risk patients.[47-49]

The fraction of older patients treated with systemic antileukemic therapy in Sweden has increased over the years.[81] This more generous attitude towards administering intensive treatment to elderly probably explains the improvements seen in patients 61-80 years of age after the mid-eighties. However, the RSRs in this age group were substantially lower than in younger age groups. Thus, age remains an important predictor of prognosis. Many elderly patients are not fit for intensive induction

chemotherapy due to comorbidities and/or poor performance status. Post remission therapy is often restricted and allo-SCT is rarely a treatment option in patients older than 60 years of age contributing to a higher relapse rate. In addition, there is an aggregation of adverse prognostic factors such as high risk cytogenetics, antecedent MDS or MPD, and overexpression of genes involved in drug resistance in elderly patients with AML.[79-80, 188]

Our findings differ slightly from the observations made in two population-based studies on AML survival performed in USA[78] and England.[12] Pulte *et al.* found evidence of increasing five- and ten-year survival in AML patients younger than 75 years with no preceding diagnosis of cancer or hematologic malignancy.[78] Interestingly, five- and ten-year RSRs in our cohort were higher in all age categories, despite the fact that patients with a preceding diagnosis of cancer or hematological malignancy were included. Survival appears also to be superior to that reported in a population-based study performed in Southeast England.[12] In addition, we found that one-year RSRs increased in all age groups, also in patients older than 80 years. There are several potential explanations for the observed superior survival in the present cohort. First, Sweden has a well established government-funded public health care system where all residents by law are entitled to equal access to health services. Second, patients with AML are almost exclusively diagnosed, treated, and followed clinically by physicians at non-private hospital-based hematology units. As a consequence, treatment decisions including allo-SCT are based on patient and disease related factors only without any financial consequences for the individual. Superior survival in elderly patients may be explained by a higher proportion of patients receiving treatment with curative intent in Sweden compared to most other countries. In 1997-2001 54% of Swedish AML patients 70-79 years old received treatment with a curative intent[81] while in a report based on SEER data 1991-1999 49% of patients 65-74 years of age received any kind of chemotherapy, not specified whether for palliation or with a curative intent.[189]

In conclusion, we found that AML patients younger than 80 years have gained significantly from the developments in management of the disease during a 33-year period. However, there are still few long-term survivors among patients older than 60 years. Thus, age remains an important predictor of prognosis. Innovative agents and procedures suitable for the older patient are greatly needed. In addition, more individualized management based on accurate risk stratification will hopefully significantly improve the outlook for the whole AML patient population.

3.2.2 Survival in acute myeloid leukemia and multiple myeloma in relation to socioeconomic status (II)

We identified a total of 9,165 and 14,744 patients with a first cancer diagnosis of AML and MM, respectively. The median age at diagnosis was 69.2 years in patients with AML and 71.8 years in patients with MM. The SES distribution was similar among AML and MM patients, respectively. The majority of patients were blue-collar (37.9; 39.5%) and lower white-collar workers (30.7; 30.9%). The distribution of the SES groups remained stable, with a predictable decrease in the proportion of farmers over calendar time.

In AML and MM respectively, self-employed, farmers, blue-collar workers, and retired had an overall significantly higher mortality compared to higher white-collar workers (Table 7). Lower white-collar workers had a significantly higher mortality than higher white-collar workers in MM but not in AML.

Table 7. Relative risk of death in acute myeloid leukemia and multiple myeloma according to socioeconomic status based on all-cause mortality*

	AML (n=9,165)		Myeloma (n=14,744)	
	No. of deaths	**Hazard Ratio (95% CI)**	**No. of deaths**	**Hazard Ratio (95% CI)**
Socioeconomic status**				
Higher white-collar worker	477	1.00 (ref)	713	1.00 (ref)
Lower white-collar worker	2,545	1.14 (0.99-1.31)	3,907	1.22 (1.09-1.36)
Self-employed	590	1.20 (1.07-1.36)	993	1.09 (1.00-1.20)
Farmer	704	1.18 (1.07-1.30)	1,334	1.11 (1.02-1.20)
Blue-collar worker	3,141	1.22 (1.11-1.34)	5,076	1.15 (1.07-1.25)
Retired	447	1.19 (1.05-1.34)	734	1.12 (1.02-1.23)
		p=0.005		p<0.005

*Analyses were adjusted for SES, age, sex, calendar period of observation and region of residence; CI denotes confidence interval.
**Most recent classification before diagnosis, excluding individuals with unknown occupation
P-values obtained using Wald Chi-squared test

Relative risk of death in relation to SES and calendar period is shown in Table 8. Among AML patients no association between SES and mortality was found during the first calendar period (1973-1979). However, during the last three periods (1980-1989, 1990-1999, and 2000-2005), a consistently higher mortality was observed in blue-collar compared to higher-white collar workers. In MM, mortality did not differ between the SES groups in the first two calendar periods (1973-1979 and 1980-1989), but in the third calendar period (1990-1999), self-employed, blue-collar workers, and retired had a significantly higher mortality compared to higher white-collar workers. In the fourth calendar period (2000-2005) blue-collar workers had significantly higher mortality compared to higher white-collar workers (Table 8).

Probably several factors contribute to the differences in survival according to SES observed in our study. These can, although with some overlap, be separated into patient-related, tumor-related, and factors related to the health care provider. Among patient-related factors an income or economic barrier contributing directly to our findings is quite unlikely given the equal access to health care in Sweden. One potential reason for the observed SES associated differences in survival is patient's delay in seeking medical attention, which has been noted in other malignancies.[85] This factor may to a certain extent contribute to the observed differences in outcome in MM patients. However, it is unlikely to affect the observed differences in AML, which is a disease that comes to medical attention within a short period of time. Patients with

comorbid conditions are less likely to receive or tolerate intensive therapy, which may reduce survival in both MM and AML.[83, 101, 190] In addition, life-style factors, physical activity, overweight, tobacco or alcohol use, factors which are influenced by SES[191-194] may also have impact on the patient's tolerance to applied therapy.

Table 8. Relative risk of death in acute myeloid leukemia and multiple myeloma in relation to socioeconomic status, by calendar period*

	1973-1979	1980-1989	1990-1999	2000-2005
AML (n=9,165)				
Socioeconomic status**		Hazard Ratio (95% CI)		
Higher white-collar worker	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Lower white-collar worker	1.04 (0.76-1.42)	1.14 (0.95-1.38)	1.29 (1.10-1.51)	1.14 (0.92-1.40)
Self-employed	1.19 (0.84-1.70)	1.29 (1.03-1.62)	1.14 (0.93-1.39)	1.06 (0.80-1.39)
Farmer	1.10 (0.78-1.53)	1.11 (0.89-1.37)	1.32 (1.07-1.62)	1.29 (0.96-1.74)
Blue-collar worker	1.10 (0.80-1.50)	1.26 (1.05-1.51)	1.23 (1.05-1.45)	1.28 (1.04-1.57)
Retired	1.00 (0.71-1.42)	1.20 (0.93-1.54)	1.57 (1.19-2.08)	1.54 (1.00-2.39)
Multiple myeloma (n=14,744)				
Socioeconomic status**		Hazard Ratio (95% CI)		
Higher white-collar worker	1.00 (ref)	1.00 (ref)	1.00 (ref)	1.00 (ref)
Lower white-collar worker	0.95 (0.73-1.24)	1.12 (0.96-1.30)	1.08 (0.96-1.22)	1.18 (0.96-1.44)
Self-employed	1.07 (0.80-1.44)	1.02 (0.85-1.21)	1.18 (1.02-1.37)	1.13 (0.87-1.46)
Farmer	0.92 (0.70-1.22)	1.06 (0.90-1.25)	1.16 (1.00-1.35)	1.15 (0.88-1.52)
Blue-collar worker	0.95 (0.73-1.24)	1.12 (0.96-1.30)	1.18 (1.04-1.32)	1.31 (1.07-1.60)
Retired	1.07 (0.81-1.43)	1.15 (0.95-1.39)	1.45 (1.16-1.80)	1.40 (0.90-2.17)

*Analyses were adjusted for SES, age, sex, calendar period of observation and region of residence; CI denotes confidence interval.
**Most recent classification before diagnosis, excluding individuals with unknown occupation

Tumor-related factors, such as stage or tumor burden at diagnosis have been suggested as possible explanations for the differences in cancer survival according to SES[195]. Again, due to the rapid course of the disease, this is probably not the case in AML. In MM on the other hand, patients with lower SES may be more likely to present with an advanced disease stage.[196] It has also been hypothesized that there may be differences in tumor biology characteristics depending on the patient's SES. In breast cancer the morphologic type is a prognostic factor and has been shown to vary by SES.[85] Given the poor prognosis of AML secondary to cytotoxic treatment, it is a possibility that AML related to cigarette smoking also may be more aggressive. Thus, the fact that smokers are overrepresented in lower SES groups[194] could contribute to the observed survival differences. Unfortunately, no information on tumor or patient

characteristics, including life-style factors, was available to us while performing this study.

The health care provider's attitude towards management may differ according to SES. Interestingly, regional differences in survival of patients 70 to 80 years of age were observed in the previously mentioned population-based study of AML patients in Sweden.[81] This finding probably reflects differences in attitudes towards aggressive induction treatment in the elderly patients[81] and it is possible that physicians also may be more prone to start intensive treatment in patients with higher SES. Procedures such as allo-SCT have been introduced and, as demonstrated in study I, has increased in number over the study period. It has been reported that patients with comorbidities are less likely to survive after allo-SCT.[101] Hypothetically, Swedish AML patients of lower SES may undergo allo-SCT less frequently and/or tolerate the procedure less well.

The SES related differences in survival in AML was observed during the last three periods (1980-1989, 1990-1999, and 2000-2005). As demonstrated in study I, survival in adult patients was very short during the first calendar period (1973-1979). Thus, the differences related to SES are actually seen in the time periods when cure has become a realistic therapeutic goal. Interestingly, the relative risk of death for blue-collar workers in relation to higher white collar workers was the same between 1980 and 2005, which was a period of continuous improvement in survival among patients younger than 80 years of age.

In summary, in AML the observed differences in survival between SES groups can probably not be explained by patient's- or doctor's delay. Comorbidities resulting in fewer patients receiving or tolerating intensive therapy and differences in attitudes toward aggressive treatment including allo-SCT between the different SES groups may to some extent explain the survival differences. Further studies are needed to better define the underlying factors of our findings.

4 STUDIES ON THE PROGNOSTIC IMPACT OF THE LEUKEMIC CELL IMMUNOPHENOTYPE AT DIAGNOSIS AND MINIMAL RESIDUAL DISEASE DETERMINATION IN ACUTE MYELOID LEUKEMIA (III, IV)

4.1 PATIENTS AND METHODS

We included patients diagnosed with AML at Karolinska Hospital and Danderyd Hospital in Stockholm between 1994 and 2001. A total of 165 patients were diagnosed with non-APL AML during this time period. After exclusions due to up-front palliative treatment (n=13), incomplete flow cytometry (FC) analysis (n=6) and lack of detailed clinical information (n=17), 129 patients receiving treatment with a curative intent were included a study of the prognostic impact of the leukemic cell immunophenotype at diagnosis (III).

Out of the original 165 patients, 62 were 60 years of age or younger. Fifty-three of theses patients achieved morphological CR. However, a total of eight patients were excluded due to uninformative immunophenotypes (n=3), lack of sufficient clinical data (n=2) or incomplete FC analysis (n=3). Thus, follow-up MRD information was available for 45 patients ≤60 years of age with morphological CR and these patients were included in a study of the effect of allo-SCT in relation to MRD (IV). The median age was 64 years (range 19-85 years) and 47 years (range 19-60 years) in studies III and IV, respectively.

The primary diagnosis of AML was established according to the FAB classification.[4] For the purpose of these studies each case was reclassified according to the WHO classification of 2001.[6] The results of cytogenetic analysis were available in 112 patients (43 patients in study IV) and risk groups were defined according to Grimwade et al.[68] No analysis regarding molecular genetic changes such as *NPM1*- or *CEBPA*- mutations or *FLT3-ITD* was performed at this time. CR was defined as described in section 1.2.5.[26] A recurrence of AML was established when there was a reappearance of leukemic blasts or manifestation of extra-medullary leukemia in patients with previously documented CR.

The patients received induction treatment based on cytosine arabinoside with the addition of an anthracycline or the anthracenedione mitoxantrone according to five main protocols.[31, 42, 197-198] All but three patients younger than 60 years received idarubicin, cytosine arabinoside, and etoposide (ICE) induction therapy.[197] In all protocols two or three consolidation courses were administered. Allo-SCT[199] or auto-SCT[197] was performed in first CR in 16 and 15 patients, respectively. The results of the MRD analyses were not available to the treating physician.

4.1.1 Immunophenotyping

The immunophenotyping was performed on whole bone marrow by three-color FC, which was the standard method at the time. A stain and lyse/wash technique was used

for surface markers. FACS-lysing solution [Becton Dickinson (BD), San Jose, CA, USA] was applied for erythrocyte lysis. For the detection of intracellular antigens, fixation and permeabilization with Permeafix [Ortho, Raritan, NY, USA (Ortho)] or (since 1999) IntraStain [Dako, Glostrup, Denmark (Dako)] was applied according to the manufacturers' instructions. A standard panel consisting of 17 triple combinations of monoclonal antibodies (MoAb) was used to define the immunophenotypes of the leukemic population at diagnosis (Table 9).

Table 9. Standard panel of monoclonal antibodies for immunophenotyping of acute myeloid leukemia at diagnosis

Triple combinations
1. Simultest control
2. CD61/GPA/CD45
3. CD19/CD34/CD45
4. CD10/CD19/CD13
5. CD15/CD33/CD20
6. CD7/CD5/CD3
7. CD65/CD2/HLADR
8. CD15/CD34/HLADR
9. CD15/CD117/CD14
10. CD2/CD56/CD33
11. CD7/CD13/CD19
12. CD65/CD11b/CD4
13. CD4/CD8/CD3
14. lambda/kappa/CD19
15. CD22/CD5/CD20
16. CD34/CD38/CD19
17. CD15/CD33/CD34

For all leukemic samples, 10 000 – 15 000 events were acquired. The expression of various markers was analyzed after gating of the leukemic blasts defined by Forward Scatter (FSC) and Side Scatter (SSC) characteristics. The position of leukemic blasts in the FSC/SSC plots was confirmed by CD45/SSC gating, which was performed using MoAb combinations 2 and 3 (Table 9). Immunophenotype patterns (study III) were defined on the basis of expression of CD33 and CD15 recorded from MoAb combinations 5 and 17 (Table 9). Based on immunophenotypes at diagnosis, phenotypic abnormalities (LAIPs) were defined and used in the investigation of MRD follow-up (study IV). At follow-up, at least $30x10^3$ cells were analyzed in each tube. Live-gate analysis was used in five cases. Detectable MRD was defined as a distinct cluster of 15-20 dots with specific LAIP and blast scatter characteristics. Sensitivity levels were determined as 1) 0.1% if 30 000 events were acquired, 2) 0.05% if 30 000 events were acquired in cases with a highly aberrant LAIP, and 3) 0.015% if the live-gate approach was used. MRD levels were determined at two time-points: at first

morphological CR after induction treatment and at the end of post-remission chemotherapy or before SCT.

Bone marrow samples from 13 adult patients (median age 73, range 20-87 years) with immune thrombocytopenic purpura (n=4), secondary anemia (n=6) and lymphoma without bone marrow involvement (n=3) were used as a reference to establish patterns of expression of CD33 and CD15 in the non-leukemic bone marrow. In the non-leukemic bone marrow samples a four-color MoAb combination CD15/CD33/HLA-DR/CD34 was applied. During myeloid differentiation, it has been described that CD34+ cells first acquire HLA-DR, then CD33. Subsequently CD34 is lost and CD15 is acquired.[200] Our results of the FC analysis of non-leukemic bone marrow in the hospital control group support these findings.

4.1.2 Statistical methods

Overall survival (OS) was measured from the date of diagnosis until death due to any cause or end of follow-up for surviving patients. Relapse free survival (RFS) was measured from the date of CR to the date of relapse or death due to any cause. Observations were censored for patients in first CR by end of follow-up. The median observation time of surviving patients was 76 months (range 56-115 months). For descriptive statistics, median and range or percentages of cases were calculated. Categorized variables were compared between different groups using the χ^2 test and continuous variables by Student's *t*-test. Spearman rank correlation was used to assess correlations between continuous variables. RFS and OS were estimated according to the Kaplan-Meier method and differences between groups were analyzed using a log-rank test. The Cox proportional hazards model (dependent variables RFS and OS) was used for multivariate analysis. All reported p-values are two-sided.

4.2 RESULTS AND DISCUSSION

4.2.1 Prognostic impact of the expression of CD33 and CD15 (III)

Based on the expression patterns of CD33 and CD15 during normal differentiation of myeloid cells, we identified five distinct AML categories (patterns I-V, Table 10, Figure 5).

Table 10. Acute myeloid leukemia categories defined by CD33 and CD15 expression

	I n=18	**II** n=43	**III** n=10	**IV** n=50	**V** n=8
CD15	-	-	Heterogeneous	+	+
CD33	-	+	++	+	-
CD34	+ (15) - (3)	+ (25) - (18)	+ (4) - (6)	+ (36) - (14)	+
HLA-DR	+	+ (24) - (19)	+	+ (46) - (4)	+
Scatter	Myeloblastic	Myeloblastic	Myeloblastic	Myelo- or monoblastic	Myelo- or monoblastic

Pattern I was defined by the lack of CD15 and CD33 expression and corresponds to the most immature cells (n=18, Table 10, Figure 5a). Leukemic blasts in all but three samples in this group were positive for CD34. Patterns II-IV represent intermediate

stages in maturation. In pattern II (n=43), leukemic blasts were positive for CD33 and negative for CD15 (Table 10, Figure 5b). CD34 was expressed in 25 samples. The third pattern was defined by a very strong expression of CD33 with heterogeneous expression of CD15 showing a spectrum from negative through weakly positive to clearly CD15 positive cells within the same sample (n=10, Table 10, Figure 5c). Four pattern III samples showed CD34 positivity. Pattern IV (n=50) was characterized by the strong expression of both CD15 and CD33 (Table 10, Figure 5d). This pattern differed from pattern III in that the leukemic blast cells had a normal expression of CD33 as opposed to the high expression seen in pattern III. Also, in pattern IV, all cells in the blast gate expressed CD15. Leukemic blasts from 36 patients expressed CD34. In pattern V (n=8, Table 10, Figure 5e) blasts expressed CD15 but not CD33. Pattern V represents an aberrant immunophenotype due to the overexpression of CD34 in all samples and lack of CD33 expression.

Patient and AML characteristics differed between the defined immunophenotypic patterns (Table 11). Pattern V patients were the youngest and patients within pattern II the oldest with median ages of 50 and 72 years, respectively. The highest frequency of patients with adverse risk cytogenetics and patients with secondary AML were found in groups II and IV. Only three patients (2%) presented with favorable cytogenetic features and they were all found in group IV. This is a lower rate than reported in other studies but in good accordance with what has previously been reported in Swedish patients.[201] Patients ≤60 years of age in category III (n=4) all underwent allo- or auto-SCT. The proportion of SCTs did not differ between the other categories.

CR was achieved in 88 patients (68%) and in 68 patients ≤60 years (84%). The lowest CR rate was observed in pattern II patients, the group with the highest median age. Importantly, also patients ≤60 years belonging to pattern II, had a low CR rate (64%). Patients within patterns III and V had the highest CR rates (Table 11). However, differences in CR rates did not reach statistical significance.

Fifty-two of 88 (59%) CR patients had a recurrence of AML. Indeed, all pattern V patients experience a relapse despite their low median age. The relapse rate was also high in patients within pattern I, where only one patient presented with adverse cytogenetics. In contrast, relapse was observed in only 33 % of the patients in pattern III.

The overall median RFS was 15 months. The median OS for all patients was 15 months and CR patients had a median OS of 35 months. The immunophenotype pattern significantly predicted RFS and OS in univariate ($p=0.048$ and $p<0.0001$, respectively) and multivariate analysis ($p=0.015$ and $p=0.024$, respectively). The prognostic significance of the immunophenotype classification was retained in patients ≤60 years of age (n=51; $p=0.023$).

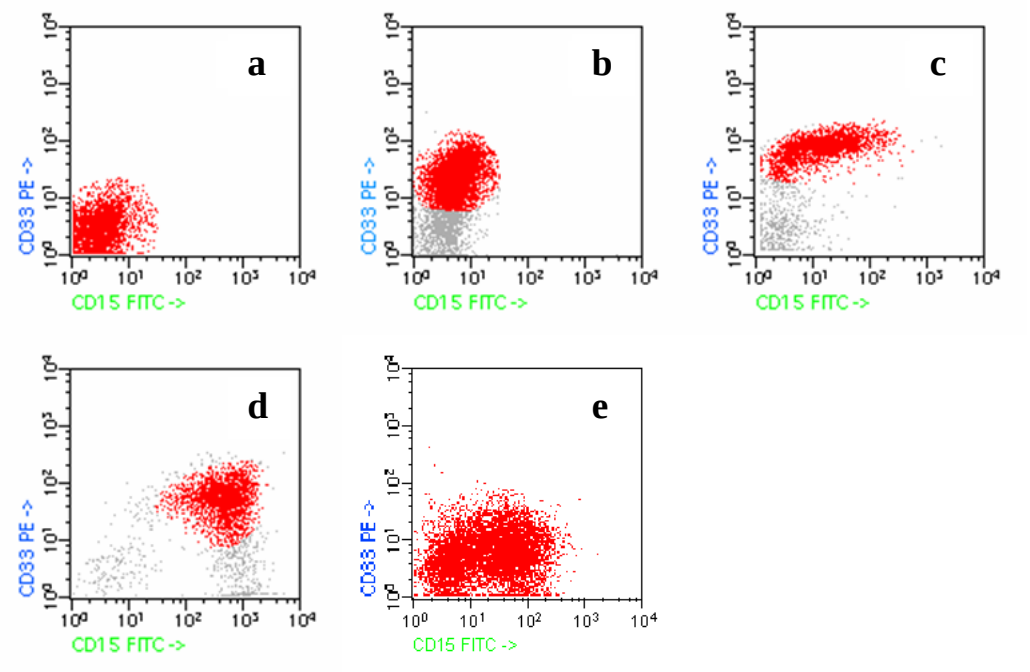

Figure 5. Flow cytometry plots showing the expression of CD33/CD15 in five immunophenotypic categories of acute myeloid leukemia
Plot (a) illustrates the lack of CD33 and CD15 expression in pattern I. Plot (b) shows pattern II with blast cells expressing CD33 but not CD15. Plot (c) illustrates the positivity for CD33 and dim expression of CD15 in pattern III. Plot (d) shows the expression of both CD33 and CD15 in pattern IV. Plot (e) illustrates pattern V where blast cells expressed CD15 but not CD33.

The two most numerous categories differed in the expression of CD15 (pattern II; CD33+/CD15-; pattern IV; CD33+/CD15+) and CD15 as a single marker was a significant predictor of longer OS (log rank test; p=0.012). Therefore, one could argue that the observed survival differences between immunophenotype categories actually are explained by differences in CD15 expression. However, in an analysis where patients with immunophenotype pattern II were excluded, there was no significant difference in the survival between the other four patient groups, of which one was CD15 negative and the others were CD15 positive. We therefore suggest that the expression pattern is more important in predicting prognosis than single antigen expression.

In summary, our classification of AML according to immunophenotype is based on two commonly expressed antigens: CD33 and CD15, which makes it less complicated than previously suggested classifications according to immunophenotype.[124-126] In addition, the defined patterns of antigen expression may reflect the biology of the leukemic cells and may therefore be more relevant than single antigens in determining prognosis in AML patients. In conclusion, immunophenotype patterns based on CD33 and CD15 expression has the potential to improve management of patients with AML. Future studies are needed to establish the clinical use of this immunophenotype classification and the potential connection between antigen expression patterns and specific cytogenetic and molecular genetic changes.

Table 11. Clinical characteristics of acute myeloid leukemia patients defined according to immunophenotype

	I*	II	III	IV	V	Total
Patients (n)**	18 (8)	43 (11)	10 (4)	50 (23)	8 (5)	129 (51)
Age (years, median and range)	63, 43-77	72, 23-84	63, 44-80	62, 19-83	50, 39-84	64, 19-84
Favorable cytogenetics (n)	0	0	0	3 (1)	0	3 (1)
Unfavorable cytogenetics (n)	1(0)	10(1)	0	11(2)	3(2)	25(5)
Cytogenetics not performed	5 (2)	3 (0)	1 (0)	8 (2)	0	17 (4)
Secondary leukemia (n) #	2 (0)	13 (4)	0	8 (1)	0	23 (5)
CR (%)	78 (88)	53 (64)	90 (100)	70 (87)	88 (100)	68 (84)
Relapse (% of patients in CR)	79 (57)	57 (29)	33 (0)	51 (40)	100 (80)	59 (42)
Auto-SCT in first CR (n)	3 (3)	1 (1)	2 (2)	6 (6)	2 (2)	14 (14)
Allo-SCT in first CR (n)	1 (1)	4 (4)	2 (2)	6 (6)	0	13 (13)
RFS (months, median)	22 (23)	9 (13)	59 (75)	14 (58)	8 (14)	15 (23)
OS (months, median)	30 (61)	8 (9)	60 (76)	23 (49)	14 (19)	15 (36)

* For definition of immunophenotype categories see Table 10 and Figure 5

** Results for patients ≤60 years of age within brackets

Secondary leukemia include therapy related AML and MDS-AML

4.2.2 The clinical significance of determining minimal residual disease by flow cytometry in acute myeloid leukemia (IV)

Forty-five patients ≤60 years of age in morphological CR were included. MRD analysis was performed in first CR in 43 patients, at the end of consolidation treatment in 31 patients, and at both time-points in 30 patients. MRD was detectable in 32 patients (74%) and in 17 patients (55%) at CR and at the end of consolidation treatment, respectively. Change of MRD status occurred in five patients; one patient was MRD-negative at CR and MRD-positive after consolidation treatment, while four MRD-positive patients at CR turned MRD-negative after consolidation treatment. This relatively low rate of change in MRD status is in accordance with a previous report.[130]

MRD status did not correlate significantly with age or cytogenetic risk group. However, the five patients presenting with an unfavorable karyotype were all MRD-positive at CR. Two of these patients were allografted and one was autografted and none of these three patients experienced a relapse. In contrast, the two patients with adverse karyotype treated with conventional chemotherapy only, had a recurrence of AML. Only two patients had favorable karyotypes and one of them, who remained MRD-positive at the end of post-remission therapy, suffered a relapse. Within the group of patients assigned to the intermediate cytogenetic risk group, patients with no detectable MRD after induction treatment had significantly longer five-year RFS than MRD-positive patients (90% vs. 49%; p=0.041). OS was also prolonged in MRD-negative patients, but this difference did not reach statistical significance. A prognostic significance of MRD within the cytogenetic risk groups has been observed in other studies.[98-99, 134]

The median duration of RFS was 36 months (range 2-105 months). Twenty patients died and the median OS was 42 months (range 4-107 months). The cause of death was

AML relapse in 13 patients, MDS in one patient, allo-SCT related complications in three patients and non-leukemia related factors in the remaining three patients.

Detectable MRD at the first time point did not predict either RFS or OS when analyzed by the Cox proportional hazards method, but there was a trend for longer RFS in patients with no detectable MRD at the second time point (p=0.061). Both RFS and OS were longer in patients who underwent SCT than in the remainder (p<0.001 and p=0.001, respectively).

As a consequence of the finding that SCT, but not MRD status, was a significant prognostic factor in this group of AML patients ≤60 years of age, we decided to investigate the influence of SCT therapy on survival in MRD-positive and -negative patients. In the first analysis patients were divided in to four groups: 1) no detectable MRD and allo-SCT; 2) detectable MRD and allo-SCT; 3) no detectable MRD and no allo-SCT; and 4) detectable MRD and no allo-SCT (Table 12). Patients who were not allografted and had detectable MRD at the first and/or second time-point had the worst outcome (five-year RFS 24% and 20%, respectively; and OS 34% and 35%, respectively). MRD-positive patients who underwent allo-SCT had similar RFS to patients with no detectable MRD. Similar OS rates at five years were also observed in allografted patients with detected MRD (67% and 75% for the two time-points, respectively) and patients with no MRD and no allo-SCT (70% and 75% for the two time-points, respectively). In the group of allografted patients with no detectable MRD after consolidation treatment, the observed five-year OS was actually lower (40%). The differences between the four groups were significant for both MRD time-points regarding RFS (p=0.002 and p=0.006, respectively) but not for OS (p=0.134 and p=0.069, respectively).

Table 12. Five-year relapse-free and overall survival in acute myeloid leukemia patients ≤60 years of age in relation to presence of minimal residual disease and allogeneic stem cell transplantation

	MRD in first CR		**MRD after consolidation treatment**	
	RFS (%)	OS (%)	RFS (%)	OS (%)
MRD neg; allo	100	67	100	40
MRD pos; allo	83	67	80	75
MRD neg; no allo	80	70	83	75
MRD pos; no allo	24	34	20	35

In addition, the potential impact of auto-SCT was analyzed in MRD-positive patients in relation to treatment with conventional chemotherapy and allo-SCT. The five-year OS rate was significantly better in allo-SCT recipients than in the autografted group or those who received conventional chemotherapy (67%, 46%, and 31% for the first time-point [p=0.021] and 75%, 53%, and 25% for the second time-point [p=0.046], respectively; Figure 6).

Ninety-four % of the patients in the present study expressed one or more LAIPs at diagnosis which is in line with previous reports.[130-131] Thus, monitoring of MRD by FC is possible in virtually all AML patients. However, there is concern about change in immunophenotype, since this phenomenon may lead to false negative results.[202-203] We experienced frequent changes in immunophenotype at relapse. Major immunophenotype changes (i.e. the loss of or gain of an antigen) were found in AML

blasts from nine of 15 patients (60%) in whom FC analysis was performed at relapse. However, immunophenotype changes resulted in false negative MRD measurements in only one of the relapsed patients. In six of the nine patients with immunophenotype changes, the prognostic power was not affected by changes in LAIP because other studied LAIPs remained aberrant. In three patients the change of antigen occurred in the most aberrant LAIP used for MRD follow-up but MRD was still detectable in two of these three patients, since a small population expressing the original LAIP persisted during treatment and relapse. A similar experience was reported by Voskova *et al.* who found LAIP changes in 24% of patients, but using several LAIPs, relapse could be detected in almost all patients.[204]

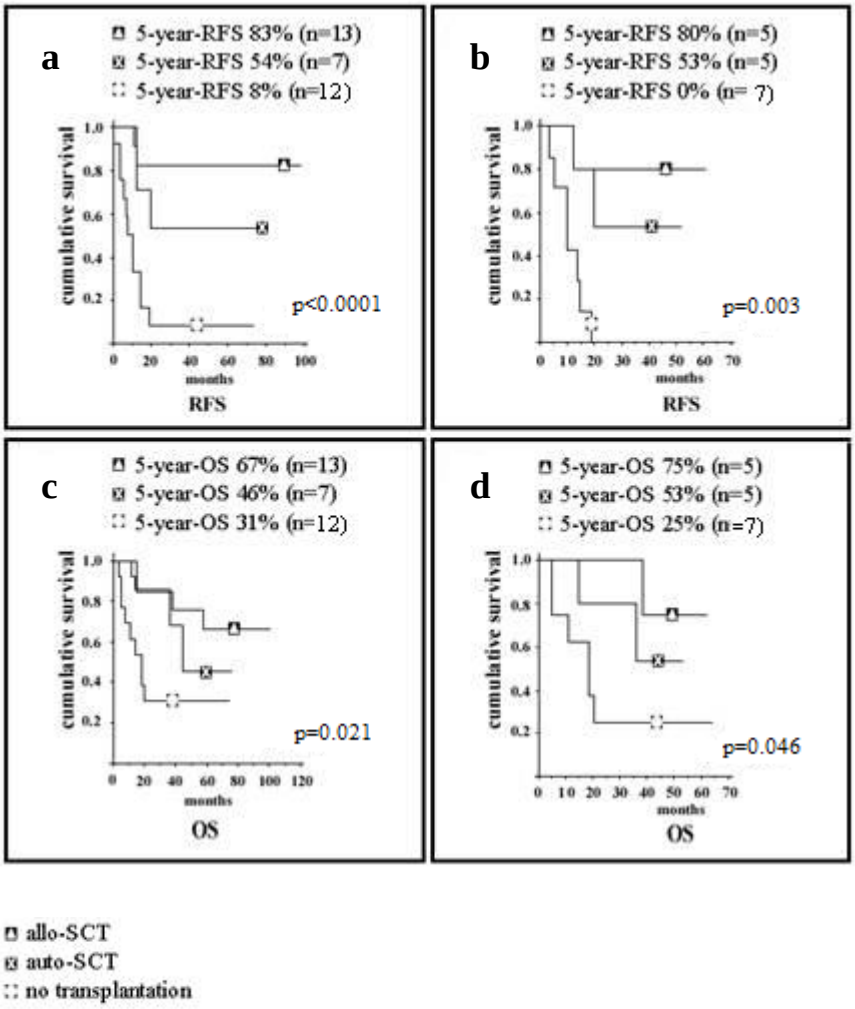

Figure 6. Relapse-free survival (RFS; a, b) and overall survival (OS; c, d) in young adult patients with detectable MRD after induction treatment (a, c) and after consolidation treatment (b, d) in relation to post-remission treatment

In summary, the FC method to monitor MRD is applicable in virtually all AML patients and immunophenotypic change at relapse is a minor problem making it a reliable method. A relatively high fraction of patients included in the present study of younger AML patients were treated with SCT (35% and 33% underwent allo- and auto-SCT, respectively). Due to the age-dependent differences in post-induction therapy we chose not to analyze the results in older and younger AML patients together. In contrast to previous reports, MRD status did not significantly predict prognosis in this group of

patients ≤60 years of age. However, when patients over the age of 60 years were included in the analysis, which is the approach often used when investigating the prognostic significance of MRD[99, 130-131, 135], MRD status after induction treatment and after consolidation treatment were significantly predictive for both RFS and OS.

The results from the present study indicate that MRD status may not be critical for the outcome of allografted AML patients. This is in accordance with the results published by Italian investigators, who observed no improvement in survival in MRD-positive patients treated with auto-SCT[138] but reported lower relapse rates if MRD-positive patients were allografted.[136] Also Feller *et al.* reported that MRD-positive patients can be cured by allo-SCT.[135] In conclusion, MRD analysis by FC may be used for refining the selection of therapeutic strategies and improving outcome in individual patients. Studies on larger series of patients are needed to confirm our findings and the optimal time-point for MRD measurement needs to be established.

5 TREATMENT RELATED RISK FACTORS FOR TRANSFORMATION TO ACUTE MYELOID LEUKEMIA AND MYELODYSPLASTIC SYNDROMES IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS (V)

5.1 PATIENTS AND METHODS

In a first step a cohort of 11,039 MPN patients diagnosed in Sweden from 1958 to 2005 was established. The majority of the patients were identified from the Swedish Cancer Registry. In addition, we retrieved information on MPN patients through our national MPN network (the Swedish Myeloproliferative Disorder Study Group), which include all major hematology/oncology centers in Sweden. By taking this approach, we could identify and add MPN patients that were not reported to the Swedish Cancer Registry.[146] In a second step, patients in the MPN cohort who developed AML (n=271) or MDS (n=21) were identified (cases; n=292) by linkage to the Swedish Cancer Registry. For each patient with a subsequent AML/MDS diagnosis, two patients from the MPN cohort without AML/MDS matched for MPN disease, age, gender, disease duration and calendar period were identified (controls; n=498). A control patient could be used for more than one case but with adjusted follow-up time. Thus, by identifying patients who transformed to AML/MDS and their matched controls from this cohort of MPN patients a nested case-control study was performed. Analysis of patients with transformation to MDS was restricted to 1993-2005 because MDS was not reported to the Swedish Cancer Registry until the early 1990's.

Cases or control patients were excluded if there was lack of relevant medical information (cases; n=51, controls; n=100), a misdiagnosis both with regard to MPN (cases; n=4, controls; n=2) and AML/MDS (cases; n=5), no proper matching (often due to a discrepancy between reported date of MPN diagnosis among cases to the Cancer Registry and true date according to medical records [cases; n=65, controls; n=142]), prior chemotherapy or radiotherapy for a non-MPN malignancy (cases; n=5, controls; n=12). Thus, the final study population in the nested case-control study consisted of 162 cases and 242 control patients.

For the purpose of this study the following information was collected from medical records: detailed information on treatment (type of therapy, cumulative dose, duration of treatment), laboratory variables at diagnosis including full blood count, bone marrow examination (at MPN diagnosis and at transformation in cases), and any other tumor preceding AML/MDS in cases, and in controls before the date of the corresponding case's AML/MDS diagnosis.

Patients with MPN, identified through the Swedish Cancer Registry only, who later developed AML (n=235) were used to determine the overall standardized incidence rate (SIR) of AML transformation. SIR was also determined in relation to MPN subtype and time after MPN diagnosis. Patients who developed MDS were not

included in this analysis due to the delayed introduction of MDS registration in the Swedish Cancer Registry.

5.1.1 Statistical methods

The risk of transformation in relation to cumulative doses of HU, P^{32} and alkylating agents was analyzed by conditional logistic regression. Relative risks were estimated as odds ratios (ORs) together with 95% confidence intervals (CIs).

5.2 RESULTS AND DISCUSSION

Among 162 cases (59% men; median age 64 years) 153 had a transformation to AML and nine to MDS, respectively. The majority had a preceding PV diagnosis (68%). Among patients who developed AML/MDS, 25% were never exposed to alkylating agents, P^{32} or HU compared to 32% among control patients. Eight % of cases and controls had received ≥1000 g of HU. In contrast, 25% of cases were exposed to ≥1000 MBq in comparison to 12% of control patients. Similarly, cumulative doses of alkylators exceeding 1.0 g were recorded in 7% of cases and 3% of control patients.

Previous exposure to HU was not significantly associated with an increased risk of AML/MDS at any cumulative dose level (Table 13). We also restricted the analysis to patients with PV and ET only, which lowered the estimates associated with HU exposure (ORs 1.03, 0.92, and 1.24 for each exposure level, respectively).

Table 13. Risk of transformation to acute myeloid leukemia or myelodysplastic syndromes in relation to exposure to hydroxyurea, radioactive phosphorous, and alkylating agents

Cumulative doses	Risk of AML/MDS (odds ratios; 95% CIs)
Hydroxyurea (g)	
0	1.00 (ref)
1-499	1.22 (0.61-2.45)
500-999	1.41 (0.58-3.40)
≥1000	1.35 (0.55-3.32) not significant
Radioactive phosphorous (MBq)	
0	1.00 (ref)
1-499	1.46 (0.65-3.29)
500-999	1.11 (0.55-2.25)
≥1000	4.60 (2.15-9.85) p<0.0001
Alkylating agents (g)	
0	1.00 (ref)
0.10-0.49	1.10 (0.53-2.26)
0.50-0.99	1.71 (0.59-4.98)
≥1.00	3.39 (1.08-10.59) p=0.036

The risk for AML/MDS transformation was strongly associated with high exposure of P^{32} (≥1000 MBq; p<0.0001) and alkylating agent treatment (≥1 g; p=0.036). However, lower exposure to P^{32} and alkylating agents were not associated with a significantly increased risk of AML/MDS (Table 13). The ORs remained virtually the same in the analysis including PV and ET patients only.

The overall SIR of AML transformation in the MPN cohort identified from the Cancer Registry only (n=235) was 36.2 (CI 31.7-41.1) and risk of transformation

increased with time following diagnosis of a MPN, SIR being approximately 60 after 15 years of observation. Among MPN subtypes PMF carried the highest risk of AML development (SIR 71.1; CI 52.9-93.5), followed by PV (SIR 33.6; CI 28.4-39.6) and ET (SIR 26.6; CI 18.9-36.3). Risk of AML transformation in relation to time after MPN diagnosis and according to subtype is graphically illustrated in Figure 7. Median survival of patients who transformed to AML was three months from time of AML diagnosis which is accordance with previous reports.[143,163]

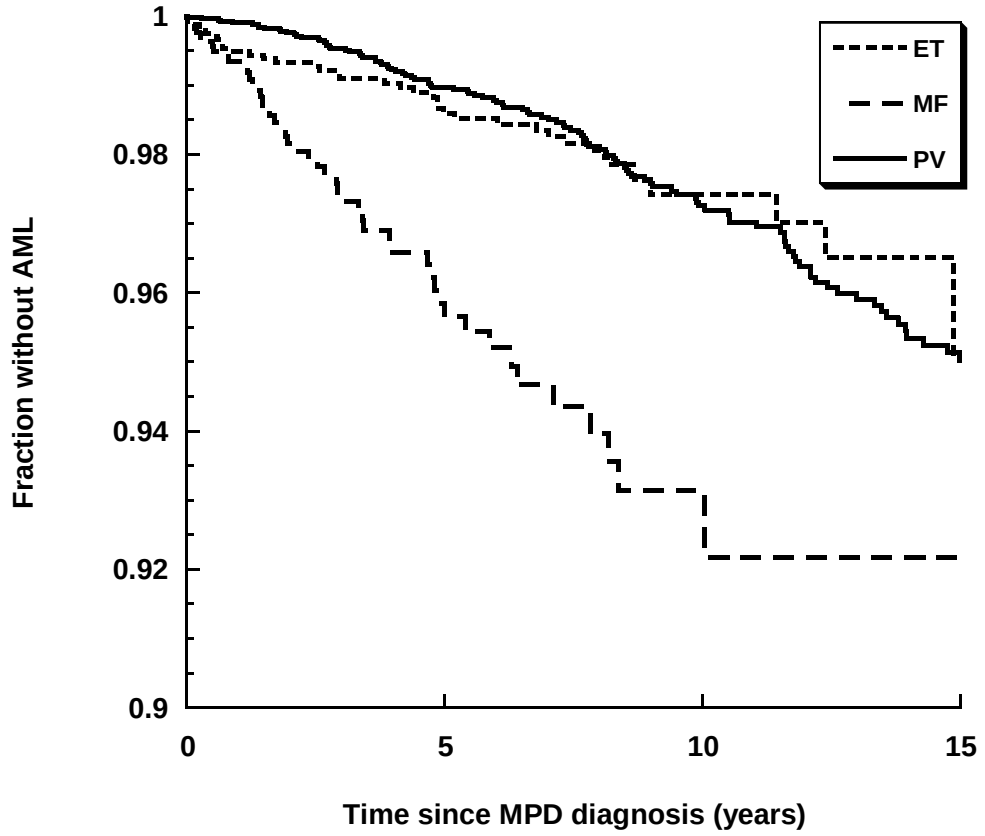

Figure 7. Risk of transformation to acute myeloid leukemia (n=235) in relation to time after diagnosis of a myeloproliferative neoplasm according to subtype

The fact that 25% of patients with a transformation to AML/MDS never were exposed to alkylating agents, P^{32} or HU, confirms AML/MDS development to be a part of the natural course of MPNs. However, the magnitude of this inherent propensity to AML/MDS transformation has been a matter of debate and exclusively been reported to be much lower, mostly ranging between 2-15% in PV and ET[164-167] and 8-23% in PMF.[148, 162-163] In addition, the potential leukemogenic effect of HU has remained a controversial issue for many years.[170] In our study, HU therapy did not significantly increase the risk of AML/MDS transformation even at very high cumulative doses. The major reasons for these discrepancies are probably related to patient selection, fewer patients under study, and shorter follow-up in previously published reports. Interestingly, among patients with a transformation to AML/MDS who never received cytoreductive therapy or were treated with HU only, the transformation occurred within five years of their MPN diagnosis. This may corroborate the notion that HU is non-

leukemogenic since the majority of patients given P^{32} and/or alkylators transformed at a later time-point. In further support for this notion is the fact that AML/MDS development in patients treated with HU for non-malignant disorders such as sickle cell anemia is a very rare event.[205-207]

Our study confirmed the increased risk of AML/MDS transformation in patients exposed to P^{32} or alkylating agents. Interestingly, the increased risk was seen only at cumulative doses above 1 000 MBq or 1 g, respectively. This finding may indicate the existence of a threshold exposure of P^{32} and alkylating agents for AML/MDS transformation in MPN.

Based on our findings we cannot totally exclude a leukemogenic effect of HU. However, if there is such an effect it seems to be very limited. These findings have important implications regarding treatments strategies in MPNs, especially in younger patients requiring decades of active treatment.

6 METHODOLOGICAL ISSUES

6.1 STUDIES USING DATA FROM CENTRAL REGISTRIES (I, II, V)

A randomized controlled clinical trial is the design of choice when comparing outcome related to different treatments. As long as the number of subjects is sufficient, randomization is an effective method for balancing confounding factors between treatment groups. However, for the purpose of determining impact of a certain therapy on outcome of a given disease in the general population, they have some important limitations. Most important is the issue of patient selection, i.e. how representative of the general population are patients included in a prospective randomized trial. Generally, patients with poor performance status and/or comorbidities, i.e. the patients with the worst prognosis, are excluded from clinical trials. In addition there are often a number of other inclusion criteria, including age, which further contributes to a skewed study population. The option of long-term follow-up is also hampered by many factors. The use of central registries, on the other hand, has in certain respects several advantages. Ideally, with a high coverage of the registry, ensuring a true population-based setting, selection of patients would be no problem. In addition, all data is reported prospectively. Long-term follow-up is feasible also enabling studies in indolent disorders where events may occur late in the disease course.

Unfortunately, very few if any registries have 100% coverage. In fact, in a validation study of the Swedish Cancer Registry performed by Åström *et al.*[208] on patients diagnosed 1987-1992 in three Swedish counties, 15% of acute leukemia patients were not reported to the Cancer Registry. Older patients (>80 years old), patients not receiving intensive induction treatment, and patient with secondary leukemia were less likely to be reported. Turesson *et al.*[175] recently investigated the accuracy and completeness of the Swedish Cancer Registry with focus on patients with lymphoproliferative disorders diagnosed 1964-2003 and found an overall diagnostic accuracy of 98% and completeness of 90%. Interestingly, the patients less frequently reported to the Cancer Registry more often had indolent diseases (mainly Waldenström's macroglobulinemia and chronic lymphocytic leukemia). Thus, there is likely to be some degree of selection of patients in studies I, II, and V, which may potentially affect our results. If there indeed is a higher proportion of patients with poor prognosis among unnotified cases, we would overestimate relative survival ratios (RSRs) in study I. However, the main finding of the study is the improvement in survival over the years. The fraction of reported AML patients has most probably increased during the study period, which would imply that a higher proportion of patients with poor prognosis was included in later as compared to earlier calendar periods. Consequently, the observed improvement in RSRs would actually be an underestimation. Hypothetically, patients with lower SES may present with more comorbidities (precluding intensive induction treatment) than patients with higher SES and therefore patients with lower SES could be overrepresented among unnotified cases (II). Consequently, the observed differences in survival among different SES groups could be underestimated.

We computed relative survival ratio (RSR) estimates as measures of AML survival (I). A major advantage of working with RSRs estimates is the fact that specific information on the cause of death is not required, which circumvents difficulties with inaccuracy or lack of death certificates. The crucial assumption in working with RSR estimates is that one can accurately estimate expected survival. For most cancers (including AML), patients are representative of the general population, so their expected survival can be estimated using general population survival rates.

The observed superior survival in patients with higher SES, may be explained by an earlier diagnosis than in patients with lower SES (lead-time bias), during an early phase of multiple myeloma (II). Lead-time bias in AML is likely to be of less or no importance with regard to our findings since the presentation is mostly acute/subacute.

We were not able to adjust for baseline differences in mortality between the SES groups, which can be expected given differences in life expectancy according to SES in the reference population. It is not inconceivable that such baseline differences are substantial. However, applied to the AML and MM populations where the absolute mortality is many times higher than in the general population, the shorter life expectancy among the lower SES groups would have but little impact on a relative scale. An advantage with our study is the use of occupation as a proxy for SES because this method assigns individuals to their "true" SES group in comparison to the use of zip-codes/neighborhoods as proxies.

Limitations in studies I and II also include the lack of individual clinical data such as laboratory analyses including cytogenetics and details on given treatment. In addition, it would be of great interest to have information on possible confounders, for example comorbidities and life-style factors such as smoking status, in different SES groups.

It is likely that indolent disorders, such as MPNs, are reported less frequently to the Swedish Cancer Registry in parallel with the findings regarding lymphoproliferative disorders of Turesson *et al.*[175] Therefore, in study V, we also included patients through the national MPN network as described previously. However, this study also relies on the report of AML/MDS secondary to MPN to the Swedish cancer registry where again some patients probably are missing. In fact, 2.6% of the patients in the MPN cohort had a transformation according to the Cancer Registry, which is a lower number than expected.[148, 162-167] However, the aim of the study was to assess the risk for transformation to AML/MDS in relation to therapy and this was done using a nested case-control design, which validity does not depend on identifying all cases. The case-control design is the method of choice when trying to determine causes for a rare disease such as secondary AML. Even though a number of cases and control patients had to be excluded (mainly due to incomplete medical records and no proper matching due to discrepancies between reported date of MPN diagnosis to the Cancer Registry and true date according to medical records) we believe that our study still is fully apt to define treatment related risk factors for transformation to AML/MDS. We have no reason to believe that the excluded cases and controls differ in exposure status or disease activity. A common issue with the case-control design is recall bias, due to the fact that cases tend to report a higher grade of exposure than controls.[209] In this study recall bias was avoided since information was gathered from medical records. The long

observation time (patients were diagnosed between 1958 and 2005) is clearly advantageous when studying diseases with a long and indolent course and late appearing events of interest.

6.2 CLINICAL STUDIES ON THE PROGNOSTIC IMPACT OF IMMUNOPHENOTYPE AT DIAGNOSIS AND MINIMAL RESIDUAL DISEASE FOLLOW-UP IN ACUTE MYELOID LEUKEMIA (III, IV)

In studies III and IV flow cytometry analyses were performed at diagnosis and in study IV also during follow-up. Clinical data was collected retrospectively from medical records which may be less reliable than prospectively assembled data. However, the information needed was easily retrieved in most patients.

AML is a relatively rare disease. In the study of immunophenotype and prognosis (III) 129 patients were included. Some of the immunophenotypic categories were very small and observed differences in survival between the different categories was mainly due to differences between the two largest groups. With more patients included, more firm conclusions regarding the other categories could have been made as well. In the MRD study (IV) only 45 patients ≤60 years of age in CR were available for inclusion. Notwithstanding this, we observed significant differences in outcome in relation to allo-SCT and MRD status.

When aiming at assessing the benefit of allo-SCT there is always the issue of patient selection. First, the patient has to be alive and free of relapse for a long enough time period to undergo the procedure including the search for a donor. Second, the patient's performance status has to be rather good to tolerate the treatment. As a result, the positive effect of allo-SCT can easily be overestimated because the patients with the worst prognosis will never be transplanted. In prospective studies aiming at assessing the value of allo-SCT in AML patients, the presence or absence of a donor can be used as a surrogate for randomization and the survival analysis is often made as an intention-to-treat analysis. Thus, part of the selection bias can be avoided, but instead the effects of allo-SCT may be underestimated if a low fraction of patients with a donor actually receive the transplant planned. In studies performed retrospectively, a possibility is to include only patients surviving for a certain amount of time in the analysis. In our study, the MRD status was not revealed to the treating physician, and thus, was not available when making treatment decisions. No specific analysis was performed on patients surviving a certain amount of time. However, among 32 patients with detectable MRD at CR, 13 were allografted and seven autografted. Three of the remaining 12 patients relapsed within four months of their achievement of CR. Thus, 16 of 19 (84%) patients not allografted were free from relapse during a long enough time in order to have undergone an allo-SCT.

During the time when patients were recruited for this study information on the *FLT3-ITD, NPM1,* and *CEBPA* mutations was not available. It would of course be of great interest to investigate a potential association between these mutations and the presented immunophenotype classification and/or presence of MRD.

7 SUMMARY AND CONCLUSIONS

7.1 EPIDEMIOLOGICAL STUDIES ON SURVIVAL IN ACUTE MYELOID LEUKEMIA (AML; I, II)

Survival in AML patients has improved substantially since the 1970's. Younger patients have gained most from the therapeutic advances made, while the prognosis in the very elderly remains poor. Intensification of induction and consolidation treatment, an increasing rate of allografted patients, a continuous improvement in supportive care measures, and a more precise risk stratification of patients are probably the most important factors contributing to the overall improvement.

AML and multiple myeloma (MM) patients with higher socioeconomic status (SES) survive longer than those with lower SES. The superior survival is most evident after 1980 in AML and after 1990 in MM. Differences in comorbidities, management, and life-style factors are likely to explain the observed survival differences.

7.2 STUDIES ON THE PROGNOSTIC IMPACT OF THE LEUKEMIC CELL IMMUNOPHENOTYPE AT DIAGNOSIS AND MINIMAL RESIDUAL DISEASE (MRD) DETERMINATION IN AML (III, IV)

AML patients can be divided into five categories depending on the expression of the antigens CD33 and CD15 on their leukemic blast cells at diagnosis. Patient and disease characteristics differ between the defined immunophenotypic patterns. The immunophenotypic category may be of use when predicting prognosis in AML patients.

Flow cytometry is a reliable technique to use with the aim to assess MRD in AML. Patients ≤60 years of age with detectable MRD in first CR or after post-remission therapy seem to live longer if allografted than if treated with conventional chemotherapy only.

7.3 TREATMENT RELATED RISK FACTORS FOR TRANSFORMATION TO AML AND MYELODYSPLASTIC SYNDROMES (MDS) IN CHRONIC MYELOPROLIFERATIVE NEOPLASMS (MPNs; V)

Patients with MPNs have an increased risk of AML/MDS compared to the general population. Twenty-five % of MPN patients with a transformation of the disease to AML/MDS were never given cytoreductive treatment, confirming that AML/MDS development is part of the natural course of MPNs. The risk of transformation is further increased by treatment with high doses of radioactive phosphorus and alkylating agents. Hydroxyurea, on the other hand, did not prove to be leukemogenic.

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