Population-based studies on acute leukemias

- lessons from the Swedish Adult Acute Leukemia Registry

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2011

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ISBN 978-91-628-8327-0
http://hdl.handle.net/2077/25488

Printed by Geson Hylte Tryck, Göteborg, Sweden 2011

Abstract

Acute leukemia (AL) is a rare, potentially curable, aggressive neoplasm of hematopoietic origin. AL is a heterogeneous disease and is further subdivided according to clinical and biological features.

The aims were to investigate: i) the incidence and survival of adult AL in regions with socioeconomic differences, ii) the outcome of acute promyelocytic leukemia (APL) with particular emphasis on the course of disease during the first weeks of diagnosis, iii) the disease characteristics and survival in patients aged 10-30 years, with acute myeloid leukemia (AML).

We have investigated these issues in population-based materials; the first two studies were based on data from the Swedish Cancer Registry and the other four studies were based on data from the Swedish Adult Acute Leukemia Registry (SAALR). Comparisons were made with Estonia on incidence and survival of AL and with the Nordic Society for Paediatric Haematology and Oncology (NOPHO) and adult registries in Denmark and Norway for young AML patients.

The incidence of *de novo* AL was higher in western Sweden than in Estonia for patients aged ≥ 65 years. The 5-year relative survival for AL in patients aged 16-64 years was better in western Sweden than in Estonia and there was a significant improvement in outcome in western Sweden during 1982-1996. The differences in survival between the regions had decreased during the period 1997-2001; a dramatical improvement of survival was seen in Estonia, while no further improvement was recorded in western Sweden.

In a population-based study of APL, 29% of patients died within 30 days from diagnosis, 41% due to hemorrhage. The early mortality was higher than described in randomized trials.

There were no differences in survival for young AML patients whether treated according to pediatric or adult treatment protocols. Age was not found to be an independent prognostic marker for outcome.

Studies from population-based materials provide real world data, an important complement to data from randomized trials. Observational studies from population-based registries with high coverage can improve the epidemiological knowledge and can also describe unknown problems that need further investigation in randomized trials.

Key words: Acute leukemia, population-based, incidence, survival, acute myeloid leukemia, acute lymphoblastic leukemia, acute promyelocytic leukemia, outcome, early death

Original papers

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

I. **Wennström L,** Juntikka EL, Safai-Kutti S, Stockelberg D, Holmberg E, Luik E, Palk K, Everaus H, Varik M, Aareleid T, Kutti J. The incidence and survival of acute de novo leukemias in Estonia and in a well-defined region of western Sweden during 1982-1996: a survey of patients aged 16-64 years.
Leuk Lymphoma 2004; 45:915-21

II. Luik E, Palk K, Everaus H, Varik M, Aareleid T, **Wennström L,** Juntikka EL, Safai-Kutti S, Stockelberg D, Holmberg E, Kutti J. The incidence and survival of acute de novo leukemias in Estonia and in a well-defined region of western Sweden during 1982-1996: a survey of patients aged ≥ 65 years.
J Intern Med 2004; 256:79-85

III. Palk K, Luik E, Varik M, Viigimaa I, Vaht K, Everaus H, **Wennström L,** Stockelberg D, Safai-Kutti S, Holmberg E, Kutti J. The incidence and survival of acute de novo leukemias in Estonia and in a well-defined region of western Sweden during 1997-2001: a survey of patients aged ≥ 65 years.
Cancer Epidemiol 2010; 34:24-8

IV. **Wennström L,** Stockelberg D, Safai-Kutti S, Holmberg E, Palk K, Luik E, Varik M, Viigimaa I, Vaht K, Everaus H, Kutti J. The incidence and survival of acute de novo leukemias in Estonia and in a well-defined region of western Sweden during 1997-2001: a survey of patients aged 16-64 years.
Acta Haematol 2011;126:176-185 [E-pub ahead of print]

V. Lehmann S, Ravn A, Carlsson L, Antunovic P, Deneberg S, Möllgård L, Rangert Derolf Å, Stockelberg D, Tidefelt U, Wahlin A, **Wennström L,** Höglund M, Juliusson G. Continuing high early death rate in acute promyelocytic leukemia: A population-based report from the Swedish Adult Acute Leukemia Registry.
Leukemia 2011;25:1128-34

VI. **Wennström L**†, Edslev PW†, Abrahamsson J, Nørgaard JM, Fløisand Y, Forestier E, Gustafsson G, Heldrup J, Hovi L, Jahnukainen K, Jonsson OG, Lausen B, Palle J, Zeller B, Holmberg E, Juliusson G, Stockelberg D, Hasle H.(† equal contribution) Acute Myeloid Leukemia in Adolescents and Young Adults in the Nordic Countries - Outcome According to Pediatric and Adult Treatment Protocols.
In manuscript

List of abbreviations

AL	Acute leukemia
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
APL	Acute promyelocytic leukemia
ATRA	All-*trans* retinoic acid
AuL	Acute undifferentiated leukemia
AYA	Adolescents and young adults
CBF	Core binding factor
CEBPA	CCAAT/enhancer-binding protein α
CMML	Chronic myelomonocytic leukemia
CNS	Central nervous system
CR	Complete remission
DS	Differentiation syndrome
ED	Early death
EFS	Event free survival
FAB	French-American-British
FISH	Fluorescence in situ hybridization
FLT3-ITD	FMS-like tyrosine kinase 3 - internal tandem duplications
GDP	Gross domestic product
ICD	International Classification of Diseases
MDS	Myelodysplastic syndromes
MPN	Myeloproliferative neoplasms
MRD	Minimal residual disease
MTX	Methotrexate
NOPHO	The Nordic Society for Paediatric Haematology and Oncology
NPM1	Nucleophosmin
OS	Overall survival
PCR	Polymerase chain reaction
PML-RARα	Promyelocytic leukemia gene – retinoic acid receptor α
RAEB	Refractory anemia with excess of blasts
RCT	Randomized controlled trial
SAALR	Swedish Adult Acute Leukemia Registry
SCT	Stem cell transplantation
6-MP	6-mercaptopurine
SNOMED	Systematized Nomenclature of Medicine
WBC	White blood cell count
WHO	World Health Organization

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Introduction

Acute leukemias

The word leukemia is derived from the Greek words leukos (= white), and haima (= blood), and means white blood.

History

The study of human blood was facilitated by improved microscopes in the seventeenth century. Van Leeuwenhoek, the Netherlands, first described the red blood cells in 1674 and Lieutaud, France, described the white blood cells in 1749[1]. In 1845 two publications on patients dying from white blood and enlarged spleens appeared[1,2]. Both authors claimed that the transformation of blood had taken place throughout the complete blood system and this was opposed to what was earlier known on pus and inflammation. The earliest published report was from Bennett, Scotland, in 1845, and only a few weeks later the very same year, Virchow, Germany, published a report where he also named the condition "leukemia"[1,2]. However, in a book published in 1844 by Donné, France, he had already described, "*several cases exist with a great excess of white blood cells*" and he suggested that "*the overabundance of white blood cells should be the result of an arrest of development of intermediate cells*"[3]. In 1857, Friedreich, Germany, described a condition with a more rapid development, acute leukemia[1]. Some years later two independent pathologists Neumann, Germany and Bizzozero, Italy, took interest in the bone marrow. The bone marrow had up to then been considered of no interest, just fat that diminished the brittleness of bones and protected the blood vessels. In 1870 and 1872 Neumann published cases of leukemia where the patients had alterations in the bone marrow[1,2]. He stated that leukemia was a disease of the bone marrow. The next advances in understanding leukemia was made by Ehrlich, Germany, who developed new staining methods, and in 1880 the white blood cells could be classified as lymphatic or myeloid cells with subdivision of neutrophils, basophils and eosinophils[1].

John Hughes Bennett (1812 –1875)

Rudolf Virchow (1821 –1902)

Definitions and diagnostic criteria

The definitions of acute leukemia (AL) have changed depending on the state of knowledge and thus during the time period covered by the present thesis (1982-2011).
In 1976, the first generally accepted uniform classification system of ALs, the French-American-British (FAB) was published[4]. It was based on morphological characteristics of the leukemic blasts in association with cytochemical reactivity patterns. This classification was in use until 2001 (after modifications in 1985[5]) when the World Health Organization (WHO) introduced a new classification[6] that also took into account medical history, cytogenetic and immunophenotypic findings. The WHO classification was updated in 2008[7].

1976-1985, the FAB classification of AL, January 1976
AL is according to the FAB criteria[4] separated into myeloid and non-myeloid leukemias. There are six myeloid leukemias, M1-M6 and three "lymphoblastic" leukemias, L1-L3. There were quotes for lymphoblastic in the publication since not all subgroups had been shown to possess surface markers of lymphoid nature. AML was distincted from the two dysmyelopoietic syndromes, refractory anemia with excess of blasts (RAEB) and chronic myelomonocytic leukemia (CMML), both had less than 30% blasts in the bone marrow.

1986-2001, the revised FAB classification of AML, October 1985
AML was in 1985 subgrouped into eight entities[5]; AML M1, M2, M3, M4, M4 with eosinophilia, M5a, M5b and M6. All forms of AML requested more than 30% blasts in the bone marrow. Amendments in 1985[8] and 1991[9], added two entities of AML, M7 and M0. In the revised FAB-criteria of 1985, rare cases, 1- 2% of AL that did not fit exactly in the categories ALL (L1-L3) or AML (M1-M6) were discussed in the publication.

2002-2008, the WHO classification of AL, 2001
The blast threshold for diagnosis of AML is reduced from 30% to 20% blasts in the blood or marrow. However, patients with the recurrent cytogenetic abnormalities (t(8;21)(q22;q22), inv(16)(p13.1q22) or t(16;16)(p13.1;q22), and t(15;17)(q22;q12)) should be considered to have AML regardless of blast counts. AML is divided into four main groups: AML with recurrent cytogenetic abnormalities, AML with multilineage dysplasia, therapy-related AML and other AML. The former AML M3, now defined as AML t(15;17)(q22;q12), is renamed and referred to as acute promyelocytic leukemia (APL)[6,10].
ALL is divided into three main groups: precursor B lymphoblastic leukemia, precursor T lymphoblastic leukemia and Burkitt leukemia. The precursor B and T leukemias could both earlier be found within ALL L1 and L2 of the FAB-classification. The blast threshold in the WHO-classification is 25%, if lower blasts and signs of a mass lesion they should instead be considered as lymphomas[6].
AL of ambiguous lineage is defined as AL in which the morphologic, cytochemical and immunophenotypic features of the blasts lack evidence to classify as of myeloid or lymphoid origin or which have features of both myeloid and lymphoid cells or of both B and T lineages. These AL account for less than 4% of total AL and are divided into undifferentiated, bilineal and biphenotypic AL[6].

2008-2011, the revised WHO classification, October 2007
The current classification of AL is given in Table 1[7].

ACUTE MYELOID LEUKEMIA AND RELATED PRECURSOR NEOPLASMS

Acute myeloid leukemia with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFC-MYH11*
- Acute promyelocytic leukemia with t(15;17)(q22;q12); *PML-RARA*
- AML with t(9;11)(p22;q23); *MLLT3-MLL*
- AML with t(6;9)(p23;q34); *DEK-NUP214*
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q25.2); *RPN1-EVI1*
- AML (megakaryoblastic) with t(1;22)(p13;q13); *RBM15-MKL1*
- *Provisional entity: AML with mutated NPM1*
- *Provisional entity: AML with mutated CEBPA*

Acute myeloid leukemia with myelodysplasia-related changes

Therapy-related myeloid neoplasms

Acute myeloid leukemia, not otherwise specified
- Acute myeloid leukemia with minimal differentiation
- Acute myeloid leukemia without maturation
- Acute myeloid leukemia with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic and monocytic leukemia
- Acute erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyleosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferations related to Down syndrome
- Transient abnormal myelopoiesis
- Myeloid leukemia associated with Down syndrome

Blastic plasmacytoid dendritic cell neoplasm

ACUTE LEUKEMIAS OF AMBIGOUS LINEAGE
- Acute undifferentiated leukemia
- Mixed phenotype acute leukemia with t(9;22)(q34;q11.2); *BCR-ABL1*
- Mixed phenotype acute leukemia with t(v;11q23); *MLL* rearranged
- Mixed phenotype acute leukemia, B/myeloid, not otherwise specified
- Mixed phenotype acute leukemia, T/myeloid, not otherwise specified
- *Provisional entity: Natural killer (NK) cell lymphoblastic leukemia/lymphoma*

PRECURSOR LYMPHOID NEOPLASMS

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); *BCR-ABL1*
  - 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 (hypodiploid ALL)
  - B lymphoblastic leukemia/lymphoma with t(5;14)(q31;q32); *IL3-IGH*
  - B lymphoblastic leukemia/lymphoma with t(1;19)(q23;p13.3); *E2A-PBX1 (TCF3-PBX1)*

T lymphoblastic leukemia/lymphoma

MATURE B-CELL NEOPLASMS
- Burkitt lymphoma/leukemia

Table 1. Classification of acute leukemias according to the World Health Organization, October 2007 (adapted from reference 7)

Incidence

AL is divided into primary (*de novo)* and secondary disease. Secondary to either previous hematological disease, mainly myelodysplastic syndromes (MDS) or myeloproliferative neoplasms (MPN), or to previous treatment with chemotherapy or radiotherapy. The WHO-classification[6] takes this division into account. Old data on incidence are often imprecise because of the lack of congruity between diagnostic codes (according to International Classification of Diseases) of leukemia and diagnostic definitions. As described above definitions have changed and diagnostic codes used for reports have not changed at the same pace. In incidence reports from different registries one often see leukemia incidence reported, and this refers to chronic and acute leukemia jointly. In more recent reports incidence of ALL, AML and APL is reported separately, while primary and secondary AL is often jointly reported. AML accounts for the majority of AL in adults; the reverse is true in children were ALL accounts for the majority of AL. The age distribution of AML and ALL differs, as seen from data retrieved from Surveillance Epidemiology and End Results (SEER) Cancer Statistics[11] (Table 2). The incidence of ALL peaks at the age of 2-4 years and is then stable with a slight increase of incidence in the last decades of human life. The incidence of AML gradually increases with age and peaks at the age > 80 years.

AML Age-Specific Incidence Rates, 2004-2008

Age at Diagnosis	All Races		
	Total	Male	Female
<1	1.6	1.6	1.7
1-4	0.9	1.0	0.8
5-9	0.4	0.5	0.4
10-14	0.7	0.7	0.6
15-19	0.8	0.8	0.9
20-24	0.9	0.8	1.0
25-29	1.1	1.1	1.0
30-34	1.3	1.2	1.3
35-39	1.3	1.3	1.3
40-44	1.7	1.8	1.6
45-49	2.3	2.4	2.3
50-54	3.2	3.5	2.9
55-59	4.4	5.0	3.7
60-64	6.2	7.4	5.1
65-69	9.5	11.5	7.8
70-74	14.3	17.8	11.5
75-79	19.0	25.4	14.3
80-84	22.5	31.0	17.1
85+	22.2	31.8	17.7

ALL Age-Specific Incidence Rates, 2004-2008

Age at Diagnosis	All races		
	Total	Male	Female
<1	2.3	2.7	1.8
1-4	7.7	8.5	6.9
5-9	3.4	3.7	3.0
10-14	2.3	2.6	2.0
15-19	1.8	2.4	1.2
20-24	1.0	1.3	0.7
25-29	0.7	0.9	0.6
30-34	0.7	0.8	0.6
35-39	0.7	0.8	0.5
40-44	0.7	0.8	0.5
45-49	0.7	0.8	0.6
50-54	0.9	0.9	0.9
55-59	0.9	0.9	0.9
60-64	1.1	1.1	1.1
65-69	1.4	1.4	1.4
70-74	1.4	1.6	1.3
75-79	1.6	2.2	1.1
80-84	1.7	1.8	1.6
85+	1.8	2.5	0.5

Table 2. Age-specific incidence rates per 100 000 inhabitants and year of AML and ALL from the SEERS database (adapted from reference 11)

From 1997 to 2006 the Swedish Adult Acute Leukemia Registry (SAALR), registered totally 3897 AL patients (both *de novo* and secondary) and 3205 (82%) of them were patients with non-APL AML, 105 (2.7%), were patients with APL, 472 (12%) were patients with ALL and 107 (2.7%) patients with acute undifferentiated leukemia (AuL) (Paper V).

Clinical signs and symptoms

AL is an aggressive disease with a rapid onset of symptoms. When blastic cells of myeloid or lymphoid origin expand in the bone marrow there is a stop in normal hematopoiesis leading to anemia, thrombocytopenia and neutropenia. The patients most often present with fatigue, bleeding and infections. Leukemic infiltration of organs can give local symptoms. Hyperleukocytosis, a high initial white blood cell count (WBC), is associated with organ failure and risk for tumor lysis syndrome. Typical for patients with APL is a pronounced coagulopathy with hemorrhage and thromboembolic events.

Prognostic factors

Acute myeloid leukemia

Patient-related prognostic factors are age[12,13], comorbidities and previous hematological disease or previous chemo/radiotherapy[14], i.e., if the leukemia is primary or secondary. Leukemia-related prognostic factors are chromosome abnormalities and gene mutations. Chromosome abnormalities are detected in 55% of adult AML patients[15] and is the strongest known prognostic factor[16,17]. Gene mutations can improve prognosis assessment in AML-patients with normal cytogenetics[15]. New chromosome abnormalities and gene mutations are discovered constantly. Knowledge of how these chromosomal abnormalities and mutations affects the prognosis is acquired through large studies[15,17] and has resulted in a risk grouping based on cytogenetic and molecular abnormalities. The cytogenetic/molecular risk groups for adult patients with AML are given in Table 3[18]. Older AML-patients have more often secondary AML and more frequent adverse cytogenetics and thus a worse prognosis[12,13]. In pediatric AML risk grouping differs from Table 3, mainly regarding MLL-rearrangements[19].

Genetic group	Subsets
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFC-MYH11* mutated NPM1 without FLT3-ITD (normal karyotype) mutated CEBPA (normal karyotype)
Intermediate-I	mutated NPM1 and FLT3-ITD (normal karyotype) wild-type NPM1 and FLT3-ITD (normal karyotype) wild-type NPM1 without FLT3-ITD (normal karyotype)
Intermediate-II	t(9;11)(p22;q23); *MLLT3-MLL* cytogenetic abnormalities not classified as favorable or adverse
Adverse	inv(3)(q21q26.2) or t(3;3)(q21;q25.2); *RPN1-EVI1* t(6;9)(p23;q34); *DEK-NUP214* t(v;11q23); *MLL* rearranged -5 or del(5q) -7 abnl(17p) complex karyotype (three or more chromosome abnormalities in the absence of one of the WHO designated recurring translocations or inversions)

Table 3. Standardized reporting of cytogenetic and molecular genetic data in AML (APL is not reported) (adapted from reference 18)

Acute promyelocytic leukemia
According to the European LeukemiaNet APL should not longer be reported jointly with other AML[18]. Indeed, the treatment and prognosis differ completely from other AML. Molecularly confirmed diagnosis is defined as a finding of t(15;17) in cytogenetic analysis, and/or positivity for promyelocytic leukemia gene – retinoic acid receptor α (PML-RARα) with fluorescence in situ hybridization (FISH) or polymerase chain reaction (PCR) analysis[7]. Further cytogenetic alterations do not affect the prognosis[20,21]. Age, comorbidity and high initial WBC[22] are adverse prognostic factors.

Acute lymphoblastic leukemia
Age and WBC at diagnosis are important prognostic factors in ALL[23,24]. In ALL, just as in AML, new cytogenetic and molecular markers affecting prognosis are constantly being reported. In ALL however, they are not yet generally accepted in all protocols as independent prognostic markers[25,26]. Philadelphia-translocation is one cytogenetic marker generally accepted as an adverse prognostic factor[24-26]. The ALL subtype (B, T or mature B) and presence of central nervous system-leukemia (CNS-leukemia) were earlier considered of prognostic importance. These factors are not longer generally accepted as prognostic markers, although some prognostic systems still include them, they do however affect choice of therapy. Treatment response, measured as complete remission[23,24] (CR) and as minimal residual disease (MRD)[27,28] at given timepoints after start of treatment has gradually become an important prognostic tool. MRD is defined as persistence of low levels of leukemic cells in a bone marrow in CR. The levels of MRD are measured with immunophenotyping or PCR methods.

Treatment

The first therapies for leukemia in the nineteenth century included arsenic, quinine, iron, and iodine[1]. During the second world war research on mustard gas resulted in alkylating agents, and research on folic acid resulted in antimetabolites and thus chemotherapy. Clinical trials in 1947-1948 on 16 children with AL showed transient remission in some patients after treatment with the antimetabolite aminopterin[29]. This was the first time when remission of AL was demonstrated in the literature. From 1960 and forward chemotherapeutic treatment for AML and ALL diverged and advances were also made in supportive care, an extremely valuable issue to get AL patients through the periods of extreme cytopenia caused by effective chemotherapy. Advances in supportive care include the ability to transfuse platelets, the continuous improvement of transfusion medicine, the introduction of broad-spectrum antibiotics, antifungal and antiviral therapies, antiemetics, parenteral nutrition and hematopoietic growth factors.

The cornerstones of AL treatment consist of; a) *induction* to induce remission, i.e., to eradicate the disease and achieve a CR, b) *consolidation* to consolidate the result achieved, c) *maintenance* to prevent relapse, and d) *allogeneic stem cell transplantation* (SCT) to prevent relapse. CR is defined as < 5% blasts in the bone marrow and hematopoietic recovery with neutrophil count of $> 1{,}0 \times 10^9$ /L and platelet count of $> 100 \times 10^9$ /L[30]. The results of treatment are often presented as overall survival (OS) at a specified time point.

Acute myeloid leukemia
Cytarabine combined with daunorubicine has been an important part of AML treatment since decades; even after more than 40 years, no other therapy has replaced this combination as standard induction therapy, which gives CR in 60-80%[18] of the patients. Changes in doses of

cytarabine[31-34], addition of a third drug as etoposide[35-37] or gemtuzumab ozogamicin[38], changes between different antracyclines[39-41] and dose escalation of the antracycline[42,43] has been tested in large clinical trials. No convincing evidence for better CR rates and OS without unacceptable toxicity has been put forward. It has clearly been demonstrated that further therapy after attaining CR is mandatory[44]. The number of consolidations and the choice of drugs are not as certain though it is well established that at least one course of high-dose cytarabine as consolidation is advantageous[45-47]. For patients with adverse risk factors for relapse, allogeneic SCT is preferred for preventing relapse[48-50]. Maintenance therapy is not generally used for AML. However, one study has shown that interleukine-2 and histamine as maintenance can prolong leukemia-free survival[51]. OS for AML at 5 years are in a recent large multicentre study reported to be 41-43% (ages < 60 years)[41], and in a recent population-based registry study 50% (ages < 50 years)[13]. Trials on allogeneic SCT for AML report higher OS results, but patients not attaining CR are often not included in these analyses.

Acute promyelocytic leukemia

The first report on all-*trans* retinoic acid (ATRA) treatment given to an APL-patient was published in an international journal in 1986[52]. ATRA causes a configuration change in a fusion gene, PML-RARα, this configuration change induces a modulation of a large number of genes and finally induces terminal differentiation of abnormal promyelocytes[53]. In 1993, a multicenter randomized trial on ATRA versus chemotherapy as treatment for APL was published[54]. Current standard treatment in adult APL-patients is ATRA combined with antracycline and/or cytarabine for induction and 2-3 consolidation courses and thereafter maintenance treatment with ATRA and methotrexate (MTX) + 6-mercaptopurine (6-MP) for 2 years. Clinical trials show CR rates of 90-95% and 10-year OS of 75-80%[55,56].

Acute lymphoblastic leukemia

Multidrug chemotherapy regimens including steroids, vincristine, anthracyclines, cyclophosphamide, cytarabine, asparaginase, MTX and 6-MP for induction, intensification and consolidation with careful scheduling of given therapy together with prophylaxis for the central nervous system are cornerstones in pediatric ALL therapy since the 1980-ties. Maintenance therapy for 2-3 years usually consists of 6-MP, vincristine, steroids and MTX. Pediatric patients in various protocols have a 10-year OS of close to 90% according to recent publications[57-59]. In adults, a widespread treatment regimen named “hyper-CVAD” with hyperfractionated cyclophosphamide, vincristine, doxorubicine and dexamethasone resulted in a long-term survival of 40% in adults with ALL[60]; other adult regimens with similar results did also exist[61,62]. However, in the early 21st century, several studies reported a better outcome among adolescents and young adults (AYA) when treated on pediatric protocols instead of adult protocols[63-65]. These studies have changed the current ALL treatment among adults up to the age of 45 years in many western countries, whereas older ALL patients are treated on less intense regimens. For patients with Philadelphia-chromosome positive ALL, tyrosine kinase receptor inhibitors are added to therapy. Treatment for Burkitt-leukemia differs from other ALL treatment, shorter chemotherapy combined with rituximab gives excellent results, 3-year OS of 89% are reported[66]. Allogeneic SCT are used for ALL-patients with high risk of relapse.

Quality registries

Since the mid eighteenth century Sweden has had a complete registration of the entire population comprising date of birth, date of death and cause of death for every citizen. The personal identification code system with a personal 10-digit-number (personnummer) for all Swedish citizens was introduced in 1947. In 1958 the national Swedish Cancer Registry was established. The registry has a dual reporting system with compulsory reporting of all pathology specimens diagnosed with cancer by pathologists/cytologists and of all patients diagnosed with cancer by clinicians.

In the nineteenth century Florence Nightingale advocated the importance of measuring and follow-up of medical care. Another pioneer was Ernest Amory Codman[67] who in the early twentieth century published data from his own hospital on procedures, results and faults. In Sweden the first medical quality registry to start was the knee prosthetic registry in 1975. A quality registry is a structured collection of data on patients, started to develop and assure quality of care. During the last decades of the twentieth century several medical quality registries have been introduced in Sweden and today more than 70 medical quality registries exist. There are registries on interventions (like knee replacement) on diagnosis (diabetes) or on prevention. There are also registries for rare disorders and malignant diseases.

The Swedish Adult Acute Leukemia Registry

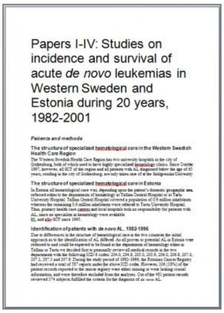
In 1997 a national Swedish Adult Acute Leukemia Registry (SAALR) was started. It was founded by the Swedish Society of Hematology, is supported by the Swedish Board of Health and Welfare, and run in collaboration with the Regional Oncology Centres in each of the six Swedish health care regions, covering populations ranging from 880 000 to 1 900 000, the total Swedish population being 9.4 million inhabitants. All patients registered Swedish citizens and aged $\geq$ 16 years are included; however, patients 16-19 years diagnosed and treated in pediatric settings are not registered within the SAALR. They are instead registered and reported within the registry of the Nordic Society for Paediatric Haematology and Oncology (NOPHO). Reporting of data by the responsible hematologist started on all patients with AL, *de novo* or secondary (blastic crisis of chronic myeloid leukemia excluded), diagnosed from 1997. The registry has 98% coverage of all AL patients when verified against the Swedish Cancer Registry. Briefly, the hematologist in clinical charge of a patient had to fill in 3 questionnaires. Thus, (a) the first form had to be filled in as soon as possible after the diagnosis of AL was established, (b) a second follow-up form had to be filled in at the latest 4 months after diagnosis, and (c) the third form 12 months after diagnosis and subsequently at yearly intervals. All this information was in a computerized fashion forwarded to the Regional Oncology Centre. Thereby, diagnostic as well as therapeutic measures (e.g., a careful past medical history, diagnostic measures regarding cytogenetics, immunophenotyping, FAB classification, chemotherapeutic regimens and stem cell transplantation) were detailed and readily available**.**

In 2007 the SAALR was divided into one registry for AML including APL and AuL, and one registry for ALL. At the same time the registries were modernized with a web-based, electronical reporting system and more diagnostic and prognostic data as cytogenetic and molecular findings at diagnosis were requested in order to more clearly integrate the registry data with the WHO-classification of AL.

Aims

The overall aim of this thesis was to investigate whether the use of a population-based registry with almost complete coverage could provide new knowledge about AL.

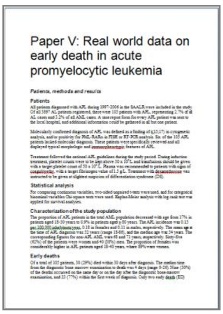
Specific aims:


Papers I-IV: Studies on incidence and survival of acute *de novo* leukemias in Western Sweden and Estonia during 20 years, 1982-2001

Patients and methods

PAPERS I-IV:

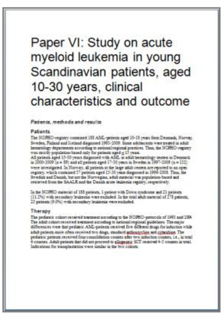
- To investigate if political and socio-economic differences between two neighbouring countries may influence the incidence and survival of AL,
- To study the influence of time trends on incidence and survival.


Paper V: Real world data on early death in acute promyelocytic leukemia

Patients, methods and results

PAPER V:

- To investigate the true outcome in APL patients by examining the proportion of patients who died very early, before diagnosis, prior to treatment or under treatment and to assess risk factors for early death.


Paper VI: Study on acute myeloid leukemia in young Scandinavian patients, aged 10-30 years, clinical characteristics and outcome

Patients, methods and results

PAPER VI:

- To investigate if outcome in young patients, aged 10-30 years, with AML differs between treatment in pediatric or adult protocols,
- To study disease characteristics within this young cohort of AML patients.

Papers I-IV: Studies on incidence and survival of acute *de novo* leukemias in Western Sweden and Estonia during 20 years, 1982-2001

Patients and methods

The structure of specialized hematological care in the Western Swedish Health Care Region

The Western Swedish Health Care Region has two university hospitals in the city of Gothenburg, both of which used to have highly specialized hematology clinics. Since October 1997, however, all SCT of the region and all patients with AL diagnosed below the age of 65 years, residing in the city of Gothenburg, are only taken care of at the Sahlgrenska University Hospital. Since the mid 1980-ties six county hospitals have had access to at least one, frequently two or three, specialists in hematology. Thereby, some non-university hospitals have been able to take care of most of their AL patients, whereas other hospitals have referred their patients. During the study period of 1982-2001 the number of AL patients referred from local hospitals to university hospitals has steadily decreased.

The structure of specialized hematological care in Estonia

In Estonia all hematological care was, depending upon the patient's domestic geographic area, referred either to the departments of hematology at Tallinn Central Hospital or at Tartu University Hospital. Tallinn Central Hospital covered a population of 0.9 million inhabitants whereas the remaining 0.6 million inhabitants were referred to Tartu University Hospital. Thus, primary health care centres and local hospitals took no responsibility for patients with AL, since no specialists in hematology were available at the different county or local hospitals. SCT were carried out in Tartu, auto-SCT since 1993, and allo-SCT since 1995.

Identification of patients with *de novo* AL, 1982-1996

Due to differences in the structure of hematological care in the two countries the initial approach as to the identification of AL differed. As all proven or potential AL in Estonia were referred to and could be expected to be found at the departments of hematology either in Tallinn or Tartu we decided first to personally review all medical records in the two departments with the following ICD-8 codes: 204.0, 204.9, 205.0, 205.9, 206.0, 206.9, 207.0, 207.2, 207.3 and 207.9. During the study period of 1982-1996, the Estonian Cancer Registry had received a total of 587 reports under the above ICD codes. However, 106 (18%) of the patient records reported to the cancer registry were either missing or were lacking crucial information, and were therefore excluded from the analyses. Out of the 481 patient records reviewed 374 subjects fulfilled the criteria for the diagnosis of *de novo* AL.

In western Sweden the Cancer Registry identified a total of 1059 patients with the above mentioned International Classification of Diseases-codes (ICD-codes) during 1982-1996. Thereafter, we scrutinized all patient records at the different hospitals. Out of these patient records, a total of 117 (11%) had to be excluded from the analyses for the same reason as above (missing or lacking crucial information). A total of 636 patients out of the remaining 942 complete patient records could be classified as *de novo* AL.

Identification of patients with *de novo* AL, 1997-2001

The SAALR was introduced in 1997, and reporting started from 1 January 1997 on all Swedish patients with AL, *de novo* or secondary. In order to evaluate the reliability of the SAALR we compared the data for all 384 patients with AL diagnosed in the Western Swedish Health Care Region during 1997-2001 with the national Cancer Registry as well as with the national Death Certificate Registry. It was shown that there was consistency between the registers, and that the SAALR had very good (100%) coverage. Since the aim was to delineate only patients with *de novo* AL we excluded 94 patients with either a history of pre-existing hematological disease, i.e., MDS and MPN, or a former malignant disorder treated with chemo- or radiotherapy. To validate the data of the SAALR a random sample comprising 29 out of the remaining 290 (10%) patient records were carefully reviewed. It was shown that only one of the 29 patients had erroneously been misdiagnosed as *de novo* AL, whereas the correct diagnosis was secondary AL. For the remaining 28 patients the diagnosis of *de novo* AL was correct. It was therefore considered that the reliability of the SAALR data was high and could be employed in the study.

For the analysis of the Estonian cohort we decided to enter all Estonian AL patients diagnosed from 1 January 1997 and onwards into a registry identical to SAALR. Therefore, in collaboration between colleagues in Gothenburg and Estonia the forms were translated word for word into Estonian. For each patient with AL the hematologist in clinical charge filled up the first questionnaire after the diagnosis of AL was established, and subsequently the other two forms were filled up as for the Swedish patients. Finally, after a meticulous review of each medical record only subjects with *de novo* AL were selected for the final analysis.

The procedures were approved by the Ethical Committee of Human Experimentation in Estonia as well as the Ethical Committee in the Western Swedish Health Care Region.

Follow-up

In the first study period, 1982-1996, the identified *de novo* AL patients were followed until 31 December 2000. In the Swedish material no patient was lost during follow-up. In the Estonian material three patients aged 16–64 years were lost during the follow-up period and were therefore excluded in the survival analyses.

In the study period of 1997-2001, the identified patients were followed until 31 December 2006. No patients were lost to follow-up either in the Swedish or Estonian materials.

Statistical Methods

The incidence in the populations was compared by use of age-standardized incidence rates. The weights of the 5 year age groups for ages 15-64 and 65-99 years in the World standard population were used as reference population. Exact confidence interval for the incidence rate ratio was calculated according to Rothmann[68].

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. Mortality data of the general population in Sweden and Estonia were used to estimate expected survival rates for the study populations. The mortality data contained the probability of death for single year age groups for both sexes in one-year calendar periods. To compare and test relative survival rates between patient groups and estimate the relative risk a Poisson regression model adjusted for year of follow-up and for age at diagnosis was used[69]. Demographic data as number of persons and deaths by age group,

sex and calendar year for the populations were based on data from Statistics Estonia and Statistics Sweden.

Results

Papers I-II: Over the years 1982-1996 the total number of *de novo* AL patients in the Estonian population aged ≥ 16 years was 374 (167 males and 207 females), the corresponding figure for the western Swedish population being 636 (322 males and 314 females). A yearly age-standardized incidence rate for *de novo* AL of 1.9 per 100 000 inhabitants for Estonia and 2.4 for western Sweden could thereby be calculated.

Papers III-IV: Over the years 1997-2001 the total number of *de novo* AL patients in the Estonian population comprising all patients ≥ 16 years was 158 (73 males and 85 females); the corresponding figure for the western Swedish population was 290 (153 males and 137 females). A yearly age-standardized incidence rate for *de novo* AL of 2.3 per 100 000 inhabitants for Estonia and 3.0 for western Sweden could thereby be calculated.

For the joint studies (Papers I-IV) the identified patients were subdivided into AML, ALL and AuL while no distinction for APL was made. At the time for the first studies 1982-1996, treatment was the same for APL and AML, since ATRA was not yet introduced. Specific treatment guidelines for APL including ATRA did not come into routine use in Sweden until the mid 1990-ties.

Incidence of *de novo* AL in the populations aged 16-64 years, 1982-2001

Table 4 shows the total number of *de novo* AL patients subdivided into AML, ALL and AuL, aged 16-64 years, studied over four consecutive 5-year periods 1982-2001 in western Sweden and Estonia.

	Western Sweden				Estonia			
Years	**Total AL**	AML	ALL	AuL	**Total AL**	AML	ALL	AuL
1982-1986	**91**	65	21	5	**76**	48	14	14
1987-1991	**86**	63	20	3	**73**	44	7	22
1992-1996	**105**	69	28	8	**88**	61	15	12
1997-2001	**125**	95	28	2	**83**	58	22	3

Table 4. Total number of acute *de novo* leukemias, in the population aged 16-64 years, during four consecutive 5-years-periods

Table 5 shows joint information regarding age-standardized incidence rates for total *de novo* AL, AML and ALL in the population aged 16-64 years over four consecutive 5-year study periods 1982-2001 in western Sweden and Estonia. It is seen that the age-standardized incidence rate was higher for the Swedish as compared to the Estonian population. There was, however, no statistical difference in incidence between the two regions. In both regions there was a statistically not significant tendency of increasing incidence of AL and AML rates over time.

	Western Sweden			Estonia		
Years	**Total AL**	AML	ALL	**Total AL**	AML	ALL
1982-1986	**1.71 (1.35-2.07)**	1.19 (0.90-1.49)	0.41 (0.23-0.58)	**1.48 (1.13-1.82)**	0.91 (0.65-1.18)	0.28 (0.13-0.43)
1987-1991	**1.61 (1.27-1.96)**	1.15 (0.87-1.44)	0.41 (0.25-0.59)	**1.39 (1.07-1.71)**	0.81 (0.57-1.05)	0.13 (0.03-0.24)
1992-1996	**1.94 (1.56-2.32)**	1.20 (0.91-1.48)	0.59 (0.37-0.82)	**1.63 (1.28-1.97)**	1.11 (0.82-1.39)	0.29 (0.14-0.45)
1997-2001	**2.17 (1.78-2.57)**	1.58 (1.25-1.90)	0.57 (0.35-0.79)	**1.77 (1.38-2.16)**	1.18 (0.86-1.48)	0.53 (0.30-0.76)

Table 5. Age standardized (to the World standard population) incidence rates (patients per 100 000 inhabitants per year) of acute *de novo* leukemias, in the population aged 16-64 years, during four consecutive 5-year periods

Incidence of *de novo* AL in the populations aged ≥ 65 years, 1982-2001

The age-standardized incidence rates of AML were considerably lower in Estonia (3.8/100 000/year) than in Sweden (6.6/100 000/year) during the study period of 1982-1996, and the incidence rate ratio between Estonia and Sweden for AML was 0.66 (99% confidence interval: 0.51–0.87). In 1997-2001 the difference in incidence rate for *de novo* AL patients between the two countries reached statistical significance (p=0.003). For AML patients the age-standardized incidence was 6.4/100 000/year for Estonian patients, and 9.2/100 000/year for the Swedish patients (p=0.052). Table 6 provides yearly incidence rates for *de novo* AL in both regions over the study periods. ´

	Total AL	
Years	Western Sweden	Estonia
1982-1986	7.03 (5.56–8.50)	3.35 (2.03–4.68)
1987-1991	7.42 (5.95–8.89)	5.83 (4.11–7.55)
1992-1996	9.51 (7.81–11.2)	6.80 (5.10–8.51)
1997-2001	10.70 (8.80-12.5)	7.18 (5.53-8.83)

Table 6. Age standardized (to the World standard population) incidence rates (patients per 100 000 inhabitants per year) of acute *de novo* leukemias, in the population aged ≥ 65 years, during the study periods

Table 7 gives the absolute numbers of patients with AL, AML, ALL and AuL for the two populations aged ≥ 65 years during the two study periods of 1982-1996 and 1997-2001, respectively.

	Western Sweden				Estonia			
Years	**Total AL**	AML	ALL	AuL	**Total AL**	AML	ALL	AuL
1982-1996	**354**	290	30	34	**137**	96	13	28
1997-2001	**165**	146	11	8	**75**	67	6	2

Table 7. Total number of acute *de novo* leukemias in the population aged ≥ 65 years during the two study periods

Relative survival for patients aged 16-64 years, 1982-2001

The details for relative survival in the whole group of *de novo* AL patients aged 16-64 years in each of the two regions and for the two different study periods (1982-1996 and 1997-2001, respectively) appear in Papers I and IV. The difference in relative survival of *de novo* AL between the two regions was highly significant ($p<0.001$) during both study periods. The survival for Estonian patients with AML and ALL were similarly inferior to the Swedish results in 1982-1996. In 1997-2001 the relative survival in ALL was rather similar for Swedish and Estonian patients (Figure 1), and the difference in the total AL material was thus caused by lower relative survival for AML in Estonia. In both regions the number of patients with AuL where to few to allow meaningful comparisons.

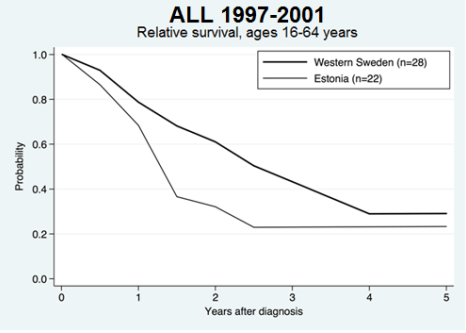


Figure 1. Relative survival for the western Swedish and Estonian ALL patients, aged 16-64 years, during the period 1997-2001

Over the three consecutive 5-year periods of the first study (1982-1996) the relative survival at 5 years after diagnosis increased significantly ($p<0.05$) in western Sweden, whereas no change in relative survival was observed in the Estonian patients over the same time period. In the study period of 1997-2001 the results were inverse with no continued improvement of relative survival in western Sweden (Figure 2), while a dramatical improvement of relative survival was evident in Estonia (Figure 3).

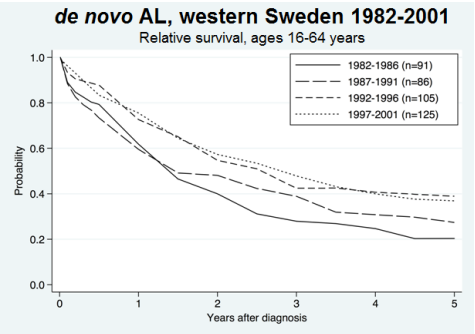


Figure 2. Relative survival for *de novo* AL patients aged 16-64 years in western Sweden during 4 consecutive 5-year periods, 1982-2001

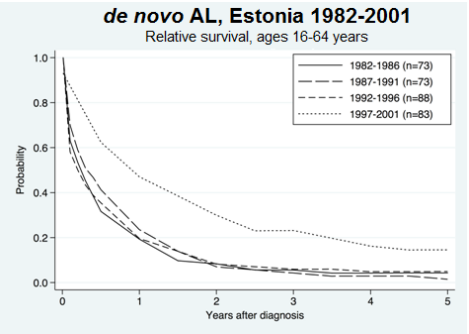


Figure 3. Relative survival for *de novo* AL patients aged 16-64 years in Estonia during 4 consecutive 5-year periods, 1982-2001

As regards treatment strategies in the two countries in the study of 1997-2001 we investigated the intention of treatment in patients aged 16-64 years (Table 6). The vast majority of patients with ALL in both regions were treated with curative intention, i.e., they were given full dose chemotherapy. With respect to relative survival the results for ALL were also rather similar in between the two countries. As compared to western Sweden, in Estonia a substantial amount of the AML patients were considered not eligible for curatively intended chemotherapy. Thus, 93% of the western Swedish AML patients were treated with curative intention compared to only 71% of the Estonian AML patients, which consequently influenced the rate of CR as seen in Table 8.

W.Sweden	Patients	Cur.int.	CR	**Estonia**	Patients	Cur.int.	CR
AML	95	88 (93%)	70 (74%)	**AML**	58	41 (71%)	25 (43%)
ALL	28	28 (100%)	25 (89%)	**ALL**	22	21 (95%)	18 (82%)
uAL	2	1 (50%)	1 (50%)	**uAL**	3	2 (67%)	1 (33%)
Total AL	125	117 (94%)	96 (77%)	**Total AL**	83	64 (77%)	44 (53%)

Table 8. The number of patients, aged 16-64 years, with *de novo* AML, ALL, uAL and total number of acute *de novo* leukemias in western Sweden and in Estonia in whom it was stated that the intention was curative (Cur.int.) chemotherapy upfront and the number of patients in whom complete remission (CR) was achieved during 1997-2001

Relative survival for patients aged ≥ 65 years, 1982-2001

The relative survival at 1 year for **AML** patients studied 1982-1996 was considerably higher for patients from western Sweden compared to Estonian patients (Table 9). It was also seen that the 1-year relative survival for the Estonian AML patients improved considerably during the last study period (1997-2001). It was also shown that the relative survival rate for the Swedish AML patients at 1 year did only improve marginally, from 25.4% to 27.7%. The relative survival at 3 or 5 years did not improve and only few patients in both regions experienced long term survival. For patients with ALL there was no difference in relative

survival during any of the study periods and at no time point. The difference of survival in total AL patients thus origins from the difference in survival of AML.

	Years after diagnosis	Relative survival rate 1982-1996		Relative survival rate 1997-2001	
		Western Sweden	Estonia	Western Sweden	Estonia
AML	1	25.4 (20.6-31.0)	8.8 (4.8-14.6)	27.7 (20.5-35.5)	16.0 (8.2-26.1)
	3	8.1 (5.3-12.3)	3.7 (1.3-10.4)	7.5 (3.7-13.3)	3.7 (0.7-11.3)
	5	5.6	1.5	3.5 (1.1-8.9)	2.1 (1.8-10.1)
ALL	1	14.2 (5.6-31.6)	16.7 (4.7-45.8)	18.9 (3.0-45.9)	17.0 (0.8-55.6)
	3	4.0 (0.7-20.1)	9.8 (1.8-42.6)	0	0
Total AL	1	22.7 (18.5-27.5)	8.5 (4.8-14.6)	25.8 (19.2-33.0)	14.2 (7.3-23.4)
	3	7.7 (5.2-11.4)	3.5 (1.4-8.7)	6.6 (3.3-11.7)	3.2 (0.6-10.1)
	5	5.4	2.1	3.1 (0.9-7.8)	1.9 (0.2-8.9)

Table 9. Relative survival rate with 95% confidence interval of acute *de novo* leukemias in the population aged ≥ 65 years during the two study periods

The intention to treat strategy was assessed in the last study period (1997-2001). Of the Swedish patients aged ≥ 65 years 64 (39%) out of the total of 165 patients were given curatively intended chemotherapy. As a total 37 (22%) of the patients achieved a CR. As regards the 75 Estonian *de novo* AL patients aged ≥ 65 years 11 patients (15%) received curative treatment and totally 6 (8%) of the Estonian patients achieved a CR.

Comments

Over the entire study period of 20 years (1982-2001) it was consistently observed that the age-adjusted incidence rates for the population in western Sweden exceeded those for Estonia, and this was true for the total group of *de novo* AL as well as for the subgroups of AML and ALL. The difference in incidence rates between the regions was particularly pronounced for AML in the elderly cohorts. Further the incidence rates of *de novo* AL appeared to increase over time.

It is evident that as regards both age cohorts in the two countries the vast majority of *de novo* AL is made up of patients with AML. Thus, the incidence rates for the total *de novo* AL are expected to mimic those for AML. The reason for the lower incidence rate for *de novo* AL and *de novo* AML in Estonia as compared to western Sweden is not well understood. The possible underdiagnosing/referral/reporting of patients from rural Estonian hospitals/health care centers should be taken into consideration; it appears probable that in Estonia not all patients were ascertained as they had to be referred to one of the two hospitals with hematology departments to be included in the study. In Sweden information from the clinical hematologist as well as from the laboratory is sent to the Cancer Registry (to which the SAALR is linked); thereby, a solid system of double reporting is obtained. The lower incidence rate in Estonia compared to western Sweden could be explained by different referral approaches to highly specialized hematology units in the two countries. Finally, it cannot be

fully ruled out that there in fact may be a true difference as regards incidence rates with respect to *de novo* AL in between the two countries.

One of the aims of the joint studies (Papers I-IV) was to investigate whether probable environmental differences between the two regions could affect the incidence of AL. It was hypothesized that there was a risk of a higher incidence of secondary AL in Sweden due to probably more cancer survivors in western Sweden and probably more patients who were followed and treated for other hematological diseases when the first study started in 1982. These assumptions were based upon the presence of a larger gross domestic product (GDP) and a more developed health care system in Sweden as compared to Estonia. As this potential difference in incidence of secondary AL could bias the studies we decided to exclude all secondary AL. Indeed, after scrutinizing the records in the study of 1982-1996 we excluded 32.5% (306 of 942) of patients in western Sweden and 22.2% (107 of 481) of Estonian patients for secondary AL.

It appears from Table 5 that the incidence rates for *de novo* AML in the younger cohorts in both western Sweden and Estonia seemed to increase over the study period of 1982-2001, i.e., over the four consecutive 5-year periods. Likewise, equivalent observations have been made by some other workers in their studies on AML epidemiology[70,71]. It appears reasonable to assume that two likely mechanisms may explain an increased incidence rate of *de novo* AML over time, i.e., the access to better diagnostic technologies and/or an improved reporting of patients. In the study of 1982-1996 we have a probable underestimation of incidence in the Estonian material of 18% and in western Sweden of 11% due to lacking or incomplete patient records. In the study of 1997-2001 this cause of underestimation of incidence was absent. This probable underestimation of incidence 1982-1996 could explain part of the increased incidence rates observed by us. However, it is also seen in Table 5 that the incidence rates for *de novo* ALL appeared fairly stable over the same study period. It is unlikely that the diagnosing and reporting procedures between patients with AML and ALL during the years of 1982-2001 could simultaneously have differed in western Sweden and Estonia. It is our intention to continue to study incidence and survival of *de novo* AL in the two regions over forthcoming 5-year periods. Thereby it may be possible to verify or reject the notion whether the tendency of increasing incidence of *de novo* AML over time is a true phenomenon.

Over the entire study period of 20 years (1982-2001) it was consistently observed that the relative survival was highly different in western Sweden as compared to Estonia both in the younger and in the older cohorts. However, relative survival at 3 years and thereafter was virtually negligible in patients aged $\geq$ 65 years of both regions. In the younger cohort of patients (aged 16-64 years) in western Sweden we observed a statistically significant gradual improvement of survival over 1982-1996 but not thereafter. In Estonia survival improvement became evident during the second study period (1997-2001) and this was true for both age cohorts.

In a major attempt on several population-based cancer registries in the USA (the SEER data), covering a population of about 30 million people, 15 638 patients aged 15 years and older with a first diagnosis of AML (and no previous cancer diagnosis) between 1980 and 2004, were followed for vital status until the end of 2004[72]. Indeed, the authors were able to report encouraging observations. It was concluded that treatment of adults with AML had changed substantially over the past two decades. Five-year relative survival improved greatly between 1980-1984 and 2000-2004 for all patients except for those aged over 75 years. Improvements were greatest for patients aged 15-34, with increases in 5-year relative survival of 34.9% in

this group. Less pronounced but still substantial improvements in relative survival were seen in 35-54 and 55-64 age groups of 19.7% and 13.2%, respectively. Another study by the same authors also using the SEER material[73] showed an increase in the corresponding survival of ALL varying from 4.3% to 20.1% in different age groups over the same periods as above. A Nordic study of relative survival at 5 years after AML showed an increase in survival over the years 1984-2003 in men aged 30-49 years where relative survival at 5 years increased from 26% to 45% in Sweden[74]. The results from the Nordic study are very similar to our results from western Sweden, but the SEER data[72] show a somewhat lower increase in relative survival for AML.

For patients with AML reported to the SEER program in 2000-2004[72] the relative survival at 5 years was 52.3% in patients aged 15-34 years, 36.6% in patients aged 35-54 years, 19.9% in patients aged 55-64 years, 9.2 % in patients aged 65-74 years and 2.5% in patients over 75 years. The results in western Sweden 1997-2001 (Paper IV) at 5 years of 40.0% for the total 16-64 years old population are thereby well comparable but the Estonian results at 5 years of 14.6% are clearly inferior. For the older patients 1997-2001 (Paper III) western Sweden had a 3.5 % and Estonia a 2.1 % relative survival at 5 years and are also comparable with the SEER data. For ALL patients 2000-2004 the SEER results[73] of relative survival at 5 years varied from 12.7% to 61.1% for the different age groups, as compared to 29.1% and 23.3% for ALL patients 16-64 years old studied in 1997-2001 by us (Paper IV).

The GDP and health expenditure of Estonia are considerably lower than of many other European countries; in 1999, the total per capita health expenditure of Estonia was seven times lower than that of Finland and 12 times lower than that of Norway[75]. Economic reasons are thus likely to explain the relative undertreatment of Estonian AML patients. In a large study of an unselected AML population based on the SAALR, 2767 AML patients diagnosed in 1997 to 2005, it was shown that standard intensive treatment for elderly patients up to 80 years of age reduces early death rates and improves the chance for survival[13]; this is also true for a younger AML population[76]. The results of Papers III-IV clearly indicate that the Estonian *de novo* AML patients as compared to the western Swedish cohort were undertreated.

In a study from the Eurocare collaboration[75] a firm relationship between GDP and the survival at 5 years was shown in all 13 participating European countries. Major economic differences between Estonia and Sweden were present. Indeed, according to the World Bank in 1997 the gross domestic product (GDP) was 3.608 USD/capita in Estonia and 28.521 USD/capita in Sweden. Another Eurocare study[77] on other cancers could show that 5-year survival was generally high in the Northern Europe and low in Eastern Europe when compared with all 15 countries in the study, where Sweden as well as Estonia contributed with data.

The collapse of the Soviet Union and the fact that Estonia as a consequence thereof in 1991 regained independence rendered major political as well as socio-economic benefits to the country. Thereby, a vigorous reconstruction of the Estonian society encompassing vivid exchange with the Western World was initiated. Indeed, over recent years considerable improvements have been made with respect to Estonian health care and welfare. We believe that the results of the current work on *de novo* AL, showing a considerable improvement with respect to relative survival, is a reflection of the achievements so far reached.

Paper V: Real world data on early death in acute promyelocytic leukemia

Patients, methods and results

Patients

All patients diagnosed with APL during 1997-2006 in the SAALR were included in the study. Of all 3897 AL patients registered, there were 105 patients with APL, representing 2.7% of all AL cases and 3.2% of all AML cases. A case report form for every APL patient was sent to the local hospital, and additional information could be gathered in all but one patient.

Molecularly confirmed diagnosis of APL was defined as a finding of t(15;17) in cytogenetic analysis, and/or positivity for PML-RARα in FISH or RT-PCR analysis. Six of the 105 APL patients lacked molecular diagnosis. These patients were specifically reviewed and all displayed typical morphologic and immunophenotypic features of APL.

Treatment followed the national APL guidelines during the study period. During induction treatment, platelet counts were to be kept above 30 x 10^9/L and transfusions should be given with a target platelet count of 50 x 10^9/L. Plasma was recommended to patients with signs of coagulopathy, with a target fibrinogen value of 1.5 g/L. Treatment with dexamethasone was instructed to be given at slightest suspicion of differentiation syndrome (DS).

Statistical analysis

For comparing continuous variables, two-sided unpaired t-tests were used, and for categorical binominal variables Chi-square tests were used. Kaplan-Meier analysis with log rank test was applied for survival analyses.

Characterization of the study population

The proportion of APL patients in the total AML population decreased with age from 17% in patients aged 18-30 years to 0.9% in patients aged $\geq$ 80 years. The APL incidence was 0.15 per 100 000 inhabitants/year, 0.18 in females and 0.11 in males, respectively. The mean age at the time of APL diagnosis was 52 years (range 18-86), and the median age was 54 years. The corresponding figures for non-APL AML were 68 and 71 years, respectively. Sixty-five (62%) of the patients were women and 40 (38%) men. The proportion of females was considerably higher in APL patients aged 18-40 years, where 89% were women.

Early deaths

Of a total of 105 patients, 30 (29%) died within 30 days after diagnosis. Early death (ED) is defined as death within 30 days of diagnosis. The median time from the diagnostic bone marrow examination to death was 4 days (range 0-26). Nine (30%) of the deaths occurred on the same day or on the day after the diagnostic bone marrow examination, and 23 (77%) within the first week of diagnosis. Only two ED patients died more than 14 days from diagnosis. Among men, the ED rate was 35% (14 of 40) and among women 25% (16 of 65) (p=not significant). The median and mean ages of the ED patients were 65 and 61 years, as compared to 45 and 48 for non-ED patients, respectively ($p<0.001$).

Hemorrhage was the most common cause of death seen in 12 (41%) of the ED patients. Hemorrhagic deaths were more common in younger patients, with fatal bleeding in 11 (53%) of the ED patients aged < 70 years. Hemorrhagic deaths occurred at a median of 4 days after diagnosis.

Of all ED patients, 6 (21%) received no antileukemic treatment. The main reason for receiving no treatment was very early ED due to CNS hemorrhage. All non-ED patients received antileukemic treatment and only 3% did not receive ATRA.

The majority of ED patients first sought help in a hospital-based emergency ward, whereas most non-ED patients first attended a primary care unit. Half of the patients treated at non-university hospitals suffered ED compared to 22% of patients treated at university hospitals ($p<0.01$).

CR rate, relapse rate and survival

Of patients surviving the first 30 days, all but two (97%) achieved CR. Twelve (16%) of the patients who achieved CR relapsed at a median of 540 days (range 120-2484) after the day of first CR. Patients who relapsed were older than CR patients who did not (mean age 59 vs. 46; $p<0.001$). Three (12.5%) men and 9 (18.8%) women relapsed (p=not significant). Of the patients treated for relapse, 4 died within 32 days from relapse. Seven (58%) of the relapsed patients achieved a second CR, and of these two patients underwent alloSCT (both alive), three are alive in second CR without transplantation, whereas two had a second relapse and died. In total, 65 (62%) of a total of 105 APL patients are still alive with a median follow up of 6.4 years. Of the patients who went into a first CR, 87% are alive, as are 50% of the relapsed patients.

Comments

The percentage of APL patients in this study was 2.7% of all AL patients and 3.2% of all AML patients, which is less compared to what is previously reported. A main factor explaining the differences in the proportion of APL patients between different studies is the age of the study population. Our study showed a high proportion of APL in younger age groups as opposed to very low percentages in the higher age groups. However, the median age at diagnosis of APL was considerably higher in our study as compared to other reports[54,78,79], which might reflect that older patients have failed to enter these studies.
Interestingly, we also found gender differences with a high proportion of women in APL patients below 40 years, not previously reported. There is no obvious explanation for this female predominance, and this needs further confirmation.

The most striking finding in this study is the high ED rate of 29% in APL-patients within the first 30 days from diagnosis. This is similar to what was reported from Brazil[80], but considerably higher compared to recent clinical trials[54,79,81-83]. The main reason for the higher ED in the SAALR compared to clinical trials is probably the inclusion of patients with very ED and of patients who were not fit or too old for inclusion in clinical trials. Sanz et al. report that half of the patients excluded from PETHEMA trials were excluded due to life threatening hemorrhages [84]. As there is no reason to believe that health care resources or the management of APL patients is inferior in Sweden as compared to other developed countries, we suggest that the ED rates reported here are more close to what can be expected in an unselected APL population, also in many other Western countries.

Similar to other reports, hemorrhages were the major cause of death and almost all cases were CNS bleedings. The proportion of non-hemorrhagic deaths is slightly higher compared to most other studies, which is consistent with older patients in this study.

An important question is how many of the deaths could have been prevented by better initial management of the patients. Some very ED are realistically very difficult to prevent since 30% of the deaths occurred on the day of diagnosis or on the day after, making the time for preventive efforts very short. Still, a substantial number of deaths, including hemorrhagic deaths, occurred later during the first weeks of treatment thereby giving more time for preventive measures. It is not clear which proportion of the ED in this study could have been prevented by better adherence to guidelines, but some examples of non-compliance to the guidelines could be identified, i.e., lack of compliance to recommendations regarding platelet and plasma transfusions. Median times between first health care contact, first contact with hematologist, diagnosis and start of treatment were short and similar between the non-ED and ED groups, but the spread was substantial and some delays in individual patients could be identified. However, the role of such delays for the death of individual cases is difficult to assess.

Patients treated at university hospitals showed lower ED rates compared to patients treated at other hospitals. Patients treated at university hospitals were somewhat younger, but also within the different age groups the mortality was lower in university hospitals. It is still an unanswered question how many of these patients could have been saved by urgent transportation to a university hospital. Some patients may have been too fragile to be moved from the local hospital. However, our data suggests that APL-patients should preferentially be treated at specialized units.

We conclude that population-based data is needed as a supplement to data from large randomized trials for information about the overall APL population. The most striking difference when studying population-based APL data is the higher ED rate, which reaches as high as approximately 30%. It is unclear how many of these deaths are preventable, but we speculate that increased knowledge about the mechanisms of the coagulopathy, increased knowledge about APL in the medical community, earlier diagnosis and initiation of ATRA therapy on the mere suspicion of APL, better compliance to existing guidelines, centralization of the treatment to highly specialized hospitals, and new guidelines with more aggressive initial management of high-risk patients could decrease ED in APL. Preventing ED is an important issue since patients who are saved from ED have a high probability of cure.

Paper VI: Study on acute myeloid leukemia in young Scandinavian patients, aged 10-30 years, clinical characteristics and outcome

Patients, methods and results

Patients

The NOPHO-registry contained 188 AML-patients aged 10-18 years from Denmark, Norway, Sweden, Finland and Iceland diagnosed 1993-2009. Some adolescents were treated in adult hematology departments according to national/regional practices. Thus, the NOPHO-registry was strictly population-based only for patients aged ≤ 15 years.
All patients aged 15-30 years diagnosed with AML in adult hematology centers in Denmark in 2000-2009 (n = 89) and all patients aged 17-30 years in Sweden in 1997-2009 (n = 132) were investigated. In Norway, all patients at the large adult centers are reported to an open registry, which contained 57 patients aged 15-30 years diagnosed in 1996-2008. Thus, the Swedish and Danish, but not the Norwegian, adult material was population-based and retrieved from the SAALR and the Danish acute leukemia registry, respectively.

In the NOPHO material of 188 patients, 1 patient with Down syndrome and 21 patients (11.2%) with secondary leukemia were excluded. In the total adult material of 278 patients, 25 patients (9.0%) with secondary leukemia were excluded.

Therapy

The pediatric cohort received treatment according to the NOPHO-protocols of 1993 and 2004. The adult cohort received treatment according to national/regional guidelines. The major differences were that pediatric AML-patients received five different drugs for induction while adult patients more often received two drugs, standard anthracycline and cytarabine. The pediatric patients received four consolidation courses after two induction courses, i.e., in total 6 courses. Adult patients that did not proceed to allogeneic SCT received 4-5 courses in total. Indications for transplantation were similar in the two cohorts.

Adult APL patients were treated according to specific APL-protocols, including ATRA in induction, consolidation, and two years of maintenance treatment. Pediatric APL patients received ATRA as supplement to NOPHO AML-protocols from 1999 during two induction courses but not during consolidation, and no maintenance was given.

Follow-up

The median time of follow-up for pediatric patients alive was 7.3 years with an interquartile range of 4.0-11.1 years. The corresponding figures for the adult cohort were 5.7 (3.8-8.9) years. Events during follow-up were registered as ED, resistant disease, death in CR, relapse or secondary malignant neoplasm.

Statistical methods

Mean crude incidence rate for the time period 2000-2009 in the population-based part of the cohort (Sweden and Denmark) was calculated by use of the numbers of cases and inhabitants per age group and country. Survival was calculated from the date of diagnosis to the date of death or date of last follow-up. Probabilities of survival were estimated using the Kaplan-

Meier method. The log rank test was used to test differences in survival. Univariate and multivariate Cox regression were used to analyze the importance of prognostic variables. A p-value less than 0.05 were considered statistically significant. The Stata software was used for all statistical analyses[85].

Patient characteristics

The total material consisted of 419 patients after excluding secondary leukemias and Down syndrome.

The yearly incidence rates in the studied age groups 2000-2009 were calculated in the complete population-based material of Denmark and Sweden. During this period the annual incidence of *de novo* AML was 4.9 per million inhabitants for the age group 10-14 years, 6.5 for 15-18 years, and 6.9 for 19-30 years.

We compared the "pediatric cohort" of 166 patients with the total "adult cohort" of 253 patients. Since patients aged 15-18 years could be treated in either adult (n = 35) or pediatric (n = 41) settings in all countries, we chose to describe and report this group both separately and as part of the respective treatment group. Patient characteristics are given in Table 10.

	NOPHO						Adults					
	10-18 years		10-14 years		15-18 years		**15-30 years**		15-18 years		19-30 years	
median age (interquartile range)	**13 (12-14)**		12 (11-14)		16 (15-17)		**24 (20-28)**		17 (17-18)		26 (22-28)	
Number of patients	**(n=166)**		(n=125)		(n=41)		**(n=253)**		(n=35)		(n=218)	
Characteristic	**No.**	**%**	No.	%	No.	%	**No.**	**%**	No.	%	No.	%
Country												
Denmark	**22**	***13.2***	21	*16.8*	1	*(2.4)*	**81**	***32.0***	18	*51.4*	63	*(28.9)*
Norway	**26**	***15.7***	24	*19.2*	2	*4.9*	**52**	***20.6***	7	*20.0*	45	*20.6*
Sweden	**83**	***50.0***	51	*40.8*	32	*78.0*	**120**	***47.4***	10	*28.6*	110	*50.5*
Finland	**31**	***18.7***	26	*20.8*	5	*12.2*	**0**	**-**	-	-	-	-
Iceland	**4**	***2.4***	3	*2.4*	1	*2.4*	**0**	**-**	-	-	-	-
Sex												
male	**96**	***57.8***	70	*56*	26	*63.4*	**124**	***49.0***	17	*48.6*	107	*49.1*
female	**70**	***42.2***	55	*44*	15	*36.6*	**129**	***51.0***	18	*51.4*	111	*50.9*
WBC count x10⁹/L												
0-19.9	**91**	***54.8***	70	*56.0*	21	*51.2*	**127**	***50.2***	18	*51.4*	109	*50.0*
20-99.9	**50**	***30.1***	36	*28.8*	14	*34.2*	**67**	***26.5***	8	*22.9*	59	*27.1*
>100	**24**	***14.5***	18	*14.4*	6	*14.6*	**20**	***7.9***	3	*8.6*	17	*7.8*
data missing	**1**	***0.6***	1	*0.8*	0	-	**39**	***15.4***	6	*17.1*	33	*15.1*
FAB subtype												
M0	**10**	***6.0***	9	*7.2*	1	*2.4*	**11**	***4.3***	0	-	11	*5.0*
M1-2	**79**	***47.6***	61	*48.8*	18	*43.9*	**88**	***34.8***	15	*42.9*	73	*33.5*
M3	**9**	***5.4***	6	*4.8*	3	*7.3*	**49**	***19.4***	9	*25.7*	40	*18.3*
M4-5	**59**	***35.6***	43	*34.4*	16	*39.0*	**77**	***30.4***	8	*22.9*	69	*31.7*
M6	**1**	***0.6***	1	*0.8*	0	-	**7**	***2.8***	0	-	7	*3.2*
M7	**1**	***0.6***	0	-	1	*2.4*	**1**	***0.4***	0	-	1	*0.5*
granulosarcoma	**2**	***1.2***	2	*1.6*	0	-	**2**	***0.8***	0	-	2	*0.9*
unclassified	**5**	***3.0***	3	*2.4*	2	*4.9*	**19**	***7.5***	3	*8.6*	16	*7.3*
CNS disease at diagnosis												
Yes	**10**	***6.0***	7	*5.6*	3	*7.3*	**7**	***2.8***	1	*2.9*	6	*2.8*
No	**152**	***91.6***	115	*92.0*	37	*90.2*	**96**	***37.9***	19	*54.3*	77	*35.3*
data missing	**4**	***2.4***	3	*2.4*	1	*2.4*	**150**	***59.3***	15	*42.9*	135	*61.9*
Cytogenetic features												
t(8;21)	**20**	***12.0***	17	*13.6*	3	*7.3*	**18**	***7.1***	3	*8.6*	15	*6.9*
inv(16)	**15**	***9.0***	13	*10.4*	2	*4.9*	**18**	***7.1***	3	*8.6*	15	*6.9*
t(15;17)	**9**	***5.4***	6	*4.8*	3	*7.3*	**48**	***19.0***	9	*25.7*	39	*17.9*
total favorable	**44**	***26.5***	36	*28.8*	8	*19.5*	**84**	***33.2***	15	*42.9*	69	*31.7*
normal	**54**	***32.5***	39	*31.2*	15	*36.6*	**45**	***17.8***	7	*20.0*	38	*17.4*
trisomy 8	**7**	***4.2***	5	*4.0*	2	*4.9*	**12**	***4.7***	1	*2.9*	11	*5.0*
t(9;11)	**10**	***6.0***	8	*6.4*	2	*4.9*	**7**	***2.8***	2	*5.7*	5	*2.3*
11q23 (other than 9:11)	**10**	***6.0***	4	*3.2*	6	*14.6*	**9**	***3.6***	1	*2.9*	8	*3.7*
t(6;9)	**2**	***1.2***	1	*0.8*	1	*2.4*	**1**	***0.4***	0	-	1	*0.5*
monsomy 7	**1**	***0.6***	0	-	1	*2.4*	**8**	***3.2***	0	-	8	*3.7*
inv(3)	**2**	***1.2***	2	*1.6*	0	-	**0**	**-**	-	-	-	-
complex	**11**	***6.6***	10	*8.0*	1	*2.4*	**20**	***7.9***	1	*2.9*	19	*8.7*
other cytogenetic abnormalities	**20**	***12.0***	15	*12.0*	5	*12.2*	**34**	***13.4***	1	*2.9*	33	*15.1*
data missing	**5**	***3.0***	5	*4.0*	0	-	**33**	***13.0***	7	*20.0*	26	*11.9*

Table 10. Demographic and clinical characteristic of patients

Outcome

The CR rate was over 90% in both the pediatric and adult setting and all age cohorts. Primary resistant disease was seen in 6.7% of the total material and the ED rate was 4.6% in the total material with no clear-cut difference between the cohorts. The relapse rate was somewhat lower in the adult cohort 31% (76/246) than in the pediatric cohort 38% (63/166) but the difference was not statistically significant (p=0.09).

No differences in OS or event-free survival (EFS) between the two cohorts were found (Figure 4).

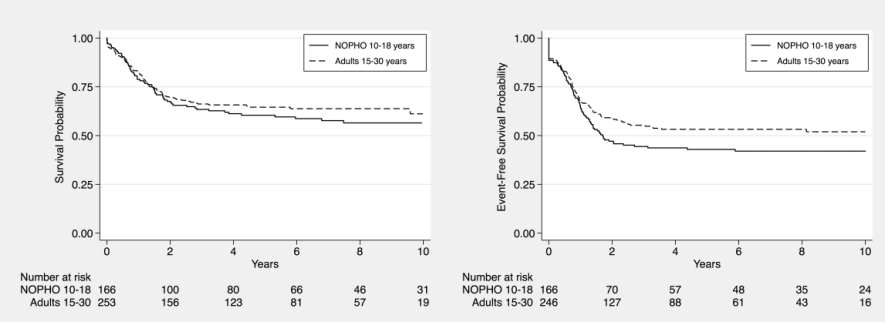


Figure 4. Overall and event-free survival for the adult and the NOPHO cohorts

The subgroup of patients aged 15-18 years at diagnosis had a lower 5-year OS in the pediatric (50.7%) than in the adult (69.9%) setting (not significant) that might be explained by differences in baseline characteristics. When APL-patients were excluded there were no differences in OS (Figure 5) between the cohorts. Moreover, we found no statistically significant difference in OS between pediatric and adult patients with APL.

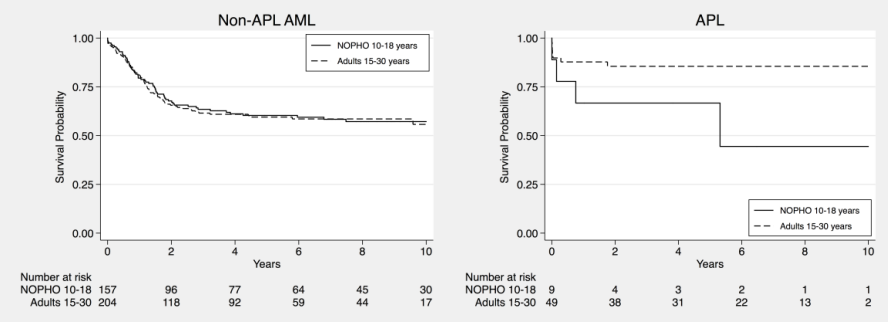


Figure 5. Overall survival in the adult and NOPHO cohort in non-APL AML and in APL

However, within the adult cohort, APL-patients had a superior OS compared to those with non-APL (p=0.003), whereas no such difference was seen in the NOPHO-cohort (p=0.45) (Figure 6).

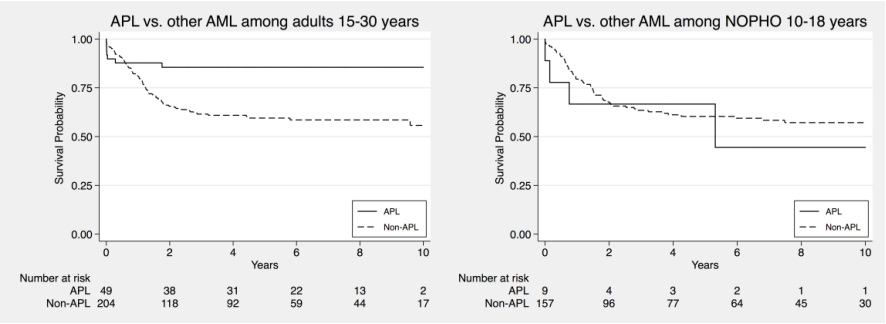


Figure 6. Overall survival in APL vs. other AML in the adult and the NOPHO cohort

Treatment group, age, or SCT in CR1, were not significant prognostic markers in this study. However, favorable cytogenetics (APL and core binding factor-leukemia (CBF-leukemia)) and low WBC at diagnosis were strong markers for better outcome. Female sex and APL were also correlated to a better outcome. Adjustment for these factors in Cox multivariate regression analysis did not change the fact that the two treatment groups had similar outcomes. In a multivariate analysis cytogenetics and WBC were the only independent prognostic factors for OS in this study.

Comments

The major differences in disease characteristics between the pediatric and adult cohorts in this study were a male predominance, a higher frequency of patients with high WBC ($> 100 \times 10^9$/L) at diagnosis, and a lower incidence of APL in the pediatric cohort. We found APL in 4.8% of patients aged 10-14 years, in 15.8% in patients 15-18 years and in 18.3% of patients 19-30 years. Our result in children is in agreement with earlier studies[86,87]. It has previously been known that the incidence of APL increases during the teen years and reaches a plateau in early adulthood[88].

Most importantly, over all we found no difference in OS or EFS between patients treated according to pediatric vs. adult protocols.

The pediatric cohort received a total of two induction courses, containing a combination of 5 different drugs. Most adult non-APL patients received induction treatment consisting of an anthracycline + cytarabine only. The intensive treatment strategy resulted in very high CR-rates in the cohorts (91.6% in pediatric patients and 91.5% in adults). Still, our study did not allow for comparison of different treatment strategies, since CR rates, EFS as well as OS was similar in the different cohorts with different treatment strategies. The very high CR rates as compared to other studies are partly explained by the age distribution, but also the exclusion of secondary AML, where CR rates are lower.

In the adult cohort patients with APL had a superior survival compared to other adult AML patients whereas there was no survival benefit for the APL-patients in the pediatric cohort. Adult APL-patients were treated with ATRA during induction and consolidation and also had an ATRA-maintenance therapy. They were most often treated within a specific APL-protocol[89]. There was no difference in the pediatric treatment protocol for APL compared to other AML patients except concerning ATRA, i.e., children with APL did receive double induction with 5 different drugs and in addition ATRA. In the pediatric patients ATRA was given from the first induction to the end of the second induction and thereafter stopped. No maintenance was given. This difference in treatment of APL could explain the difference in outcome. ATRA maintenance has been proved to improve survival[55,90]. These speculations on outcome in pediatric APL-patients are hazardous since APL was rare in the pediatric cohort of this study and the lack we see of survival benefit could be due to the small number of patients.

Cox regression multivariate analysis showed that favorable cytogenetics (APL and CBF-leukemia) and WBC independently affected OS in this AML cohort. The superior outcome with favorable cytogenetics is in line with previous studies. However, WBC at diagnosis was an independent prognostic marker for OS in multivariate analysis, with a worse survival for a continuously higher initial WBC. The pediatric cohort had a higher median of WBC at diagnosis. It is generally understood that high WBC is associated with a higher risk of ED and that it does not affect risk of relapse [91,92]. In our study high WBC at diagnosis did not translate into a higher frequency of ED instead it was thus an independent prognostic marker for OS in multivariate analysis.

Age is a prognostic marker for AML in adults[12,13]. Studies have shown that age below 10 years was a positive prognostic marker for outcome and that increasing age was a negative prognostic marker in the age group 2-30 years[93,94]. In adult population-based studies[95] OS decreases with age for every decade over the age of 30 years. Our study could not confirm age as a prognostic marker of survival in AML in this more narrow age span (10-30 years).

The promising results from the ALL-studies[63-65], where treatment according to pediatric protocols had superior outcome compared to adult protocols, inspired to similar comparison between treatment strategies in AML, but our AML-results differ from the corresponding findings for ALL. However, the treatment regimens for AML are more similar between pediatric and adult care than the corresponding ALL protocols, which could explain the results.

Finally, in the present study we could not find differences in outcome of young AML-patients according to treatment by pediatricians or by hematologists in the adult care, with different treatment protocols. In the small group of pediatric APL patients, we could not demonstrate any survival advantage over other AML-patients, this finding was unexpected, and calls for increased attention to this subset of patients with modification of treatment protocols.

General discussion

Overview of study designs used in clinical research

Clinical research studies are usually divided into experimental and observational studies[96]. In experimental studies, for example randomized controlled trials, investigators assign exposure whereas in observational studies investigators observe an existing exposure. Observational studies can be either analytical (having a comparison or control group), or descriptive (without comparison/control groups). Analytical observational studies can be further categorized into cohort studies, case-control studies and cross-sectional studies[96] (see Figure 7).

Cohort studies follow the subjects over time, information on characteristics and exposures at baseline is gathered and outcomes are studied during a given period. Case-control studies observe cases that have a specified outcome to controls (without the specified outcome) and then look back in time for an exposure and finally compare the groups. Cross-sectional studies examine the prevalence of a phenomenon, for example disease and the prevalence of an exposure in a cohort at a particular time. Descriptive studies consist of two major groups: those dealing with individuals and those mainly relating to populations[97].

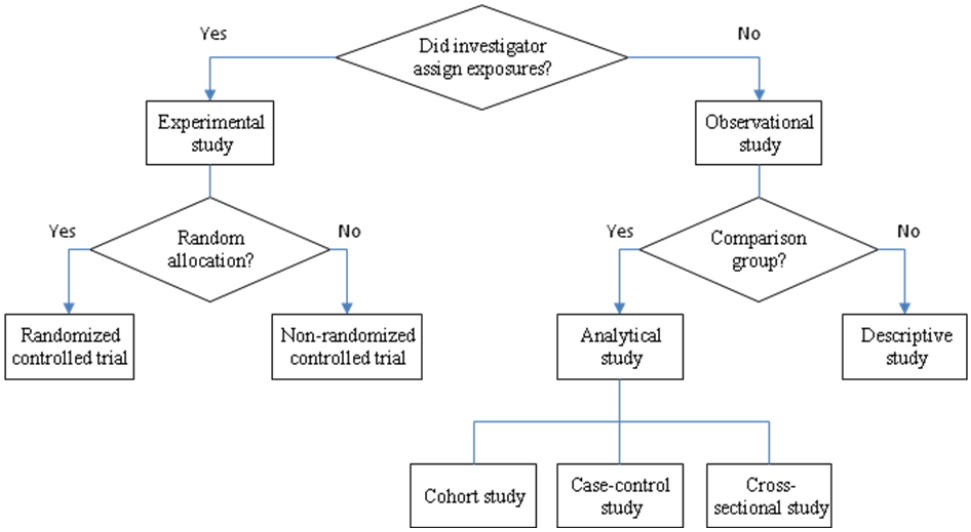


Figure 7. Algorithm of clinical research studies (adapted from reference 96)

The clinical research in the present thesis, based on data from the SAALR, could with this classification be considered as population-based, observational, analytical cohort studies (Papers I-IV and VI) and also as population-based, observational descriptive studies (Paper V).

Lessons from the SAALR

Randomized controlled trials (RCT) are regarded as the gold standard in evidence-based medicine and are considered the highest grade of evidence[98]. When evaluating effects of different treatment RCT is the design of choice. They have high internal validity; randomization is an effective measure to balance for confounding factors. However, one important limitation with RCT is the external validity[99], i.e., how representative are the patients included and thus the results of the trial for the general population. Indeed, studies show that in cancer-treatment RCT there is an under-representation of patients > 65 years of age[100] and there is also evidence for race and sex disparities in clinical cancer trials[101]. The same pattern is reproduced when assessing patients excluded from clinical trials on AML; those patients were older, had more comorbidity and different AML subtypes than those included[102]. Indeed, in this particular Swiss study[102] on inclusion of AML-patients in clinical trials, only 76 (35%) of totally 215 AML-patients were eventually included in a RCT.

In western countries more than half of all patients with AL are > 65 years of age[11,13]. However, most of the knowledge as to AL is based upon clinical trials were patients < 60 years of age are included; such trials may sometimes contain older patients who have passed various selection criteria. It is usually argued that selection bias is a problem of observational studies and that the selection bias questions the internal validity of results. Population-based observational studies eliminate the selection bias if the coverage is high. The strength of the data from the SAALR is that all Swedish AL patients regardless of age, sex, socioeconomic status, ethnicity and comorbidity are included. The coverage is 98%. The results are thus not affected by selection. Thereby, both the internal and external validity of the results is high. It could be argued that Sweden, a developed welfare country without major socioeconomic differences and a rather homogenous population of predominantly Caucasian origin, is not representative for the global population. This could implicate that the external validity is only true for comparable countries, i.e., western countries.

The problem with external validity of RCTs is very obvious when comparing our results on APL with results from clinical trials. We report that 29% of APL-patients die within 30 days and that APL has a total OS of 62% after a median of 6.4 years (Paper V). Clinical trials report a long-term survival of 75-80%[55,56]; these results are achieved after comparing treatment in randomized settings in selected patients. RCTs thus give us the impression that APL has a very favorable prognosis. Our observational descriptive study shows that patients not affected by ED have a very good prognosis. However, the problem of ED affects as many as one third of APL-patients, a problem not seen in RCTs where non-randomized patients never are reported. Our study thus generates the hypothesis that it would be more beneficial to investigate possible improvements of the clinical measures during the early phase of APL. Observational descriptive cohort studies are useful to generate hypotheses, but not to test them[97]. Other typical features of descriptive studies are that they, in accord with our APL-study, are readily available, inexpensive and frequently pose few ethical problems[97].

The SAALR has a very low loss to follow-up and also allows the possibility of a long time of observation. The registry started to include patients in January 1997, which at the present yields a long-term follow-up of close to 14 years for the first patients included. The most important result variable, date of death, is found within the Swedish population register. The data in the Swedish population register are cross-checked with the SAALR. Patients emigrating after diagnosis or after treatment can potentially be lost to follow-up, since they then escape the Swedish population register. However, emigration from Sweden is low, < 0.5% annually (Statistics Sweden).

Cohort studies allow the calculation of true incidence rates[103] and thereby information on the size of different problems. For example RCTs cannot provide the knowledge that the majority of AML-patients are aged > 65 years. Knowledge on descriptive epidemiology, i.e., the size of the problem, can help us understand which measures are important when deciding on future investments in health care and research. Changes of incidence and survival of the total patient group, is knowledge attained from observational studies as exemplified in Papers I-IV.

The mere existence of a registry and the knowledge that results are measured and published can possibly improve results in participating hospitals. In a recent publication in JAMA on observational data from the Swedish Register of Information and Knowledge about Swedish Heart intensive Care Admission (RIKS-HIA) showed an initial variation in treatments according to guidelines in different hospitals which decreased over time and that the higher adherence to guidelines gradually lowered short and long-term mortality [104]. This could be part of the explanation of improved results in our comparative studies between western Sweden and Estonia (Papers I –IV).

Data from observational studies can extend support for the use of a treatment to patients not included in an original trial[105]. For example the SAALR has shown that AML patients aged 70 to 80 years, usually not included in RCT, do also benefit from standard intensive induction treatment[13]. This is in contrast to what was earlier generally recognized.

Occasionally, due to ethical considerations, issues of great interest are impossible or difficult to study in RCTs. One such issue arises in AL where it is difficult to assess the value of allogeneic SCT as part of treatment in some age and risk groups of AL patients. The SAALR did recently show that a high rate of allogeneic SCT in AML patients up to 60 years of age was associated with a better long-term survival[95]; age as such had limited influence on survival in AML of those patients who received an allogeneic SCT.

Finally, one important future task for the SAALR will hopefully be to evaluate the national guidelines both as to therapeutic and prognostic measures. To use the SAALR to evaluate national guidelines requires a continuous high coverage, preferably 100% of diagnosed patients and a good compliance to the current guidelines from all hospitals. To closely evaluate national guidelines and changes within them would give a possibility for adult hematologic care to approach pediatric care in the way improvements of results are made. The majority of pediatric cancer patients are treated at pediatric institutions and participate in studies. Sequential studies evolve from the earlier completed studies in an iterative manner in pediatric oncology. The advances made in pediatric care of for example ALL with these large sequential studies are impressing. Different pediatric collaboration groups, national or international, around all western countries do apply this system[106]; thereby almost 100% of pediatric patients are included in trials. This is in contrast to what is currently seen in adult hematologic care where the majority of patients are not included in clinical studies; this

problem is even more pronounced in elderly populations. Well-run population-based quality registries could in the future give a platform to evaluate new therapeutic and diagnostic measures in larger, more heterogeneic populations than those currently included in adult phase III-trials. The initiation of such assessments on the majority of adult AL patients may hopefully facilitate future advances in the understanding of leukemia treatment.

Conclusion

Based on the findings described above I conclude that the SAALR, a population-based registry with high coverage, does provide a basis for building new knowledge on AL and is a valuable complement to RCTs.

Sammanfattning på svenska (Summary in Swedish)

Bakgrund

Akut leukemi (AL) är en ovanlig, allvarlig men potentiellt botbar blodsjukdom. Sjukdomen är heterogen och indelas i undergrupper beroende på sjukhistoria och de sjuka vita blodkropparnas fenotyp. AL delas in i primär (*de novo*) och sekundär sjukdom; sekundär betyder att tidigare hematologisk sjukdom eller given cancer-behandling såsom cellgift och/eller strålning har utlöst leukemin. AL delas in i akut lymfatisk leukemi (ALL), akut myeloisk leukemi (AML) och akut promyelocytleukemi (APL) beroende på vilka vita blodkroppar som är drabbade. Vid AL blir benmärgen överfull av sjuka vita blodkroppar och friska blodkroppar bildas därmed i mindre utsträckning än normalt. Patienterna får anemi, brist på blodplättar och brist på fungerande vita blodkroppar. Symtom i form av trötthet och andnöd, blåmärken och blödningar samt infektionskänslighet för patienterna till vården. År 1997 startade Svensk Förening för Hematologi ett kvalitetsregister för AL, akut leukemi-registret, det första hematologiska kvalitetsregistret i Sverige och ett av de första populationsbaserade leukemiregistren i världen.

Syfte

Syftet med de här studierna har varit att i populationsbaserade material undersöka:

i) förekomst och överlevnad av AL i regioner med skillnader avseende socioekonomi,
ii) överlevnad i APL med särskilt fokus på sjukdomsförloppet under de första veckorna kring diagnos,
iii) sjukdoms-karaktäristika och behandlingsresultat hos patienter i åldrarna 10-30 år med AML, och om en eventuell skillnad i behandlingsresultat för dessa unga patienter föreligger som beror av typ av behandlingsschema och/eller vårdgivare.

Metoder

Under en transitionsperiod i Europa, 1982-2001, har vi jämfört incidens och överlevnad vid *de novo* AL i två regioner (Västra Götalands-regionen (VGR) och i Estland). Regionerna har liknande förhållanden avseende yta, befolkningsstorlek och latitud men skilda förhållanden avseende ekonomi, sociala strukturer och miljöpåverkan. De första studierna 1982-1996 gjordes retrospektivt utifrån cancerregister och sjukhusjournaler i de två regionerna. Alla patienter med *de novo* AL inkluderades, patienter med sekundär AL exkluderades. De uppföljande studierna 1997-2001 gjordes utifrån akut leukemiregistret i Sverige och utifrån sjukhusjournaler i Estland. Samtidigt skapades ett akut leukemiregister i Estland, i vilket alla fall av AL sedan 1997 och framåt registreras på ett identiskt vis som i Sverige. Vi har följt alla patienter till minst 5 år efter diagnos.

För den populationsbaserade studien om APL har vi med hjälp av akut leukemiregistret identifierat alla fall med denna diagnos 1997-2006 i Sverige. 105 fall identifierades där behandlande klinik ombads lämna kliniska uppgifter enligt en standardiserad blankett.

Ett samarbete med NOPHO (the Nordic Society of Pediatric Hematology and Oncology) och med vuxenregister för AML i Norge och Danmark har givit ett populationsbaserat material över alla *de novo* AML i åldersgruppen 10-30 år under åren 2000-2009. För detaljerade fakta om prognostiska markörer och behandling har journalgranskning genomförts.

Resultat

Incidens i *de novo* AL är högre i VGR än i Estland. Skillnaderna är endast signifikanta i åldersgruppen ≥65 år. Incidensen av AL ökar för varje 5-årsperiod under åren 1982-2001 i båda regionerna men ökningen är inte statistiskt signifikant. Överlevnaden är signifikant bättre i VGR 1982-2001. Överlevnaden förbättrades varje 5-årsperiod under de första 15 åren hos de yngre västsvenska patienterna, samtidigt sågs ingen förbättring i Estland. I studien 1997-2001 har skillnaderna i överlevnad mellan regionerna utjämnats i viss del till följd av utebliven förbättring i Sverige och en dramatisk förbättring av överlevnad i Estland.

I ett populationsbaserat material APL-patienter kunde vi visa att den tidiga dödligheten i sjukdomen var så hög som 29 %. En så stor andel av patienterna dog inom 30 dagar från diagnos, 41 % avled p.g.a. blödningar. Tidig död drabbade oftare äldre patienter. Totalt 62 % av patienterna var i livet efter median uppföljningstid på 6.4 år.

För unga patienter i åldersgruppen 10-30 år med *de novo* AML fanns inga statistiska skillnader i överlevnad beroende på om behandlingen skett inom pediatrisk vård med NOPHOs vårdprogram eller om patienterna hade behandlats inom vuxenvården i Sverige, Norge och Danmark. När materialet korrigerades för kön, vita blodkroppar och cytogenetiska avvikelser vid diagnos, fanns fortfarande inga statistiska skillnader avseende behandlingsresultat mellan grupperna.

Slutsats

Skillnaderna i incidens och överlevnad vid *de novo* AL mellan Estland och VGR är väntade med tanke på socio-ekonomiska skillnader som ger olika förutsättningar för vården. Incidensskillnaderna, som inte är statistiskt signifikanta, kan var beroende av skillnader avseende tillgänglighet till vård och möjlighet till diagnostik. Överlevnadsskillnaderna som är statistiskt signifikanta kan vara beroende av ett senare omhändertagande och knappare resurser i Estland. Förvånande är den incidensökning, dock ej statistiskt säkerställd, som ses i båda regionerna inom *de novo* AML 1982-2001. En studie av ytterligare en 5-års-period (2002-2006) planeras för att undersöka om tendensen till incidensökning är sann.

Vår studie visar att trots goda behandlingsresultat för patienter med APL förlorar vi många patienter tidigt i sjukdomsförloppet framför allt på grund av blödningar. Den tidiga dödligheten är i vårt material högre än vad som tidigare rapporterats från stora kliniska prövningar. I kliniska prövningar inkluderas sällan äldre och svårt sjuka patienter varför våra resultat troligen är mer verklighetsnära. Studier som undersöker det initiala omhändertagandet och behandlingen av dessa patienter behöver genomföras för att nå verkligt goda överlevnads-resultat vid APL.

Vid AML, till skillnad från ALL, har unga patienter samma behandlingsresultat oavsett om de behandlas med barn eller vuxenprotokoll. Tidigare studier har visat att stigande ålder är en markör för sämre prognos bland både barn och vuxna med AML. I den grupp AML-patienter 10-30 år som vi undersökt kunde inget sådant ålderssamband påvisas.

Studier från stora kvalitetsregister ger populationsbaserad information vilket kan vara ett viktigt komplement till kunskap från randomiserade studier. Observationsstudier från populationsbaserade register med hög täckningsgrad kan ge ökad epidemiologisk kunskap om ovanliga sjukdomar och kan också beskriva tidigare okända problem som kan behöva studeras närmare i randomiserade studier.

Acknowledgements

This thesis would not have been possible without the guidance and the help of several individuals who in one way or another contributed with their valuable assistance in the preparation and completion of this thesis.

First and foremost, my utmost gratitude to Dick Stockelberg, my main supervisor, clinical tutor and also the person who employed me and made me chose the field of hematology some 10 years ago. As my supervisor, your trust in me always made me believe in my abilities, helping me overcoming problems, transforming them into possibilities. An inspiration to me, as I hurdled all the obstacles in the completion of this research work.

I owe my deepest gratitude to Jack Kutti, my co-supervisor who first introduced me to research, for sharing your long experience from research, always being there listening, discussing, supporting and using lots of red ink to help me improve my English writing.

My sincere gratitude to all patients reported and all colleagues reporting to the SAALR and NOPHO over the years.

I am indebted to many of my colleagues as well as organizations, and I would especially like to pay my gratitude to;

- Erik Holmberg for collaboration, for statistical advice and support, always explaining with the greatest patience
- Gunnar Juliusson, responsible for the SAALR and valuable co-author
- The Swedish AML Group, especially Sören Lehmann and Martin Höglund
- All colleagues in Estonia, especially Ene Luik, Katrin Palk and Mirja Varik
- Soodabeh Kutti, co-author and friend
- Pernille Edslev-Wendtland, Henrik Hasle and the NOPHO AML-group
- Jan Maxwell Nørgaard and Yngvar Fløisand, the Danish and Norwegian acute leukemia registries
- The Oncological Centres in Gothenburg and in Lund
- Colleagues, nurses and secretaries in Hematology clinics all over Sweden for helping me finding "paper" patient records and explaining different electronic record systems
- P-O Andersson, head of the section of Hematology and Coagulation at Sahlgrenska for making it possible for me to finalize this thesis and for somehow finding time helping all the PhD students at the section, including me
- All colleagues at the section of Hematology and Coagulation at Sahlgrenska and especially our acute leukemia-team for doing all the clinical work when I didn´t

I wish to express my warm and sincere appreciation and gratitude to my friends and family; to my parents, for never doubting but always believing in me and my abilities; to Reine, for love, fun, it-support and for taking extra care of me, especially these last eight months…

Finally, this work was supported by "Göteborg Medical Society", "Blodcancerfonden", "Assar Gabrielsson", "ALF-medel", "FoU Västra Götalandsregionen" and "Cancer- och Trafikskadades Riksförbund". I thank them all for their support.

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