Epidemiological studies in de novo and secondary acute leukemia

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Abstract

Background

Acute leukemia (AL) is a rare blood cancer with poor prognosis in adult patients. Socioeconomic factors are known to impact cancer outcomes, but have not been adequately examined among adult AL patients. Acute myeloid leukemia (AML) secondary to another myeloid malignancy, irradiation or chemotherapy (s-AML), constitutes a quarter of AML patients and is considered to confer a poor prognosis. Still, population-based characterization of s-AML is scarce, and the role of allogeneic hematopoietic stem cell transplantation (a-HSCT) in s-AML is poorly studied.

Main aims

The main aims for this thesis were to:

i) compare the incidence and survival of adult AL between regions with major socioeconomic differences (Estonia and Western Sweden) during a quarter of a century.

ii) describe the incidence and prognostic factors in s-AML.

iii) explore the role for stem cell transplantation in s-AML in a population-based setting.

Method

We have analyzed all adult patients in the Swedish Acute Leukemia Registry, and comparable Estonian data.

Results and conclusion

During 1982-2006, relative survival for Estonian elderly AL patients has gradually improved and almost equals Western Sweden. However, few patients live after five years. For AL patients under 65, relative five-year survival has increased from almost zero to approximately 20% for Estonian and from 20 to 55% for Swedish patients during the course of our 25-years study.

S-AML constitutes approximately 25% in a large population-based setting, and has a significant negative impact on survival in younger AML patients, whereas less prognostic value among the elderly. In a nationwide population-based Swedish setting, there is virtually no long-term survival in patients with s-AML without a-HSCT. A-HSCT was superior to conventional chemotherapy in s-AML patients, and should therefore be considered for all eligible patients at diagnosis.

Keywords: Acute leukemia, ALL, AML, adult, secondary acute leukemia, MDS, MPN.

Svensk sammanfattning

Akut leukemi är en allvarlig form av blodcancer som varje år drabbar drygt 400 vuxna svenskar, med en medelålder på 70 år vid insjuknandet. Vanligast är akut myeloisk leukemi (AML), medan akut lymfatisk leukemi (ALL) drabbar cirka 60 vuxna per år. Utan behandling leder sjukdomen vanligen till döden inom några veckor. Behandling med högpotenta cellgifter syftar till att avdöda de sjuka cellerna och förhoppningsvis bota patienten från sjukdomen. I utvalda fall kan patienten genomgå en benmärgstransplantation i botande syfte.

Sekundär AML (s-AML) är en undergrupp som utgör cirka 25 % av AML. S-AML kan föregås av en tidigare blodcancer, som utvecklas till AML. Vanligen är detta myelodysplastiskt syndrom (MDS) eller myeloproliferativ neoplasi (MPN). I andra fall utvecklas AML efter cellgifts- eller strålbehandling för en annan tumörsjukdom, till exempel bröstcancer.

I denna avhandling presenteras två artiklar (I och II) med en jämförelse under 25 år mellan Estland och västra Sverige dvs. före, under och efter Sovjetunionens sammanbrott. De visar att den relativa överlevnaden bland vuxna patienter under 65 år i Estland har förbättrats, och ligger nu på drygt 20%, medan den i Sverige/Västra Götaland ligger på nästan 60%. För äldre patienter har relativ överlevnad ökat något under 25-årsperioden. Dessvärre är den fortfarande dålig för såväl svenska som estniska patienter och få äldre patienter blir botade. Artiklarna visar också tydligt att socioekonomiska skillnader spelar stor roll vid behandling av akut leukemi, och att mer resurser och bättre samarbete behövs för att förbättra överlevnaden i länder med sämre ekonomiska förutsättningar.

Vidare lägger avhandlingen fram en artikel där s-AML har karaktäriserats (III) i en stor befolkningsbaserad studie, baserat på Svenska akutleukemiregistret. Vi konstaterar att s-AML utgör drygt 25 % av all AML, patienterna är äldre och att överlevnaden för s-AML är sämre än för de novo AML. I artikel IV analyseras om benmärgstransplantation kan förbättra den dåliga överlevnaden vid s-AML.

Resultatet visar entydigt att benmärgstransplantation utgör det enda realistiska alternativet till bot för patienter med s-AML och bör därför övervägas tidigt i sjukdomsförloppet.

Contents

List of papers

I. Punab M, Palk K, Varik M, Laane E, Everaus H, Holmberg E, **Hulegårdh E**, Wennström L, Safai-Kutti S, Stockelberg D, Kutti J. **"Sequential population-based studies over 25 years on the incidence and survival of acute de novo leukemias in Estonia and in a well-defined region of western Sweden during 1982-2006: a survey of patients aged ≥65 years".** Med Oncol. 2013 Mar;30(1):487

II. **Hulegårdh E**, Punab M, Holmberg E, Palk K, Laane E, Everaus H, Wennström L, Stockelberg D. "**Acute de novo Leukemia in Estonia and Western Sweden 1982-2006:"Positive Trend in the Survival of Acute Leukemia over 25 Years."** Acta Haematol. 2016;136(3):167-73. doi: 10.1159/000446525. Epub 2016 Aug 19. PubMed PMID: 27537981.

III. **Hulegårdh* E**, Nilsson* C, Lazarevic V, Garelius H, Antunovic P, Rangert Derolf Å, Möllgård L, Uggla B, Wennström L, Wahlin A, Höglund M, Juliusson G, Stockelberg D, Lehmann S. **"Characterization and prognostic features of secondary acute myeloid leukemia in a population-based setting: a report from the Swedish Acute Leukemia Registry".** Am J Hematol. 2015 Mar;90(3):208-14. *) equally contributing.

IV. Nilsson C, **Hulegårdh E**, Garelius H, Möllgård L, Brune M, Wahlin A, Lenhoff S, Frödin U, Remberger M, Höglund M, Juliusson G, Stockelberg D, Lehmann S. **"Allogeneic hematopoietic stem cell transplantation in patients with secondary acute myeloid leukemia. A population-based study from the Swedish AML registry".** Submitted for publication.

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Abbreviations

ACE	Amsacrine, cytarabine and etoposide
aGVHD	Acute Graft Versus Host Disease
a-HSCT	Allogeneic hematopoietic stem cell transplantation
Auto-HSCT	Autologous hematopoietic stem cell transplantation
AHD-AML	AML with antecedent hematological disease
AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
s-AML	Secondary acute myeloid leukemia
APL	Acute promyelocytic leukemia
ATO	Arsenic trioxide
BCR-ABL	Break point cluster- Abelson
CAR	Chimeric antigen receptor
CBF	Core binding factor
CEBPA	CCAAT/enhancer-binding protein alpha-gene
cGVHD	Chronic Graft Versus Host Disease
CIC	Curatively intended chemotherapy
CK	Complex karyotype
CMML	Chronic myelomonocytic leukemia
CLL	Chronic lymphocytic leukemia
CNS	Central nervous system
CML	Chronic myelogenous leukemia
CR	Complete remission
CRi	Complete remission with incomplete blood count recovery
DIC	Disseminated intravasal coagulation
EBMT	European Group for bone and marrow transplant
FAB	French-American-British
FLAG-IDA	Fludarabine, cytarabine, idarubicine
FLT3	FMS-like tyrosine kinase 3
GDP	Gross Domestic Product
GvHD	Graft versus host disease
HLA	Human leukocyte antigen
HCT-CI	Hematopoietic cell transplantation comorbidity Index

MAC	Myeloablative conditioning
MAR	Missing at random
MDS	Myelodysplastic syndrome
MFC	Multiparameter flow cytometry
MLL	Mixed lineage leukemia
MM	Multiple myeloma
MPB	Matched peripheral blood
MPN	Myeloproliferative neoplasm
MRC	Medical research council
MRD	Minimal residual disease
Mtx	Methotrexate
NATO	North Atlantic Treaty Organization
NPM	Nucleophosmin
OS	Overall survival
PML-RARA	Promyelocytic Leukemia/Retinoic Acid Receptor Alpha
RCT	Randomized controlled trial
RD	Related donor
RT-qPCR	Quantitative reverse transcription PCR
SAALR	Swedish Acute Leukemia Registry
SKL	Swedish association of local authorities and regions
t-AML	Therapy related AML
TKI	Tyrosine kinase inhibitor
TLS	Tumor lysis syndrome
TP53	Tumor protein 53
URD	Unrelated donor
WBC	White blood cells
WHO	World Health Organization

Introduction

Leukemia means “white cells in the blood.” The name comes from the fact that many (but not all) leukemias, or blood cancers, present with a high white blood cell count. There are four major types of leukemias and some rarer ones. The major types are acute myeloid leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML) and chronic lymphocytic leukemia (CLL). A high proportion of very immature cells, called “blasts,” in the bone marrow or blood, defines acute leukemia (AML and ALL). When the proportion of blasts is 20% or more, acute leukemia is considered present.

If untreated, acute leukemia is rapidly fatal, most patients die within months after diagnosis. With appropriate therapy, however, the natural history of acute leukemia can be markedly altered with cure or symptom relief

Historical overview

Discovery and classification of leukemia

On June 14 in 1810, Peter Cullen, born and raised in Glasgow, examined a 35-year-old male patient. Cullen´s patient presented with pronounced abdominal pain and high fever, and was diagnosed with “splenitis acutus”. Cullen treated the patient with bloodletting five times. Interestingly, Cullen observed, that in the first three blood samples, the serum of the blood was like milk in color and consistence. However, the last two samples were normal in appearance (1). Cullen believed that the milky appearance of the blood was an emulsion, formed by the rapid absorption of fat. He treated the patient with calomel (mercury chloride), and the patient appeared to have recovered. A Dr. Hopper examined a sample of blood from Cullen´s patient, and concluded that it consisted of mostly coagulable lymph. At this time, Cullen did not realize the relationship between the enlarged spleen and the findings of the blood, but it´s most probable that he gave a very early description of chronic leukemia.

Velpau made the first accurate description of a case of leukemia in Paris in 1825. The patient was a 63-year old female florist, who had symptoms from an illness characterized by weakness, fever, urinary stones and an enormous hepatosplenomegaly (2). Moreover, Velpau interestingly reported, that his patient´s blood was "thick like gruel such that one might have asked if it were not rather laudable pus, than blood". Velpau's hypothesis was that the blood's peculiar appearance was due to white blood cells and that the cause was anything other than an infection.

A few years later, a pathologist from Edinburgh, John Hughes Bennett, published a report of a patient, John Meredith, aged 28. Treatment included the application of leeches, purgatives and potassium iodide, and the patient´s status improved and he was discharged (3). However, Bennet´s patient was seriously ill and died later with changes in the "color and consistency of their blood" after a few months. Although he did not find any infectious etiology for the changes in the blood, he attributed these changes to "purulent material", and introduced the term "leucocytemia" (4).

The second case of leukemia, published only 6 weeks later, was published by Virchow, a demonstrator in pathological anatomy at the Charité Hospital in Berlin(5). Virchow described a similar case with enlargement of the spleen. By microscopic examination, he concluded, that the proportions between colored and colorless blood corpuscles were approximately the reverse of those in normal blood. Virchow understood that the excess of cells was not purulent matter, but instead originated in the blood. He was unsure of the etiology of his findings and was content to use a descriptive name, "weisses Blut" (white blood), and named the disorder leukemia, derived from the Greek word for white blood. Leukemia then gradually became accepted as a distinct disease, and case reports grew in number. Clinical signs and symptoms and histopathological descriptions of the disease became more detailed, and so did speculation on its etiology. The definition of leukemia was far from precise and not all the reports could be considered as being clinically correct.

Figure 1. Milky serum of the blood form a leukemic patient. The serum of the blood extracted from a leukemic patient demonstrates how the early physicians interpreted that the serum looked milky white, as pus-filled blood (1). Reprinted with permission from Elsevier.

In 1857 Nikolaus Friedreich, a pathologist in Wurzburg, reported a case with a female, aged 46. She presented with signs and symptoms of leukemia, and died 6 weeks later. The rapid progress between presentation of the disease and the patient´s death caught Friedreich´s attention. He was convinced that this was a case of *acute* leukemia, of the lymphatic type. And in 1877 Ehrlich developed a technique to use aniline-based stains on air-dried films of blood, and described differences between normal and abnormal white blood cells (WBC) (6).

From historical records of the early studies of leukemia, it is most probable that none of the physicians could be appointed as "the" discoverer of leukemia. A correct description might indeed be that the early understanding of leukemia was a gradual process. There was also a dispute between Virchow and Bennett. Both claimed they were the first and had the accurate description of the disease. Bennett´s position was, that leukemia was a disease of the blood, and caused by "purulent matter". Virchow however, examined the pus-like substance microscopically and observed a decreased amount of red blood corpuscles in contrast to an increased number of white blood corpuscles (1, 4, 7).

The knowledge then gradually increased with important findings but it was not until 1869, when an important breakthrough in the understanding of leukemia was made, and the disease was connected to the bone marrow by Neumann (1, 8). First attempts to obtain a bone marrow sample by surgical trephine for diagnostic reasons were undertaken in Italy in 1903 by Pianese (9). He punctured the top part of the epiphysis of a femur, and also reported of a case of anemia caused by Leishmania (9).

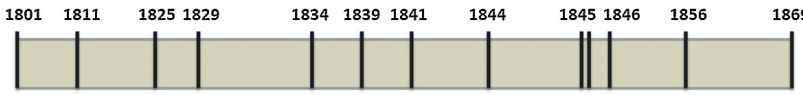
The initial diagnostic difficulties for the physicians in the mid- 19th century might very well be explained by the heterogeneity of leukemia. In fact, there are four major subtypes of leukemia: chronic lymphoid leukemia, chronic myeloid leukemia, acute lymphoid leukemia, and acute myeloid leukemia. These forms can nowadays easily be distinguished based upon morphological differences in maturation stages and lineage commitment. They can also be divided into different risk groups: good, intermediate and poor prognosis, based on genetic aberrations.

Chronic leukemias are characterized by infiltration of the inner organs: hepatomegaly, splenomegaly, and lymphadenopathy. Many of the early case reports, display organ infiltration, which might reflect a chronic leukemia. Sometimes the physician found enlargement of the liver and spleen in combination with the milky appearance of the patient's blood, while in others they only observed that the blood looked pus-filled. This might indeed explain why it took so long before the pieces of the puzzle fell into place.

Early treatment of leukemia

Efficient treatment options were not at all available at the time leukemia was first described. Early attempts included bloodletting. Other therapeutics in the armory of the physician included quinine for fever, morphine and opium for diarrhea and pain, iron for anemia, and iodine for external use as an antibacterial. Arsenic was also used in the form of Fowler´s solution, 1% solution of arsenic trioxide. The first report of the use of arsenic in the treatment of leukemia was by Lissauer, a German physician who administered it to a woman with chronic myeloid leukemia in 1865. She was temporarily restored to health for some months (4, 10). After the discovery of X-rays in 1895 by Wilhelm Röntgen, X-rays was used as a new treatment for leukemia, with initial similar results as those produced by arsenic. Mionot performed an assessment of the efficacy of X-rays in 1924. It showed that x-rays was best used in patients with chronic leukemias and lymphomas. All acute leukemias and a proportion of the lymphomas proved resistant to radiation treatment (11). Since leukemia was now understood be a disease of the blood, the

first case of blood transfusion of a patient with leukemia was carried by Callendar in 1873 at St. Bartholomew´s Hospital in London (4, 12).



1801 **Bichat**; defined leucocytosis
1811 **Cullen** (1st)
1825 **Velpeau**; accurate description of leukemia with the associated symptoms (2nd)
1829 **Collineau** (3rd)
1834 **Duplay** (4th)
1839 **Barth** (published in 1856) (5th)
1841 **Craigie** (published in 1845) (6th)
1844 **Donné**; accurate microscopic examination of the blood from a leukemia patient (histological figures were included in the Atlas published in 1845 7th)
1845 **Bennett**; termed the disease Leucocythemia (with additional figures of the colorless corpuscles) (8th)
1845 **Virchow** (termed leukämie in 1847) (9th)
1846 **Fuller**; patient diagnosed with leukemia during life for the first time (10th)
1856 **Virchow**; distinction between splenic and lymphatic leukemia
1869 **Neumann**; physiology of leukemia connected to the bone marrow

Figure 2. Historical overview. Early reports associated to leukemia, representing the highlights in the early understanding of leukemia(1). Reprinted with permission from Elsevier.

Clinical presentation of acute leukemia

As the early descriptions of acute leukemia by Cullen, Velpau and others, AML presenting symptoms and signs are related to failure of normal hematopoiesis. The leukemic cells have a competitive growth advantage and thus impairs normal hematopoiesis, which eventually leads to bone marrow failure.

This typically results in anemia, neutropenia and thrombocytopenia. One common complaint can be nonspecific fatigue or unspecific malaise, which has been present for a couple of months. Anemia causes pallor and weakness. Fever is common, and

can be caused by an infection secondary to neutropenia, or by the disease itself. Petechiae, epistaxis and ecchymoses are generally caused by thrombocytopenia, and it can be aggravated by disseminated intravascular coagulation (DIC), which is most common in acute promyelocytic leukemia (APL). Organomegaly and lymphadenopathy can be found in AML, but is more common in ALL. Gum and skin infiltration are more common in monocytic variants of AML.

Other extramedullary manifestations of the disease can be found, such as infiltration of the cerebrospinal fluid and central nervous system, which is mostly found in ALL. Other extramedullary manifestations are rare, but one exception is a mediastinal mass in T-ALL, with a risk for acute compression of intramediastinal structures such as the trachea or vena cava superior.

Pathophysiology of acute leukemia

As the molecular mechanisms of acute leukemia are studied, it becomes clear that AL is a heterogenic disease with respect to morphology, immunological phenotype, cytogenetic profile and molecular abnormalities. More recently, findings are differences in methylation profile and microRNA expression (13-15). This heterogeneity is reflected in substantially different responses to therapy.

The molecular pathogenesis of AML is far from fully understood. However, in approximately 40 % of cases there is evidence that the initiating event is acquisition of a balanced chromosomal abnormality (i.e. translocation or inversion). This event is initiated in an hematopoietic progenitor cell and chimeric oncoproteins induce further leukemic transformation and additional cooperating mutations accumulates (13).

The majority of studies of etiology concerns childhood ALL. The cause of ALL is considered multi-factorial. That includes exogenous or endogenous exposures, genetic susceptibility, and chance (16). Epidemiological studies show some support of a hypothesis on early life viral infection influence on leukemogenesis (17, 18).

Risk factors

AML

In the majority of AML cases, there is no direct cause of the disease. There is association with irradiation, chemical exposure (benzene) and obesity (19-21). Interestingly, smoking is not an established risk factor for AML (22). One of the strongest risk factors is age over 65 years, and a vast majority of AML cases are diagnosed in patients aged over 60 years. Other myeloid malignancies, which includes mainly myelodysplastic syndromes and myeloproliferative neoplasms, enhance the risk of disease evolution to secondary AML.

Another important risk factor is previous treatment with chemotherapy (Table 1). Alkylating agents (e.g., cyclophosphamide, melphalan, and nitrogen mustard) predisposes for AML. The latency is 4-8 years and is associated with chromosome 5 and/or 7 abnormalities (23-26). Topoisomerase inhibitors such as etoposide inhibit DNA repair and predispose for AML with a latency of 1-3 years, and are associated with chromosome 11q23 (MLL gene) abnormalities(25).

Table 1. A summary for risk factors for the development of therapy-related AML (t-AML).

Alkylating agent therapy- 5q or 7q deletion, bad prognosis. 4-10 years latency. Often preleukemic MDS.
DNA-topoisomerase II inhibitor therapy (epipodophyllotoxins and anthracyklines). Short latency (2-4 years). MLL translocation. Often no preleukemic phase.
Intense therapy High doses of chemotherapy for prolonged periods as in therapy for Hodgkin's disease and non-Hodgkin's lymphoma Direct correlation between intensity of original therapy and latency period to development of myelodysplasia
High-energy beta-emitters: 32P for polycythemia rubra vera. Similar to alkylating-related AML.
Occupational exposure: benzene, xylene(27)

ALL

Only a very small minority of ALL cases (<5%) are associated with predisposing inherited syndromes such as Down syndrome, Bloom syndrome, Ataxia telangiectasia and Nijmegen breakage syndrome. However, the underlying cause for ALL is in most cases not known. Although tobacco or alcohol use, exposure to pesticides or solvents have all been proposed, but only ionizing radiation has been clearly linked to increased risk of developing ALL (16, 28, 29). Ionizing radiation has been established as a causal exposure for childhood ALL after the 1945 atomic bombs in Japan (30). Other suggested causal exposures include hair dye use and, interestingly paternal but not first suggested causal cause of ALL. The infection theory suggests that ALL may result from an abnormal response to common infection, and children with genetic susceptibility might eventually develop ALL (16).

Incidence

AML

Acute myeloid leukemia, primarily a disease of the elderly, has an incidence of 2-3 per 100,000 per annum in children but rises to 15 per 100,000 with increasing age. Approximately 350 Swedish patients are diagnosed with AML annually. The disease can occur in all ages, but it has its peak incidence in elderly patients in the seventh decade. The mean age at diagnosis is 71 years in Sweden. The gender distribution is equal, but there is a slight male predominance in elderly patients. The incidence does not seem to rise, however since the population is ageing the number of cases will rise and increase the need to take care of AML patients in our healthcare system(34).

ALL

If AML is a disease of the elderly, ALL is primarily a disease of childhood, and the majority of cases occurs in children under the age of 10 at diagnosis. An estimated 100 new cases of ALL occur annually in Sweden. The disease is rare in adults with

an incidence of 0,5 per 100,000 per annum. It is slightly more common in boys and men (explained by T-ALL). The incidence increases somewhat after the age of 40 years, but not as much as for AML. Mean age at diagnosis is 5 years in children, and 51 for adults according to Swedish registries (35).

Classic disease classification of AML

An excess of primitive blast cells in the bone marrow or blood confirms the disease. Originally, the French-American-British (FAB) classification required the blast percentage to be at least 30%, but 20% is the current threshold. The FAB-classification has traditionally been used to develop a common vocabulary, but has very little predictive value since the introduction of genetic markers. Cytochemistry, cytogenetics and immunophenotyping is used to further enhance valuable diagnostic information (36).

The FAB classification has since 2001 been superseded by a new classification by the World Health Organization (WHO) (37, 38). These new classifications are based on accumulating knowledge of cytogenetic and molecular characteristics of the disease. The leukemic blasts may demonstrate aberrant immunophenotype and/or mutations that can discriminate distinct entities of AML. They can also provide prognostic information, and in some cases, define response to treatment.

Advances in cytogenetics and molecular genetics for AML

Over the last decades, great progress has been made in deciphering genetic abnormalities in AML. Approximately 60% of AML-cases have acquired chromosomal abnormalities, which define different subsets of the disease (14, 34, 39).

These genetic abnormalities include balanced chromosomal rearrangements, such as translocations and inversions, which often affect genes that encode hematopoietic transcription factors. Among them Retinoic Acid receptor Alpha due to (15:17(q22;q21), Core binding factor (CBF) complex due to t (8;21)(q22;q22) and

inv(16). Another molecular abnormality is that of epigenetic regulators (e.g. KMT2A [MLL]) due to rearrangements of 11q23(14).

However, adult patients have not a predominance of balanced rearrangements. Instead, particularly in older adults, complex karyotypes predominate. During the last decades, much effort and knowledge has been gained in understanding the molecular basis of AML lacking balanced chromosomal abnormalities, as well as in detail study the 40 % of AML with a normal karyotype (13). An important consequence of this is that the original morphology-based classification of AML is no longer suitable, since entities of the disease are recognized based on cytogenetic and molecular genetic characteristics. Even the current blast threshold of 20% is quite arbitrary, and patients can enter treatment with 10-15 % blasts (high-risk myelodysplastic syndrome). Other consequences are that patients with specific chromosomal rearrangement, e.g. t(15:17)/PML-RARA or t(8;21) can get an AML diagnosis irrespective of marrow blast percentage (13).

Classic disease classification of ALL

A bone marrow aspiration is mandatory and the bone marrow is typically infiltrated with leukemic blasts (>20% of nucleated cells required for diagnosis). It is important to rule out blast transformation of chronic myeloid leukemia, since the distinction between Philadelphia-positive ALL (Ph+-ALL) can be very challenging. In these cases, molecular investigations can be of help- presence of the p190 BCR-ABL1 transcript suggests de novo ALL. The finding of the p210 BCR-ABL1 transcript is less helpful, since it occurs in both scenarios (13, 31). Historically, ALL was classified by the FAB classification, based on morphology. However, this classification was of no prognostic importance. The 2008 World Health Organization (WHO) classification (Table 2) of ALL is based on the cell origin, i.e. B or T cell (40). B-cell disease is further classified in subgroups based on cytogenetic abnormalities.

Table 2. Classification of ALL according to WHO 2008.

B lymphoblastic leukemia/lymphoma
B lymphoblastic leukemia/lymphoma, not otherwise specified
B lymphoblastic leukemia/lymphoma with recurrent genetic abnormalities
B lymphoblastic leukemia/lymphoma with t(9;22)(q34;q11.2), BCRABL1
B lymphoblastic leukemia/lymphoma with t(v;11q23); MLL rearranged
B lymphoblastic leukemia/lymphoma with t(12;21)(p13;q22) TEL-AML1 (ETV6-RUNX1)
B lymphoblastic leukemia/lymphoma with hyperdiploidy
B lymphoblastic leukemia/lymphoma with hypodiploidy
B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32) IL3-IGH
B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3) TCF3-PBX1
T lymphoblastic leukemia/lymphoma

Treatment of AML and ALL

Intentions of treatment and strategy

AL-patients form a heterogeneous group with respect to age-distribution. In addition, especially in elderly patients, comorbidity can affect the treatment decision. First, it must be decided goals of treatment in every patient. In young people, there is often little doubt that the patient will benefit from an intensive approach. On the other hand, in elderly patients, where comorbidity and less responsiveness of the disease per se play a greater role, the clinician must consider

palliative treatment as the most suitable approach for the patient. The impact of age is a well-established factor (41-43).
The initial treatment strategy is to apply chemotherapy in order to induce complete remission (CR). CR is characterized by a bone marrow that appears normal under the microscope, and is functional enough to produce a normal number of circulating cells (13). The common definition of CR for AL is based on five promises: less than 5% blast cells in a cellular bone marrow, a peripheral neutrophil count of at least 1×10^9/L and a platelet count above 100×10^9/L, no signs of extramedullary disease and no need of blood transfusion(13, 14, 34).

In many cases these criteria are met, but the bone marrow shows signs of dysplasia under the microscope. The prognostic impact of this finding is not clear. Other patients show a normal bone marrow after induction treatment, but do not fulfill the criteria for regeneration of peripheral blood count. This subgroup of AML, which is called CRi (CR with incomplete count recovery) might have a poorer prognosis (13). The lack of regeneration of peripheral blood cells can represent a pre-existing dysplastic condition-which can have an adverse effect on prognosis. Another cause of CRi for a particular patient is overtreatment of that patient, which can represent optimum treatment of the underlying leukemia (13).

The development of new cytogenetic and molecular techniques has resulted in more sophisticated methods to detect a low level of remaining leukemia. When all conventional criteria for complete remission are met, it is still possible to detect residual disease i.e. minimal residual disease (MRD).

The two dominating techniques in clinical practice for this purpose is real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR) and multiparameter flow cytometry (MFC), which are capable of detection leukemic cells at a level of 1 in 10^4 - 1 in 10^5 cells.

Use of MFC in order to detect aberrant phenotype in leukemic cells can be used in most patients for characterization of the disease. Molecular markers however, are

not present in all cases of leukemia. The use of these markers is dependent on skilled expertise and high-quality labs, in order to perform the analyses. However, the use of these sophisticated methods has already changed the way we define acute leukemia, remission, response to therapy and prognosis.

Treatment details for AML

The common backbone for treatment of AML for the last decades has been combination chemotherapy with daunorubicin and cytarabine. The normal dosage for daunorubicin is 60 mg/m^2 for three days. In Sweden according to the national guidelines, cytarabine is given at a dose of 1g/m^2x2 for five days for the first two treatment courses (34). The courses given before CR is achieved is normally termed "induction treatment", while the following courses are called "consolidation". Treatment course 3 is normally daunorubicin 60g/m^2 for two days with cytarabine 1g/m^2x2 for five days. The fourth and last course normally consists of cytarabine 1g/m^2 for five days.

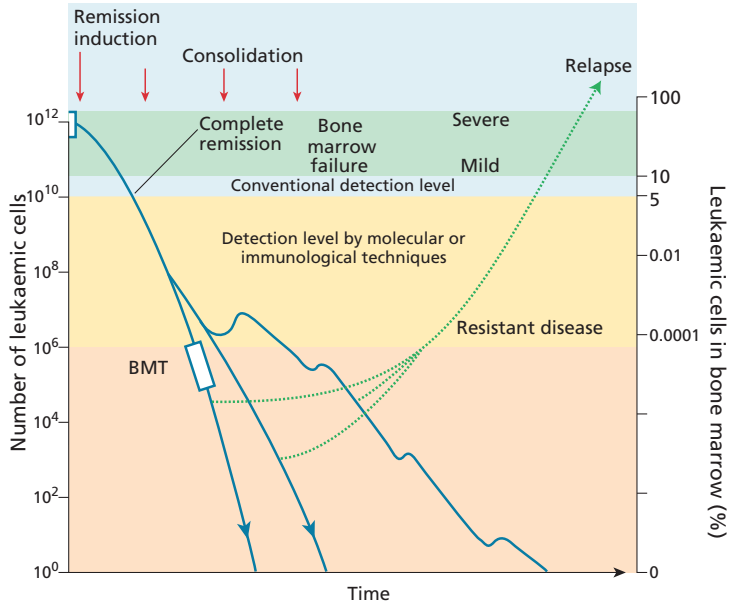


Figure 3. Schematic overview of the treatment of AML. The number of leukemic cells and percentage in bone marrow are illustrated in remission induction and consolidation. TBI-total body irradiation. Reprinted with permission from Wiley.

The goal is to get the patient in complete remission (Figure 3). However comparison between different induction treatments can, besides the rate of complete remission, also be described as the degree of cytoreduction. By achieving greater cytoreduction, at the same rate of complete remission, a therapy can, in theory, result in fewer subsequent relapses. Consequently, adding a third drug in induction treatment has been evaluated. This might be thioguanine or etoposide. However, there is no evident advantage of adding these drugs in combination with cytarabine. In conclusion- little progress has been made for the last decades regarding new and more efficient drugs for effective induction of AML (13, 44-46).

In general, patients who enters remission will do so after one course of treatment. For patients who do not enter complete, or almost complete remission after one course of daunorubicin and cytarabine, should be considered refractory to the drugs. Under these circumstances, an alternative treatment schedule is normally

considered. There is no obvious choice of therapy in these cases (34). One of these alternative regimens is FLAG-Ida (fludarabine/Ara-C/G-CSF and idarubicin), and another is a combination of, cytarabine and etoposide (ACE) (34).

The treatment of most cases of APL differs from usual AML treatment. Initial treatment includes the non-chemotherapy drug all-trans-retinoic acid (ATRA), which is combined with an anthracycline chemotherapy drug (daunorubicin or idarubicin) and arsenic trioxide (34).

Induction results for AML

With the treatment approaches which are outlined above 60 % (cytogenetic high risk)-95% (cytogenetic low risk) of patients under 60 will achieve morphological complete remission. Older patients will enter CR in approximately 50-60% (13, 34, 43). Age is the dominant risk factor as well as performance score at diagnosis. A well-known fact is also that a large proportion of older patients will have a disease characterized by poorer risk biology (includes poor cytogenetics, secondary AML, and drug resistant phenotype). Concurrent occurrence of other serious diseases, comorbidity, is another risk factor for early death (34). For patients who have had an antecedent hematological disorder, e.g. myelodysplastic syndrome (MDS), myeloproliferative disease or the t-AML remission rate will be approximately 20% points lower than in age-matched groups (47).

Consolidation of treatment

Having achieved remission, the focus is to prevent relapse. For younger patients the strategy is normally two or three courses of consolidation treatment. Younger patients in the cytogenetic intermediate or poor risk group, if comorbidity and access to a suitable donor permits, are usually further consolidated with allogeneic stem cell transplantation. There is strong evidence that the most effective way to prevent relapse among these patients is a-HSCT with a Human leukocyte antigen (HLA)-compatible sibling donor, or as shown in recent years, a well matched

unrelated donor (14, 34). It is important to point out the fact that some of the survivors treated with a-HSCT have morbidities that survivors of chemotherapy may avoid, such as loss of fertility, graft-versus-host disease (GvHD) and serious infections.

Factors influencing relapse risk for AML

Cytogenetics and molecular genetics

It is now apparent that AML is a heterogeneous disease with respect to the risk of relapse. In a multivariable analysis, there are a number of factors that can predict the risk of relapse, independent of treatment schedules and the use of allogeneic stem cell transplantation (Table 3). AML-patients with core binding factor (CBF) leukemia, with t(8;21) and inv(16) are characterized by a better prognosis, including higher remission rate, lower risk of relapse and also a higher chance of a second remission after relapse. The patients have a 5-years survival rate of 65-75% in younger patients (13, 48, 49). The same good risk applies to AML with mutated nucleophosmin 1 (NPM1) without mutated FMS like tyrosine kinase 3 internal tandem duplications (FLT3-ITD) and biallelic mutation of the transcription factor CCAAT/enhancer-binding protein alpha-gene (CEBPA) (14).

Patients with acute promyelocytic leukemia, characterized by t (15;17), is a separate entity and uniquely sensitive to treatment with all-*trans*-retinoic-acid (ATRA) and arsenic trioxide (ATO) and is associated with a favorable prognosis. These two good-risk patient groups comprise approximately 25% of patients under the age of 60. In older patients they account for a smaller proportion, but also in this age group is associated with a survival of approximately 35%, as opposed to the over-all survival of only 15-20 % in patients over 60 years (Figure 4).

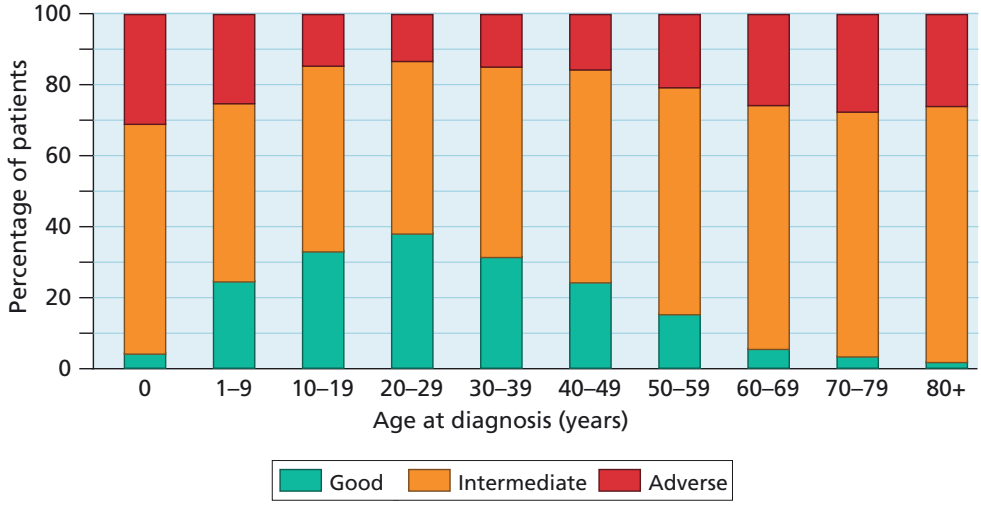


Figure 4. Age specific incidence of cytogenetic risk group in 12,000 patients. Based on data from 6 trials (AML 10-16) in the United Kingdom. Good-risk leukemia is rare in older adults. Reprinted with permission from Wiley.

In younger adult patients, approximately 15 % have cytogenetic abnormalities associated with adverse risk, with lower remission rates as well as a higher risk of relapse. These cytogenetic alternations includes -5/del(5q), -7(del7q), inv (3), t(9;22) and complex karyotype (defined as more than three unrelated changes)(13, 14, 34). It is of most importance that these patients are identified early, since if remission is achieved, it is short-lived. With current chemotherapy regimens, transplantation is the only realistic treatment option, although even that is associated with a high risk of relapse (14, 34). Patients who do not fall into good or poor risk are regarded as standard risk, with a five-year survival of only about 45% in patients under 60 (13).

Table 3. European leukemia Net (ELN) risk stratification 2017 for AML (14).

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); RUNX1-RUNX1T1
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); CBFB-MYH11
	Mutated NPM1 without FLT3-ITD or with FLT3-ITDlow
	Biallelic mutated CEBPA
Intermediate	Mutated NPM1 and FLT3-ITDhigh
	Wild-type NPM1 without FLT3-ITD or with FLT3-ITDlow (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); MLLT3-KMT2A
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); DEK-NUP214
	t(v;11q23.3); KMT2A rearranged
	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); GATA2,MECOM(EVI1)
	t(9;22)(q34.1;q11.2); BCR-ABL1
	-5 or del(5q); -7; -17/abn(17p)
	Complex karyotype (>3 abnormalities), monosomal karyotype
	Wild-type NPM1 and FLT3-ITD high
	Mutated RUNX1 (transcription factor in hematopoiesis)
	Mutated ASXL1 (chromatin remodeling)
	Mutated TP53 (tumor suppressor)

Age and other patient related risk factors

Increasing age, from children to the elderly is strongly associated with a poorer chance of remission as well as relapse and survival. Indeed this is also true considering the obvious differences in comorbidities and a higher proportion of high-risk cytogenetics in older patients (13, 34, 50). Other well-known patient related risk factors include performance status, comorbidities and general health-factors that affect the tolerance to intensive chemotherapy (13, 14, 34, 36). Another important risk factor is AML that is preceded by another malignant hematological disease such as myelodysplastic syndrome , chronic myelomonocytic leukemia (CMML), i.e. antecedent hematological disease AML (AHD-AML), or exposure to cytotoxic therapy or radiation for other disorders (therapy related AML, t-AML) (47).

Treatment details for ALL

Treatment of ALL typically spans over 2,5 years for younger patients, comprising 3 phases: remission-induction, intensification (or consolidation), and maintenance with courses. Interestingly, most of the drugs used were developed before 1970. Allogeneic hematopoietic stem-cell transplantation is a treatment option for patients at very high risk (35). An important feature of ALL treatment is the administration of central nervous system-directed therapy to prevent CNS-relapse.

According to Nordic guidelines (51, 52), induction is typically based on combinations of dexamethasone or prednisolone, anthracyclines and asparaginase. The backbone of consolidation consists of cyclophosphamide, cytarabine, high dose methotrexate, vincristine and mercaptopurine. As mentioned above, CNS therapy and prophylaxis is given frequently with intrathecal methotrexate, sometimes in combination with cytarabine and steroids.

The treatment of Ph+ ALL has been revolutionized by addition of selective tyrosine kinase inhibitors (TKI). These non-chemotherapy agents, such as imatinib and dasatinib, bind to the ATP-binding site of the Abelson tyrosine kinase on the break point cluster region (BCR) on the BCR-ABL1 fusion protein that is characteristic of this ALL-subtype. When this agent is added to traditional chemotherapy, complete remission rates are >90% and event-free survival is superior to the pre-TKI-era (35, 53-57).

Induction results for ALL

Complete remission is obtained in approximately 90 % of ALL-patients. However, almost 40 % of patients will eventually relapse (35, 58) . Risk of ALL recurrence is increasing with age at diagnosis, and prognosis is dismal, particularly in those relapsing after a-HCST. A majority of patients (90%) relapse in the BM, while CNS is the most common extramedullary relapse site (59). After relapse, long-term OS is seen only in 7‑12% of patients, of which a majority is younger (31, 35, 60).

Factors influencing relapse risk for ALL and prognosis

Prognosis in ALL is based on clinical and biological risk factors (28). They are especially useful in deciding postremission treatment strategy. There are some established risk factors for a poor prognosis with current treatment options, as shown in Table 4. Three year survival for different age groups in Sweden are shown in table 5, which illustrates a distinct improvement 1997-2014 , especially in younger patients.

Table 4. Established and emerging risk factors for survival in ALL.

Factor	
Age	>60 years
WBC count	>30x10^9 (B-cell), >100x10^9 (T-cell)
Cytogenetics	t(4;11)(q21;q23) and other MLL rearrangements t(9;22), Philadelphia chromosome. Hypodiploidy (<44 chromosomes).
Therapy response	
MRD	>0.01% at 3-6 months after initiation of therapy
Performance status	WHO poor
Emerging prognostic factors for survival in ALL	
Immunophenotype	CD20 +
Molecular	BAALC FUS ERG IKZF1 Ph-like ALL

Table 5. Three-year survival for ALL in different age groups in Sweden. Number of patients and fraction surviving after three years (35).

Age group	*1997-2001*	*2002-2006*	*2007-2014*
<45 years	96 (51 %)	81 (60%)	175 (77 %)
45-60 years	59 (32 %)	55 (41 %)	76 (51 %)
>60 years	93 (8 %)	88 (15 %)	173 (20 %)
Total	248 (30 %)	224 (37 %)	424 (49 %)

Common strategies for supportive care for AML and ALL

In the majority of cases where full induction treatment is given, the chemotherapy will clear most of the leukemic blasts. However, this potent regimen comes with a cost- often 3-4 weeks of pancytopenia. This substantially increases the risk of infection, mainly bacterial and fungal, and intracerebral hemorrhage. Another cause of death during induction is tumor lysis syndrome (TLS), which is characterized by the massive cell death of leukemic cells due to treatment. The lysed cells´ content is released into the bloodstream causing hyperkalemia, hyperphosphatemia andhyperuricemia. Ultimately acute uric acid nephropathy, seizure, cardiac arrhythmias and, in some cases, death will follow.

It is therefore crucial to support the patient during this period of marrow suppression in order to reduce the number of severe infections and induction death (34). Approximately 10% of patients die within 30 days from start of induction (4-26% depending on age-group) (44). Among the most important is careful monitoring of organ function- renal as well as liver. It is also of importance to regularly monitor coagulation parameters. A central venous line and high quality blood products (mainly erythrocytes and thrombocytes) that are readily available are also important prerequisites to guide the patient safely through the cytopenic phase of induction treatment. TLS is normally prevented by prophylactic oral allopurinol (a xanthine oxidase inhibitor, which inhibits uric acid production) and adequate intravenous hydration. In some cases with a high risk for TLS intravenous

rasurbicase, a synthetic urate oxidase, is given and acts by degrading uric acid (34, 61, 62).

According to Swedish guidelines, antibiotic prophylaxis is recommended with quinolones during the neutropenic phase (neutrophil count $<0,5x10^9/L$). Antiviral prophylaxis with acyclovir against Herpes Simlex virus, as well as antifungal therapy with fluconazole, or posakonazol in case of high risk for Aspergillus infection, is also recommended in neutropenic phase (34).

Aims

This thesis is based on Swedish and Estonian population-based data and includes four publications with three main aims:

First, in paper I and II, the specific **aim** was to compare the incidence and prognosis between Western Sweden and Estonia regarding AL, and the impact of major differences in financial resources for the survival of AL.

The *second* focal point was secondary acute myeloid leukemia. Patients with s-AML often escape inclusion in clinical trials and thus, population-based studies are crucial for its accurate characterization.

In paper III and IV, **aim** was to in-depth explore this disease regarding incidence, prognostic factors and the role for stem cell transplantation in s-AML treatment in a large population-based setting. In paper III, we explored and characterized s-AML in, thus far, the largest population based setting.

Third, to what extent a-HSCT influences survival in s-AML is not thoroughly examined, although S-AML constitutes more than one fourth of AML. No large population-based study on the role of a-HSCT has been performed.

Therefore, in paper IV, the **aim** was to make a profound analysis mainly on how a-HSCT might improve outcome in a large real life study.

Methods

The Swedish acute leukemia registry

"… every hospital should follow every patient it treats, long enough to determine whether or not the treatment has been successful, and then to inquire 'if not, why not?' with a view to preventing similar failures in the future." E. Amory Codman, 1916 (63).

Quality registration to improve medical results is attributed to the Boston surgeon Ernest Amory Codman (1869-1940). Dr. Codman advocated a systematic and prolonged follow up, the so called End-Result Idea (63). However, it was not until several decades later that cancer registries with survival outcome became reality in Scandinavia, the United States and in the United Kingdom (41, 43). Additionally, quality registries on performed procedures were launched, the first one in Sweden was on knee surgery and launched in 1975 (43) (41).

The Swedish Society of Hematology, together with the Regional Tumor Registries, founded the Swedish Adult Acute Leukemia Registry (SAALR) in 1997. The SAALR covers the Swedish population, currently of about 10 million (64-66).

The Swedish Association of Local Authorities and Regions (SKL) supports the registry, and ethics review boards have approved data registration and analysis. Reporting of data on all newly diagnosed adult patients with acute leukemia, de novo or secondary (excluding blastic phase of chronic myeloid leukemia), is compulsory, with three separate registrations (pathology, clinical report to national cancer registry and report to leukemia registry). In 2007, the SAALR was digitalized and separated into the Swedish AML-registry and the Swedish ALL-registry and has constantly evolved to include more specific data on cytogenetics, mutational examinations and choice of treatment. The clinician in charge of the patient register details at diagnosis, after treatment and then a yearly follow up survey for patients still alive. In the case of a-HSCT, the clinician have to report details about this treatment.

Pediatric patients are excluded and reported to the Nordic Society of Pediatric Hematology and Oncology (NOPHO) database.

The AL registry contains 98% of all adult patients diagnosed when compared to the Swedish national cancer registry and includes basic parameters such as performance status (PS) and intention-to-treat (intensive versus no or palliative therapy), risk profile, response to induction therapy and survival follow-up.

Strengths and limitations on population-based registries

The **strength** of my study and other population-based studies is that it gives accurate incidence and mortality rate and numbers. They are also considered useful as a compliment to clinical trials to support clinical decisions in individual patients by analyzing important prognostic factors. Population-based registries have some major advantages to clinical trials (67):

1. To minimize patient selection- i.e. elderly, comorbidity, residence and socioeconomic factors.
2. To answer questions that cannot be answered in a clinical trial (such as incidence and survival trends).
3. Provide additional information by linking to other quality registries.
4. To provide information much cheaper than in randomized controlled trials (RCT).
5. To compare treatment results between different regions and countries.
6. To generate hypotheses that later can be tested in a RCT or in the lab.
7. Provide comparative benchmark reports to hospitals and other caregivers, patients, authorities and funders.
8. No industry sponsorship or conflicts of interests.

There are also some known **limitations** and disadvantages of population-based registries:

1. The details on treatment and outcome is often limited.
2. Monitoring can be scarce or absent due to economical limitations.

3. Reporting to the registry can be slow.
4. Absent or historical control group.
5. Limitations in registry data to correct for confounding factors
6. Missing values or misclassification.

Patients and methods- paper I-II

Papers **I** and **II** are a study on the incidence and survival of acute de novo leukemia in Western Sweden and Estonia for a quarter of a century, 1982-2006 (68, 69).

Important Facts on Estonia

Estonia is a small country in northeast Europe with a population of 1.3 million inhabitants in 2011 and comprises an area of 45,000 km^2. Estonia has been dominated by foreign powers through much of its history. It regained its independence in 1991 after five decades of occupation by the Soviet Union. Estonia set about transforming its government into a parliamentary democracy and reorienting its economy toward market capitalism. Nowadays, the majority of enterprises are privately owned. It sought integration with greater Europe, and in 2004, it joined the North Atlantic Treaty Organization (NATO) and the EU. The Estonian health care system is built on the principle of compulsory solidarity-based insurance, and in about 80% financed through health insurance taxes. Life expectancy (according to Eurostat) in year 2010 was 76 years in total, and 71 for males and 81 for females, respectively. During 2002–2006, about 5% of the gross domestic product (GDP) was spent on health care in Estonia (69).

Important Facts on the Health Care Region in Western Sweden

The Western Swedish health care region has a population of approximately 1.7 million inhabitants in 2011 in an area of 27,000 km^2. Western Sweden has a 1,000-year-long continuous history as a sovereign region, but its borders often changed until 1809. Today, Sweden is a constitutional monarchy with a well-established parliamentary democracy that dates from 1917. Sweden's per capita GDP is among the highest in the world. Of the health care sector, over 95% is financed through national and regional/local taxes and comprises approximately 9% of Sweden's

GDP. However, a growing part of the health care sector is in private or collective management but financed by taxes. Most enterprises are privately owned and market oriented. Roughly, three fifths of the GDP pass through the public sector, including transfer payments for pensions, sick pay, and child allowances, as well as health care and education. Government involvement in the distribution of national income, however, diminished over the last two decades of the 20th century. The total GDP is much higher in Sweden than in Estonia (70). It can therefore be assumed that the quality of Swedish health care in general is higher. As a consequence of the higher Swedish GDP, the total health expenditures per capita are many times higher than in Estonia(69, 71). Life expectancy (according to Eurostat) in 2010 was 82 years in total, and 80 for males and 84 for females, respectively.

Definition of de novo ALs

Patients with secondary AL, i.e. a history of pre-existing myelodysplasia, polycythemia vera, essential thrombocythemia, idiopathic myelofibrosis, chronic myeloid leukemia, or leukemia secondary to chemo-/radiotherapy were excluded from the study. The same patient parameters were registered in identical registers in both countries, and all the identified de novo AL patients were followed until December 31, 2011, i.e. all patients were followed for at least five years. No patients were lost to follow-up in neither the Swedish nor the Estonian cohorts (68, 69, 72-74).

Statistical methods for papers I and II

The incidence in the population was compared with age-standardized incidence rates. The World standard population was used as reference (68, 69).

In order to better compare outcome in the two neighboring countries, survival analyses were carried out by estimating relative survival. The relative survival is the ratio between the observed survival of the patients and the expected survival of a comparable group from the general population. In this way, it is possible to carry out a more leukemia-specific survival analysis. Mortality data of the general

population in Sweden and Estonia were used to estimate expected survival rates for the study populations. For the patients aged 16-64 years, internal age standardizing of the relative survival rates was done using the age distribution of all individuals in the two cohorts (69).

The strs-macro in Stata developed by P. Dickman, E. Coviello, and M. Hills was used for the calculation of the relative survival. A two-sided $p < 0.05$ was considered statistically significant. Demographic data such as the number of persons and the number of deaths by age group, sex, and calendar year for the populations were based on data from Statistics Estonia and Statistics Sweden.

Patients and methods paper III

In paper III- "Characterization and prognostic features of secondary acute myeloid leukemia in a population-based setting: A report from the Swedish Acute Leukemia Registry" we collected data from all patients aged 17 years and above diagnosed during 1997-2006 from SAALR (47). The total Swedish population during the study period was approximately 9 million and the coverage of SAALR was 98 % (43, 44).

In order to identify s-AML cases we selected patients with presence of antecedent myeloid hematological disease, or other malignant disorder and previous treatment with chemo- or radiotherapy. Cytogenetic risk was defined according to the Medical Research Council (MRC) criteria (75, 76)

Definition of s-AML

Secondary AML is a heterogeneous AML group but is usually sub-grouped into two major categories (77, 78). AML related to previous exposure to chemotherapy or radiation (therapy-related AML; t-AML) or AML with an antecedent hematological disorder. In order to make adequate analyses of s-AML, it is vital to use a consistent classification of this rather poorly defined disease. In an attempt to uniformly classify our material, we divided the patients into three disjoint groups: de novo AML, AHD-AML, and t-AML (47).

AHD-AML was defined as patients previously diagnosed with a myeloid hematological disease known to confer an increased risk of AML, mainly including MDS and MPN.

We defined *t-AML* as AML patients previously diagnosed with a malignant or non-malignant disease that had been treated with cytotoxic therapy and/or radiation. All previous chemotherapy treatments were considered, regardless of type, including methotrexate and cyclophosphamide for rheumatic disease. Immunosuppressive treatment using no chemotherapeutic agents was not considered.

Patients developing MDS or MPN between the chemotherapy or radiation treatment for their primary disease and the diagnosis of AML were classified as t-AML. Similarly, patients treated with chemotherapy or radiation for their MPN or MDS were classified as AHD-AML.

Patients and methods paper IV

In order to elucidate the impact and role of allogeneic hematopoietic stem cell transplantation in s-AML, we identified 5661 non-APL AML patients who were diagnosed between 1997 and 2013 (79). Data from the Swedish AML registry (including data from the Swedish acute leukemia registry 1997-2006) was used and merged with data from the Swedish Cancer registry (80, 81). Since patients with APL have a specific treatment and better prognosis, these patients were excluded in this study. To further sharpen our analysis, additional detailed information from local registries and medical records was obtained from all the six centers in Sweden that perform a-HSCT. Survival was updated from the Swedish Population Registry in May 2014.

Statistical methods (paper III and IV)

We used the Mann–Whitney U-test for comparing continuous variables and the Pearson's chi-squared test for categorical data.

Survival was estimated using Kaplan–Meier method and compared using log-rank tests. To test for bias due to baseline variables missing at random (MAR), a sensitivity analysis was performed using multiple imputation for all variables with missing data. Multiple imputation is a general approach to the problem of missing data, which is present in almost all clinical studies (82).We used a predictive mean matching Cox proportional hazards model for multivariable analyses of survival and CR. In order to avoid immortal time bias, a day 200 landmark was used (83).

Results

Paper I:

Incidence

During 2002-2006, the total number of patients over 65 years was 140 in Western Sweden and 114 in Estonia (Table 6). The patients were divided into three groups- AML, ALL and those with a non-classifiable undifferented AL (uAL).The aged standardized incidence rate for AL was similar in both countries, and there was no statistical significance.

Table 6. Total number and age adjusted incidence rates in Western Sweden and Estonia. Rates (per 100,000 inhabitants per year) with 95 % confidence interval of acute de novo leukemia in the population aged >65 years during 2002–2006 in western Sweden and Estonia. Age adjustment to the world standard population was applied.

	Total number		**Age-standardized incidence rate**	
	W. Sweden	Estonia	W. Sweden	Estonia
AML	129	108	7.3 (5.9–8.8)	9.0 (7.2–10.8)
ALL	4	4	0.3 (0.0–0.6)	0.3 (0.0–0.6)
uAL	7	2	0.3 (0.1–0.6)	0.2 (0.0–0.6)
Men	79	47	10.7 (8.1–13.2)	12.2 (8.7–15.8)
Women	61	67	5.9 (4.1–7.6)	8.3 (6.2–10.4)
Total	140	114	7.9 (6.4–9.4)	9.5 (7.7–11.3)

The age-adjusted incidence rates for AL tend to be slightly higher in Western Sweden during the initial 20 years of the study (1982-2001). However, this difference is not present during the last study years 2002-2006 (68).

Treatment

Out of the 140 de novo AL patients from Western Sweden diagnosed at the age > 65 years, 50 (39 %) of the 129 AML patients received curatively intended chemotherapy (CIC). None of the 4 ALL patients and only 1 (14 %) of the 7 u-AL were reported to receive CIC. Complete remission was obtained in 29 (23 %) of the

AML patients and in 1 (14 %) of the uAL subjects(68). As regards the 114 Estonian de novo AL, 25 (23 %) of the 108 AML patients received CIC and 13 (12 %) achieved CR. None of the 4 ALL and 2 uAL patients were reported to receive CIC. Nevertheless, 3 out of 4 (75 %) ALL patients obtained CR.

Relative survival

Relative survival rate at 5-years for AL patients in western Sweden and Estonia was 3,4% and 3,5 %, respectively. In both countries the number of ALL and uAL patients was too few to draw further conclusions.

Table 7 depicts the relative 5-year survival (with 95% confidence intervals) for AML-patient during the 25-years study period. As shown, survival in both countries is low, and even absent in Estonia during the period 1982-1991(68). However, in the last study period there is no difference in relative survival between the countries.

Table 7. Relative survival at 5 years (percent, with 95 % confidence intervals) for de novo AML patients in western Sweden and Estonia diagnosed at the age >65 years 1982–2006.

Western Sweden		Estonia	
1982–1986	0	1982–1986	0
1987–1991	6.3 (2.3–13.4)	1987–1991	0
1992–1996	7.2 (3.2–13.3)	1992–1996	1.8 (0.1–11.0)
1997–2001	2.9 (0.9–7.1)	1997–2001	1.5 (0.1–8.4)
2002–2006	3.7 (1.2–8.6)	2002–2006	3.7 (1.0–9.7)

Paper II:

Incidence

During 2002-2006, the total number of patients under 65 years was 110 in Western Sweden and 87 in Estonia (Table 8). The aged standardized incidence rates were similar for this younger cohort. Age-standardized incidence rates over the years 1982-2001 tended to be slightly higher in Western Sweden, but this finding was absent in the last period 2002-2006(68, 73, 74, 84).

Table 8. Total number of patients and age-standardized incidence rates (per 100,000 inhabitants per year) with 95% CIs of de novo AL in the population aged 16–64 years during 2002–2006 in western Sweden and Estonia (age adjusted to the World standard population).

	Total number		Incidence rate	
	W. Sweden	**Estonia**	**W. Sweden**	**Estonia**
AML	79	69	1.2 (0.9–1.5)	1.4 (1.0–1.7)
ALL	30	18	0.6 (0.4–0.8)	0.4 (0.2–0.6)
uAL	1	0		
Men	58	42		
Women	52	45		
Total	110	87	1.8 (1.4–2.1)	1.8 (1.4–2.1)

Treatment

In the Swedish cohort during 2002-2006, 98 % of patients received CIC, and the total rate of complete remission was 87%. In Estonia, 81% of patients were treated with curative intention, and the total CR rate was 64% (69).

In Western Sweden, 23 (29%) AML patients underwent a-HSCT. Of the Western Swedish ALL patients, 14 (47%) were treated with a-HSCT and two patients underwent auto-SCT. In Estonia, 14 (20%) AML patients and 4 (22%) of the ALL patients were treated with a-HSCT; 1 ALL patient received auto-SCT (69).

Relative survival

For AL patients in western Sweden and Estonia, a total relative survival rate over 5 years during 2002-2006 was calculated to 56 % and 22%, respectively (69).

The corresponding figures as regards AML for western Sweden and Estonia were 58 % and 22%. Similar differences in relative survival for AL were seen when dividing the cohort in three age groups.

For the whole period of 25 years from 1982 to 2006, relative 5-year survival for AL in Sweden increased from 20 to 56 % (Table 9). The Estonian AL-patients showed a corresponding increase from 3 to 22% (69). The survival difference was statistically significant.

Table 9. Relative 5-year survival (%) at 5 years for AL patients in Western Sweden and Estonia aged 16-64 years at diagnosis.

Year	W.Sweden	Estonia
1982-1986	20,4	3,4
1987-1991	26,1	1,8
1992-1996	38,4	4,6
1997-2001	38,9	14,2
2002-2006	55,9	21,5

Results (paper III)

In total, 3,363 patients diagnosed with AML during a 10-year time period between 1997 and 2006 were included. Of these, 2,474 (73.6%) were classified as de novo AML, 630 (18.7%) as AHD-AML, and 259 (7.7%) as t-AML, resulting in 889 (26.4%) cases of secondary AML (Table 10). Gender distribution was uniform in de novo AML, whereas AHD-AML showed a male predominance and t-AML a female predominance (47).

Table 10. Characteristics of 3,363 AML-patients diagnosed between 1997-2006.

	Total	De novo	AHD-AML	t-AML
N	3363	2474 (74%)	630 (19%)	259 (8%)
Gender				
Female	1668 (50%)	1236 (50%)	267 (42%)	165 (64%)
Male	1695 (50%)	1238 (50%)	363 (58%)	94 (36%)
Age				
Median	71	70	73	70
Mean	67.6	66.7	71.4	66.9

The median age at diagnosis in the whole cohort was 71 years (range 17-98 years). There was no statistically significant difference in age between de novo and t-AML (median 70 years). However, patients with AHD-AML were significantly older than de novo AML (73 vs. 70 years). Before the age of 40 years, t-AML constituted about 5% of the AML cases and increased to 10% at ages of 40 and above. AHD-AML is rare below 40 years, but increases to reach its maximum of 25% in patients between the age of 70 and 79 years.

Apart from AHD-AML, which had a slightly poorer PS compared to t-AML and de novo AML, PS within the groups were similar. High-risk cytogenetics was seen in almost half of the patients with t-AML (46%) and was also clearly overrepresented in patients with AHD-AML (40%) compared to de novo AML (26%) ($P<0.001$ in both comparisons). Low-risk cytogenetics was only reported in 2 cases of AHD-AML.

Patients with AHD-AML.

Among 630 patients with AHD-AML (Table 11), the primary disease was MDS in about 2/3 (n=404) and MPN in approximately 1/3 (n= 187). Due to lack of data, we were unable to identify type of AHD-AML in 39 (6%) of cases. As regards MPN patients, 77 (41%) were PV, 44 (24%) ET and 66 (35%) other types, which most probably include myelofibrosis.

The median time between diagnosis of the antecedent hematological disease and diagnosis of AML was 1,6 years overall. In MDS the median latency was 1,1 years and in PV 7,3 years. For ET, the latency before onset of AML was 7,6 years.

Table 11. Type of antecedent hematological disease preceding AML. The predominant AHD is MDS, followed by MPN.

AHD-AML (n=630)		n	%	Median latency in years (range)
MDS		404	(64%)	1.1 (0 - 26.8)
MPN		187	(30%)	
	PV	77	(12%)	7.3 (0.6 - 36.8)
	ET	44	(7%)	7.6 (1.0 - 18.3)
	MPN uns.	66	(10%)	
Unspecified and others		39	(6%)	

Patients with t-AML

Of the 259 patients with t-AML, 222 (86%) had a previous malignancy and 34 (13%) had a history of rheumatoid diseases (Table 12). The most common diagnoses were breast cancer (n=55) and non-Hodgkin lymphomas (n=50), together constituting approximately 40% of the patients with a malignant disease. Multiple myeloma (MM) is the second most common blood cancer in Western countries and accounted for 17 cases of s-AML. Treatment related factors including the use of melphalan have been considered the main cause of the observed elevated risk (85, 86). Female genital cancers such as cervical and ovarian carcinoma were more common compared to male cancers such as prostate and testicular cancer. Some common types of malignancies such as lung cancer and prostate cancer were heavily underrepresented considering their high frequency in the general population (87). Median time to diagnosis of s-AML was 6.2 years overall and 5.8 and 14.3 years for malignancies and nonmalignant diseases, respectively.

Table 12. Overview of diseases preceding t-AML. Median latency between diagnosis of primary disease and AML in years.

THERAPY RELATED AML (n=259)	n	(%)	Median latency in years (range)
Malignancies, n=222 (86%)			**5.8 (0.7 - 49.1)**
Breast ca	55	(21%)	
Non-Hodgkin lymph. incl CLL	50	(19%)	
Uterine/cervical ca	18	(7%)	
Myeloma	17	(7%)	
Hodgkin's lymphoma	13	(5%)	
Colon/rectal/anal ca	11	(4%)	
Ovarial/tubar ca	10	(4%)	
Bladder/kidney ca	7	(3%)	
Skin ca incl m melanoma	7	(3%)	
Testicular ca	5	(2%)	
Lung ca	5	(2%)	
Prostate ca	4	(2%)	
Others	20	(8%)	
Other diseases, n=34 (13%)			**14.3 (0.4 - 44.3)**
Rheumatoid arthritis	18	(7%)	
Vasculitis incl Wegener's	9	(3%)	
Others	7	(3%)	
Not reported, n=3 (1%)			

Treatment and complete remission rate

A total of 1,967 (58%) patients were given intensive induction chemotherapy with the intent to obtain a CR. Curatively intended chemotherapy was significantly less common in t-AML and AHD-AML patients than for de novo AML. This finding was consistent in younger as well as older patients. Intensive treatment was considerably more common in younger patients aged < 65, where it was given to 94% of patients with de novo AML, 69% in AHD-AML and in 82% of t-AML patients.

In patients who received intensive treatment, CR rates were significantly lower for both AHD-AML (39%) and t-AML (54%) compared to de novo AML (72%), as shown in Figure 5.

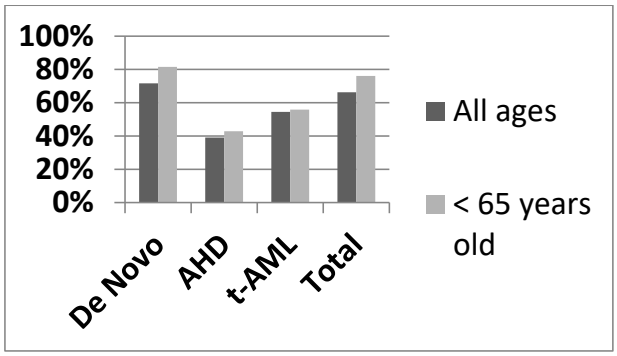


Figure 5. CR rates in patients receiving intensive treatment, all ages and patients <65 years. De novo AML has higher CR rates than AHD and t-AML in both groups.

Survival

Survival rates were significantly worse for AHD and t-AML compared to de novo AML regardless of treatment and age. AHD-AML generally showed worse prognosis than t-AML.

The impact of secondary AML on survival was highly dependent on age. Median survival in de novo AML was 158, 16, and 7 months for patients aged <55, 55–74, and >75 years, respectively, but 7, 7, and 6 months in AHD-AML, and 14, 9, and 8 months in t-AML. Thus, in contrast to de novo AML, where younger patients do fairly well, survival was very poor in younger secondary AML patients.

Both types of secondary AML showed inferior survival compared to de novo AML in each of the three cytogenetic risk groups, indicating that poor outcome in secondary AML is independent of karyotype, as shown in Figure 6.This finding was consistent when analyzing only patients who achieved CR (47).

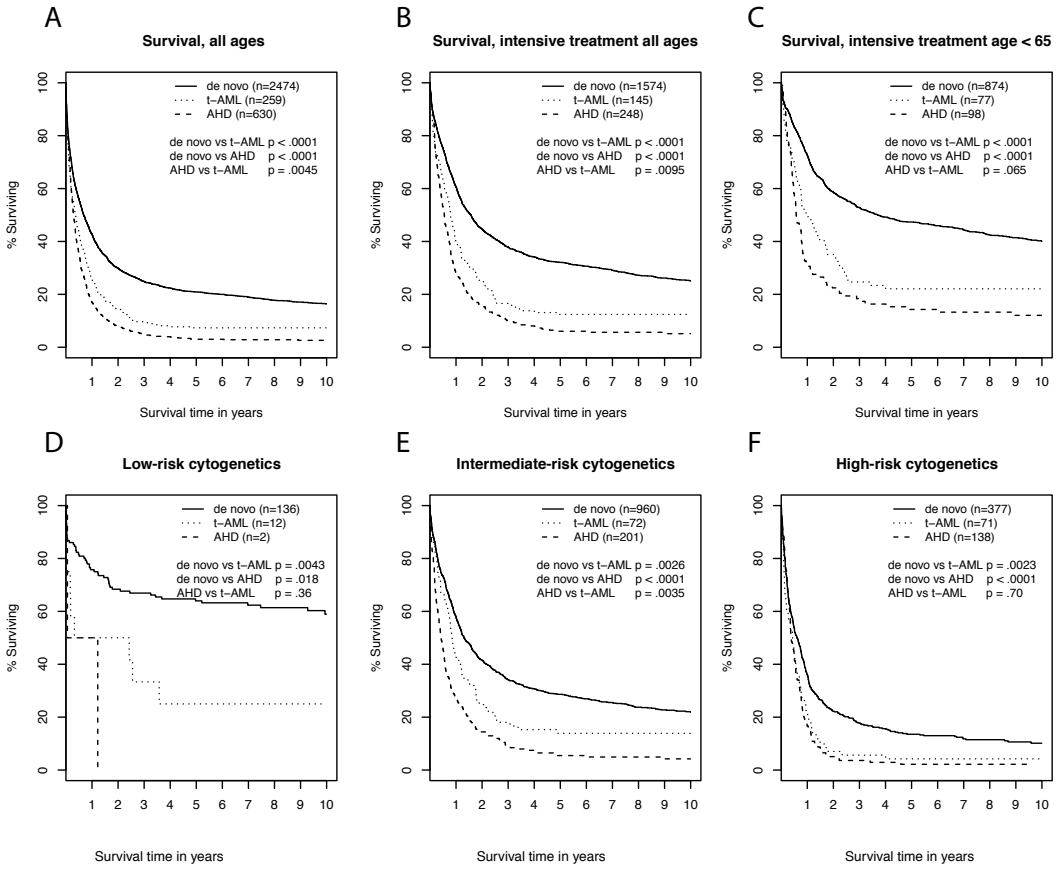


Figure 6. Overall survival. (A) OS irrespective of treatment and age. (B) OS in patients given intensive treatment irrespective of age. (C) OS in patients <6 5 years given intensive treatment. (D–F) OS according to cytogenetics irrespective of treatment.

A multivariable Cox regression analysis showed that both AHD-AML and t-AML were independently associated to poor survival, with t-AML displaying a slightly higher hazard ratio (HR 1.72; 95% CI 1.38–2.15) compared to AHD-AML (1.51; 1.26–1.79).

We also performed a subgroup analysis to evaluate the impact of secondary AML on survival in relation to other prognostic factors. In younger patients (<55 years), secondary AML had a striking and independent effect on survival, whereas in elderly patients, the fact that the patient had secondary AML did not add much prognostic information

Results paper IV

Of the 5661 non-APL patients, we selected the 3337 patients who received intensive induction therapy for further analysis.

Of these patients, 2613 (78%) had de novo AML, 282 (8%) t-AML and 442 (13%) AHD-AML, of which 130 (4%) MPN-AML and 311 (9%) MDS-AML. The median age at diagnosis for intensively treated patients was highest for AHD-AML with 68 years compared to 63 years for *de novo* AML and 65 years for t-AML. The gender distribution in *de novo* AML was 53% males, while there was a male predominance (62% males) in AHD-AML and a female predominance (57% females) in t-AML. De novo AML patients were more likely to achieve CR, with a CR rate of 72% compared to 60% in t-AML and 45% in AHD-AML.

Characteristics of the transplanted s-AML cohort

Of the 3337 intensively treated non-APL patients, 707 (21%) underwent a-HSCT at any stage of the disease. Among *de novo* AML, 576 (22%) underwent a transplant and among AHD-AML and t-AML, 74 (17%) and 57 (20%), respectively.

Of transplanted s-AML patients, 100 (76%) were transplanted in CR1, as were 55 (74%) of AHD-AML and 45 (79%) of t-AML patients (Table 13). Remaining transplants were performed in refractory or relapsed status or in later CRs. The proportion of patients in CR1 that underwent a-HSCT in CR1 was similar between *de novo* AML, AHD-AML and t-AML, i.e. 23%, 28% and 27%, respectively (79).

The cytogenetic risk profile for transplanted patients differed between the groups; intermediate risk patients constituted the majority of the de novo AML patients while adverse risk patients was most common in AHD-AML and t-AML, constituting half of those transplanted patients. It is also notable that we found more favorable cytogenetics in de novo AML (11%), compared to 5% in s-AML.

Of the s-AML patients who were allografted in CR1 39 % received a graft from a related donor (RD) and 61% from an unrelated donor (URD), 37% received a myeloablative conditioning regimen and 63% a non-myeloablative. Stem cell source was peripheral stem cells in 89% of cases. There was no significant difference between AHD-AML and t-AML as regards donor type, conditioning, stem cell source, female donor to male recipient, EBMT score (88) or time from CR1 to a-HSCT.

Table 13. Characteristics of patients with secondary AML who underwent allogeneic stem cell transplantation in first remission. AHD patients are older and adverse cytogenetic risk patients are most common in s-AML patients compared to de novo AML.

		AHD-AML	**t-AML**	***de novo* AML**	**P**
n		55	45	426	
Sex (%)	Male	30 (55)	17 (38)	210 (49)	0.228
	Female	25 (45)	28 (62)	216 (51)	
Age (median [range])		58 [28, 77]	51 [18, 68]	48 [17, 72]	**<0.001**
Cytogenetic risk (%)	Adverse	25 (50)	22 (50)	138 (36)	**0.006**
	Intermediate	22 (44)	17 (39)	236 (61)	
	Favorable	3 (6)	5 (11)	14 (4)	
WBC (mean (SD))		19 (30.9)	19(31.9)	41 (60.8)	**0.023**
Age ≤ 65 (%)	Yes	47 (85)	41 (91)	410 (96)	**0.002**

Survival in transplanted s-AML patients compared to non-transplanted-crude survival

We first aimed to asses "real-world" data on crude survival for s-AML patients. Strikingly, at 5 years after diagnosis, there were no survivors among MPN -AML patients that had not undergone a-HSCT and only 2% and 4% of MDS-AML and t-AML patients had survived without a-HSCT at 5 years. Thus, in patients with s-AML, there is virtually no long-term survival without a-HSCT. A direct comparison of survival rates from time of diagnosis or time of CR between patients being treated with a-HSCT or not at a later point in time is misleading due to immortal time bias (89). Still, we can conclude that the large majority of s-AML patients that are long-term survivors have undergone a-HSCT and the 5-year survival for s-AML patients that have undergone a-HSCT at any time point or disease stage was 32%, 18% and 25% for AHD-AML, MDS-AML and t-AML, respectively. This indicates that there is a significant fraction of long-term survivors among s-AML patients that have been subjected to transplantation.

Landmark analysis comparing HCT with chemotherapy consolidation

Immortal time bias has been found to be quite prevalent in survival studies (89). It is created when there exists a period during which the outcome of interest (e.g. death or relapse) for one of the cohorts cannot possibly occur. To analyze if crude survival reflected a real benefit for transplanted s-AML patients, we performed a landmark analysis. We selected patients <65 years in CR1 and excluded patients with a favorable karyotype. In this landmark analysis, follow up started at 200 days after diagnosis. Patients who died, relapsed or was lost to follow up before the landmark time were excluded. Patients who were transplanted before day 200 were assigned to a-HSCT-group. Patients who had not undergone a-HSCT by day 200 (or never were transplanted), were classified as non a-HSCT.

The comparison favored a-HSCT in both *de novo* and s-AML (Figure 7). S-AML patients with postremission therapy without a-HSCT had a 20% OS at 5 years post

landmark, as compared to 39% in patients who received a-HSCT. In *de novo* AML patients, the corresponding figures were 45% and 60% respectively.

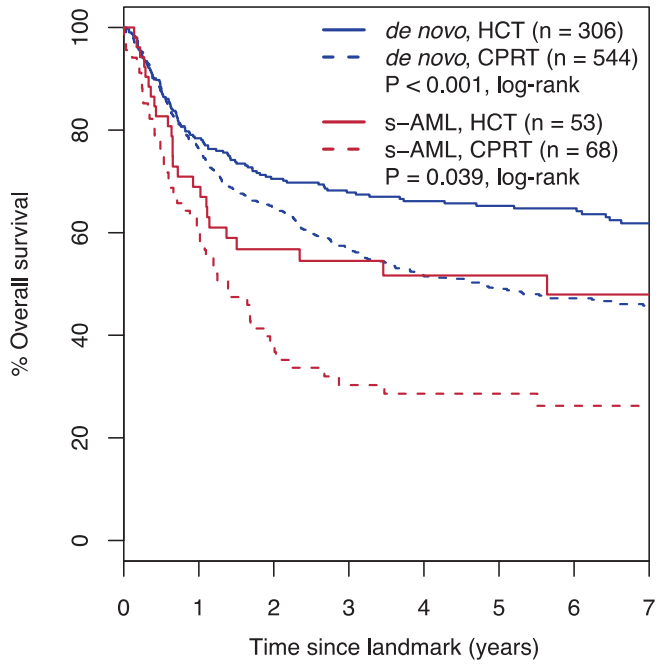


Figure 7. Overall survival after landmark day 200. Allogeneic hematopoietic cell transplantation (HCT) compared to conventional postremission therapy (CPRT) in de novo AML and s-AML patients. Patients older than 65 years and patients with a favorable karyotype are excluded.

Multivariable analysis comparing a-HSCT with chemotherapy consolidation

In the same patient group, a Multivariable Cox regression analysis with a-HSCT as a time-dependent variable and adjusted for the subtype of AML, cytogenetic risk and sex (age was included as a stratum variable) showed an overall mortality hazard ratio for a-HSCT of 0.73 (95% CI 0.64-0.83). Additional independent factors for survival were the AML subtype and the cytogenetic risk (Figure 8A). The impact of

a-HSCT on survival in relation to different subgroups in s-AML is shown in Figure 8B. A-HSCT was beneficial in both t-AML and AHD-AML and had a seemingly stronger survival benefit in patients with adverse risk cytogenetics, male gender and younger age.

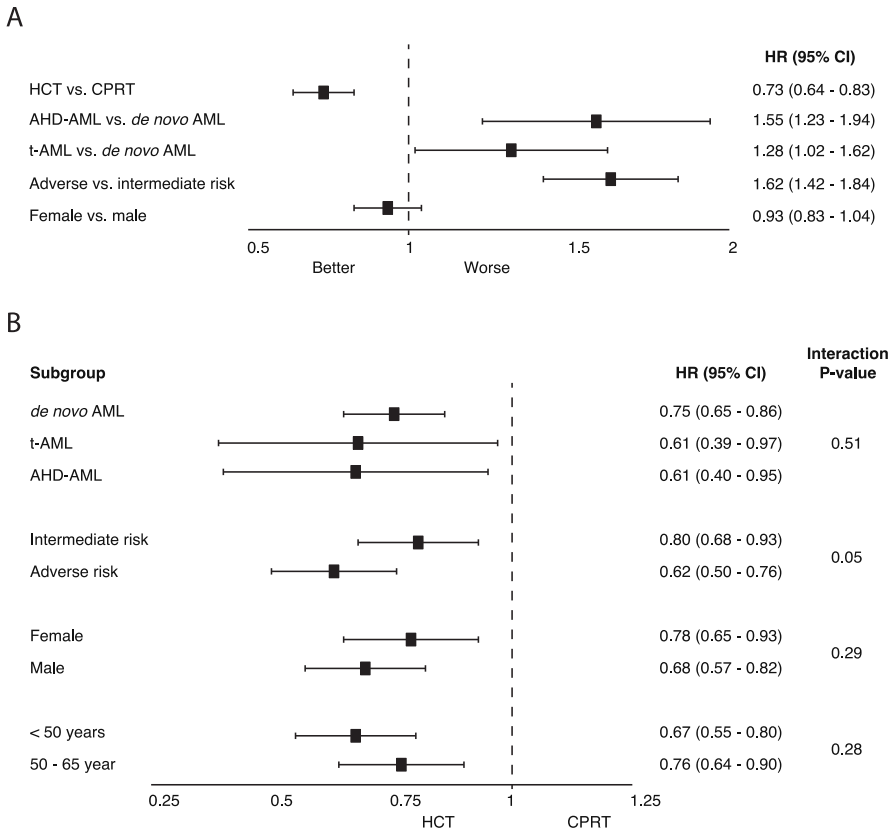


Figure 8. ***Survival hazards analysis and subgroup analysis.*** *Patients older than 65 years and patients with a favorable karyotype are excluded. Multivariable Cox regression analysis in de novo and s-AML combined (A). Forest plot showing the impact of allogeneic hematopoietic cell transplantation (HCT) vs. conventional postremission therapy (CPRT) in subgroups of patients (B). HR, hazard ratio; AML, acute myeloid leukemia; s-AML, secondary AML; t-AML, therapy-related AML; AHD-AML, AML with antecedent hematological disorder.*

Propensity score matching analysis between HCT and chemotherapy consolidation in s-AML

To further validate the comparison between a-HSCT and conventional postremission therapy in patients with s-AML, we performed a *propensity score matching analysis* adjusting for major confounding factors. Patients with CR1 shorter than 90 days were excluded.

Our model matched 45 patients undergoing a-HSCT versus 66 cases treated with conventional postremission therapy (supplemental table S2). Our model confirmed our findings since the projected 5-year survival rate was significantly higher in the a-HSCT group, with 48% compared to 20% in the CPRT group, and we found similar results for relapse-free survival (Figure 9 A and B).

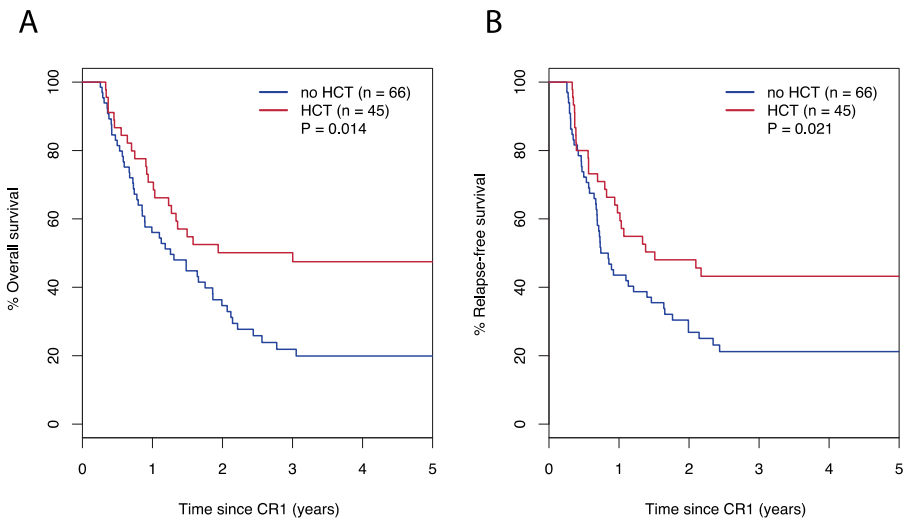


Figure 9. ***Overall survival*** *(A) and relapse-free survival (B) in matched secondary AML patients in first complete remission (CR1) comparing patients undergoing allogeneic hematopoietic cell transplantation (HCT) versus conventional postremission therapy (characteristics of matched patient groups (supplementary table in IV).*

Prognostic factors for outcome after a-HSCT in secondary AML

We analyzed prognostic factors that possibly could predict outcome for the 100 s-AML patients who were transplanted in CR 1 in a multivariable analysis (Table 14). Only the presence of mild cGvHD compared to no cGvHD and absence of aGvHD above grade 1 was significantly associated with better survival.

Table 14. Prognostic factors for 100 s-AML patients transplanted in CR 1. Only patients alive after 100 days were included in the analysis.

Factor	Hazard Ratio	P
Time period 2005-2014 vs. 1997-2004	0.92 [0.32, 2.67]	0.881
URD vs. RD	1.11 [0.48, 2.56]	0.812
MPB vs. BM	2.16 [0.38, 12.22]	0.383
Acute GvHD, grade > 1	3.24 [[1.47, 7.13]	***0.003***
Chronic GvHD, mild vs. none	0.19 [0.06, 0.61]	***0.005***
Chronic GvHD, moderate/severe vs. none	0.49 [0.17, 1.43	0.192

Discussion

Discussion on papers I-II

These two papers reflect how incidence rate, treatment and survival of AL are influenced by profound political and economic changes in two neighboring countries during a quarter of a century. Western Sweden- with a long history and tradition of a democratic society with a market economy, and Estonia, a state that has been under control by the Soviet sphere from 1939 until 1991. This study is unique since it covers a transitional period in Estonia´s history- from planned economy to market economy since its liberation from the Soviet bloc in 1991.

There has been a marked increase in Estonia´s GDP from 5600 USD in 1992 to 20400 USD in 2010. Corresponding figures for Sweden from 19666 USD to 39300 USD. There is also a corresponding large increase in health expenditures per capita from 170 USD to 850 between 1995 and 2010 (70). Corresponding figures for Sweden is an increase from 2287 to 4710 USD.

During the study period for 25 consecutive years 1982-2006 there has been no major changes in the overall structure of specialized hematological care in the two countries(68, 73, 74, 90). Since it is a challenge to standardize data collection and analyses of data, we have had good communication and meetings with our colleagues in Estonia, where we have discussed our material together. The same parameters were used for data collection in the two countries. It was decided that all treatment had to be at the discretion of the hematologist in clinical charge of the patients in the two countries. Other than reporting curative and palliative intention and the use of HSCT, no efforts were made to compare therapeutic strategies between western Sweden and Estonia. In addition, the use of supportive care, such as blood and platelet transfusions, antibiotics and intensive care was not systematically reported or analyzed. Another limitation of this study is that there is no analysis of cause of death, although the use of relative survival partly correct for this.

At the beginning of this 25-years study, we decided to exclude secondary AL, since we found it plausible that there was a higher risk of t-AML in Sweden due to a higher proportion of cancer survivors (73, 74). In addition, it was likely that there were more patients in Sweden who were followed and treated for other hematological diseases such as MPN and MDS, who subsequently developed AHD-AML, which would cause incomparable cohorts.

The age-standardized incidence in Estonia for de novo AL is slightly lower than in Western Sweden during the first 5 years of the study, 1982-1986, for patients 65 years and above. During the following five-year study period this difference gradually disappears, and is completely absent in the last period 2002-2006. The probable explanation for the lower incidence rates in Estonia is thought to be underreporting and underreferral from more rural Estonian hospitals and health centers before the fall of the iron curtain (68). For the younger cohorts aged 16-64 years, there tends to be a higher reported incidence in Western Sweden during the initial years of the study, although this is not statistically significant. Indeed, it seems that Estonia, although having smaller resources than western Sweden, is able to find and diagnose younger AL patients at a lower cost (69).

In the older cohort, it is clear that relative survival in both countries is at a very low level. To illustrate this, we found that there was no relative survival at five years for de novo AML during 1982-1986 in Estonia. In Western Sweden, relative 5-year survival was 0 % in 1982-1986 and 6 % during the years 1987-1991. From 1992-2006 relative survival in both countries was, as expected, very low in both countries, with a slight advantage to survival in Western Sweden. Although the prognosis for AL in elderly patients is known to be dismal, there have been some major improvements in 1- year survival in both countries since the first study period 1982-1986 (68, 72, 84). The interpretation of the poor survival in both these countries, despite the huge difference in wealth and health expenses, is that it is still extremely difficult to cure acute leukemia in the elderly patient. This applies regardless of a country, such has Sweden, has more financial resources.

From our analysis of age-standardized 5-year relative survival rates for AL patients aged 16–64 years, we conclude that Estonia has done real progress with an increase in relative survival from 2 to 22% during this 25-years period. However, survival in Western Sweden has increased from 19% to 58% for the same time-period (69). It is encouraging that survival has increased in Estonia as well as in western Sweden, but a big difference in prognosis for the two countries persists. The distance between Stockholm and Tallinn is approximately 37 miles, and the difference in relative survival for AL patients is 39 percentage points, for the last five-year period 2002-2006. Having discussed this issue, we can identify three main reasons for this appalling difference:

Firstly, curatively intended therapy is given in at least 97% of the ALL patients in both countries, but only in 75% of AML patients in Estonia as compared to 99% in western Sweden. This difference is explained partly by the fact that even in the period 2002– 2006, Estonian patients aged 60 and above were considered too old for curatively intended chemotherapy, which was not the case in Sweden(69).

Secondly, 37 patients (34%) in Sweden and 18 (21%) patients in Estonia were transplanted with a-HSCT, a gap that also might contribute to the differences in survival between the two countries. One important reason for the lower number of a-HSCT in Estonia was the lack of donors, since up to 2005, only family donors could be used.

Thirdly, in scientific discussions with our Estonian colleagues, it became clear that there was a good supply of antibiotics and blood components in both countries. However, access to antifungal treatment was restricted in Estonia, which might contribute to higher mortality. During the years 2002–2006, Estonia could use fluconazole, amphotericin B (not liposomal), and itraconazole. Novel agents such as caspofungin and voriconazole became available later. This might have caused excess mortality in avoidable fungal infections. Further studies, which also cover the use of supportive care and cause of death, might be able to address this question in detail.

Similar studies, which compare cancer survival in Europe, have shown clear variations by geographical area in Europe and the rest of the world, mainly due to socioeconomic factors (91-94). One study of particular interest compares cancer survival in Eastern and Western Germany in the early twenty-first century, after the fall of the iron curtain (95, 96). Prior to the German reunification, cancer survival was significantly lower in East Germany. The authors concluded survival estimates for Eastern and Western Germany to be quite comparable in the second decade after the German reunification. These findings are in contrast with other Eastern and Western European countries, which showed persistent lower survival in Eastern Europe compared to Western Europe during the same time period (92). These latter results are consistent with the differences between Sweden and Estonia. The authors conclude that health differences often result from unequal distribution of education, unemployment, income and other socioeconomic factors (95). The German reunification provided a rapid assimilation of the East German political health care system towards the West German system. The authors of this German study find it encouraging, that if resources are added to an old and ineffective system, comparable levels of cancer survival can be achieved in a decade (95).

To conclude, the age-standardized incidence rate was slightly higher for AL in Western Sweden during the early part of our 25-years study, but it now equals Estonian AL-incidence. Prognosis for elderly patients is disheartening in both countries. For patients aged 16—64 there is improvement in Estonia during our 25-years study period, but a considerable survival-gap persists compared to Western Sweden.

Discussion on papers III-IV

Our studies on s-AML give the first detailed description of AHD-AML and t-AML in a large population-based AML cohort **(III).** They also provide new, important, information of the efficacy and role of a-HSCT in "real-life" (**IV**).

One key finding is the age-dependent prognostic impact of AHD-AML and t- AML. It seems that there is a considerable negative prognostic impact of secondary AML in younger patients. This finding contrasts to a lack of independent prognostic impact in elderly patient. We could also show that a-HSCT surpasses conventional chemotherapeutic consolidation and constitutes the only realistic curable treatment alternative in subgroups of s-AML such as AHD-AML.

The high median age of approximately 70 years in paper III and IV demonstrates their true population-based nature with a median age substantially higher when compared with previous reports on secondary AML-studies (26, 97, 98). One illustrating example of this is a study from Denmark with 630 AML patients with a median age of 67 years, compared to the median age of 71 in our study (47, 97). We also note a slightly higher proportion of t-AML, which can be explained by or quite broad inclusion of the primary disease, including non-malignant diseases treated by cytotoxic chemotherapy or irradiation. In addition, there was no requirement for a minimal time or dosage in order to classify a patient as t-AML. Thus, patients with short exposure or exposure of low doses, e.g. patients with rheumatoid diseases, there is a possibility that there is no causality between the exposure and subsequent development of AML. The association between methotrexate (Mtx) therapy and the development of lymphoma and pseudolymphoma is well established(99). However, the association between low weekly Mtx-doses and t-AML remains unclear, although there is some evidence in the literature (99, 100). Clinical signs in t-AML often includes unexplained pancytopenia and karyotypic abnormalities in marrow cells in patients who received chemotherapy and/or radiation therapy for another disease be pathognomic of

preleukemia. T-AML can in some cases be preceded by t-MDS. These signs can help the clinician to establish a diagnosis of t-AML in unclear cases (101).

In conclusion, occurrence of AML in patients with rheumatoid arthritis in the setting of Mtx-therapy can be coincidental rather than indicative of a causal relationship, which is a limitation of our study that can be investigated in more detail in future work in this field.

Another important finding in paper III is that CR rates were significantly lower in both types of s-AML. This is regardless age, performance status and cytogenetic risk group. We also conclude that these poor CR rates are not explained by higher early death rates for s-AML, since early death was similar in de novo and s-AML. In comparison with a German study, our reported CR rates differ somewhat. We found CR rates of 72% and 54% for de novo and t-AML, as compared to 67% and 63% in the German study (102). However, the German study was not population-based, included younger patients and fewer patients with high-risk cytogenetics.

We also conclude that survival for AHD-AML as well as t-AML was poorer than de novo AML. It is particularly striking, that median survival for younger patients with s-AML equals that of elderly patients with s-AML. This is in clear contrast for de novo AML, where younger patients have a substantially better prognosis. The reason for this might be that s-AML in younger patients is more similar to de novo AML of the elderly. Indeed, this is true in the aspect that high-risk cytogenetics is more common in both s-AML and older patients. However, our multivariable analysis shows that other factors than cytogenetics must contribute to the adverse prognosis in s-AML- i.e. s-AML is not a confounding factor.

Another limitation of our study is that it is partly based on existing data during 1997-2006. Therefore, there is no good coverage on mutational data for NPM1, FLT3 and CEBPA. Consequently, more recently discovered mutations that carry important prognostic information is lacking in this cohort, and constitutes an important opportunity to explore further in clinical and experimental studies.

Another somewhat problematic area in our and other studies on s-AML that needs to be discussed is the rather wide definition that exists regarding s-AML. Secondary leukemia is poorly defined; it is not clear whether the term ‘‘secondary’’ implies causality or merely a sequence in time (103-106). The term secondary leukemia is most common for AML that evolves from a preceding phase of known MDS. However, the required latency interval from the diagnosis of MDS to the debut of AML is rarely stated (14, 77). AML with trilineage dysplasia often occurs after a known myelodysplastic syndrome, and is recognized by the WHO-classification of hematologic malignancies as a clinically important subset of AML (38, 40).

Topoisomerase II inhibitors can lead to MLL gene rearrangements and development of AML 2-3 years after exposure. The same is true for alkylating agents, which cause alterations of chromosome 5 or 7, typically within 5-7 years (14, 77, 107). Classically, blood marrow findings resemble those in MDS, with greater dysplasia(108). In these cases, the term “therapy-related” is descriptive and a severe late complication following cytotoxic therapy. A causal relationship is implied, and genetic pathways have been suggested but the mechanism remain to be proven (109).

The poor survival in s-AML is partly because it is difficult to get the patient into CR. We also show that for patients who achieve CR, the poor outcome persists regardless of cytogenetic risk profile. We conclude that primary treatment resistance is a major reason for the poor outcome in s-AML. It has been shown that s-AML is associated with a poor prognosis (102, 110-112). A population-based Danish study on s-AML raised questions on the negative impact of s-AML (97). In order to explore causality and the independent, prognostic impact of s-AML, it is vital to use multivariable models (97). In clear contrast to the Danish study, our study establishes the independent prognostic impact of both AHD-AML and t-AML, particularly in younger patients.

Another striking feature in our characterization of s-AML is the female predominance found in t-AML. The probable cause of this is that breast cancer, the most common female cancer, has a very good long-time prognosis. It is well known

that t-AML is a side effect after treatment of breast cancer (113-115). Numerous studies have reported an increased risk of AML after treatment of breast cancer. Support for causality has been strengthened by signature chromosomal aberrations and evidence of a dose–response relationship after exposure to alkylating and topoisomerase II-targeted agents(28, 116). Radiation treatment, with or without chemotherapy and granulocyte colony-stimulating growth factors have been reported to be associated with an increased risk of AML in breast cancer survivors (114, 117-119).

Other common cancers are underrepresented due to less intense chemotherapy (e.g., prostate cancer) or high mortality, where lung cancer is an illustrating example. There is an increased risk for patients with MM and monoclonal gammopathy of undetermined origin (MGUS) to develop AML, as previously described (85, 86). During the last decade, many new drugs have been introduced for MM, and the long term risk of developing t-AML in this new era has to be elucidated (85, 86, 120).

Since our characterization of s-AML clearly showed that it is a disease that is, independently, associated with poor prognosis, we decided to in **paper IV** in detail study the efficacy of a-HSCT. At present, recommendations for transplant of s-AML patients are unclear and not included in the current ELN risk stratification (14). In the treatment of AML, it is known that allogeneic stem cell transplantation is the most potent postremission treatment (121-125). However, due to the risks in form of complications that is associated with this procedure, it is important to choose wisely when deciding whom to transplant. This is a decision typically based on cytogenetic and molecular risk, age, comorbidity and the availability of a suitable donor. I have shown in paper III that s-AML is an independent predictor of poor outcome but the extent to which transplantation influence survival in this group is not very clear, since s-AML patients normally are excluded from clinical trials (126). One major strength of our study of the efficacy of a-HSCT is that it includes all Swedish adult patient during 17 years 1997-2013.

Our most important finding as regards a-HSCT is that it is the only realistic curable treatment alternative for patients who develop s-AML. Only a small percentage of s-AML survive more than five years without a-HSCT. Transplanted patients have a crude survival of 18%-32%. In the cohort of t-AML, patients have a minimal chance for cure without a-HSCT (4 %). This contrast to t-AML patients who undergo a-HSCT, of which 25% could be cured with transplantation.

These figures represent survival in real life, in a large population of adult patients. An obvious weakness of this crude survival benefit of a-HSCT versus non-a-HSCT is that these groups are incomparable due to selection. Fit patients who tolerate treatment are selected to a-HSCT, and patients with comorbidities and poor performance status are excluded from this possibility of cure, because of the high transplantation related mortality and morbidity associated with a-HSCT.

To correct for this, we undertook a number of measures to define comparable groups, as has previously performed by Stelljes (127). Our analyses demonstrate that the improvement in outcome for a-HSCT patients remains in multivariable analysis and matching pair models. This is also true for patients in CR1 with non-favorable cytogenetics who had been in CR for at least 90 days. The beneficial effect of a-HSCT for s-AML was significantly better for transplanted patients in our matched analysis of s-AML, with a five year overall survival of 48% vs. 20% for CPRT.

In our study of transplantation related factors that predicted better outcome of a-HSCT in s-AML was the presence of mild cGvHD and absence of severe aGvHD. However, the associations between GvHD and survival should be interpreted with caution, since we lack the debut of GvHD. Somewhat surprising, we found no patient- or AML-related factors such as cytogenetics and age to be significant prognostic markers. This indicates transplantation-related factors as key elements in survival of transplanted AML-patients. A limitation in our transplant-studies is the relatively small number of patients with s-AML who are transplanted. This reduces the statistical power.

Other smaller studies have investigated the role of a-HSCT in s-AML (128-130). Alam et al also found unrelated donor and adverse cytogenetics to be significant risk factors for survival in 65 patients with t-AML/MDS. One limitation of this study is that only 31 of them were given induction treatment and went to CR before a-HSCT (130). Michaelis et al studied 180 de-novo AML patients with 84 s-AML patients transplanted in CR1. Increasing age, higher HCT-CI score, unrelated donor and time of transplant before 2005 identified as negative prognostic variables. However, this Canadian study found no difference in survival between de novo and s-AML (129). A recent large study by Sengsayadeth et al investigates a large cohort of 4997 s-AML patients who received a-HSCT from 2010-2016 (126). Some patient variables such as active disease, adverse cytogenetics, old age, poor performance status and CMV seropositivity were associated with poorer outcome. Although being a large study, it is not entirely population based and does not include a control group of de novo AML patients for comparison. There is also no group that only received chemotherapy, which is another limitation.

The dismal prognosis in s-AML was independent of the overrepresentation of poor risk cytogenetics in s-AML (131). This suggests that s-AML has properties not conveyed by cytogenetics, such as specific mutations that we were unable to analyze. Studies on the role of mutational data for a-HSCT in s-AML are warranted. One important study has shown that evolution of secondary AML is a dynamic process shaped by multiple cycles of mutation acquisition and clonal selection (107). A recent study on MDS and s-AML suggests a particularly poor outcome in patients with *TP53* or *RAS* pathway mutations in combination with complex karyotype (CK) or myelodysplastic/myeloproliferative neoplasms (132). For patients with mutated TP53 or CK alone, long-term survival could be obtained with stem cell transplantation (132). This is important information as it can help the patient and physician make an informed decision, if there is a realistic chance of being cured by transplant.

In summary, we have made two large population-bases studies on the rather heterogeneous disease s-AML. Our first study conclude that s-AML comprises a significant proportion of AML cases. Secondary AML constitutes a high-risk subtype, especially in younger patients, and remains a therapeutic challenge. In our second study on HCT, our data suggests that a-HSCT is the only realistic curative treatment for these patients. A-HSCT should therefore be considered already at diagnosis of s-AML, in order to not lose valuable time.

Conclusions and future perspectives

It is clear that AML is a heterogeneous disease that, in most cases, is a challenge to treat. This is regardless of whether it occurs de novo or secondary to an antecedent malignancy or its treatment. From our studies on acute leukemia in Estonia and Western Sweden, we conclude that socioeconomic factors play a vital role in the management of this malignant disease. In order to improve wellbeing and survival, it is important to intensify international co-operation between countries. Not least to improve survival in countries and regions with low healthcare budgets. The rapid rise in cancer survival in former Eastern Germany after the German reunification shows that this is possible. One example of constructive and fruitful cooperation is treatment of young adults with ALL (51, 52). The Nordic Society of Pediatric Hematology and Oncology ALL 2008 protocol is used for Philadelphia-negative ALL with good results in the Nordic and Baltic countries as well as Iceland. The outcome for these patients is generally favorable, also in the Baltic region. This type of multinational cooperation is something that should be pursued largely in the future.

It is also obvious that healthcare systems in developed countries are struggling with rising healthcare costs (133-135). Thus, there is a need to establish and develop cost-effective systems in order to develop the quality of healthcare as well as efficiency (136). Randomized controlled clinical trials remain the best way to evaluate optimal treatment in acute leukemia, but many clinical questions cannot be answered in such studies. Limited patient numbers, resource availability and conflicts of interest in industry-sponsored trials are just some downsides of randomized controlled trials.

Therefore, population-based studies will still have an important role to play in future studies of acute leukemia. My studies of acute leukemia show that there is room for improvement of the Swedish Acute Leukemia Registry. One important improvement is automatic transfer of vital patient data from medical records to quality registries. Another point to improve is that it should be easier to cooperate

and validate registers, nationally and internationally. A good example of this is the NOPHO-cooperation as regards ALL. This is likely to be particularly important in relatively unusual diseases, such as AML and ALL, in order to reach enough patients to draw true conclusions. The lack of external, independent validation is an issue that the SAALR has to address in future work. A third improvement area is to make it easier to simplify the process to extract data from the different quality registries, while retaining personal integrity for the patient. Finally, it would be a welcome development if it could be easier to link the patient record with information from new molecular methods used in the lab to classify and prognosticate acute leukemia.

For s-AML, I believe that further molecular characterization of the disease is a key to explain the differences in outcome as compared to de novo AML, and might help in selecting treatment modality. Further studies to investigate the optimal conditioning regime, where myeloablative might be the treatment of choice for eligible patients. Optimization of disease control pre-transplant and post-transplant and pre-emptive therapy to reduce the risk of relapse might further increase the prognosis for these patients.

Despite the advances in our understanding of the pathobiology of de novo AML and s-AML, the chemotherapy directed management of these diseases has remained largely unchanged for the last 40 years (137-139). However, the prognosis, especially for elderly patients is dismal, and very few patients are, still today, cured. As is clearly shown in paper I and II, there is also only a small difference in survival between Western Sweden and Estonia in the elderly. Therefore, since the limitations of conventional cytotoxic therapy is clear, there is a need to translate our understanding of the biology of this disease into novel chemotherapeutic and a-HSCT strategies. Important steps in this process is to develop new and more accurate predictors of high-risk disease by utilizing techniques such as next generation sequencing and MRD analysis.

There is also an urgent need to study and incorporate novel targeted therapies for AL. At present, there are more than 20 ongoing trials for new therapies in AML (46, 140-147). However, despite the increase in clinical trials there is a minimal change in current standard of care (36, 148). Among the more promising drugs is isocitrate dehydrogenase (IDH) inhibitors, FLT-3 inhibitors and monoclonal antibodies, such as anti–CD33 (Vadastuximab). Some progress for prognosis and adverse advents for patients above 60 years has also been reported with the liposomal formulation of daunorubicin and cytarabine (CPX-351). This finding was particularly strong in s-AML (36, 140).

Cancer immunotherapy—the science of mobilizing the immune system to kill cancer—has been pursued for more than a century. Yet only recently has this powerful tool finally taken center stage in mainstream oncology and hematology (149-152). On the basis of dramatic results, autologous T cells engineered to express a chimeric antigen receptor (CAR) specific for the CD19 B lymphocyte molecule, have recently been approved in the USA for treatment of refractory pre-B cell acute lymphoblastic leukemia and diffuse large B cell lymphoma (153).

New therapeutic agents hold promise to improve mortality and morbidity for future treatment of AL; however, it is unlikely that any of these compounds, when used as a single drug, will cure the disease. A major challenge is to identify predictors for a response to specific agents, which will allow for the rational design of studies with new combinatorial therapies.

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