Survival and complications in patients with chronic lymphocytic leukemia in the pre-ibrutinib era

Vilhjálmur Steingrímsson

Thesis for the degree of Philosophiae Doctor

Supervisor:
Sigurður Yngvi Kristinsson

Doctoral committee:
Magnús Gottfreðsson, Magnus Björkholm, and Ola Landgren

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Lifun og fylgikvillar í langvinnu eitilfrumuhvítblæði

Vilhjálmur Steingrímsson

Ritgerð til doktorsgráðu

Leiðbeinandi:
Sigurður Yngvi Kristinsson, prófessor

Doktorsnefnd:
Magnús Gottfreðsson, Magnus Björkholm og Ola Landgren

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ORCID ID: https://orcid.org/0000-0002-9385-2960

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Ágrip

Inngangur: Langvinnt eitilfrumuhvítblæði (CLL) er sjúkdómur sem leggst helst á eldra fólk, meðalaldur þeirra sem greinast er um 72 ár. Langt fram eftir 20. öldinni var helsta meðferðin við sjúkdómnum chlorambucil, en í kringum aldamót breyttist meðferðin með tilkomu fludarabin. Seinna meir bættust rítúxímab og cýklófosfamíð við þá meðferð. CLL svaraði betur þessum lyfjum en engu að síður gekk illa að sýna fram á aukna lifun í lyfjarannsóknum. Einnig var gagnrýnt að meðalaldur sjúklinga í lyfjarannsóknunum var talsvert lægri en meðalaldur sjúklinga með CLL almennt. Fáar rannsóknir hafa metið hvernig fylgikvillar, lifun og dánarorsakir í CLL hafa breyst síðan um aldamót og þær hafa flestar verið smáar í sniðum. Við rannsökuðum þessar mikilvægu breytingar í lýðgrundaðri rannsókn til að meta þær meðferðarbreytingar sem áttu sér stað um síðustu aldamót.

Aðferðir: Þýðið var samansett úr sjúklingum sem voru skráðir með CLL samkvæmt sænsku krabbameinsskránni árin 1982-2013. Fyrir hvern CLL sjúkling var af handahófi valinn samanburðareinstaklingur af sama kyni og svipuðum aldri og búsetu. Upplýsingar um dánardag og dánarorsök var fengin frá sænsku dánarmeinaskránni. Upplýsingar um fyrri sjúkdóma, alvarlegar bakteríusýkingar og tækifærissýkingar fengust úr sænsku sjúklingaskránni. Eftirfylgd náði til 1. janúar 2014. Umframdánartíðnihlutfall (EMRR) og hlutfallsleg lifun (RS) voru reiknuð í líkönum sem leyfðu breytilega hlutfallsáhættu með 95% öryggisbilum (95% CI). Byrði fylgisjúkdóma var metin með Charlson fylgisjúkdómaskori (CCI). Líkan til að meta áhættu á bakteríusýkingum reiknaði áhættuhlutfall (HR) og leyfði endurtekna atburði. Áhætta á tækifærissýkingum var metin með nýgengishlutfalli (IRR) og horfur eftir sýkingu metnar með tilfella-dánarhlutfalli (CFR).

Niðurstöður: Þýðið samanstóð af 13,009 CLL sjúklingum. Samanborið við 1982-1992 var EMRR 0.72 (95% CI 0.66-0.77) árabilið 1993-2002 og 0.53 (0.48-0.58) árabilið 2003-2013. Lifun jókst jafnt og þétt yfir rannsóknartímabilið að yngsta aldurshópnum undanskildum. Hjá yngstu karlkyns CLL sjúklingunum minnkaði lifun lítillega til aldamóta en jókst eftir það (5 ára RS 0.87, 0.84 og 0.89 fyrir sjúklinga greinda 1982, 1992 og 2002, í sömu röð). CLL var aðaldánaorsök í 41-44% af þeim sem létust og var óbeint tengt allt að 70% af andlátum. Áhættan af því að deyja úr CLL minnkaði yfir rannsóknartímann (HR 0.78, 95% CI 0.75-0.81 fyrir hver 10 ár). Áhættan af

því að deyja úr öðrum blóðsjúkdómum og sýkingum minnkaði einnig (HR 0.60, 95% CI 0.55-0.65 og HR 0.62, 95% CI 0.52-0.73 í sömu röð). Aukin byrði fylgisjúkdóma var tengd verri horfum, hvort sem dánarsökin var tengd CLL (HR 1.35, 95% CI 1.25-1.45 og HR 1.47, 1.37-1.57 fyrir 1 og 2+ CCI stig, í sömu röð) eða ótengd CLL (HR 1.45, 1.30-1.63 og HR 2.09, 1.90-2.30 fyrir 1 og 2+ CCI stig, í sömu röð).

Nýgengi (IR) innlagna vegna bakteríusýkinga var 15 á 100 sjúklingaár. Algengustu sýkingarnar voru lungnabólgur (IR 10.5), blóðsýking (IR 3.4), og húðsýkingar (IR 1.0). Þegar nýgengið var borið saman við viðmið, var áhættan mest fyrir blóðsýkingar (HR 6.91, 95% CI 6.46-7.39) og lungnabólgur (HR 5.91, 5.64-6.18). Áhættan af því að leggjast inn vegna bakteríusýkinga minnkaði á rannsóknartímanum (HR 0.87, 0.81-0.94 árin 1992-2002 og HR 0.76, 0.70-0.82 árin 2003- 2013, samanborið við 1982-1992). Alls voru 829 innlagnir vegna tækifærissýkinga í 8,989 sjúklingum sem greindust með CLL árin 1994-2013. Algengasta sýkingin var lungnabólga vegna *Pneumocystis jirovecii* (IR 4.03 á 1,000 sjúklingaár) og áhættan var einnig mest samanborið við viðmið (IRR 114, 95% CI 58.7-252). Alvarlegar herpes zoster sýkingar voru næst algengasta sýkingin (IR 2.94). Næst komu sveppasýkingar vegna Candida (IR 1.66) og Aspergillus (IR 1.20) og höfðu þær sýkingar mjög slæmar horfur (CFR 33% og 42%, í sömu röð).

Ályktanir: Í stórri lýðgrundaðri rannsókn höfum við sýnt að lifun CLL sjúklinga hefur aukist undanfarna áratugi. Þegar dánarorsakir voru skoðaðar kom í ljós að þetta var að stórum hluta vegna bættrar lifunar m.t.t. CLL. Þessar niðurstöður styrkja fyrri niðurstöður um gagnsemi þeirrar CLL lyfjameðferðar sem var tekin upp um aldamót. Fylgikvillar jukust ekki á rannsóknartímanum, þvert á móti var lægri innlagnatíðni vegna alvarlegra bakteríusýkinga og á því að deyja úr sýkingum. Þessar niðurstöður eru mikilvægar því meðferðarárangur í lyfjatilraunum þarf að staðfesta í lýðgrunduðum rannsóknum á sjúklingum sem njóta almennrar þjónustu.

Lykilorð:

Langvinnt eitilfrumuhvítblæði, Lifun, Dánarorsakir, Fylgisjúkdómar, Sýkingar

Abstract

Introduction: Chronic lymphocytic leukemia (CLL) is a disease of the elderly and the median age of newly diagnosed patients is 72 years. CLL was traditionally treated with chlorambucil, but it was replaced at the turn of the century with fludarabine based chemo- and chemo-immunotherapy. These therapies offered better response rates, however, most clinical trials failed to show improved overall survival and elderly patients were underrepresented in those trials. Infections cause a great burden for CLL patients and the increased risk is both due to the disease itself and the treatment. Few studies have estimated temporal changes in survival, causes of death and complications of CLL and the literature is largely based on clinical trials and single-center studies. To evaluate the effect of the treatment changes that occurred at the turn of the century, we performed a nationwide study on survival, causes of death and infections in CLL patients.

Methods: Information on CLL patients diagnosed 1982-2013 was obtained from the Swedish Cancer Registry. For each CLL patient, four controls matched for age, sex and place of residence were randomly allocated. Information on date and cause of death was obtained from the Swedish Cause of Death Registry. The Swedish Patient Register was used to obtain information on serious inpatient bacterial infections, opportunistic infections and on previous diagnoses for calculation of Charlson comorbidity index (CCI). End of follow-up was January 1st 2014. In the survival and cause of death analysis, flexible parametric models were used to assess excess mortality rate ratios (EMRR), relative survival (RS) and hazard ratios (HR) with 95% confidence intervals (95% CI). The risk of serious bacterial infections was estimated in a recurrent event analysis. The risk of opportunistic infections in CLL patients compared to matched controls was estimated with an incidence rate ratio (IRR) and the impact was estimated with a 60-days case fatality ratio (CFR).

Results: In the Swedish CLL cohort; 13,009 patients; survival increased over time. Compared to the calendar period 1982-1992, the EMRR adjusted for age and sex was 0.72 (95% CI 0.66-0.77) for 1993-2002 and 0.53 (95% CI 0.48-0.58) for 2003-2013. The improvement was continuous over the study period for all age groups except for the youngest CLL population. In young male patients, survival trends were relatively static until 2000, after

which there was a continuous improvement in survival. The 5-year RS in males aged 50 years and younger was 0.87 (95% CI 0.78-0.92), 0.84 (95% CI 0.78-0.89), and 0.89 (95% CI 0.84-0.93) for 1982, 1992, and 2002, respectively. In general, CLL was the primary cause of death in 41-44% and CLL was related to nearly 70% of the mortality. Over time, CLL decreased as a cause of death (HR 0.78, 95% CI 0.75-0.81 for 10-year increase in calendar year). Furthermore, other hematological diseases (HR 0.60, 95% CI 0.55-0.65) and infections (HR 0.62, 95% CI 0.52- 0.73) decreased as a cause of death. Higher CCI was associated with increased risk of CLL-related mortality (HR 1.35, 95% CI 1.25-1.45 and HR 1.47, 1.37-1.57 for 1 and 2+ CCI points, respectively) and increased risk of CLL unrelated mortality (HR 1.45, 1.30-1.63 and HR 2.09, 1.90-2.30 for 1 and 2+ CCI points, respectively).

The incidence rate (IR) of serious bacterial infections in the CLL cohort was 15 admissions per 100 patient years. The most common infections were pneumonia (IR 10.5), septicemia (IR 3.4) and skin infections (IR 1.0). The risk compared to matched controls was highest for septicemia (HR 6.91, 95% CI 6.46-7.39) and pneumonia (HR 5.91, 5.64-6.18). The risk of infections decreased over time (HR 0.87, 0.81-0.94 in 1992-2002 and HR 0.76, 0.70-0.82 in 2003- 2013, compared to 1982-1992). In total, 829 opportunistic infections occurred in 8,989 CLL patients diagnosed 1994-2013. The most common infection was *Pneumocystis jirovecii* pneumonia (PCP, IR 4.03 per 1,000 patient years), and relative to matched controls, risk of PCP was also highest (IRR 114, 95% CI 58.7-252). Herpes zoster infections were the second most common opportunistic infections (IR 2.94). Finally, fungal infections with Candida and Aspergillus had an incidence of IR 1.66 and IR 1.20, respectively, and were associated with abysmal prognosis (CFR 33% and 42%, respectively). Other opportunistic infections were rarer.

Conclusions: In these population-based studies we have established that survival improved in CLL patients during the study period. This was largely due to decreased CLL-related mortality. Importantly, admissions and mortality due to infections decreased significantly over time. Population-based studies are essential to evaluate the benefit and complications of new treatments in real-world patients.

Keywords: Chronic lymphocytic leukemia, Survival, Causes of Death, Comorbidity, Infections.

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Contents

List of abbreviations

CCI	Charlson comorbidity index
CLL	chronic lymphocytic leukemia
CMV	cytomegalovirus
EMRR	excess mortality rate ratio
FC	fludarabine and cyclophosphamide
FCR	fludarabine, cyclophosphamide, and rituximab
HR	hazard ratio
IPR	the Swedish Inpatient Register
IR	incidence rate
IRR	incidence rate ratio
MBL	monoclonal B cell lymphocytosis
OPR	the Swedish Outpatient Register
PCP	pneumocystis pneumonia
PML	progressive multifocal leukoencephalopathy
SCR	Swedish Cancer Registry
95% CI	95% confidence interval

List of figures

Credits:

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Figure 1 B by Dr Graham Beards - Own work, CC BY-SA 3.0, https://commons.wikimedia.org/w/index.php?curid=20525320

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List of original papers

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals (I-III):

I. **Steingrímsson V,** Lund SH, Dickman PW, Weibull CE, Björkholm M, Landgren O, Kristinsson SY. Survival, Causes of Death, and the Prognostic Role of Comorbidities in Chronic Lymphocytic Leukemia in the pre-ibrutinib era. A Population Based Study. Manuscript, 2021.

II. **Steingrímsson V**, Gíslason GK, Aspelund T, Turesson I, Björkholm M, Landgren O, Kristinsson SY. A population-based study on serious inpatient bacterial infections in patients with chronic lymphocytic leukemia and their impact on survival. European Journal of Haematology. 2020 Nov;105(5):547-54.

III. **Steingrímsson V**, Gíslason GK, Þorsteinsdóttir S, Rögnvaldsson S, Gottfreðsson M, Aspelund T, Turesson I, Björkholm M, Landgren O, Kristinsson SY. A nationwide study on inpatient opportunistic infections in patients with chronic lymphocytic leukemia in the pre-ibrutinib era. European Journal of Haematology. 2020 Nov 19.

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Declaration of contribution

See *Author Contributions* in Paper I and *Acknowledgements* in Paper II-III.

1 Introduction

1.1 History

The first cases of chronic leukemias are reported as early as in the 19th century, and before 1850 Wirchow already classified leukemia into `splenic' with granular leukocytes and segmented nuclei and `lymphatic' with a small round nuclei (Hamblin 2000). Ernst Neumann pioneered the idea that leukemia was a disorder of the bone marrow around 1870 (Hamblin 2000) and the division to acute and chronic form of leukemia was set by Ebstein around 1889 (Rai 1993). At the end of the century, Paul Ehrlich developed the triacid staining that made it possible to distinguish the nucleus and cytoplasm in detail and distingish myeloid and lymphoid cells (Rai 1993, Hamblin 2000). Following this, Türk published critera for diagnosing CLL and emphasized the similarities of CLL and lymphoma (Hamblin 2000). In the 1920s Minot observed, in what is considered the first well described CLL cohort, that X-radiation shrinked lymph nodes, although it did not affect survival (Rai 1993). The benefits of chlorambucil and steroid treament were observed in the 1950s and 1960s and at the same time thourough description of the clinical features of CLL was established (Hamblin 2000). It was noted that survival was highly variable, and subsequently, stratification by predefined clinical features was implemented. In the 1960s Dalton and Dameshek, seperately but almost simultaneously, concluded that the underly pathology in CLL was related to prolonged survival and accumulation of dysfunctional lymphocytes, rather than rapid proliferation (Rai 1993).

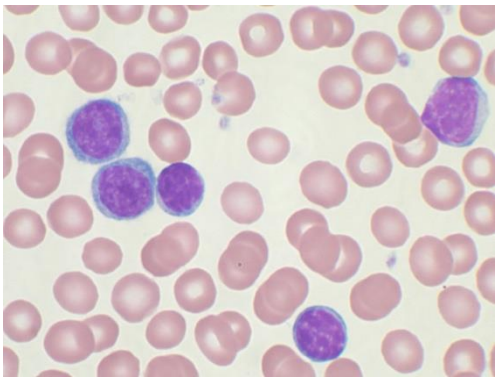
1.2 Chronic lymphocytic leukemia

1.2.1 Definition and pathogenesis

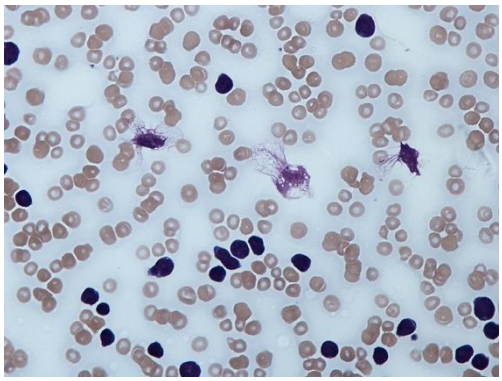
The definition of CLL has changed over time and in older studies, many diseases were included in what we now do not consider CLL, for example lymphosarcoma cell leukemia, t(14;18) follicular lymphoma, pro-lymphocytic leukemia, hairy cell leukemia, and mantle cell lymphoma (Hamblin 2000).

CLL involves growth of mature clonal B-cells within the bone marrow, lymph nodes, and spleen (Hallek 2019). In most cases CLL diagnosis is made with blood counts with differentials, blood smear, flow cytometry, and occasionally genetic features of the lymphocytes (Hallek, Cheson et al. 2018).

The diagnosis requires a consistently more than 5,000 B lymphocytes/μL for at least three months (Hallek, Cheson et al. 2018). The presence of increased clonal B lymphocytes but less than 5,000 B lymphocytes/μL and no signs of lymphadenopathy, organomegaly or bone marrow failure constitutes the diagnosis of monoclonal B cell lymphocytosis (MBL). It is thought that approximately 1% of patients with MBL will progress to CLL yearly (Rawstron, Bennett et al. 2008). The B cells in CLL are also clonal, and this must be established by immunoglobulin light-chain restriction in a flow cytometry analysis. The blood smear depicts uniform mature lymphocytes with small cytoplasm and dense nucleus (Figure 1 A). Smudge cells, representative of cellular debris, are often present (Figure 1 B).



A



B

Figure 1 Blood smears from a patient with CLL, showing **A** uniform mature lymphocytes **B** smudge cells

CLL cells express the T-cell CD5 antigen, in addition to B-cell antigens CD19, CD20, and CD23 (Hallek, Cheson et al. 2018). Furthermore, the immunoglobulins CD30 and CD79b are usually low compared to normal B cells. The panel of CD19, CD5, CD20, CD23 and clonal κ or λ is used to establish the diagnosis, but aided with the antigens CD43, CD79b, CD81, CD200, CD10, and ROR1.

The pathogenesis in CLL is related to the B-cell receptor, especially the Ig-heavy chain variable genes and whether mutations have occurred in the germinal center (Delgado, Nadeu et al. 2020). The CLL cells multiply mainly in the lymph nodes where the complex signaling from the microenvironment is important (such as the presence of notch ligands) and relation to other cells of the immune system (such as T-cells mediated with MYD-88

mutations) (Herndon, Chen et al. 2017, Delgado, Nadeu et al. 2020). Deletions on chromosome 13, especially del(13q14), are the single most common genetic abnormality observed in CLL cells and are indicative of a benign disease if occurring in isolation (Landau, Tausch et al. 2015, Hallek 2019). Other common abnormalities include del(11q), trisomy 12, del(17p), and TP53 mutation (Hallek 2019). It has been speculated that the CLL cells might derive from hematopoietic stem cells (Kikushige, Ishikawa et al. 2011).

1.2.2 Epidemiology

CLL is the most common leukemia in Western countries, with an age-adjusted incidence of approximately 4 diagnoses per 100,000 inhabitants (Ries LAG 2008). The median age is ~72 years and there is a male predominance with approximately 60% of diagnosed patients being male (Kristinsson, Dickman et al. 2009, da Cunha-Bang, Simonsen et al. 2016). In familial studies, close relatives of CLL patients have been found to be at increased risk of developing CLL (Goldin, Björkholm et al. 2009, Berndt, Camp et al. 2016, Delgado, Nadeu et al. 2020). CLL is more common in North-America and in people of European descent (Miranda-Filho, Piñeros et al. 2018, Yang, Varghese et al. 2020). No definite occupational and enviromental risk factors have been established in CLL (Brandt 1985, Talibov, Auvinen et al. 2017) although some agricultural factors and benzene exposure have been suggested (Vigliani 1976, Burmeister, LIER et al. 1982, Blair and White 1985). Observations of settlers support that the genetic variation is more important regarding incidence than enviromental factors (Gale, Cozen et al. 2000). As discussed in this thesis, CLL is associated with immunosuppresson, however, some have speculated that infections may also predispose to CLL (Anderson, Landgren et al. 2009).

Survival in CLL is discussed in chapter 1.4. In population-based studies on CLL, one must consider the possibility of lead time bias (see chapter 3.2.2.) if patients with no or minimal symptoms are diagnosed earlier due to an increase in routine blood testing. A single center study from Barcelona observed that patients were being diagnosed with earlier disease over time (Abrisqueta, Pereira et al. 2009). Similar median age over recent calendar periods argues against this being a considerable effect in Sweden (Kristinsson, Dickman et al. 2009), although other large population-based studies have indicated that there might be a slight increase in CLL incidence (da Cunha-Bang, Simonsen et al. 2016, Lenartova, Johannesen et al. 2016). In a validation study on the Swedish Cancer registry, the data did not imply that there was an increased diagnosis of early-stage CLL over time (Turesson, Linet et al. 2007).

1.2.3 Staging of chronic lymphocytic lymphoma and prognostic factors

In the mid 1970s, Rai et al. introduced clinical staging (1975) that correlated well with survival and shortly after Binet et al. proposed a new staging system (1977, 1981). These clinical staging systems are summarized in Table 1.

Table 1 The Rai and Binet staging systems

Rai	Clinical Stage	Binet	Clinical stage
0	Bone marrow and lymphocytosis	A	Less than three enlarged lymph nodes
I	Enlarged lymph nodes	B	More than three enlarged lymph nodes
II	Enlarged liver or spleen	C	Anemia and/or thrombocytopenia
III	Anemia		
IV	Thrombocytopenia		

In 1987, the Rai staging system was simplified to three stages: low risk (0), intermediate risk (I/II), and high risk (III-IV) (Rai and Montserrat 1987). Since then, these staging systems have been used unchanged clinically although numerous additional prognostic factors have been identified in CLL (Hallek, Cheson et al. 2018). TP53 mutationsm del(11q), and del(17p) have been linked to worse prognosis although this might be changing with newer therapy. Furthermore, patients with expression of CD49d, ZAP-70 and CD38 in flow cytometry as well as dominant unmutated immunoglobulin variable heavy chain (IGHV) gene have inferior prognosis. Higher levels of the serum marker β2-microglobulin is indicative of high tumor burden and is associated with worse prognosis. The presence of more than 30% mature lymphocytes on bone marrow aspiration also indicates high tumor burden, although this procedure is usually performed to establish cause of cytopenias, if necessary, and to establish complete remission.

More recently, the international prognostic index was introduced which uses molecular and clinical data at the time of CLL diagnosis to predict survival (2016). The index constitutes of age, clinical stage, TP53 status, IGHV mutational status, and β-2 microglobulin level. The index was developed from clinical trials before the era of chemoimmuntherapy where

the patients were relatively young and fit compared to CLL patients in general. However, it was validated in a population based study in the era of chemoimmunotherapy (da Cunha-Bang, Christiansen et al. 2016).

1.2.4 The immunosuppression associated with chronic lymphocytic leukemia

Arguably the most important complication of CLL is immunosuppression. The pathogenesis is multifactorial, and in part due to the CLL pathogenesis but compounded by the treatment. Up to 80% of patients with CLL will develop a serious infection, and this was even true before the era of chemo-immunotherapy (Molica 1994). The increased risk of infections was recognized in the 1930s and in the 1950s when it was observed that hypogammaglobulinema was common in CLL (Hamblin 2000, Wadhwa and Morrison 2006). The risk and severity of hypogammaglobulinemia is associated with the time since CLL diagnosis and the disease stage (Itälä, Helenius et al. 1992, Molica, Levato et al. 1993). Hypogammaglobulinemia appears to mainly predispose patients to infections with encapsulated bacteria, such as *Streptococcus pneumoniae* and *S. pyogenes, Neisseria meningitidis, Klebsiella pneumoniae, Haemophilus influenzae type b, Pseudomonas aeruginosa,* and encapsulated yeasts such as *Cryptococcus neoformans (Wadhwa and Morrison 2006).* Deficiencies in IgA and IgG4 predispose patients to respiratory tract infections (Tadmor, Welslau et al. 2018) and, interestingly, deficiency in IgA is in some studies associated with the highest risk of infections and lower survival (Rozman, Montserrat et al. 1988, Aittoniemi, Miettinen et al. 1999). Additionally, it has been speculated that not only quantitative, but also qualitative defects in immunglobulins play a role in the immunosuppression (Wadhwa and Morrison 2006).

Studies have also shown other defects in the immune system in CLL patients, such as decreased function of helper T cells, increased T-cell suppressor function, inversed CD4/CD8 ratio, decreased number of NK cells, abnormal complement activity, and enzyme deficiencies and ineffective chemotaxis in neutrophils (Wadhwa and Morrison 2006). The clinical importance of these changes in the context of the immunosuppression is, however, unclear. Furthermore, it has been shown that CLL patients that have progressed and need therapy are at increased risk of infections (Moreira, Rabe et al. 2013, Visentin, Compagno et al. 2015). Similarly, unmutated IgHV, abnormal p53 and CD38 positivity have been associated with increased risk of infections (Morrison 2010).

The immunosuppression associated with each treatment will be disussed seperately.

1.3 Treatment for chronic lymphocytic leukemia

1.3.1 Indication for treatment

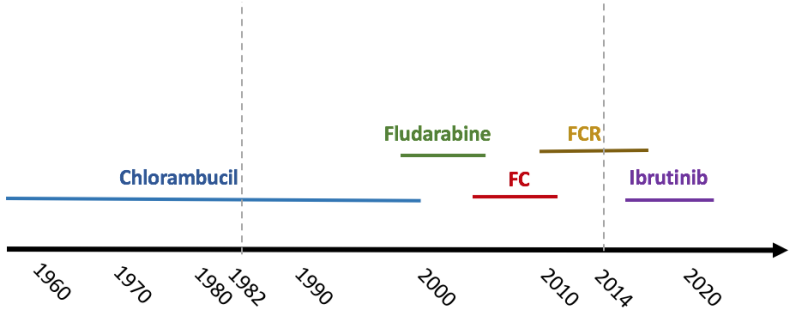
Conventionally, treatment for CLL is withheld in asymptomatic patients without signs of disease progression, i.e. stages RAI 0 and Binet A (Hallek, Cheson et al. 2018). This remains true, even with the new therapy which is well tolerated. Binet stage B or Rai intermediate risk patients can also be observed. Indication for treatment include:

i. Evidence of progressive bone marrow failure
ii. Massive, progressive or symptomatic splenomegaly or lymphadenopathy
iii. Rapidly progressive lymphocytosis
iv. Autoimmune complications, such as hemolytic anemia or thrombocytopenia, that do not respend to corticosteroids
v. Symptomatic extranodal disease, unexplained weight loss, fatique (ECOG higher than one), prolonged fevers or night sweats

Decision to initiate second- or subsequent line treatment follow the same principle.

1.3.2 Historical treatment and associated immunosuppression

Alkylating therapy was replaced by purine analogues in the 1990s and later with chemo-immunotherapy in the first decade of the century. With some simplification, immunosuppression associated with chemo-immunothepry can be divided to two phases (Tadmor, Welslau et al. 2018). The early phase is related to neutropenia which mainly exposes patients to bacterial infections, and then the delayed phase which is related to T- and B-cell depletion, which exposes patients to viral and other opportunistic infections, in addition to more common infections. Prolonged neutropenia in relation to fludarabine and the delayed neutropenia associated with FCR, can also expose patients to opportunistic infections (Tadmor, Welslau et al. 2018).



Dashed lines mark the study period.
FC: Fludarabine and cyclophosphamide
FCR: Fludarabine, cyclophosphamide, and rituximab

Figure 2 Historical overview of treatment in CLL.

1.3.2.1 Alkylating agents: Chlorambucil and bendamustine

In the 1950-1960s, the alkylating agent chlorambucil was introduced as the primary agent in treatment for CLL (Galton, Wiltshaw et al. 1961). Although this treatment reduced the disease burden, it did not improve overall survival (Rai 1993, Dighiero, Maloum et al. 1998). In general, chlorambucil was very well tolerated and the most severe infections occuring in treated patients was with common bacterial pathogens (Travade, Dusart et al. 1986).

In the first decade of the century, bendamustine was compared to chlorambucil in previously untreated CLL patients (Knauf, Lissichkov et al. 2009). The treatment response was significantly better, although there was increased toxicity and no overall survival benefit. Furthermore, it was comparable to fludarabine as single agent therapy in relapsed CLL (Niederle, Megdenberg et al. 2013).

1.3.2.2 Fludarabine

Treatment with fludarabine was introduced in the early 1990s (O'Brien, Kantarjian et al. 1993) and generally implemented in clinical practice as first line treatment around 2000 (Rai, Peterson et al. 2000). Compared to chlorambucil, fludarabine showed higher response rates, longer remissions, and progression free survival. Profound immunosuppression resulting from fludarabine treatment was observed early on, with high frequency of opportunistic infection such as PCP and Listeria sepsis (O'Brien, Kantarjian et al. 1993). However, this could also be explained in part by concomitant

steroid use (Wadhwa and Morrison 2006). The immunosuppressive effects of fludarabine is believed to be largely due to prolonged abnormal qualitative and quantitative T cell function in treated patients, although some patients show improvement in hypogammaglobulinemia (Wijermans, Gerrits et al. 1993, Keating, O'Brien et al. 1998, Wadhwa and Morrison 2006). In addition to serious bacterial infections, a high frequency of opportunistic infections has been observed in patients treated with fludarabine, including Listeria, Nocardia, mycobacteria, disseminated Candida and Aspergillus, PCP, Varicella Zoster, CMV, and hepatitis B reactivation (Wadhwa and Morrison 2006).

1.3.2.3 Anti-CD20 agents: rituximab, obinutuzumab and ofatumumab

Rituximab was usually used in combination with fludarabine and single agent treatment has not been associated with a high risk of infections, although it causes a transient reduction in B cell lymphocytes (Hainsworth, Litchy et al. 2003, Wadhwa and Morrison 2006, Morrison 2010). Ofatumumab and obinutuzumab are humanised anti-CD20 antibody that have shown activity in relapsed and refractory CLL disease (Hallek 2019).

1.3.2.4 Cytostatic combination therapy and chemo-immunotherapy

Several combination regimens have been studied in CLL. A combination of fludarabine and cyclophosphamide was taken into general practise around 2006 as it showed improved response and longer treatment free survival compared to fludarabine in young and healthy patients (Eichhorst, Busch et al. 2006, Flinn, Neuberg et al. 2007). A higher frequency of neutropenias was observed compared to fludarabine alone, however, this did not result in increased risk of severe infections (Hallek 2019).

A number of preclinical and clinical studies observed a synergy in the combination of fludarabine and rituximab early in 2002-2005, and the addition of cyclophosphamide added to the response of the treatment (Hallek 2019). The combination of fludarabine, cyclophophamide, and rituximab became the standard therapy in those eligible, folllowing the results from a large randomized controlled trial, where it was demonstrated to significantly improve the response in CLL patients (Hallek, Fischer et al. 2010). It, however, also increased the incidence of serious neutropenia. This study, as many previous studies, has later been criticized as the cohort had a median age of 61 year and comorbidities were underrepresented compared to the

general CLL population. In FCR, major infections occur in up to 16% of treated patients and was a reason for early termination of therapy in 1/3 of those patients (Morrison 2010).

Treatment with bendamustine and rituximab is effective as second line treatment and has less side effects compared to FCR (Hallek 2019). It can also be considered for elderly patients or those with previous history of infections, although severe bacterial, viral, and fungal infections have been observed with this treatment (Tadmor, Welslau et al. 2018). In addition, some combination therapies with chlorambucil, rituximab, obinutuzumab, and ofatumumab have had a minor role in CLL therapy. The addition of af an anti-CD20 antibody to chlorambucil showed improved overall survival compared to chlorambucil alone in CLL patients with coextising conditions (Goede, Fischer et al. 2014) and it did not appear to increase the risk of infections despite an increased risk of neutropenia (Tadmor, Welslau et al. 2018).

CD-52 is an antigen expressed on the surface on mature and malignant lymphocytes, NK cells and monocytes (Wadhwa and Morrison 2006). In 2007, therapy with the anti-CD52 antibody, Alemtuzumab, showed a greatly improved response compared to chlorambucil (Hillmen, Skotnicki et al. 2007). However, Alemtuzumab, is associated with profound short- and long-term disturbance of the immune system, including neutropenia as well as depletion in B, T, and NK-cell count (Morrison 2010). Opportunistic infections including CMV (very common), toxoplasmosis, acanthamoebiasis, Aspergillus, Zygomycetes, Candida, Listeria, and PCP have been reported (Keating, Flinn et al. 2002, Martin, Marty et al. 2006). As with previous treatments, infections appear to be more common in patients who have been previously treated and those with incomplete response (Wadhwa and Morrison 2006).

Table 2 Important clinical trials on CLL treatment during the study period

Clinical trial	Year	Therapy	Median age	Comorbidities	Results
Rai et al.	2000	F vs. Cl	62-64	Underrepresented	F+
Eichorst et al.	2006	F vs FC	58-59	Underrepresented	FC+
Hillmen et al.	2007	A vs. Cl	59-60	Underrepresented	A+
Knauf et al.	2009	Cl vs. B	63-64	Underrepresented	B+
Eichorst et al.	2009	F vs. Cl	70-71	Slightly underrepresented	F ~ Cl
Hallek et al.	2010	FC vs. FCR	61	Underrepresented	FCR+

F: Fludarabine, Cl: Chlorambucil, C: Cyclophosphamide, A: Alemtuzumab, B: Bendamustine, R:Rituximab

1.3.3 Current treatment options

Ibrutinib is an oral Bruton tyrosine kinase inhibitor which downregulates the overactive B cell receptor signaling in CLL cells and induces apoptosis (Herman, Gordon et al. 2011). Early trials showed good response to ibrutinib in CLL patients with relapsed and refractory CLL, even when compared to ofatumumab (Hallek 2019). Furthermore, it was effective in CLL disease with TP53 mutation (Eichhorst, Busch et al. 2009). Therapy with ibrutinib is generally very well tolerated (Eichhorst, Busch et al. 2009). Acquired resistance is becoming a better recognized long term consequence of the treatment (Woyach, Furman et al. 2014) and the main reason for discontinuation of treatment is disease progression, often related to resistance (Hallek 2019). Recent trials on ibrutinib as a single agent or in combination with rituximab or obinutuzumab have further underlined the high effectiveness and good tolerability of this new treatment, especially considering that the trials included patients with a median age of 70-73 years (Eichhorst, Busch et al. 2009, Hallek 2019).

Other targeted agents such as venetoclax and idelalisib can be considered for first line treatment as well as treatment for relapsed and refractory disease in CLL patients (Hallek 2019). Currently first line treatment include agents such as ibrutinib, combination of venetoclax and obinutuzumab, FCR, bendamustine and rituximab or chlorambucil and

obinutuzumab (Hallek 2019). The treatment regimen depends on disease stage, del(17p), p53 and IGVH mutation status, and general fitness. In second line treatment, agents such as lenalidomide, alemtuzumab and allogenic stem cell transplant are considered.

Importantly, the targeted therapy has shown good response in TP53 mutated CLL disease, in which chemo-immunotherapy had shown to be of limited efficiency (Eichhorst, Busch et al. 2009, Hallek 2019).

In general, ibrutinib is thought to be less immunosuppressive than the previous chemo-immunotherapy, and may even lead to improvement in immune responses against infections (Sun, Tian et al. 2015). However, it has also been shown that patients treated with ibrutinib have higher risk of infections compared to treatment-naïve patients and when used as salvage therapy, it has higher risk of infections than previous therapy (Williams, Baran et al. 2018). However, longer follow-up may be needed to show improvement in immune system (Byrd, Furman et al. 2015). Ibrutinib has recently been thought to confer to an increased risk of opportunistic infections compared to treatment-naïve patients, for example PCP (Ahn, Jerussi et al. 2016). Treatment with ibrutinib had a higher rate of infections compared to ofatumumab in relapsed/refractory CLL, although the risk of serious infections were similar (Tadmor, Welslau et al. 2018). The risk of infections associated with ibrutinib treatment appears to be highest early in therapy and decreases over time, although late infections have also been described, even opportunistic infections (Tadmor, Welslau et al. 2018).

1.3.4 Treatment in elderly CLL patients

From the beginning of the century, fludarabine, cyclophosphamide and later with the addition of rituximab was that standard treatment for younger and non-frailty CLL patients without important comorbidities.

The median age of CLL patients at diagnosis is more than 70 years (Kristinsson, Dickman et al. 2009). However, the median age of included CLL patients in many of the earlier clinical trials was around 60-65 years and furthermore, most patients had few or none comorbid diseases (Eichhorst, Hallek et al. 2016). Of the large clinical trials in CLL before 2010, only one included patients with a median age over 70 years (Eichhorst, Busch et al. 2009). The trial observed that chlorambucil was non-inferior to fludarabine in elderly patients.

It has been established that around half of CLL patients will have one or more major comorbidity at diagnosis, however, the prevalence of geriatric

syndromes (immobility, sarcopenia, malnutrition, etc.) is unrecognized (Eichhorst, Busch et al. 2009). Both comorbidities and geriatric syndromes are likely to have independent prognostic value in CLL (Eichhorst, Busch et al. 2009).

Early in the century; chlorambucil (with or without anti-CD20 antibody), dose attenuated FCR, and the combination of bendamustine and anti-CD20 antibody were feasible options for elderly CLL patients with or without comorbidities (Eichhorst, Busch et al. 2009).

1.3.5 Supportive management in relation to immunosuppression

1.3.5.1 Risk assessment for infections

Currently there are no formal guidelines to assess risk of infections in patients with CLL (Tadmor, Welslau et al. 2018). Known risk factors include advanced age, disease progression, relapsed/refractory disease, comorbidities, time with neutropenia, treatment, hypogammaglobulinemia, and low CD4 count (Tadmor, Welslau et al. 2018). Prophylaxis with antivirals and antibiotics should be considered in patients with multiple risk factors (Tadmor, Welslau et al. 2018). Recently, an explainable machine learning algorithm was introduced to predict the risk of infections in treatment naïve CLL patients, based on multifactorial variables (Agius, Brieghel et al. 2020). One aim was to perform a clinical trial with treatment to modulate the innate CLL immunosuppression in high risk patients.

1.3.5.2 Vaccination

CLL patients show a suboptimal response to important vaccinations, such as *Haemophilus influenza* and *Streptococcus pnemoniae* (Sinisalo, Aittoniemi et al. 2003). Routine vaccinations are recommended before initation of treatment if possible, although support for this approach from randomized controlled studies is lacking. (Hallek, Cheson et al. 2018). Influenza vaccines are recommended (Tadmor, Welslau et al. 2018), however live vaccines are contraindicated.

1.3.5.3 Immunoglobulin therapy

Important randomized controlled trials have shown decreased risk of infections (mainly bacterial) and admissions among high risk CLL patients treated with immunoglobulin prophylaxis (Gale, Chapel et al. 1988, Chapel, Dicato et al. 1994, Jurlander, Geisler et al. 1994, Molica, Musto et al. 1996). However, the cost-effectiveness of this treatment has been disputed,

especially as it has not been compared to prophylactic antibiotics and, furthermore, it is unlikely to be as effective among patients receiving highly immunsuppresive treatment with fludarabine and alemtuzumab (Wadhwa and Morrison 2006). Studies have not shown improved survival, and therefore treatment is reserved for patients with repeated infections and hypogammaglobulinemia (Hallek, Cheson et al. 2018, Tadmor, Welslau et al. 2018).

1.3.5.4 Antimicrobial prophylaxis

There has been a lack of uniform guidelines and randomized controlled trials regarding antimicrobial prophylaxis in CLL treatment and the guidelines often supported by anecdotal experience and clinical trials on CLL therapy (Morrison 2010, Tadmor, Welslau et al. 2018). However, many national associations have published guidelines in specific circumstances (Tadmor, Welslau et al. 2018).

Table 3 Examples of guidelines on antimicrobial prophylaxis recommended in different treatment regimens.

CLL treatment	Circumstances*	Prophylaxis
F	Elderly, low CD4 count	Antivirals
F	High doses of steroids	PCP prophylaxis
FC(R)	-	Antivirals and PCP prophylaxis
A	-	Antivirals and PCP prophylaxis
A	Consideration	Antifungals and CMV prophylaxis
B or Cl+R	Consideration	PCP prophylaxis

*Not including patients with repeated infections and patients with neutropenia.
F: Fludarabine, Cl: Chlorambucil, C: Cyclophosphamide, A: Alemtuzumab, B: Bendamustine, R:Rituximab

Antibiotics and/or antivirals can be used in patients with recurrent infections, however, one must consider the increased risk of pathogen resistance (Tadmor, Welslau et al. 2018). Regarding treatment with fludarabine, some recommend antivirals in elderly patients and those with low CD4 count to prevent herpesvirus infections (Morrison 2010). PCP prophylaxis should be considered in patients receiving fludarabine and high doses of steroids (Keating, Flinn et al. 2002). The addition of cyclophosphamide (FC or FCR), however, warrants antiviral and PCP prophylaxis (Morrison 2010, Tadmor,

Welslau et al. 2018). The use of Alemtuzumab also warrants both antiviral and PCP prophylaxis and a consideration of antifungal prophylaxis (Morrison 2010). CMV prophylaxis with ganciclovir has been recommended by some for Alemtuzumab (O'Brien, Ravandi-Kashani et al. 2005). Others have recommended weekly PCR although treatment/prophylaxis for asymptomatic patients with positive PCR is controversial (Tadmor, Welslau et al. 2018). Before therapy, it is often recommended to screen for status of hepatitis B, hepatitis C, CMV, and HIV infections (Tadmor, Welslau et al. 2018). Regarding alkylator based therapy, PCP prophylaxis can be considered with bendamustine and chlorambucil with anti-CD20 therapy if CD4+ cell drop below 200/μL (Tadmor, Welslau et al. 2018).

1.3.5.5 Other

The use of G-CSF to reduce the risk of febrile neutropenia is used in CLL patients according to general guidelines (Smith, Bohlke et al. 2015).

1.4 Survival in CLL

As discussed above, treatment with chlorambucil did not result in improved survival. A large population-based study on Swedish CLL patients showed improved survival from 1978 to 2003, although the survival had not improved in the youngest patients since the 1980s (Kristinsson, Dickman et al. 2009).

The survival in a Danish population-based study on more than 10,455 CLL patients showed a consistently increased survival over time from 1978-2013 for both genders and in all age groups, although the difference between the most recent calendar periods was not significant (da Cunha-Bang, Simonsen et al. 2016).

The improved survival of elderly patients in these population-based studies indicate that factors additional to the changes in treatment have also improved survival, including supportivie therapy and possibly increased lead-time bias.

1.4.1.1 Comorbidities, survival, and cause of death

CLL is a disease of the elderly and a large part of patients will not require treatment during the first years (Molica, Mauro et al. 2010, Bulian, Tarnani et al. 2011, Wierda, O'Brien et al. 2011). Therefore, it is valuable to compare the burden of CLL to the burden of comorbidities in newly diagnosed CLL patients. However, the studies on the prognostic value of comorbidities and cause of death analyses have been sparse.

In a large single-center study of unselected CLL patients, nearly 90% had a comorbid condition and nearly half had a major comorbid condition (Thurmes, Call et al. 2008). The comorbid condition, however, had minimal effect on survival compared to age and CLL stage. A retrospective analysis from two clinical trials observed that comorbid conditions negatively affected survival, but durable remission with chemoptherapy appeared to be highly important to improve survival in these patients (Goede, Cramer et al. 2014).

A population-based Danish study observed that CLL patients had a 50% increased risk of dying from infections compared to matched controls. (da Cunha-Bang, Simonsen et al. 2016). The authors observed that improved survival in CLL in recent decades was mainly explained by decreased mortality from hematological malignancies. The risk of death from infections was stable and even a slight increase was observed in oldest patients in the most recent calendar period. Importantly, the Danish study did not observe an increasing risk of death from other malignancies over time. One difficult bias to overcome in cause of death analyses is the possibility of registration bias, where CLL is registered as a cause of death due to the significance of the disease, even though it was unrelated to the mortality.

Strati et. al investigated the causes of death and related to Charlson comorbidity index in a large single-center study in 1,143 CLL patients (2017). They observed that 93% of CLL patients had a comorbidity at diagnosis and they negatively affected survival. However, almost half of the patients died directly from CLL or from an infection and the risk of CLL related mortality was unrelated to that comorbidity status at diagnosis.

2 Aims

The success and limitations of the treatment introduced since the 1990s has sparsely been evaluated in real-world practice using population-based studies. Due to inconclusive results of the clinical trials and the underrepresentation of elderly comorbid patients, the success observed in the clinical trials is not necessarily generalizable to the use in real-world CLL patients.

Our aim was to estimate temporal changes in survival and complications of CLL and relate to changes in treatment, especially the introduction of FC and FCR.

2.1 Specific aims

- To assess temporal changes in survival and causes of death in CLL patients
- To assess the prognostic role of comorbidities in newly diagnosed CLL patients and their association with different causes of death
- To assess the incidence and risk of serious bacterial infections in CLL patients compared to controls and estimate the temporal changes in the risk
- To assess the impact of serious bacterial infections on survival in CLL patients
- To assess the incidence and risk of serious opportunistic infections in CLL patients
- To assess temporal changes in the risk of serious opportunistic infections
- To assess the impact of different opportunistic infection on survival

3 Materials and methods

3.1 CLL patient cohort and registers

Each Swedish citizen is provided a unique national registration number. This allows an accurate linkage between registries to obtain information on CLL diagnoses, baseline characteristics and outcomes.

3.1.1 The Swedish Cancer Registry

Access to health care is ensured to all inhabitants of Sweden. The Swedish Cancer Registry (SCR) was founded in 1958 and every physician and pathologist in Sweden are required to report each incidence of cancer to the registry. Furthermore, lymphoproliferative diseases are almost exclusively diagnosed and treated at few hematology and oncology centers and not in private practice. These centers typically provide both outpatient and inpatient service. SCR has been validated and has been found to have high completeness and accuracy (Turesson, Linet et al. 2007). In the case of CLL the accuracy was 83.7% and completeness 87.9%, both of which is somewhat lower than for many other lymphoproliferative diseases. In most cases of wrong diagnosis, the revised diagnosis was another lymphoproliferative disease, of which well-differentiated lymphocytic lymphoma was most common. The lower completeness was likely related to the fact that early-stage disease does not require therapy and this was supported by the observation that underreporting was more common for early stage disease compared to advanced disease. Importantly for the consideration of lead time bias, the authors noted that there were no indications of increased reporting of early-stage CLL over time. Finally, in general, underreporting was more common for older patients.

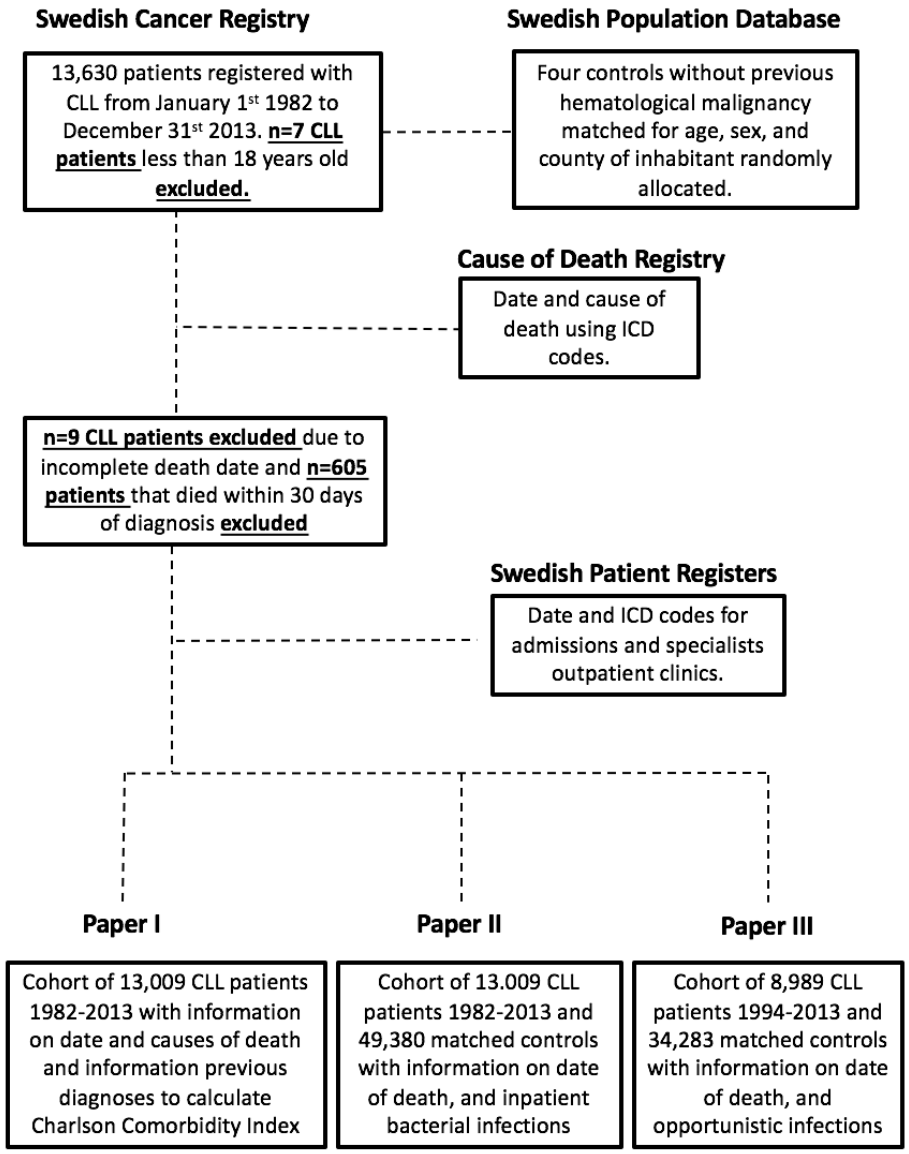


Figure 3 The linkage of information from the Swedish registers

3.1.2 The Swedish Patient and Cause of Death Registers

The Swedish Inpatient Register (IPR) was founded in 1964. IPR lists discharge diagnoses using the ICD system and has had complete coverage of hospitals in Sweden since 1987. In 1983, roughly 83% coverage of

somatic disorders was achieved by the IPR. Since 2001, outpatient visits to the hospitals have also been registered in the Swedish Outpatient Register (OPR). The IPR has been validated (Ludvigsson, Andersson et al. 2011). In relation to the study period of the thesis, the IPR used ICD-8 coding in 1982-1986, ICD-9 coding in 1987-1996, and ICD-10 coding in 1997-2013.

The Swedish Cause of Death Register provides information on causes and date of death on electronical form for research purpose since 1952. The register has, in practical terms, complete coverage of all mortality in Sweden. In the study period, the register has used the same ICD coding over time as the IPR (listed above). The register lists the primary cause of death in addition to secondary causes of death. The Cause of Death Register has also been partly validated and the accuracy has been found to be high for malignancies (Brooke, Talbäck et al. 2017).

3.1.3 Exclusion criteria

CLL is sometimes diagnosed in patients with other acute or serious illnesses where the patient dies and never receives traditional CLL follow up. As our aim was to evaluate the effect of changes in CLL treatment over time on survival and complications, patients who died within a month of CLL diagnosis were excluded.

For papers I and II, 13,630 patients were diagnosed with CLL in the years 1982-2013. In total, n=9 patients were excluded due to incomplete death date, n=7 patients who were diagnosed under the age of 18 were excluded, and n=605 were excluded as they died within a month of CLL diagnosis.

For paper III, 9,244 CLL patients were diagnosed 1994-2013, n=9 patients had incomplete death date, n= 5 were younger than 18 years of age, and n=241 died within a month of diagnosis.

3.1.4 Matched controls

For each CLL patient included in the study, four population based controls were randomly obtained from the *Swedish Population* database and had to be alive at the inclusion date, without previous history of hematological malignancies, and matched by age, sex, and county of residence. Controls that developed hematological malignancies after inclusion were not excluded. For some patients, it was not possible to find four controls. These numbers can be observed in the subtext of Table 1 in paper II and in the methods section of paper III.

3.2 Definitions and statistical analysis

3.2.1 Paper I: Survival, causes of death, and Comorbidities

Survival was defined as the number of days between CLL diagnosis and date of death or end of follow-up (1.1.2014). The causes of death categories were defined corresponding to the ICD blocks with the exception that organ specific infections were included in the infection category. Detailed definitions can be observed in Supplementary Table 1 in Paper I. Charlson comorbidity index at CLL diagnosis was calculated using information from the IPR, OPR, and SCR. The exact codes and the values assigned to them can be observed in Supplementary Table 2 in Paper I.

The statistical methods are discussed in details in the methods sections of Paper I. Survival was estimated with relative survival (RS) and derived estimates (Dickman and Coviello 2015). The strength of relative survival analyses is that they do not rely on accurate diagnoses of causes of death and thus eliminate the speculation of whether a specific cause of death is directly or indirectly related to CLL. Crudely, it is calculated by dividing the observed survival in the CLL cohort with the observed survival in Swedish population, adjusted for calendar year, age and sex. Thus, if the current 10-year observed survival for 80-year old men with CLL was 30% and the current general survival for 80-year old men was 50%, the relative survival would be 0.3/0.5 = 0.6, or 60%. RS is of limited value in cohort subjected to selection bias (such as in clinical trials) as the comparison with the population cohort becomes skewed.

3.2.2 Paper II: The risk of bacterial infections and impact on survival

The definitions of serious inpatient bacterial infections are depicted in Supplementary Table 1 of Paper II and the details on the statistical analysis are discussed in the methods sections of Paper II.

To account for the possibility of multiple admissions due to infections, the risk of infections in CLL patients compared to matched controls was estimated in a recurrent event analysis using the marginal means method (Amorim and Cai 2014). This is an extension to the traditional Cox model. In the Cox model, due to the independence assumption, it is only possible to model the time to the first event. The marginal means method importantly accounts for correlation between the events, i.e. that being admitted with an infection affects the probability of being admitted again with an infection.

To estimate the risk of infections with regards to time after CLL diagnosis we used a time dependent coefficient. This was done to address the violation of the proportional hazard assumption (Zhang, Reinikainen et al. 2018). The follow-up period was split into 10 one-year periods, and a stratified Cox analysis performed on each period, assuming proportional hazards for each period.

To estimate the risk of mortality after infections, one must allow for the covariate of infection to change over time. If the binomial variable of infection would simply be applied to the Cox model, the results would be affected by immortal time bias as the patients who were admitted due to an infection had to survive long enough to be predisposed to the infections. One way of solving this problem is to incorporate a time dependent covariate in the Cox model (Zhang, Reinikainen et al. 2018). Then, however, the issue that survival after an infection does not fulfill the proportional hazard assumption is encountered and time dependent coefficients cannot be incorporated to time dependent covariates. To address this, we performed the nested matched analysis described in the methods sections of Paper II and III.

3.2.3 Paper III: Risk of opportunistic infections and impact on survival

The definitions of inpatient opportunistic infections are depicted in Supplementary Table 1 of Paper III. The details on the statistical analysis are discussed in the methods sections of Paper III.

Opposed to Paper II, it was not necessary to allow for recurrents events when observing opportunistic infections, as they are much rarer and the information of the extremely rare recurrent opportunistic infection is limited. Furthermore, as the CLL cohort and controls were matched to baseline characteristics no adjustments for those covariates was necessary. Therefore, to assess the risk of opportunistic infections compared to controls, the incidence rate ratio was calculated using the EpiR package in R (Stevenson, Stevenson et al. 2020).

To assess temporal trends, the risk of infections in the calendar period 2002-2008 was compared to 1994-2001. In the analysis, follow up was limited to five years to minimize any possible effect of the non-propiortionality of the risk of opportunistic infection after CLL diagnosis. Competing risks were adjusted for as CLL patients are at increased risk of death and this precludes them from being able to be admitted because of opportunistic infections. This is in violation of one of the assumption of the Cox model. To

address this we estimated the risks ajdusting for competing risk using the Fine-Gray method (Zhang 2017). Using a competing risk analysis decreases the incidence of events of interest (Satagopan, Ben-Porat et al. 2004). One can consider this as such: Traditional Kaplan-Meier Curves are used to estimate cumulative hazard. Given enough time, it is possible to achieve 100% risk. However, using competing risk, two curves are produced (similar to Figure 3 in Paper I), where each event of a competing risk reduces the resulting cumulative incidence of the risk of interest compared to the traditionaly Kaplan-Meier approach. This subsequently affects all calculations of HR and confidence intervals. How much the competing risk influences the assessment goes in hand with how much the event of interest and competing risk are correlated.

3.2.4 Important limitation of the Cox proportional hazard model

To understand the choice of statistical methods in all three papers, it is very important to understand three assumptions of the Cox proportional hazard model.

1. Observations should be independent (Amorim and Cai 2014), i.e. knowing information about one study subject does not inform us about the outcome of another subject. This is for example not true for modelling repeated infections where there is a within individual correlation.
2. Censoring should be independent of outcome (Satagopan, Ben-Porat et al. 2004), i.e. non-informative. This is for example not true in a cause of death analysis, where censoring due to other causes of death is informative.
3. Stratified survival functions by covariates should be proportional over time (Zhang, Reinikainen et al. 2018). This is for example not true for estimation of survival in CLL patients stratified on the occurrence of an infections, where the risk of death is highest early after the infection.

3.2.5 Important biases to consider

Lead-time bias (Tripepi, Jager et al. 2008) occurs when diagnosis of a disease is different between groups of patients in the study cohort such that one group is systematically diagnosed at earlier stage than the other. This affects any evaluation on the survival between the groups. In our example, CLL patients diagnosed more recently could have been diagnosed earlier in the disease course due to more frequent blood testing in general.

Immortal time bias (Ho, Dion et al. 2013) occurs when, for at least a part of the cohort, there is a period of time when an event (usually death), cannot occur. In our study, patients that were admitted because of an infection after the CLL diagnoses, could not die in the period between the CLL diagnosis and the admission. Any comparison of the survival from CLL diagnosis between those who were admitted and other CLL patients could therefore become biased.

Selection bias (Hernán, Hernández-Díaz et al. 2004) is an umbrella term for many different biases. In our papers, possible selection bias arises from the difference of those CLL patients reported to the SCR compared to those who were not (see chapter on SCR above). However, the effect of selection bias to our cohort is very small compared to the clinical trials discussed above (Table 2) which increases the generalizability of our results.

4 Results and discussions

4.1 Temporal changes in survival

As observed in the 13,009 CLL patients included in Paper I, survival increased in general during the study period estimated with EMRR adjusted for age and sex (Table 2, Paper I). Compared to the calendar period 1982-1992, the EMRR was 0.72 (95% CI 0.66-0.77) for 1993-2002 and 0.53 (95% CI 0.48-0.58) for 2003-2013. The temporal changes in survival by age groups were estimated (Figure 1 and Supplementary Table 3, Paper I). Observed from Figure 1, the improvement appears to be continuous for CLL patients aged 51 years and older. For the youngest CLL population, especially male patients, survival trends were relatively static until the turn of the century, after which there is improvement in survival. This is confirmed in Supplementary Table III, Paper I, where the 5-year RS in males aged 50 years and younger was 0.87 (95% CI 0.78-0.92), 0.84 (95% CI 0.78-0.89), and 0.89 (95% CI 0.84-0.93) for 1982, 1992, and 2002, respectively.

These findings support previous studies (Kristinsson, Dickman et al. 2009, da Cunha-Bang, Simonsen et al. 2016) and suggest that the introduction of purine analog based therapy and chemo-immunotherapy resulted in improved survival. However, other changes in therapy have also contributed to this improvement, such as changes in diagnostics, risk stratification and treatment of complications (Hallek 2019). This could explain the improvement occurring in the study period before the introduction of the fludarabine-based therapies. Previous study from the Swedish CLL cohort had observed that the survival of the youngest CLL population had not improved (Kristinsson, Dickman et al. 2009). Interestingly, we observed a sharp improvement in the latest calendar period, corresponding to the introduction to the fludarabine-based therapies. This emphasizes the role of these treatment changes in the improvement of survival in recent decades.

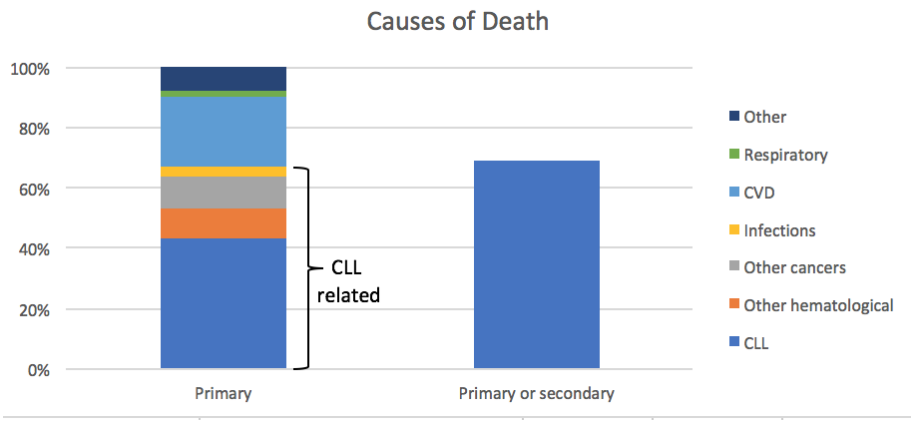


Figure 4 Causes of death in the CLL cohort, 1982-2013.

4.2 Temporal changes in causes of death

In general, CLL was the primary cause of death in 41-44% of the mortality in CLL patients during the study period (Table 3 in Paper I and Figure 4). Other causes of death related to CLL (other hematological diseases, other cancers, and infections) accounted for ~25%. Thus with this approach CLL is related to nearly 70% of the mortality, and the results is similiar when CLL is estimated as primary or secondary cause of death (Table 3 in Paper I and Figure 4). Over time, CLL decreased as a cause of death in a model adjusted for age and sex (Table 3 in Paper I and Figure 5, HR 0.78, 95% CI 0.75-0.81). Of the causes of death related to CLL, other hematological diseases (HR 0.60, 95% CI 0.55-0.65) and infections (HR 0.62, 95% CI 0.52-0.73) decreased as a cause of death, whereas other cancers remained stable over time.

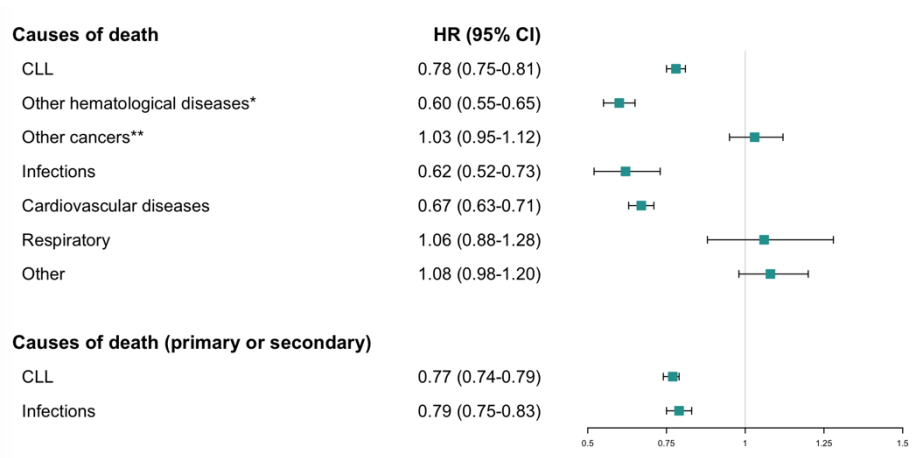


Figure 5 Hazard ratios (HRs) and 95% confidence intervals (95% CIs) for a 10-year increase in calendar year of CLL diagnosis.

As discussed above, CLL is a disease of the elderly and a large part of patients will not require treatment during the first years after diagnosis (Molica, Mauro et al. 2010, Bulian, Tarnani et al. 2011, Wierda, O'Brien et al. 2011). However, more than 40% of the mortality in the CLL cohort was directly related to CLL and nearly 70% directly or indirectly related to CLL, which shows that CLL is a considerable burden for patients. The decrease in CLL related mortality over time supports the notion that the improved survival in recent decades is in large part due to the introduction of fludarabine based therapies. This is further supported by the sharp decrease in CLL related mortality in the youngest CLL populations concomitant with these changes in treatment. These results add to the currently sparse literature on causes of death in CLL. The results were largely in concordance with a Danish population-based study, although the statistical methods differ substantially and infections did not decrease as a cause of death in the Danish cohort (da Cunha-Bang, Simonsen et al. 2016). Chemotherapy has been associated with complications such as infections and secondary cancers and therefore the observation that other hematological malignancies and infections have decreased as a cause of death and the stable non-hematological cancers is important.

4.3 Association of comorbidities and poor survival in CLL

In total, 38.1% of the CLL patients had 1+ CCI points at diagnosis (Table 1, Paper I). There was increased risk of death in patients with 1 and 2+ CCI points (HR 1.37 and 1.65, respectively, Table 4 in Paper I and Figure 6), both due to CLL (HR 1.35 and 1.47, respectively) and CLL-unrelated death (HR 1.45 and 2.09, respectively), adjusted for age, sex, and calendar year.

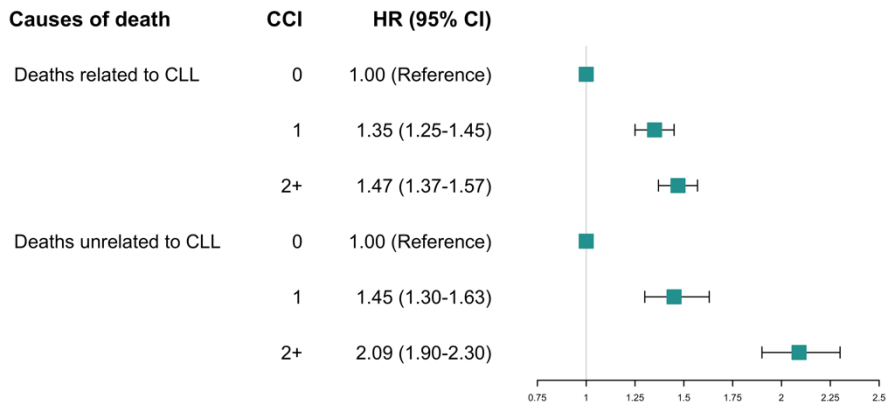


Figure 6 The hazard ratios (HR) with 95% confidence intervals (95% CI) for the risk of CLL-related and CLL-unrelated death in patients with increased Charlson comorbidity index (CCI).

Paper I is the first population-based study to estimate the association of comorbidities at CLL diagnosis and prognosis. Consideration of this association in CLL is important as comorbidities have been the key determinants on the choice of CLL treatment (Eichhorst, Busch et al. 2009). However, the results show that this increased risk of death is not entirely due to diseases unrelated to CLL and thus emphasizes the importance of available CLL treatment options in patients with comorbidities. Fortunately, with the introduction of targeted therapy, the arsenal has expanded and highly effective treatments are now considered in patients that would have been ineliegible to earlier fludarabine-based therapy (Hallek 2019).

4.4 The incidence, risk and temporal changes of serious bacterial infections

The incidence rate of serious bacterial infections in CLL patients was 15 admissions per 100 patient years (Figure 1, Paper II). The most common infections were pneumonia and other lung infections (IR 10.5 admissions per 100 patients years), septicemia (IR 3.4), and skin infections (IR 1.0). The risk compared to matched controls was highest for septicemia (HR 6.91, 95% CI 6.46-7.39) and pneumonia (HR 5.91, 95% CI 5.64-6.18) but lowest for musculoskeletal infections (HR 1.67, 95% CI 1.31-2.12). The risk of infections decreased over time in the CLL patients, where compared to 1982-1992 the HR was 0.87 (95% CI 0.81-0.94) in 1992-2002 and HR 0.76 (95% CI 0.70-0.82) in 2003-2013 (Table 2, Paper II and Figure 7). Importantly, the results were essentially the same in a sensitivity analysis where the follow-up was limited to 5-years (see Paper II) to address any possible effect from a non-proportional hazard (and additionally the proportional assumption seems to hold well considering Figure 2, Paper II).

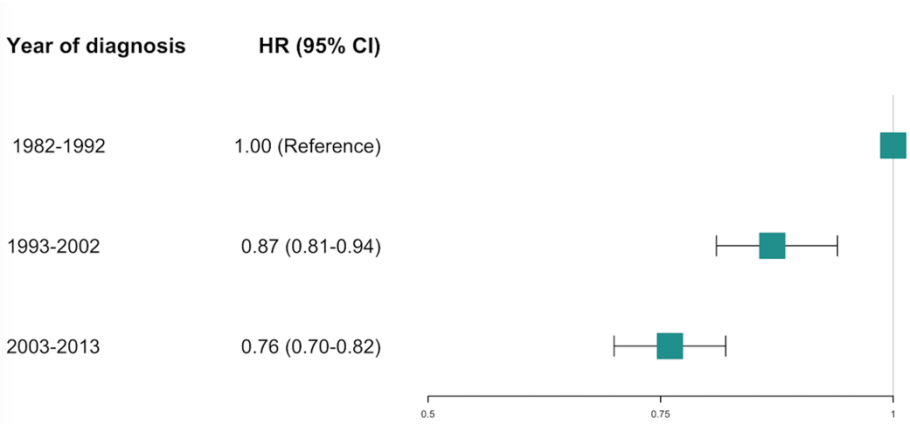


Figure 7 The temporal changes in the risk of serious bacterial infections

We observed a high risk of lower respiratory infections in CLL patients compared to controls and a low risk of muskuloskeletal infections. Studies with categorization of infections in CLL are rare but give an insight to possible mechanism of the immunpsuppression and can aid in the decision of prophylaxis. A recent single-center study on 263 patients observed an incidence rate of 20 major infections per 100 patient years with 7.79 lower respiratory infections per 100 patients years (Williams, Baran et al. 2018). This is in accordance with our study, although they were not able to compare

the incidence to matched controls.The high risk of pneumonia is interesting in the context that IgA deficiency has been shown to be an important risk factor for infections in CLL (Andersen, Eriksen et al. 2018).

The decreased risk of infections over time complemented the earlier results form Paper I where infections decreased as a cause of death. This is, to our knowledge, the first analysis of temporal trends of CLL infections and the decreasing trend is in harmony with an earlier study that reported decreased rate of positive blood cultures (Kjellander, Björkholm et al. 2016). As we concluded from paper I, this supports that the changes in CLL treatment, risk stratification and treatment of infections has resulted in improved survival without an increase in complications.

4.5 The impact of serious bacterial infections on survival

There was a high early risk of mortality after a serious bacterial infection, HR 5.48 (95%CI 5.11-5.90) for the first 12 months. The risk gradually decreased with time since infection, however, there was still a significantly elevated risk in the period beyond 60 months after the bacterial infections (HR 1.65, 95% CI 1.50-1.81). The risk of death after the infections was similar in different calendar periods, although the early risk was higher in 2003-2013 (HR 6.80, 95% CI 5.82-7.96) compared to the earliest period, 1982-1992 (HR 5.03, 95% CI 4.49-5.64).

Although expected, it was important to confirm the poor short and long term prognosis after a bacterial infection. This depicts that although most bacterial infections can be treated with antibiotics, they are associated with poor prognosis. Earlier studies have estimated the impact of infections. Andersen et al. reported ~10% 30-day mortality after a positive blood culture (2018). In another analysis, the same group observed a decreased survival in CLL patients with infections prior to treatment, where they adjusted for possible immortal time bias by plotting Kaplan-Meier curves in a landmark analysis set one year after CLL diagnosis (Andersen and Niemann 2018). A single-center study reported a poor prognosis after a major infection although it is not clear whether immortal time bias was adjusted for (Visentin, Imbergamo et al. 2017).

The impact of serious bacterial infections was similar between calendar periods, although the early risk of death was increased in the latest calendar period. This is unexpected but correlates with a study on blood stream infections in CLL patients, where the risk of death increased over time (Kjellander, Björkholm et al. 2016). Possible explanations are discussed in Paper II.

4.6 The incidence, risk and impact of opportunistic infections

In total, 829 opportunistic infections occurred in the 8,989 CLL patients diagnosed 1994-2013. Our study is the first population-based study to evaluate opportunistic infections in CLL patients.

The most common infections was PCP (200 infections, IR 4.03 per 1,000 patient years), and relative to matched controls, the risk of PCP was also highest (IRR 114, 95% CI 58.7-252). The 60-days case-fatality ratio was 20% (95% CI 14-26%). The incidence of PCP in our study was higher than in previous studies (Francis, Karanth et al. 2006, Moreira, Rabe et al. 2013), where the incidence was ~1 PCP infections per 1,000 patient years. These studies were single center-studies with less than 1,000 patients and relatively few PCP events which might explain the discrepance. Furthermore, PCP prophylaxis was quite commonly used in at least one of the studies (Francis, Karanth et al. 2006). In addition, as we could not confirm that the PCP diagnoses in our cohort were diagnosed microbiologically, the incidence might be an overestimation (see strength and limitations). The high incidence and up to 20% mortality from PCP emphasize the importance of monitoring them as side-effects of newer treatment and to have guidelines on prophylaxis. The risk of PCP is becoming recognized in patients treated with ibrutinib (Lee, Nayernama et al. 2017) although recent papers suggest that prophylaxis might not be necessary (Ryan, Cheng et al. 2020).

Herpes zoster infections were the second most common opportunistic infection (146 infections, IR 2.94). We included only serious infections, such as CNS herpes zoster, disseminated herpes zoster, or admissions where herpes zoster was the primary diagnosis (Supplementary Table 1, Paper III). This is reflected in the 15% 60-days CFR observed in patients with these herpes zoster infections. Of great interest, there was a significant decrease in herpes zoster infections between calendar periods, despite the increased risk associated with fludarabine. This might indicate that use of antiviral prophylaxis (Morrison 2009, Melchardt, Weiss et al. 2013) counterweighs the increased risk of herpes zoster infections associated with the CLL treatment. There appears to be increased risk of herpes zoster infections with ibrutinib therapy (Giridhar, Shanafelt et al. 2017) although there is not enough evidence to warrant prophylaxis. It is, however, recommended for idelalisib treatment (Wierda, Byrd et al. 2020).

Fungal infections with Candida and Aspergillus had an incidence of IR 1.66 and IR 1.20 per 1,000 person-years in the CLL cohort, respectively. This

incidence is comparable to earlier reports of various CLL cohorts (Francis, Karanth et al. 2006, Pagano, Caira et al. 2006, Moreira, Rabe et al. 2013, Visentin, Gurrieri et al. 2017, Andersen, Moser et al. 2019). Candida and Aspergillus infections were associated with abysmal prognosis, where 33% and 42% of CLL patients died within 60-days of the infections. During the era chemo-immunotherapy, only treatment with alemtuzumab warranted antifungal prophylaxis. Our results emphasize the importance of studying possible antifungal prophylaxis in high risk CLL patients receiving treatments known to predispose to these infections. However, one must also consider the number needed to treat to prevent rare events.

Other opportunistic infections were rarer. The high incidence and risk of Legionella infections was unexpected and they have, to our knowledge, not been described in CLL patients except anecdotally (Nunnink, Gallagher et al. 1986, Hendrick 2001, Ziemer, Ebert et al. 2009, Sivagnanam, Podczervinski et al. 2017). Finally, we observed seven cases of PML with abysmal prognosis, where all the patients died within a year of the PML diagnosis. PML has been described in patients receiving rituximab (Carson, Evens et al. 2009) and the poor prognosis observed in our study has been described in a case report of three CLL patients with PML (D'Souza, Wilson et al. 2010).

4.7 Strength and limitations

The major strength of the current thesis is the prospectively gathered population-based data from validated Swedish registers with negligible loss to follow-up. Furthermore, there is general access to modern medical care for all Swedish citizens. In all three papers, robust but diverse statistical analyses were performed, especially designed to address possible biases due to non-proportionality of the risk and to address immortal time bias (see Papers I-III for details).

The major limitation of the current thesis is the lack of information on staging and treatment, information which has only been available in the SCR since the first decade of the century. Due to this limitation, lead time bias is of concern, although, Turesson et al. did not observe increased early diagnosis in an validation study of the SCR (2007). Additionally, the age-adjusted incidence has not risen during the period (2015) which argues against a large effect of lead time bias. As we lacked information on individual treatment status, findings regarding survival and infections could not be directly related to specific treatment agents. However, in the temporal trend analyses, we sought to relate the findings to general changes in CLL treatment taking

place. Of course, medical practice and registration have also changed over time and this could influence the results. This was addressed to some level by comparison to matched controls in most analyses in addition to mainly including primary diagnoses. As discussed above (chapter 3.1.1.) the completeness and accuracy of the CLL was 84-88%, which was lower than most common lymphoproliferative diseases. The sensitivity of infections diagnoses in the IPR is highly variable (Ludvigsson, Andersson et al. 2011), although probably high for rare and well defined infections (see Discussions in Paper III). Finally, an important limitation was that, as the end of follow-up was January 1st 2014, the follow-up for CLL patients diagnosed late in the study period was short.

5 Conclusions

In this population-based cohort from Sweden, survival continued to improve during the era of chemo- and chemoimmunotherapy and this was largely attributed to decreased CLL-related death. The youngest CLL population had lagged behind, however, there was a sharp improvement in survival after the introduction of the fludarabine-based therapy.

Although CLL is a disease of the elderly, many of whom will not require treatment during the first years, the current thesis has depicted that CLL causes a heavy burden for the patients where it was related to almost 70% of the mortality in the cohort. Comorbidities at diagnosis were associated with increased risk of CLL-related, CLL-unrelated, and all cause mortality. The association of comorbidities with increased risk of CLL-related death further emphasizes the importance of available treatment options for CLL patients with comorbidities.

Non-CLL hematological diseases and infections decreased as a cause of death over time and non-hematological cancer were stable. This is important as chemotherapy has been associated with secondary cancers and infections.

The most common bacterial infections observed in the CLL cohort were lower respiratory infections, sepicemia, and skin infections. The risk of bacterial infections compared to a control cohort was more than five-fold, the highest for septicemia, lower respiratory infections, and CNS infections.

The risk of serious bacterial infections decreased over time, which complemented the results from the cause of death analysis. There was a more than five-fold early risk of mortality after a serious bacterial infections and there was around twofold risk beyond five years.

In the CLL cohort, there were ~17 opportunistic infection per 1,000 patient years. The most common were PCP; Herpes zoster, Pseudomonas, Candida, and Aspergillus infections. Of the most common opportunistic infections, the poorest prognosis was for the fungal infections. There was an unexpectedly high incidence and risk of Legionella infections and additionally five CLL patients were diagnosed with PML with abysmal prognosis.

Taken together, information from a Swedish population-based CLL cohort was used to observe important findings regarding survival and complications of CLL. Many of these findings were new, some have not been shown in a population-based studies and others supported the few previous population-based studies in CLL. The statistical analysis was robust and was designed to avoid biases that can cause errors in studies on infections, survival, and causes of death. The stage has been set for future population-based studies, where individual information on CLL stage, treatment, and detailed characteristics can be accounted for in analysis on survival and complications. Studies in real-world patients are essential to evaluate the overall benefit and complications of new treatment regimens.

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Original publications

Paper I

Survival, Causes of Death, and the Prognostic Role of Comorbidities in Chronic Lymphocytic Leukemia in the pre-ibrutinib era. A Population Based Study.

Short title: Survival, Causes of Death, and Comorbidities in CLL

Vilhjálmur Steingrímsson[1], Sigrún Helga Lund[1], Paul W Dickman[2], Caroline E. Weibull[2], Magnus Björkholm[3], Ola Landgren[4], Sigurdur Y. Kristinsson[1,3]

[1]Faculty of Medicine, University of Iceland, [2]Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Sweden, [3]Department of Medicine, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden, [4]Myeloma Program, Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL

Corresponding author: Sigurdur Y Kristinsson, MP, PhD, Faculty of Medicine, University of Iceland, Sturlugata 8, 101 Reykjavik, Iceland; email: sigyngvi@hi.is, tel: +354-6967371

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Key Points

Survival in chronic lymphocytic leukemia (CLL) continues to improve in the era of chemo-immunotherapy and there is decreasing mortality due to CLL itself, other hematological malignancies, and infections. Comorbidities at CLL diagnosis are associated with an increased risk of CLL-related and CLL-unrelated mortality.

Abstract

Few clinical trials on chemo-immunotherapy in chronic lymphocytic leukemia (CLL) have showed improved overall survival and elderly patients have been underrepresented. We evaluated temporal trends in survival and causes of death in a nationwide cohort consisting of 13,009 Swedish CLL patients diagnosed 1982-2013. Survival was estimated with excess mortality rate ratios (EMRR) and relative survival (RS) with 95% confidence intervals (CIs). This was calculated using flexible parametric survival models. In a cause of death analysis, hazard ratios (HRs) and 95% CIs were estimated as the linear effect of 10-year increase in year of diagnosis. The impact of Charlson Comorbidity index (CCI) at diagnosis was assessed. The excess mortality decreased over calendar time (EMRR=0.53, 95% CI: 0.48-0.58 comparing 2003-2013 to 1982-1992). The 5-year RS increased between 1982 and 2012 for all patients aged 51 years at diagnosis or above and improved for patients younger than 51 years of age after 2002. The rate of CLL-specific deaths (HR=0.78, 95% CI: 0.75-0.81), other hematological malignancies (HR=0.60, 95% CI: 0.55-0.65), and infections (HR=0.62, 95% CI: 0.52-0.73) decreased significantly over time. Compared to patients with no comorbidity, patients with 1 and 2+ CCI points had HR=1.35 (95% CI 1.25-1.45) and HR=1.47 (95% CI 1.37-1.57) for CLL-related mortality, respectively; and HR=1.45 (95% CI 1.30-1.63) and HR=2.09 (95% CI 1.90-2.30) for CLL-unrelated mortality, respectively. Taken together, survival in CLL patients improved in the era of chemo-immunotherapy and this was largely explained by reduced CLL-related mortality. The increased risk of CLL-related mortality in patients with comorbidities emphasizes the importance of treatment options for this patient group, which were introduced in the era of targeted therapy.

Introduction

Historically, chronic lymphocytic leukemia (CLL) was treated with chlorambucil.[1] Response rates improved with the introduction of purine analogs, monoclonal antibodies, and immunomodulatory drugs at the turn of the century.[2-5] The results from clinical trials of these drugs are, however, inconsistent where most[6-9], but not all[10, 11], research groups failed to show improvement in overall survival. In addition, it has been a major concern that elderly patients with comorbidities were heavily underrepresented in earlier clinical trials on CLL[6, 10-19], although this has been addressed in some of the more recently published clinical trials.[20, 21]

The inconclusive results and selection bias of important earlier trials underline the role of population-based studies to confirm clinical benefit observed in clinical trials. Few population-based studies on the trends of CLL survival over time have been published[15, 22-25], and have observed an improved survival in recent decades, although the improved survival in the youngest patients seems to have lagged during the era of chemotherapy[5], especially for young male patients.[26] A recent study on CLL patients diagnosed 1989-2016 in the Netherlands, and thus in part covering the era of targeted therapy, observed an improved survival in all age groups between the latest calendar periods.[27]

CLL is typically a disease of the elderly[28] where comorbid conditions are common[29] and many patients will not require CLL therapy for years following the diagnosis.[30-32] Therefore, it is important to evaluate the burden of CLL itself in relation to comorbidities. The literature on cause of death analyses and the impact of comorbidities in CLL on survival is, however, quite scarce. A prospective single-center study (n=1,143) depicted that CLL progression was the cause of death in nearly half the patients[29] and in a Danish population-based analysis based on 10,455 CLL patients, improved survival in recent decades was mainly due to decreased risk of death from CLL and hematological malignancies.[26] The prognostic role of comorbidities in CLL has not been established in population-based studies and results from clinical trials and single-center studies have either shown minimal[13] or negative[29, 33] effect on survival.

To establish the temporal changes in survival, causes of death, and the prognostic role of comorbidities in real-world CLL patients, we conducted a population-based study with more than 13,000 CLL patients diagnosed from 1982 to 2013 in Sweden.

Methods

Patient cohort and outcomes

Data was retrieved from Swedish nationwide registers. Linkage between registers was possible using the national registration number issued to all Swedish residents. CLL patients diagnosed from 1982 to 2013 were identified in the Swedish Cancer Register (SCR).[34] The SCR was started in 1958 and includes information on type of cancer, diagnosis date, and patient characteristics for all primary malignancies diagnosed in Sweden. Reporting is done by a physician and/or pathologist and is mandatory by law. Dates and causes of death were retrieved from the Cause of Death Register. Categories for causes of death (CLL, other hematological diseases, other cancers, infections, cardiovascular diseases, respiratory disease, and other causes) were defined by using the corresponding chapters/block in the ICD classification (see supplementary Table 1). Infections ascribed to a specific organ (meningitis, intracranial abscess, endocarditis, myocarditis, pneumonia, lung abscess, skin infections/fascitis, osteomyelitis, and pyelonephritis) were included in the infection category, not within the organ-specific category. To enable adjustment for comorbidities in terms of the Charlson comorbidity index[35] (CCI, see Supplementary Table 2 for details), the CLL cohort data was linked to the Swedish patient register which has complete nationwide coverage since 1987 for all inpatient visits, and since 2001 for all specialist visits. In addition, information of previous cancers was obtained from the SCR. Patients with incomplete death date (n=9) and aged below 18 at time of diagnosis (n=7), were excluded from the study. CLL is sometimes diagnosed during other acute illnesses, and the patient dies before ever receiving CLL specific follow-up and treatment. As one of our main aims was to assess how changes in CLL treatment affected survival and causes of deaths, patients dying within 30 days of diagnosis (n=605) were excluded from the study.

Changes in CLL treatment during study period

During the study period, treatment strategies for CLL patients in Sweden have closely followed international guidelines. Chlorambucil was the standard initial treatment for

CLL patients.[1] It was replaced in the late 1990s with fludarabine as primary therapy.[6, 7, 36] In the next years, the combination of fludarabine and rituximab was found to be effective as first line treatment[37, 38] and the addition of cyclophosphamide (FCR) was found to further improve responses.[39] Most Swedish patients in the years 2007-2014 were treated with fludarabine and cyclophosphamide (FC), FCR, or chlorambucil in those patient ineligible to fludarabine therapy.[40] Alemtuzumab alone and bendamustine together with rituximab accounted for approximately 10% of treatment options in 2007-2014 in Sweden.[40] Allogenic stem cell transplantation has been a therapeutic option in young and healthy patients with high risk profile.[41]

Statistical methods

Data was analyzed using time to event analysis. The underlying time scale was time since diagnosis, and all patients entered at 30 days after diagnosis (i.e. delayed entry/left-truncation). Patients were followed until date of death, December 31, 2013, or ten years after diagnosis, whichever came first.

Trends in cancer-related mortality were analyzed using relative survival methods. Expected mortality rates, stratified on year, sex, and age, were retrieved from the human mortality database (www.mortality.com). For estimation of excess mortality rate ratios (EMMR) and 95% confidence intervals (CIs), a flexible parametric relative survival model[42] with 5 degrees of freedom (df) for the baseline, assuming proportional excess hazards was used. Both univariable and multivariable; adjusted for age (≤50, 51-65, 66-80, ≥81), sex, and calendar year (1982-1992, 1993-2002, 2003-2013); models were fitted. To estimate temporal trends, the assumption of proportional excess hazards was relaxed and a more complex model was used. This model included sex, age, and calendar year (modelled using splines), two-way interactions between all three variables, and interactions between all mentioned variables and the time scale (df=3).

For each competing cause of death, HRs and 95% CIs for a ten-year increase in calendar year were estimated using a cause-specific flexible parametric survival model with 5 df for the baseline rate, assuming proportional hazards and adjusted for

age (categories as above) and sex. For CLL, hematological cancers, other cancers, and all other causes of death combined, cumulative probabilities of death were predicted from a model with age and year modelled as splines, adjusted for sex, including two-way interactions between all three variables, and with time. Due to convergence issues, the df's for the time-varying effects were 2 for CLL deaths and 1 for the remaining.

All analyses were performed using Stata version 15 software (StataCorp, College Station, TX). Modelling and predictions were done using the stpm2[43] and multistate[44] packages.

Approvals were obtained from the the Ethical Review Board in Stockholm.

Results

Demographic features

In total, 13,009 patients diagnosed between January 1, 1982 and December 31, 2013, were included for the analysis. Median age at diagnosis was 72 years and males constituted 60.9% of patients. The baseline characteristics stratified by calendar periods are shown in Table 1. Also, shown in Table 1, is the distribution of comorbidities at CLL diagnosis where 61.9% had no comorbidity at diagnosis, 15.5% had 1 CCI point and 22.6% had 2+ CCI points.

Survival and excess mortality

Comparing the calendar periods 1993-2002 and 2003-2013 to 1982-1992, there was a significant decrease in mortality adjusted for age and sex with EMRR=0.72 (95% CI 0.66-0.77) and EMRR=0.53 (95% CI 0.48-0.58), respectively (Table 2). Increasing age was associated with increasing risk of mortality with EMRR=1.45 (95% CI 1.20-1.75), EMRR=2.72 (95% CI 2.27-3.26), and EMRR=5.68 (95% CI 4.67-6.92) for the age groups, 51-65 years, 66-80 years, and ≥81 years of age, respectively, compared to the age group ≤50 years of age.

Comparing temporal trends in the five-year relative survival, there was an improvement in RS in all age categories for both sexes during the study period (Figure 1 and Supplementary Table 3). The improvement was significant in males 51-65 years old with RS=0.83 (95% CI 0.81-0.85) in 2002 and RS=0.75 (95% CI 0.72-0.78) in 1992 and in males 66-80 years old with RS=0.73 (95% CI 0.70-0.75) in 2002 and RS=0.61 (95% CI 0.57-0.64) in 1992. Furthermore, the improvement was significant in females 66-80 years old with RS=0.80 (95% CI 0.77-0.83) in 2002 and RS=0.73 (95% CI 0.69-0.76) in 1992.

Cause of death analysis and the prognostic role of comorbidities

CLL was the primary cause of death in 41.5-44.2% of the mortality observed in the cohort, stratified by calendar periods (Table 3). CLL decreased significantly as a cause of death over time estimated in flexible parametric survival model (HR=0.78, 95% CI 0.75-0.81 as primary cause of death and HR=0.77, 95% CI 0.74-0.79 as primary or secondary cause of death). Other hematological malignancies (HR=0.60, 95% CI 0.55-0.65), infections (HR=0.62, 95% CI 0.52-0.73 and HR=0.79, 95% CI 0.75-0.83 as primary or secondary cause of death), cardiovascular disease (HR=0.67, 95% CI 0.63-0.71) decreased significantly as a cause of death, whereas other cancers and respiratory diseases did not.

Stratified by age groups, there was a sharp decrease in five-year CLL-related mortality in patients younger than 50 years of age in the late 1990s (Figure 2-3). In the age groups 51-65 and 66-80 years of age, the decrease was more continuous throughout the study period. In the oldest age group, older than 81 years of age, there was a rise in the cumulative CLL related death until the mid 1990s where the risk decreases again.

The percentage of mortality related to CLL was 71.2%, 69.2%, and 62.5% for patients with 0, 1, and 2+ CCI points at CLL diagnosis, respectively (Table 4). In an adjusted model, the risk of overall mortality, CLL-related and CLL-unrelated mortality increased with increasing CCI score. Compared to the risk of CLL-related mortality in patients with 0 CCI points at CLL diagnosis, patients with 1 and 2+ CCI points had HR=1.35 (95% CI 1.25-1.45) and HR=1.47 (95% CI 1.37-1.57), respectively. For CLL-unrelated mortality the risk was HR=1.45 (95% CI 1.30-1.63) and HR=2.09 (95% CI 1.90-2.30) for 1 and 2+ CCI points, respectively.

Discussion

In this large population-based study, observing more than 13,000 CLL patients diagnosed in Sweden, survival continued to improve in the era of chemo-immunotherapy. Importantly, CLL related deaths decreased over time and, furthermore, the risk of infections related mortality decreased, despite the increased risk of infections associated with chemo-immunotherapy. Our results underline that the improvement in CLL therapy introduced at the beginning of the century resulted in improved survival in real-world CLL patients.

We found a steady improvement in survival in CLL patients aged 51 years and older over the study period. A slightly decreased survival was observed in the youngest CLL patients early in the study period, however, the survival improved after 2002. The overall improvement in survival is likely to reflect the chemotherapy introduced in the 1990s and the chemo-immunotherapy introduced in the first decade of the century. The survival improvement in elderly patients early in the study period was mainly due to decreased risk of death not related to CLL. The improvement observed later in the study period, however, might in part represent the introduction of treatments such as dose reduced FCR and bendamustine.[45, 46] Apart from changed treatments regimens in CLL, other improvements in management have also occurred, such as improved staging systems[47], improved management of infections, and increased specialization.[48] Our findings of improved survival in this population-based cohort support earlier observations from clinical trials of a clinical benefit of fludarabine based therapies.[6-10]

CLL was the primary cause of death for more than 40% of the observed mortality and diseases related to CLL (other malignancies and infections) accounted for additional ~24% of the mortality. These findings were in harmony with earlier findings reported from a single-center cohort.[29] Our results emphasize the burden of CLL even though it is disease of elderly patients with comorbidities[13] where a large part will not require treatment early in the disease.[30-32] The risk of CLL-related death decreased over time which underscores the role of more effective treatment in the improved survival in recent decades. Infections and hematological diseases decreased as a cause of death,

and non-hematological cancers were relatively stable over time. This is important, as a rise in these causes of death could have been expected with the introduction of chemotherapy and chemo-immunotherapy. The temporal changes were largely in accordance with a recent Danish population-based study, although it did not depict decreased risk over time from infections[26] as observed in the current study. Our results from the cause of death analysis, establish that the improved survival in recent decades is largely due to decreased risk of CLL-related mortality.

Comorbidities at CLL diagnosis were significantly associated with increased risk of CLL-related, CLL-unrelated, and overall mortality. Previous studies have been conflicting regarding the prognostic value of comorbidities in CLL[13, 29, 33], and this is the first population-based study to estimate this. In the era of chemotherapy, many patients were not considered for standard therapy due to comorbid conditions. The association of a higher CCI score with an increased risk of CLL-related mortality is in contrast with a previous single-center study where no association was found adjusted for age, sex, and CLL stage at diagnosis.[29] Our results underline the importance of considering effective treatment in CLL patients despite known comorbidities and fortunately these approaches have improved with the new targeted therapy.[49]

The main strength of the study is the generalizability of the real-world data due to its population-based setting in Sweden, where there is a general access to standardized up-to-date medical care. The data was prospectively gathered in high quality nationwide registers[34, 50, 51] over a long study period where there was negligible loss to follow-up. Robust statistical analysis was performed where relative survival was used to estimate the improvement in survival independent of the exact cause of death and those results complemented by a cause of death analysis. This study has some limitations. Lead-time bias is of concern, as we did not have information on CLL staging at diagnosis. However, there was no evidence that reporting of early-stage CLL had been increasing in a validation study of the Swedish Cancer Registry.[34] Another limitation is the 88% completeness of CLL in the SCR and the diagnostic accuracy around 84%, which is lower than for many other lymphoproliferative diseases.[34] The revised diagnosis was typically another lymphoproliferative disease, of which well-differentiated lymphocytic lymphoma was the most common. Importantly, underreporting did not seem to increase with calendar periods. Finally,

the end follow-up was 1st of January 2014 which limits the interpretation for the patients diagnosed late in the study period.

Taken together, we have in this large population-based study shown improvement in survival of CLL patients in the last decade, which was largely due to improved CLL-specific survival. This is, to our knowledge, the first population-based study to estimate the prognostic role of comorbidities in CLL patients and its association to causes of death. In the next decade, population-based studies must evaluate the effects of targeted therapy on survival and causes of death in real-world patients.

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Authorship Contributions

V Steingrímsson, SH Lund, and SY Kristinsson designed the study. SY Kristinsson and O Landgren obtained data. C Weibull performed the statistical analyses. V Steingrímsson and SY Kristinsson wrote the report. All the authors were involved in the analyses and the interpretation of the results. All authors read, gave comments, and approved the final version of the manuscript.

Disclosure of Conflicts of Interest

The authors have no conflict of interests relevant to this paper.

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TABLE 1 Demographic features of 13,009 patients diagnosed with chronic lymphocytic leukemia (CLL) in Sweden between 1982 and 2013, stratified by calendar period of diagnosis.

	1982-1992	1993-2002	2003-2013	Total
Total, freq. (row %)	3,643 (28.0)	4,230 (32.5)	5,136 (39.5)	13,009 (100)
Age at diagnosis, freq. (col %)				
≤50 years	159 (4.4)	185 (4.4)	235 (4.6)	579 (4.5)
51-65 years	837 (23.0)	1,090 (25.8)	1,391 (27.1)	3,318 (25.5)
66-80 years	2,031 (55.8)	2,182 (51.6)	2,466 (48.0)	6,679 (51.3)
≥81 years	616 (16.9)	773 (18.3)	1,044 (20.3)	2,433 (18.7)
Median age (range)	72 (24-102)	72 (23-102)	71 (23-98)	72 (23-102)
Sex, freq. (col %)				
Male	2,225 (61.1)	2,540 (60.0)	3,157 (61.5)	7,922 (60.9)
Female	1,418 (38.9)	1,690 (40.0)	1,979 (38.5)	5,087 (39.1)
Charlson Comorbidity Index				
0 points	2,516 (69.1)	2,705 (63.9)	2,838 (55.3)	8,059 (61.9)
1 point	575 (15.8)	612 (14.5)	827 (16.1)	2,014 (15.5)
2+ points	552 (15.2)	913 (21.6)	1,471 (28.6)	2,936 (22.6)

Due to rounding, not all percentages add up to 100%

TABLE 2 Excess mortality ratios and 95% confidence intervals (CIs) during first 10 years after chronic lymphocytic leukemia (CLL) diagnosis by calendar period, sex, and age at diagnosis.

	EMRR[a]	(95% CI)	EMRR[b]	(95% CI)
Calendar period of diagnosis				
1982-1992	1.00	*ref*	1.00	*ref*
1993-2002	0.70	(0.65-0.77)	0.72	(0.66-0.77)
2003-2013	0.50	(0.45-0.55)	0.53	(0.48-0.58)
Age at diagnosis				
≤50 years	1.00	*ref*	1.00	*ref*
51-65 years	1.39	(1.15-1.68)	1.45	(1.20-1.75)
66-80 years	2.60	(2.16-3.12)	2.72	(2.27-3.26)
≥81 years	5.05	(4.14-6.15)	5.68	(4.67-6.92)
Sex				
Male	1.00	*ref*	1.00	*ref*
Female	0.75	(0.69-0.81)	0.68	(0.63-0.73)

[a] Estimated from univariable flexible parametric relative survival main effects models, assuming proportional excess hazards.
[b] Estimated from a multivariable flexible parametric relative survival main effects model assuming proportional excess hazards.

TABLE 3 Frequencies and proportions of causes of death within the first 10 years after diagnosis among 13,009 patients diagnosed with chronic lymphocytic leukemia (CLL) in Sweden between 1982 and 2013, stratified by calendar period of diagnosis. Hazard ratios (HRs) with 95% confidence intervals (CIs) estimating the linear effect of every 10-year increase in calendar year of diagnosis of a specific cause of death, estimated from a flexible parametric survival model (df=3) adjusted for age at diagnosis and sex, assuming a proportional effect of calendar year over follow-up.

	Number of deaths (col%)			
Cause of death (primary)	**1982-1992**	**1993-2002**	**2003-2013**	**HR (95% CI)**
Any cause	2,770 (100)	2,855 (100)	1,888 (100)	-
CLL	1,189 (42.9)	1,263 (44.2)	783 (41.5)	0.78 (0.75-0.81)
Other hematological malignancies*	327 (11.8)	284 (10.0)	129 (6.8)	0.60 (0.55-0.65)
Other cancers**	228 (8.2)	331 (11.6)	273 (14.5)	1.03 (0.95-1.12)
Infections	92 (3.3)	60 (2.1)	61 (3.2)	0.62 (0.52-0.73)
Cardiovascular disease	726 (26.2)	626 (21.9)	380 (20.1)	0.67 (0.63-0.71)
Respiratory	49 (1.8)	72 (2.5)	56 (3.0)	1.06 (0.88-1.28)
Other	159 (5.7)	219 (7.7)	206 (10.9)	1.08 (0.98-1.20)
Cause of death (primary or secondary)				
CLL	1,917 (69.2)	1,964 (68.8)	1,273 (67.4)	0.77 (0.74-0.79)
Infections	862 (31.1)	920 (32.2)	614 (32.5)	0.79 (0.75-0.83)

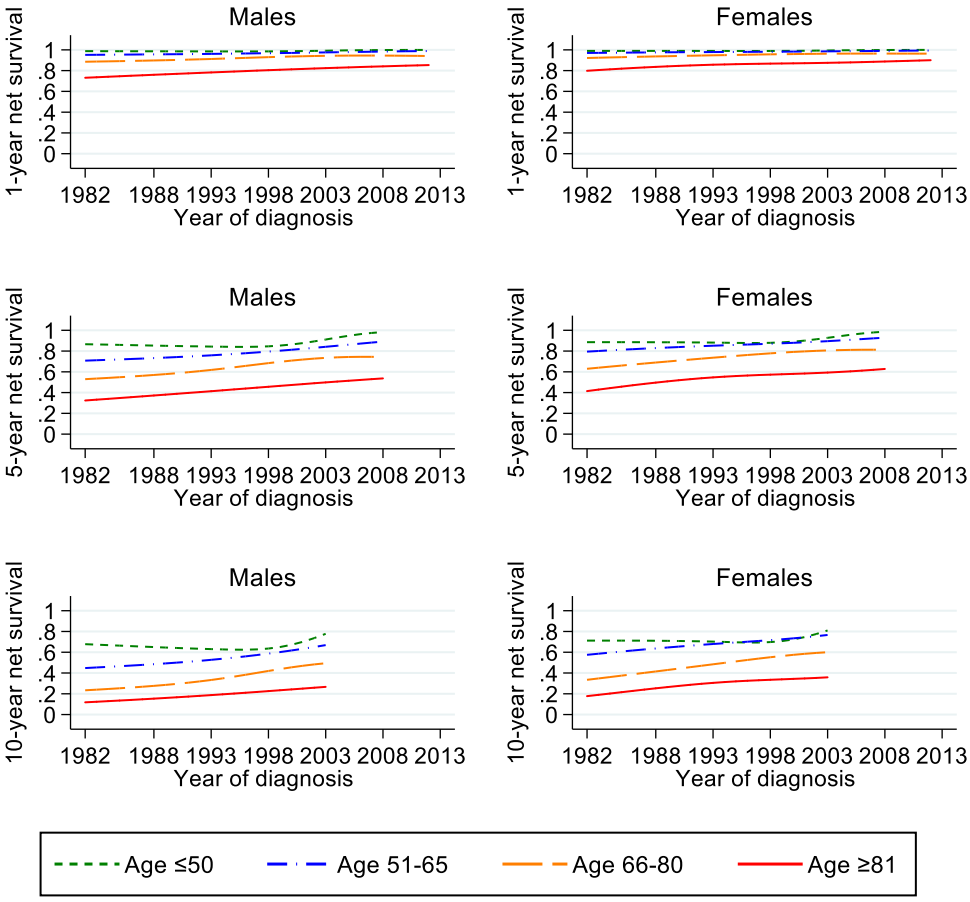
* Not including CLL.
** Not including any hematological malignancies.

TABLE 4 Charlson comorbidity index (CCI) distribution among chronic lymphocytic leukemia (CLL) patients dying within the first 10 years after diagnosis, stratified by whether the death was related to CLL (death certificate with CLL as primary or secondary cause of death) or not.

	Deaths related to CLL		**Deaths unrelated to CLL**		**All deaths**	
CCI	**N (%)**	**HR* (95% CI)**	**N (%)**	**HR* (95% CI)**	**N**	**HR* (95% CI)**
0	3,018 (71.2)	1.00	1,221 (28.8)	1.00	4,239	1.00
1	924 (69.2)	1.35 (1.25-1.45)	412 (30.8)	1.45 (1.30-1.63)	1,336	1.37 (1.29-1.46)
2+	1,212 (62.5)	1.47 (1.37-1.57)	726 (37.5)	2.09 (1.90-2.30)	1,938	1.65 (1.56-1.75)
Total	5,154 (68.6)		2,359 (31.4)		7,513	

Due to rounding, not all percentages add up to 100%
* Estimated from a flexible parametric survival model (df=5) adjusted for calendar year, age at diagnosis, and sex.

FIGURE 1 Temporal trends in 1-, 5-, and 10-year net survival for patients diagnosed with chronic lymphocytic leukemia (CLL) in Sweden, diagnosed between 1982 and 2012 (1-year



estimate), 2008 (5-year estimate), and 2003 (10-year estimate).

FIGURE 2 Temporal trends in the 5-year cumulative probability of death due to CLL among male (top panel) and female (bottom panel) patients diagnosed with CLL 1982-2008, by age at diagnosis (≤50/51-65/66-80/>80 years).

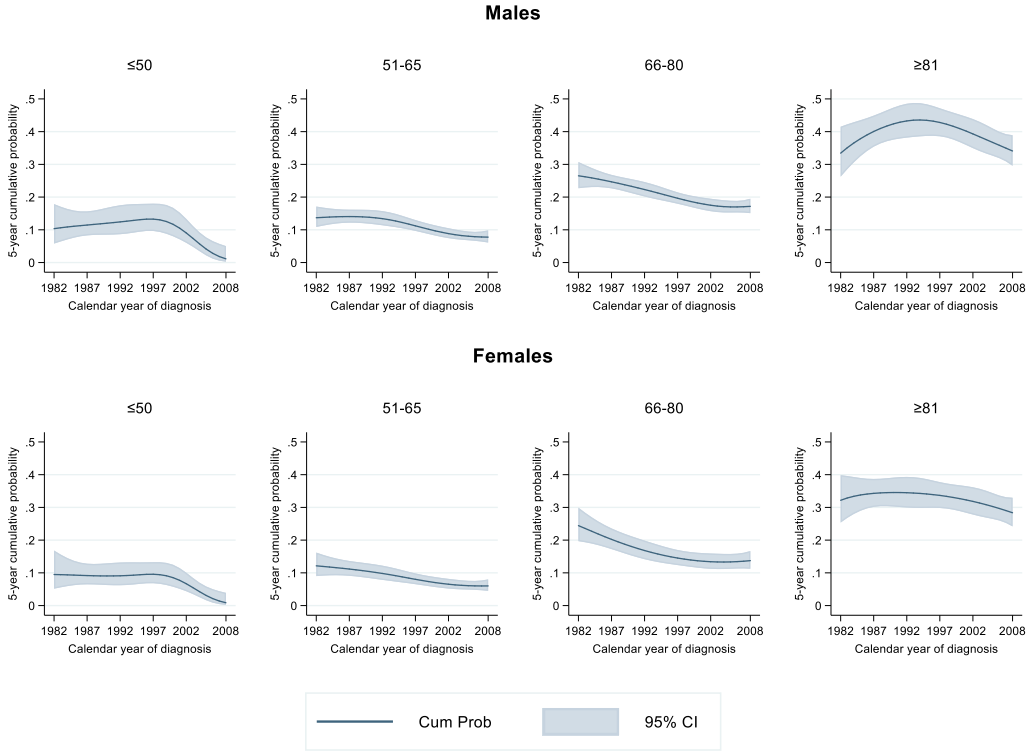
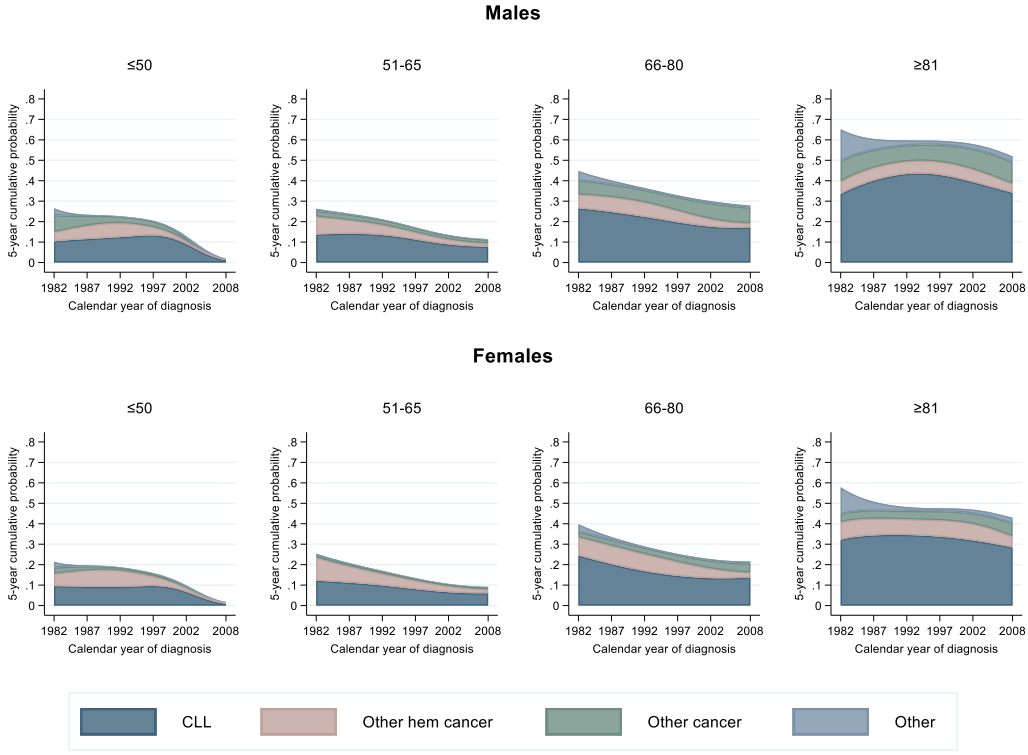


FIGURE 3 Stacked cumulative probabilities of death due to CLL, other hematological cancer, other cancer, and other deaths among male (top panel) and female (bottom panel) patients diagnosed with CLL 1982-2013, by age at diagnosis (≤50/51-65/66-80/>80 years).



SUPPLEMENTARY TABLE 1 ICD codes used for cause of death categories.

	ICD 10	**ICD 9**	**ICD 8**	**Note**
Chronic lymphocytic leukemia	C91.1	204.1	204.15	
Hematological malignancies	C81-C96, D45-D47	200-208	200-209	Without CLL
Other Cancer	C00-C80, C97	140-199	140-199	Without other hem
Infections	A00-B99	001-139	001-139	
Cardiovascular diseases	I00-I99	390-459	390-458	
Respiratory	J00-J99	460-519	460-519	
Organ specific codes assigned to infections:				
Meningitis	G00-G02, G04.2,	320-321	320	
Intracranial abscess	G06-G07	324	322,324	
Endocarditis	I33, I38, I39.8	421	421	
Respiratory tract infections	J00-J22	460-466, 475, 480-487	460-486	
Lung abscess	J85-86,	513	513	
Skin infections/fascitis	L00-08, M72.6,	680-686	680-686	
Osteomyelitis	M46.2, M86,	730.0,	720.0	
Pyelonephritis	N10,	590	590	

SUPPLEMENTARY TABLE 2 ICD codes used for the calculation of Charlson Comorbidity index (CCI). Information on previous cancer obtained from the Swedish Cancer Registry (SCR)

	ICD 10	ICD 9	ICD 8	CCI value
Myocardial infarction	I21-22, I252	410, 412	410, 412	1
Congestive heart failure	I50	428	427.00, 427.10	1
Peripheral vascular disease	I71, I73.9, I79.0, R02, Z95.8-9	441, 443W, 443X, 785E, V43E	441, 443.99	1
Cerebrovascular disease	I60-66, I67.0-2, I67.4-9, G45.0-2, G45.4, G45.8-9, G46, I68.1-2. I68.8, I69	430-438	430-438	1
Dementia	F00-F02, F05.1	290	290	1
Chronic lung disesase	J40-47, J60-67	490-496, 500-505	490-493, 515-518	1
Connective tissue disease	M32, M33.2, M34, M35.3, M35.8-9, M06.0, M06.3, M06.9, M05.0-3	710A-B, 710E, 714A-C, 714W, 725	734.00-01, 734.09, 716.10, 734.10, 695.40, 712.00, 712.10, 712.20, 712.38-39, 712.50	1
Peptic ulcer	K25-28	531-534	531-534	1
Chronic liver disease	K70.2-3, K73, K71.7, K74.0, K74.2-6	571C, 571E-G	571.90, 571.98-99	1
DM without complications	E100-101, E109, E110-111, E119, E130-131, E139, E140-141, E149	250A-C	250.00, 250.09	1
Hemi- or paraplegia	G04.1, G81, G82.0-2	342, 344B	343.00-01, 343.08-09, 344.00-03,344.08-09	2
Kidney disease	N01, N03, N05.2-6, N07.2-4, N18-19, N25	582-583, 585-586, 588	582-583	2
DM with end organ damage	E10.2-8, E11.2-8, E13.2-8, E14.2-8	250D-H, 250X	250.01-8	2
Severe liver disease	K72.1, K72.9, K76.6, K76.7	572C-E, 572W	573.02-03, 573.08-09	2
HIV	B20-B24	042-044	-	6
Malignancy	SCR	SCR	SCR	2
Metastatic tumor	SCR	SCR	SCR	3

SUPPLEMENTARY TABLE 3 Estimated 1-, 5-, and 10-year relative survival (RS) with 95% confidence intervals (CIs) for patients diagnosed with chronic lymphocytic leukemia (CLL) in Sweden, 1982-2102. Top panel shows results for male patients, bottom panel for female patients.

Males		**1-year RS (95% CI)**	**5-year RS (95% CI)**	**10-year RS (95% CI)**
≤50 years	1982	0.99 (0.97-1.00)	0.87 (0.78-0.92)	0.68 (0.52-0.79)
	1992	0.99 (0.96-0.99)	0.84 (0.78-0.89)	0.63 (0.53-0.72)
	2002	0.99 (0.97-1.00)	0.89 (0.84-0.93)	0.74 (0.65-0.81)
	2012	1.00 (0.99-1.00)		
51-65 years	1982	0.95 (0.94-0.96)	0.71 (0.65-0.76)	0.45 (0.37-0.52)
	1992	0.96 (0.95-0.97)	0.75 (0.72-0.78)	0.52 (0.47-0.56)
	2002	0.97 (0.97-0.98)	0.83 (0.81-0.85)	0.65 (0.61-0.69)
	2012	0.99 (0.98-0.99)		
66-80 years	1982	0.89 (0.86-0.90)	0.53 (0.47-0.58)	0.23 (0.18-0.29)
	1992	0.91 (0.90-0.92)	0.61 (0.57-0.64)	0.32 (0.28-0.36)
	2002	0.94 (0.93-0.95)	0.73 (0.70-0.75)	0.48 (0.44-0.52)
	2012	0.94 (0.92-0.96)		
≥81 years	1982	0.73 (0.65-0.79)	0.32 (0.22-0.43)	0.12 (0.05-0.21)
	1992	0.78 (0.73-0.82)	0.41 (0.33-0.48)	0.18 (0.12-0.25)
	2002	0.82 (0.78-0.85)	0.49 (0.42-0.55)	0.26 (0.19-0.34)
	2012	0.85 (0.79-0.90)		
Females				
≤50 years	1982	0.99 (0.97-1.00)	0.89 (0.79-0.94)	0.71 (0.53-0.83)
	1992	0.99 (0.97-1.00)	0.88 (0.82-0.92)	0.70 (0.59-0.79)
	2002	0.99 (0.98-1.00)	0.91 (0.86-0.94)	0.77 (0.68-0.85)
	2012	1.00 (1.00-1.00)		
51-65 years	1982	0.97 (0.96-0.98)	0.79 (0.74-0.84)	0.58 (0.49-0.65)
	1992	0.98 (0.97-0.98)	0.85 (0.82-0.87)	0.67 (0.62-0.72)
	2002	0.98 (0.98-0.99)	0.89 (0.87-0.91)	0.75 (0.71-0.79)
	2012	0.99 (0.99-1.00)		
66-80 years	1982	0.92 (0.90-0.94)	0.63 (0.57-0.68)	0.33 (0.27-0.40)
	1992	0.95 (0.93-0.95)	0.73 (0.69-0.76)	0.47 (0.43-0.51)
	2002	0.96 (0.95-0.97)	0.80 (0.77-0.83)	0.59 (0.55-0.64)
	2012	0.96 (0.95-0.98)		
≥81 years	1982	0.80 (0.74-0.85)	0.41 (0.31-0.52)	0.18 (0.09-0.28)
	1992	0.85 (0.82-0.88)	0.54 (0.47-0.61)	0.30 (0.22-0.38)
	2002	0.87 (0.84-0.90)	0.59 (0.52-0.65)	0.35 (0.27-0.43)
	2012	0.90 (0.85-0.93)		

Paper II

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ORIGINAL ARTICLE

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A population-based study on serious inpatient bacterial infections in patients with chronic lymphocytic leukemia and their impact on survival

Vilhjálmur Steingrímsson[1] | Gauti K. Gíslason[1] | Thor Aspelund[2] | Ingemar Turesson[3] | Magnus Björkholm[4] | Ola Landgren[5] | Sigurdur Y. Kristinsson[1,4]

[1]Faculty of Medicine, University of Iceland, Reykjavik, Iceland

[2]Centre for Public Health Sciences, University of Iceland, Reykjavik, Iceland

[3]Department of Hematology and Coagulation Disorders, Skane University Hospital, Malmö, Sweden

[4]Department of Medicine, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden

[5]Myeloma Service, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Correspondence
Vilhjálmur Steingrímsson, Faculty of Medicine, University of Iceland, Sturlugata 8, 101 Reykjavik, Iceland.
Email: vilhjalmur.steingrimsson@gmail.com

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Abstract

Objective: Infections in chronic lymphocytic leukemia (CLL) have been thoroughly investigated in the setting of clinical trials and single-center studies. However, large cohort studies on real-world data and studies on temporal trends are lacking. We performed a nationwide study on serious bacterial infections in CLL.

Methods: Using high-quality Swedish government-based registries, 13 009 CLL patients diagnosed in 1982-2013 and their 49 380 matched controls were included.

Results: Overall, CLL patients had an increased risk of serious inpatient bacterial infections with a hazard ratio (HR) 5.32 and 95% confidence interval (95% CI) 5.11-5.53, and the highest risk was observed for septicemia (HR 6.91, 95% CI 6.46-7.39) and lung infections (5.91, 5.64-6.18). The risk of serious inpatient bacterial infections decreased overtime with HR 0.87 (0.81-0.94) and HR 0.76 (0.70-0.82) in 1993-2002 and 2003-2013, respectively, compared to 1982-1992. CLL patients had an increased risk of death following a serious inpatient bacterial infection compared to matched CLL patients, and the risk was highest in the first 12 months after the infection (HR 5.48, 95% CI 5.11-5.90).

Conclusion: We have, in this nationwide study, characterized the risk of serious bacterial infections in CLL patients and, importantly, depicted that the risk has decreased overtime.

KEYWORDS
chronic lymphocytic leukemia, immunology and infectious diseases, lymphoproliferative diseases

1 | INTRODUCTION

Chronic lymphocytic leukemia (CLL) is a disease of the elderly, and long-term survival has increased considerably in recent decades.[1,2] Infections are a common cause of morbidity and mortality in CLL patients, and they are attributed both to the immunodeficiency associated with the disease and side effects from CLL therapy.[3] CLL patients have been reported to have 50% increased risk of death from infections compared to matched controls.[1]

Our knowledge on the incidence and risk of infections in CLL patients is largely based on clinical trials on CLL therapy[4,5] where patients are not necessarily representative for the CLL patients population in general.[6] Few large cohort studies have investigated the risk of infections in CLL.[7-14] Earlier cohort studies have shown that there is a susceptibility to infections in patients with monoclonal B-cell lymphocytosis, a precursor to CLL,[15] and the risk is even similar to the risk in treatment naïve CLL patients.[9] In a recent nationwide Danish study, where the outcome was first blood culture after CLL diagnosis, it was established that increased age, male gender, Binet stage B or C, high beta-2 microglobulin, and low IgA levels were associated with increased risk of infection in treatment naïve CLL patients.[7] In another nationwide Danish study on more than 4000 CLL patients diagnosed 2004-2017, a model using machine learning algorithm to identify CLL patients at a high risk for infections was introduced.[14] The generalizability from such large cohort studies is highly valuable in defining the risk of infections in CLL.

In a recent study, based on 263 CLL patients, infections were categorized by site and the incidence estimated in relation to previous therapy.[8] Overall, the highest incidence was found for respiratory and skin infections. This categorization of infections by organs is important as it gives information on the mechanism of immunosuppression in CLL and can be used to aid decision making in prophylaxis[16] and early empiric therapy.[17]

The literature on temporal patterns of infections in CLL patients is limited, although this knowledge is imperative to evaluate the effects of treatment regimens on infections. In a study on bloodstream infections in 275 CLL patients, a significantly lower percentage of positive bloodstream infections was reported after 2000 and it was speculated to be due to more effective CLL treatment.[10]

Motivated by these studies and considering the lack of large cohort studies on the risk of infections in CLL patients, we performed a nationwide study on serious inpatient bacterial infections in CLL patients using ICD discharge codes from the Swedish Patient Registry. We evaluated the risk of the infections in comparison with matched controls, estimated temporal changes and the effect of the infections on patient survival.

Novelty statement

Few nationwide studies have been conducted on infections in CLL, and temporal trends and categorization by sites have not been depicted before in such studies. In our study, there was a more than five-fold increased risk of serious inpatient bacterial infections in patients with CLL and the risk decreased between calendar periods. Changes in CLL treatment and supportive therapy have not resulted in increased risk of serious bacterial infections, and different risk of infections by sites can guide antibiotic prophylaxis and empiric treatment.

2 | METHODS

2.1 | Study population

Information on CLL patients diagnosed 1982-2013 was obtained from the Swedish Cancer Registry. The data did not include information on CLL stage or treatment. Vital status was retrieved from the Cause of Death registry and the end of follow-up was January 1, 2014. Patients diagnosed with CLL younger than 18 years old (n = 7) were excluded (as misclassification). Furthermore, as CLL is occasionally diagnosed during treatment for other serious illnesses where the patient dies before receiving traditional CLL specific treatment, we excluded CLL patients who died within 30 days from CLL diagnosis but included them in a sensitivity analysis.

For each CLL patient (n = 13 009), four random population-based controls without previous history of hematological malignancy and matched by gender, year of birth and place of inhabitant were identified (n = 49 380). The matched controls could develop hematological malignancies, including CLL, after the date of inclusion.

For each CLL patient and matched controls, information on all inpatient infections was obtained from the centralized Swedish Patient Registry. The patient registry has almost complete coverage of national hospital admissions with high accuracy.[18] Discharge ICD codes that were indicative of serious bacterial infections were included in the study, and the infections were categorized by site (Table S1).

2.2 | Statistics and approvals

Incidence was estimated with the number of serious inpatient bacterial infections per 100 person-years. Significance of the difference between CLL patients and the matched controls was assessed with hazard ratio (HR) and 95% confidence interval (95% CI) in a recurrent event Cox model, using the marginal means method.[19] We additionally calculated the incidence per 2-month periods in relation to time since CLL diagnosis and assessed the significance with the time-dependent coefficient and 95% CIs from the recurrent event analysis.

To assess the risk of mortality after the first serious inpatient bacterial infection in the CLL patients, a nested matched control analysis was performed. For each CLL patient with a first serious inpatient bacterial infection, the time of the infection was set $t = 0$ and two other CLL patients of the same gender, similar age and year of diagnosis (±4 years) and exact same duration of CLL but without a serious inpatient bacterial infection up to that time, were

identified (Table S3). The time of the infection, $t = 0$, was also used as time point of reference for the two matched CLL controls. Time-dependent coefficient in a Cox proportional hazards model was calculated to estimate the difference in mortality between CLL patients with an infection compared to the matched CLL patients.

All statistical analysis was performed in RStudio, version 1.0.136. Approval was obtained from the Swedish Ethical Review Authority for this study. Due to the large population-based design of the study over a long period, informed consent was not possible. This is in accordance with our prior studies and covered by the Ethical committee.

3 | RESULTS

3.1 | Baseline characteristics

A total of 13 009 CLL patients and 49 380 matched controls were included in the study. In total, 11 787 serious inpatient bacterial infections occurred in 5757 CLL patients and 14 747 serious inpatient bacterial infections occurred in 9922 matched controls during follow-up (Table 1). CLL patients with a serious inpatient bacterial infection had the same median age (median age 72 years, range 23-102) as those without an infection (72 years, 23-99). CLL patients with a serious inpatient bacterial infection had a mean follow-up of 2396 days (median 1964 days) compared to 2047 days (median 1518) in those without an infection. The mean number of serious inpatient bacterial infection in CLL patients with an infection was 2.05, compared to 1.46 in matched controls with an infection.

3.2 | The risk of serious inpatient bacterial infections

Overall, CLL patients had a significantly increased risk of serious inpatient bacterial infections compared to matched controls (Figure 1, HR 5.32, 95% CI 5.11-5.53). Furthermore, CLL patients were at a significantly increased risk of all infections categories studied. The

TABLE 1 Demographic features of patients with chronic lymphocytic leukemia and matched controls grouped by occurrence of serious inpatient bacterial infections

	Patients with chronic lymphocytic leukemia		Matched controls	
	n = 13 009		n = 49 380	
	No infections	Admissions due to infections	No infections	Admissions due to infections
Number of infections	-	11 787	-	14 474
Number of patients (%)	7252 (56)	5757 (44)	39 458 (80)	9922 (20)
Age at diagnosis, number of patients (%)				
<50	348 (5)	231 (4)	1991 (5)	130 (1)
51-65	1852 (26)	1466 (25)	10 913 (28)	1736 (17)
66-80	3590 (50)	3089 (54)	19 581 (50)	5866 (59)
81 and above	1462 (20)	971 (17)	6973 (18)	2190 (22)
Median age at diagnosis (range)	72 (23-102)	72 (23-99)	71 (23-102)	74 (37-100)
Gender				
Females	42%	36%	40%	34%
Year of diagnosis, number of patients (%)				
1982-1992	1657 (23)	1986 (34)	9784 (25)	3856 (39)
1993-2002	2050 (28)	2180 (38)	12 219 (31)	3909 (39)
2003-2013	3545 (49)	1591 (28)	17 455 (44)	2157 (22)
Mean follow-up in days (median)	2047 (1518)	2396 (1964)	3207 (2,664)	3830 (3392)
Mean number of infections (median; range)	-	2.05 (1; 1-17)	-	1.46 (1; 1-20)

Note: Including all infections from 180 d prior to CLL diagnosis to end of follow-up. Patients who died within 30 d from CLL diagnosis (n = 593) and patients diagnosed younger than 18 y of age (n = 7) were excluded. Patients with incomplete follow-up date were excluded (n = 21 CLL patient and n = 86 matched controls). For 338 (2.6%) CLL patients, no matched controls were found, and for 738 (5.7%) CLL patients, there were less than four matched controls. Infections of the same category recurring within 30 d were considered readmission for the same infection.

highest risk was for septicemia (6.91, 6.46-7.39) and lung infections (5.91, 5.64-6.18) and the lowest risk for septic arthritis and osteomyelitis (1.67, 1.31-2.12).

When assessing temporal trends, there was a significantly decreased risk of serious inpatient bacterial infections in CLL patients in the most recent calendar periods compared to 1982-1992 (0.87, 0.81-0.94 and 0.76, 0.70-0.82 for 1993-2002 and 2003-2013, respectively; Table 2).

The incidence of serious inpatient bacterial infections in CLL patients was higher compared to the matched controls throughout a 10-year period after the CLL diagnosis (Figure 2A). The significance was estimated with time-dependent coefficient in the recurrent event analysis, and the risk was significantly increased throughout the 10-year period (Figure 2B).

3.3 | Mortality after first serious inpatient bacterial infection

There was a significantly increased risk of mortality in CLL patients that had a serious inpatient bacterial infection throughout the observed 5-year period (Figure 3). The risk was highest in the first 12 months after an infection (HR 5.48, 95% CI 5.11-5.90) and steadily declined throughout the 5-year period. For the period after 60 months, the risk remained significantly elevated (HR 1.65, 95% CI 1.50-1.81). Mortality was relatively similar between calendar periods (Figure 3), although there was a significantly higher early mortality in 2003-2013 (HR 6.80 for the first 12 months after an infection, 95% CI 5.82-7.96) compared to 1982-1992 (HR 5.03, 4.49-5.64).

3.4 | Sensitivity analyses

In a sensitivity analysis (Supplementary Text) where CLL patients who died within 30 days were included and in an analysis where all CLL patients without a matched control were excluded, the results were essentially the same as in the overall analysis. In the analysis using only the primary discharge diagnosis, the results were also essentially the same, except that the risk of serious inpatient bacterial infections remained stable between calendar periods (Table S2A,B).

In addition, the increased early mortality after a serious inpatient bacterial infection did not differ significantly between calendar periods. In the sensitivity analysis where follow-up was limited to 5 years, the results from the temporal trend analyses were essentially the same as in the overall analysis.

4 | DISCUSSION

In this population-based study on more than 13 000 CLL patients in the real-world clinical care and their almost 50 000 matched controls, we have shown that CLL patients have more than five-fold increased risk of serious inpatient bacterial infections and the risk has decreased overtime. The infections were associated with a high risk of early mortality. Our results are important considering the treatment changes in the study period and no standard guidelines on prophylaxis for bacterial infections.

The incidence of infections in the CLL patients observed in our study was comparable with earlier smaller studies.[20,21] The incidence was highest for lung infections, skin infections, and septicemia, and the risk, when compared to matched controls, was highest for septicemia, lung infections, and central nervous system infections. Few studies have categorized the risk of infection in CLL patients by site; however, Williams et al[8] reported in a study based on 263 CLL patients that the incidence rate for major infections was 20 per 100 patient years, with the highest rate for lower respiratory infection. The study, however, lacked comparison with matched controls to be able to estimate where the risk was relatively highest. The high risk of pulmonary infections is interesting in light that IgA deficiency is one of the most important risk factors for infection in CLL patients[7] and that elevation of IgA following treatment with ibrutinib has been found to be protective for infections.[22] The high risk of pneumonia in our study and the high proportion of *Streptococcus pneumoniae* in positive blood cultures in earlier studies on CLL patients[10,13] stress the importance of pneumococcal vaccination, although there is decreased response in CLL patients.[23]

In the main analysis, the risk of serious inpatient bacterial infections in CLL patients decreased between the calendar periods, a trend that was not observed in the controls where the risk increased.

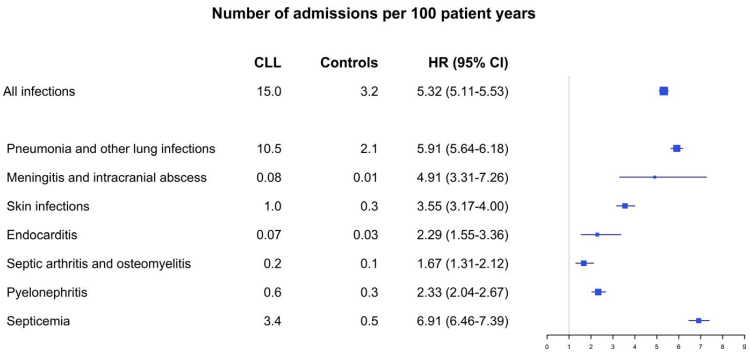

FIGURE 1 Incidence and risk of serious inpatient bacterial infections in patients with chronic lymphocytic leukemia compared to matched controls. Including all infections from 180 prior to CLL diagnosis to end of follow-up in the incidence calculation and all infections from CLL diagnosis to end of follow-up in HR calculations. CI, confidence interval; CLL, chronic lymphocytic leukemia; HR, hazard ratio [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 2 The hazard ratios for each calendar period in a recurrent event analysis with the endpoint admission due to serious inpatient bacterial infections, adjusting for age and gender

	Patients with chronic lymphocytic leukemia	Controls
Year of diagnosis	Hazard ratio (95% confidence interval)	Hazard ratio (95% confidence interval)
1982-1992	1.00 (Reference)	1.00 (Reference)
1993-2002	0.87 (0.81-0.94)	1.13 (1.07-1.19)
2003-2013	0.76 (0.70-0.82)	1.12 (1.05-1.20)

Note: Including all infections from CLL diagnosis to end of follow-up.

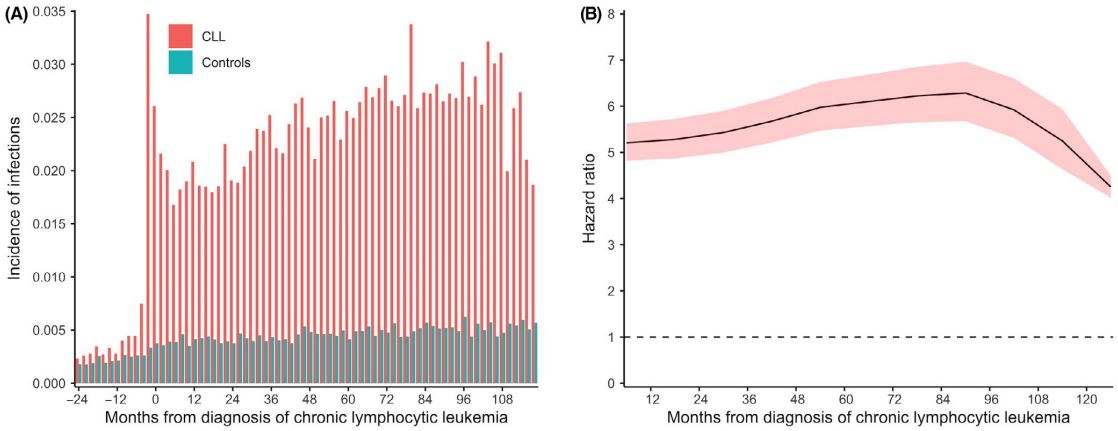

FIGURE 2 A, The incidence of admissions due to serious inpatient bacterial infections in patients with CLL and matched controls in 2-mo periods with relation to the time of diagnosis. B, Hazard ratio with 95% confidence interval using Loess regression for the risk of serious inpatient bacterial infections in CLL patients compared to matched controls by time since diagnosis of CLL [Colour figure can be viewed at wileyonlinelibrary.com]

Although the risk remained stable in the CLL patients in a sensitivity analysis using only the primary discharge diagnosis, it decreased relative to the matched controls where a significantly increased risk was observed. These results are very important as a rise in the risk of bacterial infections in CLL patients could have been expected considering the treatment changes at the turn of the century, where fludarabine and cyclophosphamide with or without rituximab replaced chlorambucil as first-line treatment in eligible patients.[24-28] To our knowledge, this study is unique in estimating temporal trends in CLL infections and supplements an important finding from an earlier study showing that infections were not increasing as a cause of death in CLL patients in 1978-2013.[1] Our results of decreasing serious inpatient bacterial infections are in line with an earlier study that showed decreasing rate of positive bacterial blood cultures,[10] and we reinforce its conclusion that more effective treatment in CLL patients probably leads to decreased incidence of serious bacterial infections in the long term.

The serious inpatient bacterial infections in CLL patients were associated with a nearly six-fold risk of death in the first year, and even though the risk decreased, it remained significant beyond the 5-year period observed in our analysis. The results of worse prognosis after infections in CLL patients are in keeping with earlier studies,[7,10,11,29] although comparison is difficult due to different definitions of infections and statistical methods. The poor prognosis following a serious inpatient bacterial infection emphasizes the importance of guidelines on risk assessment,[14] antibiotic prophylaxis,[16] and early empiric treatment[17] of serious infections in CLL patients. There has been a lack of risk assessment tools for infections in CLL patients, although recently[14] a nationwide Danish study introduced an explainable machine learning model to identify CLL patient at high risk for infection. In recent years, the guidelines on early empiric treatment in febrile neutropenia have improved[17]; however, there has been a lack of international consensus on antibiotic prophylaxis for bacterial infections,[16,30] although individual national and local institutions have recommended prophylaxis in specific circumstances.

The mortality risk in CLL patients after a serious inpatient bacterial infection was similar between calendar periods, although there was a significant increase in early mortality between the first and the last calendar period. The finding of higher early mortality in the most recent calendar period is likely of limited clinical importance, although in line with an earlier study[10] where blood stream infections were associated with worse prognosis in the most recent calendar period. One interpretation of these results is that the proportion of high-risk patients among those admitted to hospitals is increasing, while low-risk patients are more likely to be treated as outpatients. Increased antibiotic resistance or change in the bacteria

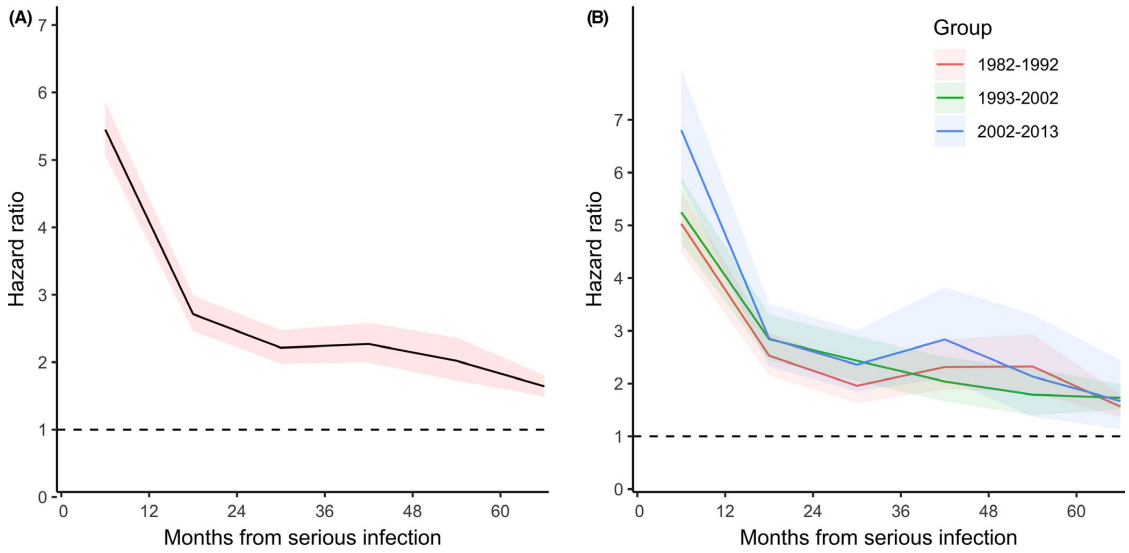

FIGURE 3 A, The hazard ratio with 95% confidence interval for mortality comparing CLL patients with an infection and their matched CLL patient controls. B, The hazard ratio with 95% confidence interval for mortality comparing CLL patients with an infection and their matched CLL patient controls, stratified by year of CLL diagnosis [Colour figure can be viewed at wileyonlinelibrary.com]

flora causing infections is an unlikely explanation as these factors have been shown to remain stable overtime.[10,31] The worse prognosis observed in the later calendar periods could imply that patients have more severe infections in the era of new chemo-immunotherapy leading to higher morbidity and mortality although further studies are needed to address this notion.

The main strength of the study is the observation of real-world CLL patients in a nationwide setting using clinically important endpoints from the validated Swedish national inpatient Registry.[18] The registry has been found to have 45.7%-95.4% sensitivity in registration of infections and a consistent high specificity 92.6%-99.7% Thus, the rate of infections in our study might be underestimated. Using robust statistical analyses, all serious inpatient bacterial infections in an individual were accounted for and the estimation of the impact of serious inpatient bacterial infections on survival in our study adds important information due to the nationwide design. Furthermore, as the risk of death after an infection is not constant (ie, the proportional assumption does not hold) and to avoid immortal time bias, we believe that our approach to depict the risk as time-dependent coefficient in a nested case-control analysis is more accurate than presenting the results with a single hazard ratio (using a time-dependent covariate) and more robust than performing a landmark analysis. Finally, several thorough sensitivity analyses were performed. The most important limitation of our study is the lack information on individual CLL stage and treatment, although temporal changes can be attributed to some extent to changes in treatment recommendations during the study period. In recent years, national cancer registries have increasingly been registering information on treatment which will in many ways allow more detailed analysis in the future. However, such an approach also has its limitations as the evaluation on the effects of specific treatments on outcomes (infections, mortality) in large registry-based studies can become biased due to different patient and disease characteristics motivating a given specific treatment. Another limitation was that we did not have information on cultures, and therefore, there was no confirmation that the infections were bacterial. A limitation to our temporal trend analysis was that diagnostic criteria and clinical practice have changed over time, which could not be completely accounted for. However, we have addressed this limitation as we could relate our findings in the CLL cohort to trends in their matched controls, and furthermore, we performed a sensitivity analysis using only the main discharge diagnosis, which is less sensitive to changes in clinical practice. The Swedish inpatient registry did not have complete coverage for somatic diseases until 1987, and the coverage was 85% in 1983.[18] Although this may impact the data, it is very unlikely to change any of the major results as the comparison between CLL patients and the matched controls should be stable, and in our temporal trend analysis, we found a decreasing trend of infections in the CLL patients. However, this might partly explain the increasing trend for infections observed in the matched controls, which emphasizes the importance of being able to compare the results in CLL patients to those observed in matched controls. A study on completeness and accuracy found that the completeness of the CLL data from the cancer registry[32] was around 88% and the accuracy 84%, which was lower than for multiple myeloma, non-Hodgkin and Hodgkin lymphoma. Although this is likely to have some impact in our analysis, it is unlikely to change any of the major results as the hazard ratios for

serious inpatient bacterial infections and mortality were found to be high with narrow confidence intervals.

Our findings from this nationwide study add important information to the literature on infections in CLL. We have established a more than five-fold risk of serious inpatient bacterial infections in CLL patients compared to matched controls. We furthermore demonstrated that the risk of serious inpatient bacterial infections has not increased overtime and that there is a high early mortality risk following an infection. Our results underline the importance of thorough and evidence-based guidelines on risk assessment, prophylaxis, and early treatment of infections.

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VS and SYK designed the study. SYK and OL obtained data. VS prepared the data and performed all statistical analysis. GKG and TA gave statistical advice. VS wrote the report. All the authors were involved in interpretation of the results. All authors read, gave comments, and approved the final version of the manuscript. All the authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

ORCID

Vilhjálmur Steingrímsson https://orcid.org/0000-0002-9385-2960
Ingemar Turesson https://orcid.org/0000-0002-4115-8010

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Paper III

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ORIGINAL ARTICLE

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A nationwide study on inpatient opportunistic infections in patients with chronic lymphocytic leukemia in the pre-ibrutinib era

Vilhjálmur Steingrímsson[1] | Gauti Kjartan Gíslason[1] | Sigrún Þorsteinsdóttir[1] | Sæmundur Rögnvaldsson[1] | Magnús Gottfreðsson[1,2] | Thor Aspelund[3] | Ingemar Turesson[4] | Magnus Björkholm[5] | Ola Landgren[6] | Sigurdur Y. Kristinsson[1,5]

[1]Faculty of Medicine, University of Iceland, Reykjavik, Iceland

[2]Department of Infectious Diseases, Landspitali University Hospital, Reykjavik, Iceland

[3]Centre for Public Health Sciences, University of Iceland, Reykjavik, Iceland

[4]Department of Hematology and Coagulation Disorders, Skane University Hospital, Malmö, Sweden

[5]Department of Medicine, Karolinska University Hospital and Karolinska Institutet, Stockholm, Sweden

[6]Myeloma Service, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Correspondence
Vilhjálmur Steingrímsson, Faculty of Medicine, University of Iceland, Sturlugata 8, 101 Reykjavik, Iceland.
Email: vilhjalmur.steingrimsson@gmail.com

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Abstract

Objective: Opportunistic infections in chronic lymphocytic leukemia (CLL) have been described in clinical trials, single-center studies, and case reports. We performed a nationwide study to estimate the incidence and impact of inpatient opportunistic infections.

Methods: The incidence rate (IR) and incidence rate ratio (IRR) for Swedish CLL patients diagnosed 1994-2013, and matched controls were calculated, as well as the case-fatality ratio (CFR).

Results: Among 8989 CLL patients, a total of 829 opportunistic infections were registered (IR 16.6 per 1000 person-years) compared with 252 opportunistic infections in 34 283 matched controls (IR 0.99). The highest incidence in the CLL cohort was for Pneumocystis pneumonia (200 infections, IR 4.03); Herpes zoster (146 infections, IR 2.94), and Pseudomonas (83 infections, IR 1.66) infections. The highest risk relative to matched controls was observed for Pneumocystis pneumonia (IRR 114, 95% confidence interval 58.7-252). The 60-day CFR for CLL patients with opportunistic infections was 23% (188/821), highest for progressive multifocal encephalopathy (5/7, 71%) and aspergillosis (25/60, 42%).

Conclusion: We have uniquely depicted the incidence of rare and serious infections in CLL patients and found a relatively high incidence of Pneumocystis pneumonia. Of the most common opportunistic infections, CLL patients with aspergillosis had the poorest prognosis.

KEYWORDS

chronic lymphocytic leukemia, immunology and infectious diseases, lymphoproliferative diseases

1 | INTRODUCTION

In recent decades, survival in patients with chronic lymphocytic leukemia (CLL) has improved considerably.[1,2] Despite this, infections cause a significant morbidity and mortality in patients with CLL, who have an 50% increased risk of death from infections compared with matched controls.[1] Furthermore, mortality from infections has been relatively unchanged while other causes of mortality have declined.[1]

Although opportunistic infections have been studied substantially in patients with CLL, the literature on this topic is primarily based on case reports,[3-5] single-center studies,[6-8] and selected patients participating in clinical trials.[9,10] The susceptibility to opportunistic infections in CLL patients is in part explained by the immunosuppression associated with CLL itself,[11-13] but compounded by treatment with corticosteroids,[5] chemotherapy,[6,7] monoclonal antibodies,[8,14] and allogeneic stem cell transplantation.[15] The immune dysfunction in treatment-naïve CLL patients is associated with shorter treatment-free and overall survival.[16-18]

Chronic lymphocytic leukemia therapy in the pre-ibrutinib era has long been known to be associated with an increased risk for opportunistic infections, and the risk is particularly high for fludarabine and alemtuzumab.[19] Chlorambucil treatment, which is associated with a relatively low risk of opportunistic infections,[3] was widely replaced as first-line treatment at the turn of the century with fludarabine, which causes quantitative and qualitative T-cell defects.[3] There has been a lack of consensus on guidelines for infection prophylaxis in CLL patients,[3] although antibiotics, antivirals, and antifungals have been proposed with some treatment regimens.[20-23]

Very few large cohort studies have investigated opportunistic infections in CLL patients. Moreira et al[13] reported 17 infections caused by Nocardia, Pseudomonas, Aspergillus, Cytomegalovirus, Pneumocystis, and Histoplasma in 174 CLL patients over a median follow-up of four years. A recent nationwide study on bloodstream infections in Danish CLL patients diagnosed 2008-2016 identified 317 bloodstream infections (including 30 infections with Candida, Pseudomonas, and Listeria) in 277 patients.[12] Delgado et al reported 216 infections (43 infections with Pseudomonas, mycobacteria, yeasts, molds, herpes viruses, and Pneumocystis) in 280 CLL patients over a median of five years and found unmutated *IgHV* gene status and clinical stage B and C to be associated with a higher risk of infections.[24] There has been an increased focus on studies on predictive risk factors for infection in CLL patients,[11] and recently, a model using explainable machine-learning algorithm was introduced to identify newly diagnosed CLL patients at high risk of infection.[25]

Motivated by these studies, we performed the first nationwide analysis focusing on inpatient opportunistic infections (viral, mycobacterial, bacterial, fungal, and parasitic) in CLL patients to determine the incidence, risk relative to matched controls, temporal changes, and the impact on survival.

Novelty statement

- This study presents nationwide results on the incidence of inpatient opportunistic infections in CLL patients and their impact on survival.
- The highest incidence was observed for Pneumocystis pneumonia; Herpes zoster, Pseudomonas, Candida, and Aspergillus infections; and of these infections, Aspergillus infections had the poorest prognosis.
- These results provide a comprehensive description of the incidence and impact of opportunistic infections in the pre-ibrutinib era and provide an important point of reference for future studies on the risks associated with CLL and its treatment.

2 | PATIENTS AND METHODS

Information on CLL patients diagnosed 1994-2013 in Sweden was obtained from the Swedish Cancer Registry,[26] and information on inpatient infections was obtained using the International Classification of Diseases (ICD) codes from the Swedish Patient Registry.[27] For each CLL patient, four random population-based controls without previous hematological malignancy were identified, matched by sex, year of birth, and county of residence. The matched controls could develop hematological malignancies, including CLL, after the date of inclusion. For 205 CLL patients (2.3%), matched controls could not be identified, and for 460 CLL patients (5.1%), less than four matched controls were identified. End of follow-up was 1st of January 2014, and date of death was retrieved from the Cause of Death Registry. In total, 25 subgroups of opportunistic infections (Table S1) were defined, using ICD codes indicative of serious infections and profound immunosuppression. Information to estimate the Charlson comorbidity index[28] at CLL diagnosis was obtained from ICD coded diagnoses in the Swedish Patient Registry and registered cancers in the Swedish Cancer Registry.

2.1 | Statistical analysis

The incidence rate (IR) of opportunistic infections per 1,000 person-years and the incidence rate ratio (IRR) with 95% confidence intervals (95% CIs) were calculated (Figure 1). The IRR adjusts for the difference in person-years at risk between the CLL cohort and the matched controls. To address possible bias due to non-proportionality in the temporal trend analysis (comparing the two calendar periods, 1994-2001 and 2002-2008), follow-up was limited to five years and patients diagnosed after 2009 excluded in this analysis. This ensured the same upper limit of follow-up for patients diagnosed in all calendar-years in the temporal trend analysis. Hazard ratios (HR) and 95% CIs were estimated using a Cox proportional hazard model adjusting for age at CLL diagnosis and sex; and controlling for the

competing risk of death using the Fine-Gray method. To estimate impact of opportunistic infections on survival, case-fatality ratio (CFR) and 95% CI were calculated at 60 days and 365 days in the CLL cohort using the exact binomial test. Patients that had shorter follow-up than 60 and 365 days were excluded from this analysis, in total 8 and 54 infections, respectively. A nested analysis with matched controls was performed to further estimate survival after an opportunistic infection. For each CLL patient who developed an opportunistic infection at the time t_I, two matched CLL controls without an opportunistic infection up to time t_I since CLL diagnosis (and were alive at time t_I) were identified. The controls were matched by sex and similar age and year of diagnosis (±4 years). Risk of death was estimated as a time-dependent coefficient in a Cox proportional hazard model.

3 | RESULTS

3.1 | Baseline characteristics

Among 8989 CLL patients diagnosed 1994-2013 (Table 1), a total of 829 inpatient opportunistic infections were registered in 690 individuals (8%). Among 34 283 matched controls, a total of 252 inpatient opportunistic infections were registered in 243 individuals (0.7%). The CLL patients who were admitted with an inpatient opportunistic infection were younger (median age 67) than those who did not develop an inpatient opportunistic infection (median age 72). Information on the Charlson comorbidity index at CLL diagnosis stratified by each opportunistic infection is shown in Table S2.

3.2 | Incidence of inpatient opportunistic infections and risk relative to matched controls

In total, the incidence of inpatient opportunistic infections in the CLL patients was IR 16.6 per 1,000 person-years (Figure 1) and IR 0.99 per 1,000 person-years in the matched controls. Thus, CLL patients had a more than 15-fold increased risk of inpatient opportunistic infections compared with matched controls (IRR 16.7, 95% CI: 14.5-19.3). The highest incidence rate per 1000 years by categories was for Pneumocystis pneumonia (PCP, IR 4.03), Herpes zoster (IR 2.94), Candida (IR 1.66), Pseudomonas (IR 1.66), and aspergillosis (IR 1.20), and relative to matched controls, the highest risk was observed for PCP (IRR 114, 95% CI: 58.7-252), legionellosis (55.9, 17.5-284), and Cytomegalovirus infection (53.4, 19.4-205). Opportunistic infections due to Hepatitis B (n = 5); bartonellosis (n = 2); actinomycosis and nocardiosis (n = 2); coccidioidomycosis; histoplasmosis, blastomycosis, and cryptococcosis (n = 3); toxoplasmosis (n = 7); *Giardia Lamblia* (n = 1); amebiasis (n = 2); and progressive multifocal leukoencephalopathy (PML, n = 7) had each less than ten events in the CLL patients and were omitted from Figure 1. Furthermore, no patients were diagnosed with cryptosporidiosis, isosporidiasis, leishmaniasis, or Strongyloides infections.

3.3 | Temporal trend analysis

When opportunistic infections in the calendar period 2002-2008 were compared to 1994-2001, there was a decreased risk for Herpes zoster infections (HR 0.59, 95% CI: 0.36-0.98, Table 2).

	CLL	Controls	
	#N (IR)	#N (IR)	IRR (95% CI)
Opportunistic infections:			
In total	829 (16.6)	252 (0.99)	16.7 (14.5-19.3)
Viral infections			
All viral	253 (5.12)	85 (0.34)	15.3 (11.9-19.7)
Herpes Zoster	146 (2.94)	66 (0.26)	11.3 (8.38-15.3)
Cytomegalovirus	42 (0.84)	4 (0.02)	53.4 (19.4-205)
Herpes simplex	40 (0.80)	9 (0.04)	22.6 (10.8-52.9)
Varicella	13 (0.26)	2 (0.01)	33.0 (7.47-301)
Mycobacterial infections			
All mycobacterial	36 (0.72)	37 (0.15)	4.94 (3.03-8.03)
Tuberculosis	25 (0.50)	32 (0.13)	3.97 (2.25-6.91)
Other mycobacterial	11 (0.22)	5 (0.02)	11.2 (3.57-41.0)
Bacterial infections			
All bacterial	147 (2.94)	56 (0.22)	13.3 (9.73-18.5)
Legionellosis	33 (0.66)	3 (0.01)	55.9 (17.5-284)
Listeriosis	27 (0.54)	4 (0.02)	34.3 (11.9-134)
Pseudomonas	83 (1.66)	47 (0.19)	8.97 (6.20-13.1)
Fungal infections			
All fungal	383 (7.66)	72 (0.28)	27.0 (20.9-35.2)
Candida	83 (1.66)	50 (0.20)	8.43 (5.87-12.2)
Aspergillosis	60 (1.20)	7 (0.03)	43.5 (19.9-113)
Other fungal	37 (0.74)	2 (0.01)	94.0 (24.2-805)
Pneumocystis pneumonia	200 (4.03)	9 (0.04)	114 (58.7-252)
Parasitic infections			
All parasitic	10 (0.20)	2 (0.01)	25.4 (5.41-238)

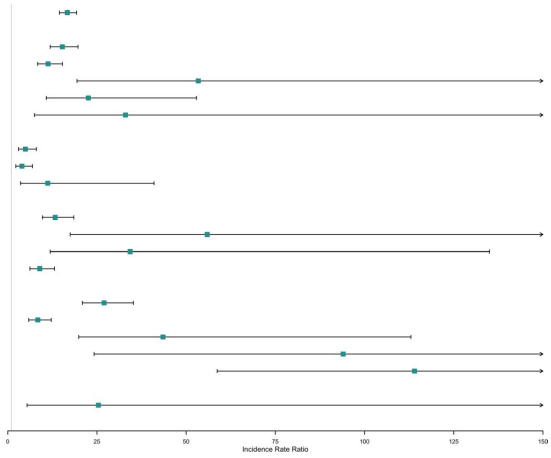

FIGURE 1 The total number and incidence rate per 1000 person-years of opportunistic infections in the 8996 patients with CLL and 34 326 matched controls [Colour figure can be viewed at wileyonlinelibrary.com]

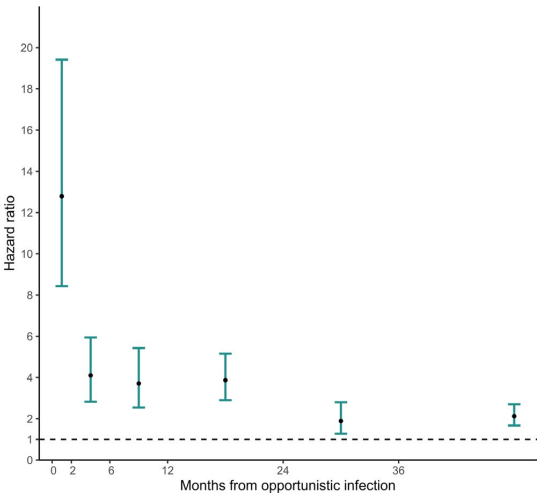

FIGURE 2 The risk of death in CLL patients after an opportunistic infection compared with matched CLL controls. IR: Incidence rate, IRR: incidence rate ratio, 95% CI: 95% confidence interval [Colour figure can be viewed at wileyonlinelibrary.com]

Other changes between the calendar periods were non-significant, although infections caused by Legionella had a borderline significant increase (2.33, 0.89-6.04; $P = .08$).

3.4 | Mortality after opportunistic infection

Eight patients had less than 60-day follow-up after diagnosis of an opportunistic infection. Of the remaining 821 cases of opportunistic infections in the CLL patients, 188 died within 60 days (CFR 23%; Table 3). The highest fatality rate was observed for the seven patients with PML (CFR 71%) and patients with aspergillosis (CFR 42%). The lowest fatality rate was observed for Cytomegalovirus infections (CFR 5%) and none of the four patients with bartonellosis, actinomycosis, and nocardiosis, nor the ten patients with parasitic opportunistic infections, died within 60 days of the diagnosis.

Compared to matched CLL controls, there was a 13-fold increased risk of death in CLL patients the first two months after an opportunistic infection (HR: 12.8, 8.43-19.4; Figure 2). The risk remained significantly elevated at twofold the baseline risk beyond 36 months (HR: 2.13, 1.67-2.70).

4 | DISCUSSION

In this first nationwide study on opportunistic infections in CLL patients, 829 inpatient opportunistic infections were observed in almost 9000 CLL patients with a median follow-up of more than five years. The most common opportunistic infections were PCP; Herpes zoster, Pseudomonas, Candida, and Aspergillus infections. Of these infections, the highest early mortality was observed for aspergillosis

TABLE 1 Baseline characteristics stratified by occurrence of an opportunistic infection

	Patients with CLL		Matched controls	
	n = 8989		n = 34,283	
	No infections	Opportunistic infection	No infections	Opportunistic infection
Total number of individuals (%)	8299 (92)	690 (8)	34 040 (99.3)	243 (0.7)
Total occurrence of infections	829			252
Age at CLL diagnosis				
<70 years old	45%	62%	46%	35%
71 years old and above	55%	38%	54%	65%
Median age at CLL diagnosis (IQR)	72 (63-79)	67 (59-74)	72 (63-79)	74 (66-80)
Sex				
Female	39%	36%	39%	36%
Year of CLL diagnosis				
1994-2001	37%	48%	38%	59%
2002-2008	37%	40%	38%	35%
2009-2013	26%	12%	25%	5%
Median follow-up in days	1641	2246	2410	3088
Mortality	54%	75%	35%	66%
<70 years old	34%	69%	14%	54%
71 years old and above	70%	85%	53%	72%

Note: Abbreviations: CLL, chronic lymphocytic leukemia; IQR, interquartile range.

TABLE 2 Results from temporal trend analysis. Hazard ratios and 95% confidence intervals for the calendar period 2002-2008 compared to 1994-2001

	2002-2008 (reference 1994-2001)
Type of infection	**Hazard ratio (95% confidence interval)**
All infections	0.99 (0.80-1.23)
Viral	0.80 (0.56-1.15)
Cytomegalovirus	1.40 (0.62-3.14)
Herpes simplex	0.75 (0.32-1.79)
Herpes Zoster	0.59 (0.36-0.98)
Mycobacterial	0.67 (0.28-1.65)
Tuberculosis	0.46 (0.16-1.32)
Bacterial	1.41 (0.84-2.35)
Legionellosis	2.33 (0.89-6.04)*
Listeriosis	1.15 (0.42-3.17)
Pseudomonas	0.93 (0.41-2.10)
Fungal	1.19 (0.86-1.64)
Candida	1.11 (0.60-2.03)
Aspergillus	1.20 (0.51-2.80)
Other fungal infections	3.00 (0.81-11.1)
Pneumocystis pneumonia	1.26 (0.82-1.93)
Parasitic	2.02 (0.37-11.0)

*$P < .10$.

(42%), followed by Candida infections (33%), Pseudomonas infections (25%), PCP (20%), and Herpes zoster infections (15%). Our results give a comprehensive description of the incidence and impact of opportunistic infections in CLL patients, most of which have merely been described in studies of few cases until now.

A high incidence of PCP was observed in the CLL cohort (IR 4.03 per 1,000 person-years) with 20% early mortality. Earlier studies have reported an incidence rate of approximately one infection per 1,000 patient-years,[13,24] although they were limited by relatively few numbers of patients. The lower incidence observed in these studies might be explained by a widespread use of PCP prophylaxis in the patient cohorts.[24] The mortality rate from PCP has been reported to be much higher in non-HIV patients compared with HIV patients[29] and was found to be 30%-40% in small studies on CLL patients treated with ibrutinib[30] and patients treated with rituximab (not exclusively CLL).[31] Our results highlight the high mortality associated with PCP in CLL patients; currently, there is no consensus on prophylaxis guidelines, although it has been recommended in certain intensive CLL treatments.[3,15,20,32]

Herpes zoster infections were the second most frequent opportunistic infection in the CLL cohort with an 15% early mortality, which in part reflects that we only included serious infections requiring hospital admission. The risk for Herpes zoster infections decreased between calendar periods which importantly suggests that the use of antiviral prophylaxis[3,33] has resulted in decreased Herpes zoster infections despite increased risk associated with fludarabine treatment.[3] This is in accordance with results from a number of clinical trials where antiviral prophylaxis completely prevented cases of Herpes zoster.[33] The newer ibrutinib and idelalisib therapy have also been associated with reactivation of Herpes zoster infections,[34] although the indications for prophylactic antivirals have not been defined conclusively in this setting.

In the CLL cohort, Candida and Aspergillus infections had an incidence rate of 1.66 and 1.20 infections per 1,000 patient-years, respectively, and were associated with a high mortality. In previous studies, the incidence of serious Candida infections in CLL patients was reported to be less than one per 1,000 patient-years,[12,35,36] whereas the incidence of yeast infections in general has been reported to be as high as 5.5 infections per 1,000-patient-years.[24] The incidence of Aspergillus and mold infections in CLL patients has consistently been reported similar to the incidence in our study.[13,24,36] Interestingly, we did not observe that the availability of improved treatment and prophylaxis for mold infections at the turn of century[23,37] coincided with decreased risk of Aspergillus infections. We have shown that a serious fungal infection, with major impact on survival, occurs in one out of every 400 CLL patients each year, which emphasizes the importance of further research to identify those patients at increased risk[25] and comprehensive guidelines on prophylaxis and treatment.[20,38]

Other opportunistic infections included in our study were less frequent. The high incidence and risk of Legionella infections was unexpected although few cases have been reported in CLL patients.[39-42] In most cases of Legionnaires' disease, a source cannot be identified, although outbreaks have been linked to residential potable water, large building water systems, and car travel.[43] Future analyses will be informative to establish whether the high risk of Legionella infections is specific to the CLL population in Sweden. Cytomegalovirus was associated with a low mortality rate compared with other opportunistic infections. This finding complements a study where the adjusted survival of Cytomegalovirus-seropositive CLL patients was not different from the survival of other CLL patients.[44] Finally, we reported seven cases of PML in the CLL cohort, all of whom died within a year from diagnosis. It is known that PML has poorer prognosis in non-HIV patients[45] and our results are in line with an earlier report of three cases of PML in CLL.[46]

The main strength of our study is the nationwide design and the high-quality registries where we could compare the results to matched controls. Our results are highly generalizable, although inevitably, treatment options vary between countries. For example, alemtuzumab alone and bendamustine together with rituximab accounted for up to 10% of treatment options in 2007-2014 in Sweden.[47] A limitation to our study was, that due the registry-based study design using data from this period, there was no information on individual disease status or treatment to which we could relate the findings. Since 2010, national cancer registries have increasingly been documenting individual disease status and treatment that can be accounted for in future population-based studies. Another limitation is that the Swedish Patient Registry was found to have highly variable sensitivity (45.7%-95.4%) in a study validating infection

TABLE 3 Case-fatality ratio within 60 days of an opportunistic infections

Type of infection	Exposed CLL patients	Fatality	Case-Fatality Ratio (%)	95% confidence interval
Viral				
Cytomegalovirus	42	2	5	1-16
Herpes Simplex	40	5	13	4-27
Varicella	13	2	15	2-45
Herpes Zoster	143	21	15	9-22
Progressive multifocal leukoencephalopathy	7	5	71	29-96
Hepatitis B	5	2	40	5-85
Mycobacterial				
Tuberculosis	25	5	20	7-41
Other mycobacterial	11	3	27	6-61
Bacterial				
Bartonellosis	2	0	0	0-84
Legionellosis	32	11	34	19-53
Actinomycosis and nocardiosis	2	0	0	0-84
Listeriosis	26	8	31	14-52
Pseudomonas	83	21	25	16-36
Fungal				
Candida	82	27	33	23-44
Coccidioidomycosis, histoplasmosis, blastomycosis, and cryptococcosis	3	1	33	1-91
Aspergillosis	60	25	42	29-55
Other fungal meningitis and pneumonia	37	11	30	16-47
Pneumocystis pneumonia	198	39	20	14-26
Parasitic				
Toxoplasmosis	7	0	0	0-41
Giardia Lamblia	1	0	0	0-98
Amebiasis	2	0	0	0-84
Total	821	188	23	20-26

registrations in the intensive care, however, a consistent high specificity (92.6%-99.7%).[27,48] The well-defined and rare condition of central nervous system infections had a high sensitivity while the more common pneumonia and sepsis had lower sensitivity.[48] We thus believe that the sensitivity is high for the rare and serious infections included in our study with the possible exception of Pseudomonas infections, where the incidence was considerably lower compared to earlier studies.[13,24] This discrepancy might be explained by underreporting of the etiology for common gram-negative infections in administrative data.[49]

Taken together, we have in this nationwide study depicted the incidence of rare and serious infections in CLL patients, including a relatively high incidence of PCP. The opportunistic infections had a major impact on survival, and fungal infections were associated with a high early mortality. Currently, ibrutinib therapy is thought to confer lower risk of opportunistic infections than the treatment regimens used in our study period[50]; however, the increased risk of certain opportunistic infections will need longer follow-up and is being more recognized.[30,51] Our results provide a comprehensive description of the incidence and impact of opportunistic infections in CLL patients in the pre-ibrutinib era and provide an important point of reference for future studies on the risks associated with CLL and its treatment.

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VS and SYK designed the study. SYK and OL obtained data. VS prepared the data and performed all statistical analysis. GKG and TA gave statistical advice. VS wrote the report. All the authors were

involved in interpretation of the results. All authors read, gave comments, and approved the final version of the manuscript. All the authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

CONFLICTS OF INTEREST

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DATA AVAILABILITY STATEMENT

Data sharing is not possible under the license of the Ethical Committee.

ORCID

Vilhjálmur Steingrímsson https://orcid.org/0000-0002-9385-2960
Sigrún Þorsteinsdóttir https://orcid.org/0000-0001-5017-3530
Ingemar Turesson https://orcid.org/0000-0002-4115-8010
Sigurdur Y. Kristinsson https://orcid.org/0000-0002-4964-7476

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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