UMEÅ UNIVERSITY

Waldenstrom's macroglobulinemia

-population based studies of familial aggregation and prognostic factors

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Tänd ljus

Låt inte mörkret hindra dig
att söka Ljuset.
Och när du funnit det,
låt andra se,
pröva, övertyga sig.

Vill du, att ljus skall leva,
tänd då hos andra
samma längtan.

Bo Setterlind

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Abstract

Background: Waldenstrom's macroglobulinemia (WM) is a rare lymphoproliferative disorder with a world-wide incidence of 3-4 patients per million persons per year. In Sweden, the incidence was about three times higher, and approximately 100 patients per year are reported to the Swedish Lymphoma Registry (SLR). Our aim was to study the WM population with focus on incidence and survival in relation to clinical prognostic factors and primary therapies (Paper I-II). We also discussed the diagnostic difficulties in patients with non-WM lymphoplasmacytic lymphoma (LPL). In Paper III-IV, we study familial WM from different aspects to better understand underlying pathogenetic factors.

Patients and methods: The patients in all four studies were collected from SLR. In papers I and II, a total of 1511 patients with WM and non-WM LPL were registered between 2000 and 2014, and medical records were retrieved for 1139 patients (75%). A retrospective review showed that 981 and 33 (after review by haematopathologist) of these patients fulfilled the World Health Organization (WHO) diagnostic criteria for WM and non-WM LPL, respectively. In Paper III and IV, we used SLR and the Northern Lymphoma Registry (NLR) for the years 1997-2011. We identified 12 families with a family history of WM, IgM monoclonal gammopathy of undetermined significance (MGUS) and/or multiple myeloma (MM).

Results: In **paper I**, the overall survival (OS) for WM improved between the two time periods, 2000-2006 and 2007-2014, with a five-year OS of 61% and 70%, respectively. Significant prognostic factors for OS at the time of diagnosis in asymptomatic patients in no need of therapy were age, poor performance status (PS), haemoglobin ≤115 g/l, and female sex. Elevated lactate dehydrogenase (LDH) level and haemoglobin ≤115 g/l were significant prognostic factors for patients receiving therapy 0-3 months after diagnosis. The level of the IgM monoclonal immunoglobulin (MI) had no significant prognostic value. Rituximab included in first-line therapy was associated with improved survival.

Paper II describes the differential diagnostic difficulties in non-WM LPL, especially with Marginal Zone Lymphoma (MZL). The non-WM LPL patients had more adverse prognostic factors as elevated LDH, anaemia, and lymphocytosis at diagnosis compared to the patients with WM. Despite this, the OS did not significantly differ between the groups (P = 0.249). The median OS for non-WM LPL was 71 months and the three-year and five-year survival was 71 % and 55%, respectively. The OS and RS were worse for males than females.

In **Paper III**, we showed that age-adjusted incidence in Norrbotten and Västerbotten for WM and non-WM LPL was higher than expected – 17.5 and 14.8

per million person and year, respectively. The corresponding figure for Sweden was 10.5 per million persons per year. Autoimmune diseases or haematological malignancies in the medical history in patients or in their relatives were reported in nine and five of the 12 families, respectively. The relatives showed a high proportion abnormal serum protein electrophoresis (SPE): 12/56 (21%) showed MGUS and 13/56 (25%) showed abnormalities in the immunoglobulin levels (i.e., subnormal levels and poly/oligoclonality).
Paper IV describes hyperphosphorylated paratarg 7 (pP-7), a target of 11% of the monoclonal immunoglobulin M (IgM) in WM and MGUS of IgM type, and distribution in Sweden and in familial WM. The frequency of pP-7 seems to be in line or lower in non-familial WM (7.1%) and higher in familial WM (16.7%) in the counties of Norrbotten and Västerbotten than in earlier published studies. Positive analysis for pP-7 was shown up to 10 years before diagnosis of WM.

Conclusion: We show that in a rare disease such as WM registry studies might bring new knowledge about incidence, disease characteristic, prognostic factors, treatments, and outcome. We also identified aggregation of families with WM in an effort to better understand the underlying pathogenesis.

Abbreviations

B2M	Beta-2-microglobuline
BR	Bendamustine and rituximab
BTK	Bruton's tyrosine kinas
CAD	Cold agglutinin hemolytic disease
DLBCL	Diffuse large B-cell lymphoma
DRC	Dexamethasone, rituximab, and cyclophosphamide
FISH	Fluorescence in situ hybridization
GWAS	Genome-wide association studies
HAS1	Hyaluronan synthase 1
IF	Immunofixations
IPSSWM	International Prognostic Scoring System for WM
LDH	Lactate dehydrogenase
LPL	Lymphoplasmacytic lymphoma
MI	Monoclonal immunoglobulin
MGUS	Monoclonal gammopathy of undetermined significance
MM	Multiple Myeloma
MYD88	Myeloid differentiation 88 gene
MZL	Marginal Zone Lymphoma
NF-κB	Nuclear factor κB

NHL	Non-Hodgkin's lymphoma
NLR	Northern Lymphoma Registry
ORR	Overall response rate
OS	Overall Survival
PCR	Polymerase chain reaction
PSF	Progression-free survival
pP-7	Hyperphosphorylated paratarg 7
R-CHOP	Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone
RS	Relative Survival
SCALE	Scandinavian Lymphoma Aetiology study
SEER	Surveillance, Epidemiology, and End Results Program
SLR	Swedish Lymphoma Registry
SNP	Single nucleotide polymorphism
SPE	Serum Protein Electrophoresis
VIP	Västerbotten Intervention project
WGS	Whole genome sequencing
WHO	World Health Organization
WM	Waldenstrom's macroglobulinemia

Sammanfattning på svenska

Bakgrund: Professor Jan Waldenström beskrev 1944 två patienter med förstorade lymfkörtlar, näsblödningar och blodbrist. Dessa patienter hade en ansamling av ett mycket stort äggviteämne i blodet som kallades makroglobulin, varför sjukdomen senare fick namnet Waldenströms makroglobulinemi (WM). Lymfocyter, en typ av vita blodkroppar som ingår i vårt immunförsvar, producerar antikroppar (även kallad immunglobuliner). Vid WM har lymfocyterna förändrats och bildar identiska antikroppar eller immunglobuliner av typ M, vilka man kan mäta i blodet som en så kallad M-komponent av IgM typ.

WM är en ovanlig sjukdom, endast 3-4 individer per en miljon invånare och år insjuknar. Orsaken till sjukdomen är inte känd. Diagnosen är vanligare hos män och i den vita befolkningen, samt ökar med stigande ålder. Patienter med autoimmuna sjukdomar, såsom olika reumatiska sjukdomar eller vissa infektioner, har en ökad risk att utveckla WM. Troligen finns även ärftliga faktorer då det förekommer enstaka familjer där flera familjemedlemmar insjuknat i WM. Sjukdomen utvecklas gradvis och föregås i de flesta fall av ett förstadium, monoklonal gammopati av oklar signifikans (MGUS), dvs. en M-komponent av IgM typ utan bakomliggande sjukdom. För att ställa diagnosen WM (enligt WHO) ska det finnas engagemang av lymfoplasmacytiskt lymfom (LPL) i benmärgen och ibland i lymfvävnad, såsom lymfknutor och mjälte, tillsammans med förekomst av en M-komponent av IgM typ, oberoende av storlek.

WM anses idag vara en kronisk sjukdom som inte går att bota och behandlas endast när den ger symptom. Symptom kan bero på infiltration av tumörceller i olika organ och kan ge exempelvis lågt blodvärde vid benmärgsinfiltration eller förstorade lymfkörtlar. M-komponenten kan i sig själv också ge symptom, exempelvis hyperviskositet (trögflytande blod), påverkan på nerver eller njurar. Slutligen kan allmänsymptom (B-symptom) såsom viktnedgång, nattliga svettningar och feber förekomma. Överlevnaden har förbättrats och antalet patienter som lever med sjukdomen har ökat. Vi har idag flera nya förbättrade behandlingsalternativ och effektivare läkemedel att tillgå.

Syfte: Syftet med avhandlingen är att studera patienter med WM i Sverige för att kartlägga hur vanlig sjukdomen är (incidens) samt studera överlevnaden och korrelera den till kliniska prognostiska faktorer och olika behandlingstyper. Vidare har vi studerat en mycket ovanlig lymfomtyp (non-WM LPL), som delar många egenskaper med WM, men saknar M-komponent av IgM typ. Vi har även

studerat familjer med flera familjemedlemmar med WM för att bättre kunna förstå de underliggande orsakerna till sjukdomsutvecklingen.

Patienter och metoder: Patienterna som ingår i alla fyra studierna är rekryterade från Svenska och Norra lymfomregistren. I artikel I och II ingick 1511 patienter diagnostiserade med WM eller non-WM LPL under åren 2000 – 2014. Serum protein elforesen (SPE) (den analysmetod som används för att mäta eventuell M-komponent och nivåer av immunglobuliner i blodet) och patologirapporten som diagnosen bygger på eftergranskades hos 1139 (75%) patienterna, av dessa uppfyllde 981 patienter de diagnostiska kriterierna för WM. Hos de 124 patienter som uppfyllde kriterierna för non-WM LPL eftergranskades först patologirapporten och sedan de diagnostiska vävnadsproverna, av dessa kunde diagnosen non-WM LPL säkerställas hos 33 patienter. I artikel III och IV identifierades genom en screening enkät och senare genom telefonkontakt 12 familjer med två eller flera familjemedlemmar med WM, MGUS och/eller Multipelt Myelom (MM) från Norrbotten och Västerbotten som registrerats under åren 1997 – 2011. MM är en närbesläktad blodsjukdom, men med en M-komponent av IgG eller IgA typ

Resultat: I **artikel I** visade vi att femårsöverlevnaden för WM under perioden 2000-2006 i jämförelse med perioden 2007-2014, hade förbättrats från 61% till 70%. Statistiskt signifikanta ogynnsamma prognostiska faktorer för överlevnad för symptomfria patienter som inte fick någon behandling vid diagnos var stigande ålder, nedsatt allmäntillstånd, lågt blodvärde och kvinnligt kön. För patienter med symptomgivande sjukdom som erhöll behandling 0-3 månader efter diagnos, var motsvarande prognostiska faktorer för överlevnad ökat laktat dehydrogenas (LD) och lågt blodvärde. M-komponentens storlek hade inget signifikant prognostiskt värde. Ökad överlevnad sågs hos patienter som behandlades med Rituximab, en tumörantikropp riktad mot ett äggviteämne (CD20) på tumörcellens yta.

Artikel II beskriver svårigheter och differentialdiagnostiska överväganden vid diagnostiseringen av non-WM LPL. Vi kunde visa att patienter med non-WM LPL hade fler negativa prognostiska faktorer, såsom förhöjt LD, lågt blodvärde och förhöjda nivåer av lymfocyter jämfört med WM. Trots detta var det ingen signifikant skillnad i överlevnad mellan patienter med WM och non-WM LPL. Däremot var överlevnaden för non-WM LPL signifikant bättre för kvinnor jämfört med män.

I **artikel III** visade vi att WM och non-WM LPL var vanligare i Sverige (10,5 individer insjuknade per en miljon invånare och år) jämfört med insjuknandet globalt. För Norrbotten och Västerbotten var incidensen ännu högre (17,5 och

14,8 individer per en miljon invånare och år). Autoimmuna sjukdomar (75%) och blodcancersjukdomar (42%) var vanligare i familjer med anhopning av WM. Familjemedlemmarna hade en hög andel avvikande SPE. Vi identifierade MGUS hos 21% och avvikande nivåer av immunglobuliner hos 25%.

Artikel IV beskriver hyperfosforylerat paratarg 7 (pP-7), som är ett målprotein (=antigen) till 11% av M-komponenterna av IgM typ (=antikropp) som finns hos patienter med WM och IgM MGUS. I familjer med familjär WM är troligen andelen familjemedlemmar som bär pP-7 högre än i icke-familjär WM. pP-7 kunde påvisas hos individer upp till 10 år innan de insjuknade i WM och även innan dessa hade utvecklat MGUS.

Sammanfattning: Vi har kunnat visa att vid en sällsynt sjukdom som WM så kan registerstudier ge ny kunskap om incidens, sjukdomsuttryck, prognostiska faktorer, behandling och prognos. I ett försök att bättre kunna förstå orsaken till WMs uppkomst, har vi studerat familjer med två eller flera drabbade familjemedlemmar.

List of included papers

I. **Brandefors, L**., Melin, B., Lindh, J., Lundqvist, K. & Kimby, E. (2018) Prognostic factors and primary treatment for waldenstrom macroglobulinemia - a Swedish lymphoma registry study. *British Journal of Haematology,* 183, 564-577.

II. **Brandefors, L**., Sander, B., Lundqvist, K. & Kimby, E. Clinical characteristic and outcome of Lymphoplasmacytic Lymphoma of non-WM type - a Swedish lymphoma registry study. *(in manuscript)*

III. **Brandefors, L.,** Kimby, E., Lundqvist, K., Melin, B. & Lindh, J. (2016) Familial waldenstrom's macroglobulinemia and relation to immune defects, autoimmune diseases, and haematological malignancies - A population-based study from northern sweden. *Acta Oncologica (Stockholm, Sweden),* **55**, 91-98.

IV. **Brandefors, L.,** Lindh, J., Preuss, K.D., Fadle, N., Pfreundschuh, M. & Kimby, E. (2019) Incidence and inheritance of hyperphosphorylated paratarg-7 in patients with waldenstrom's macroglobulinaemia in sweden. *Acta Oncologica (Stockholm, Sweden),* **58**, 824-827

Aims of the thesis

I: To study the incidence and outcome of WM in relation to clinical prognostic factors and primary systemic therapies in patients from Swedish Lymphoma Registry.

II: To describe the rare entity non-WM LPL, the clinical presentation and outcome and discuss diagnostic considerations and challenges.

III: To estimate the incidence of WM in northern Sweden and to identify and describe patients with familial WM in this area.

IV: To investigates the carrier state of pP-7 and other paratarg (=paraprotein target) proteins in WM patients from Sweden and relate it to the high incidence and familial clustering of WM in the northern counties.

Introduction / Background

History – the man behind the ”syndrome”

In 1944, the Swedish physician Jan Gösta Waldenström (1906 – 1996) published an article: ‘Incipient myelomatosis or “essential hyperglobulinemia” with fibrinogenopenia – a new syndrome? He described the symptoms of two patients with anaemia, thrombocytopenia, oronasal bleedings, lymphadenopathy, elevated serum viscosity, elevated erythrocyte sedimentation rate, and bone marrow infiltrated lymphoid cells. This syndrome was later named Waldenstrom’s macroglobulinemia.

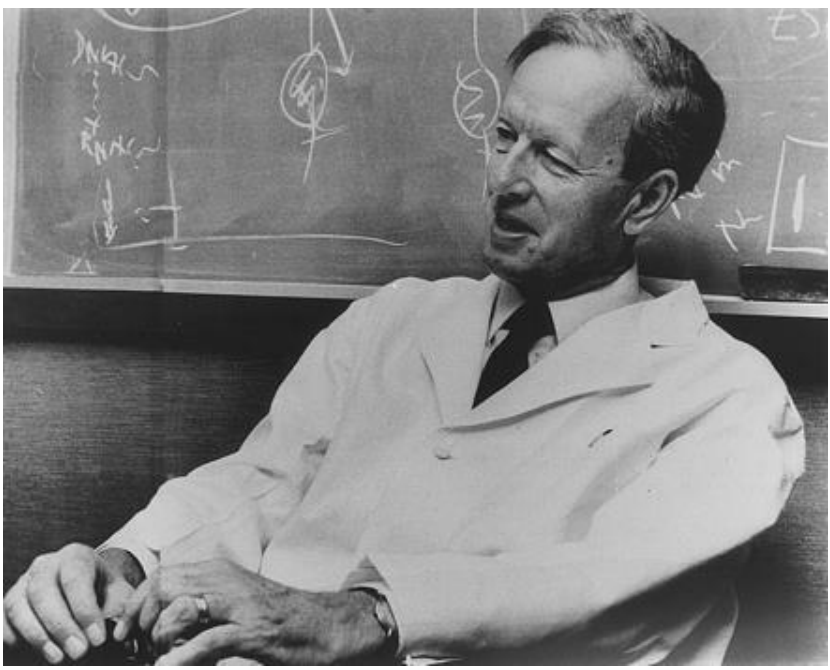


Figure 1. Jan Gösta Waldenström (1906 – 1996)
(photographer unknown)

During his long career, Waldenström made observations in several fields within haematology that provided new understanding of macroglobulinemia, the monoclonal gammopathies, and of other diseases such as hemosiderosis and porphyria. Porphyria was described in the early 1900s by Einar Wallquist, MD,

in Arjeplog, but Jan Waldenström's studies of patients with acute intermittent porphyria (described in his thesis published in 1937) had such a large international impact that the disease was called Swedish porphyria.

Jan Waldenström had connection to the northern part of Sweden through his great grandfather Erik Magnus Waldenström. He worked as a provincial doctor in Luleå between 1819 and 1862. The first years he was the only doctor in this part of the country, and he served an area as large as one-fourth of Sweden. The nearest hospital was located in Umeå 260 km away. When he first came to the northern Sweden, he lived in Sunderbyn (the location of Sunderby Hospital), but he eventually moved to Luleå, where he lived for the rest of his time in Norrbotten. Erik Magnus Waldenström was a respected doctor, well-known for his surgical skills, and he was the first doctor in this area to do cataract operations. He also travelled around his large district to meet 'far away' patients and there are descriptions of his challenging travels over rivers, mountains, and valleys on horse, with reindeer, or by boat (Lulebygdens forskarförening, September 2016)

The conditions for healthcare were different from how it is today. Or as a man from Arjeplog described it, the doctors sometime visited the sick, but most people die a natural death.

Lymphogenesis and the origin of the tumour cell

Background

The word immune is originally from the Latin *immunitas* and means 'freedom from'. Our immune system has four features; it is specific, selective, adaptive, and has a memory. That the immune system is specific means that every 'enemy' can be recognised and that immune cells and antibodies develop the ability to identify and destroy this specific 'enemy'. Selectivity means that the immune system can identify foreign agents and spare the body's own cells. An adaptive immune system means that it waits with a strong defence until the 'enemy' appears and strengthens the defence as long as the 'enemy' remains in the body. Finally, memory means that the 'enemies' that the immune system previously reacted to are remembered and that the immune system reacts more powerfully the next time it encounters these 'enemies'. The immune system is

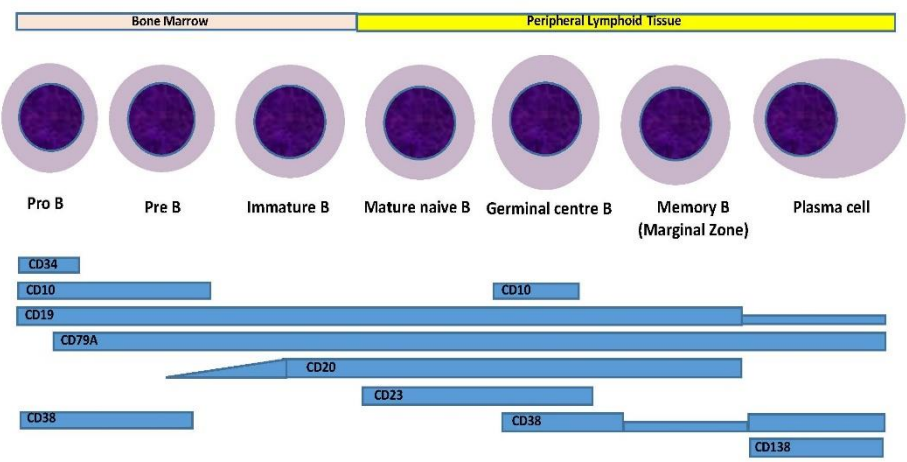
important and extensive, with thousand billions (10^{12}) lymphocytes (0.5 kg) and 10^{20} immunoglobulins (0.5 hg).

The immune system has two major parts: the innate and the adaptive. The innate immune system provides a first-line primitive defence and often plays a role in protection of mucosal and cutaneous barriers. The most important actors are phagocytes and the complement system. The adaptive immune system has a more sophisticated immune response, involving antigen specificity and memory (Bränden & Andersson, 2009, tredje upplagan).

Normal B-cell differentiation

The B-cell (or B-lymphocyte) can produce antibodies against specific antigens, and every B-cell can only produce an antibody for a specific antigen. B-cells that produce the exact same antibodies are called a B-cell clone. Only the B-cells whose antibodies bind to the foreign antigen activates the production of antibodies (clonal selection).

Figure 2. The development of the B-lymphocytes and the expression of CD markers in the different states of development



Redrawn and modified after Figure 11.03 in WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2017, Swerdlow et al.

After recovery, some of the B-cells 'rest' as more long-lived memory B-cells and are more easily to activate (immunological memory). The antibodies consist of

five isotypes. The first type of antibody produced in an antigen-antibody response is the IgM immunoglobulin, a pentamer. Later in the immune response, the antibody switch isotypes to IgG, IgA, or IgE, depending on the antigen. Another mechanism to produce more specific antibodies during the immune response is the ability of somatic mutations (i.e., random mutations in the antibody variable domain leading to a stronger affinity to the antigen, *affinity maturation).* For more details, see Figure 2.

Lymphomagenesis

Lymphomas of B-cell type appear to imitate the stages of normal B-cell differentiations (with a few exceptions such as hairy cell leukaemia) and can to some extent be classified according to the normal differentiation stage. This relationship is a major basis for lymphoma classification and nomenclature (Figure 3).

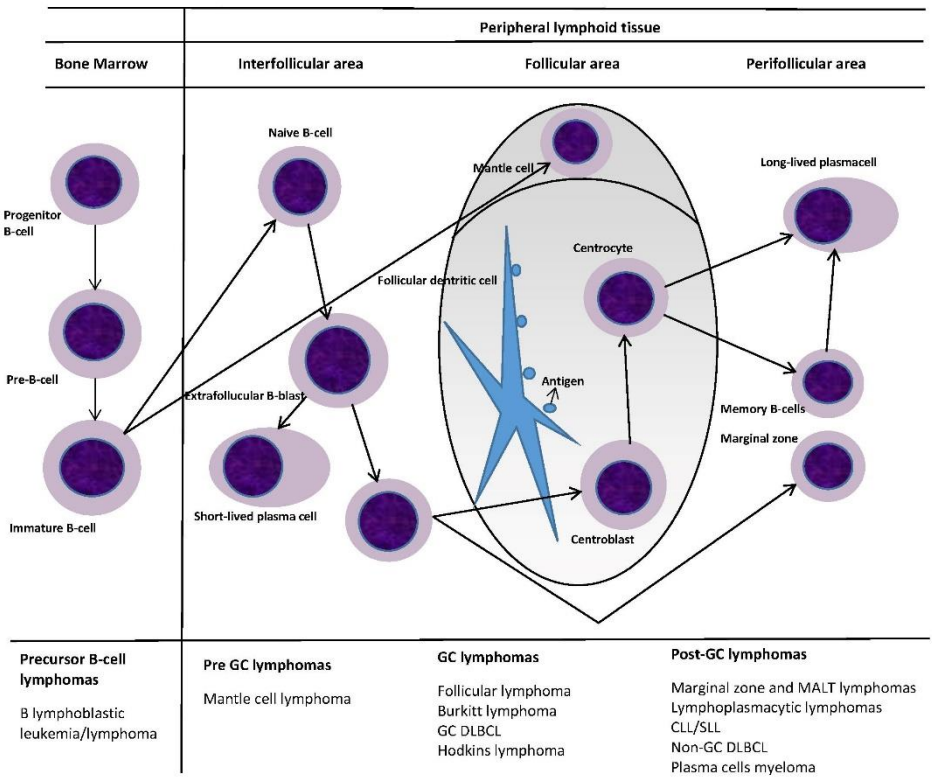
The origin of the tumour cell in Waldenstrom's macroglobulinemi

Molecular studies have shown that WM cells' postulated normal counterpart is a post-follicular B-cell that differentiates into plasma cells. It is thought to be an IgM-positive memory B cell that is largely VH3 restricted, arrested after somatic hypermutation, cannot undergo class switching, and might have bypassed the germinal centre (Garcia-Sanz *et al*, 2016, Kriangkum *et al*, 2007, Swerdlow, Campo *et al*, 2016)

Incidence

WM is a rare lymphoproliferative disease with a worldwide incidence of 3-4 patients per million person per year (Groves *et al*, 1998, Herrinton & Weiss, 1993). The incidence increases with advancing age and the median age at diagnosis is high, about 70 years. The incidence is twice as common in males than in females; the reason for this is unknown. There are differences in incidence between different ethnic groups; WM is more common in the white population compared with the African-American population in the US (Wang *et al*, 2012) and lower in some Asian countries (Japan and Taiwan)(Iwanaga *et al*, 2014). The incidence seems to be relative stable over the years.

Figure 3. Normal B-cell differentiations and its relationships to major B-cell neoplasm



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Pathogenesis

Immunological, genetic, or environment factors (or the story of the chicken and the egg)

The pathogenesis of WM is still mostly unknown, although immune-related, genetic, and environmental factors have been suggested. Several epidemiological studies support the hypothesis that various types of chronic antigenic stimulation contribute to the development of WM and other B- cell lymphoma subtypes (Koshiol *et al*, 2008, Baecklund *et al*, 2014a, Smedby *et al*, 2006). In a population-based study from Sweden, including 2470 patients with

LPL/WM and with 5710 first-degree relatives and matched controls, an increased risk for LPL/WM was associated with a personal history of systemic sclerosis, Sjögren's syndrome, or other autoimmune diseases and a personal history of specific infections such as pneumonia or septicaemia. A family history of autoimmune diseases and specific infections were also associated with an increased risk for LPL/WM (Kristinsson *et al*, 2010). Another example is The International Lymphoma Epidemiology Consortium (InterLymph) with pooled data from 374 cases and 23096 controls from 11 countries investigating associations between medical and family history, lifestyle, and occupational risk factors for LPL/WM. In multivariate analysis LPL/WM, risk was associated with history of Sjögren's syndrome, systemic lupus erythematosus, hay fever, positive hepatitis C serology, hematologic malignancy in a first-degree relative, high adult weight, duration of cigarette smoking ≥ 40 years, and occupation as a medical doctor (Vajdic *et al*, 2014a). To address environmental and clinical factors in the pathogenesis, Royal et al. designed a questionnaire-based study for WM families with multiple cases of WM, mixed families with WM, and other B-cell malignancies and sporadic WM. Data on 103 WM patients and 272 unaffected relatives were included. Familial WM patients were more likely than unaffected relatives to report a history of autoimmune disease, infections, exposure to farming pesticides, wood dust, and organic solvents (Royer *et al*, 2010).

Although the mechanism behind the above mentioned association between genetic, immune-related, and environmental factors are largely unknown, the main hypothesis for this correlation is that chronic inflammation and/or antigen stimulation leads to activation of B-cells, and ultimately leads to the development of a malignant clone (Baecklund *et al*, 2014b)pubmed

Genetics landscape of WM

Cytogenetics

The use of conventional karyotyping is restricted by the low mitotic activity of the malignant WM cell. Fluorescence in situ hybridisation (FISH) is another approach to detect cytogenetic abnormalities. Overall, up to 50% of the WM cases shows cytogenetic abnormalities by karyotyping or FISH (Nguyen-Khac *et al*, 2013). The most common chromosomal abnormality is deletion of the long arm of chromosome 6 (6q) and is seen in about 50% of the patients. Deletion of 6q is associated with adverse prognostic factors such as anaemia, hypoalbuminemia, and elevated β2-microglobulinemia, but have no impact of

survival; however, the role in pathogenesis is still unknown (Chang *et al*, 2009, Ocio *et al*, 2007). Other recurrent abnormalities are partial or whole gains in chromosome 3, 4 (8-28% of WM patients), 12 (up to 13% of WM patients), 18 and X, and losses in 11q (involving the *ATM* gene), 13q, and 17p (involving the *TP 53* gene) (Nguyen-Khac *et al*, 2013). None of the chromosomal abnormalities influence the OS. Patients with *TP53* mutation and trisomy 12 had a shorter PFS (Nguyen-Khac *et al*, 2013).

Somatic mutations

Next-generation sequencing studies have led to the discovery of highly recurrent somatic mutations in WM. Treon et al. (2012), using whole genome sequencing (WGS), identified a single-nucleotide change from T to C that resulted in a leucine-to-proline change at amino acid position 265 in Myeloid differentiation 88 gene (*MYD88* ^{L265P}) at chromosome 3p22. *MYD88* ^{L265P} – seen in more than 90% of patients with WM and more than 50% of patients with IgM MGUS – activates downstream signalling of the transcription protein complex nuclear factor kB (NF-kB), which stimulates WM cells growth and survival (Yang *et al*, 2013, Treon, Xu *et al*, 2018). *MYD88* mutations have also been found, but in lower frequencies, in other subtypes of lymphomas: ABC-type diffuse large B-cell lymphoma (DLBCL) (29%); immune privileged DLBCL (i.e., testicular and primary central nervous system lymphoma) (up to 75%); mucosa-associated lymphoid tissue lymphoma (9%); and CLL (3%) (Varettoni *et al*, 2013a, Ngo *et al*, 2011, Puente *et al*, 2011, Kraan *et al*, 2013). In IgM MM, the mutation is almost absent.

CXCR4 mutations are the second commonest mutation in WM, occurring in approximately 30-40% of the patients and exclusively accompanied with *MYD88* ^{L265P}. More than 30 nonsense and frameshift mutations in the C-terminal domain of *CXCR4* have been described and are similar to the mutation described in the germline in patients with WHIM syndrome (Wart, hypogammaglobulinemia, infection, and myelokathexis). *CXCR4* mutations promote AKT and ERK-1/2 signalling and drug resistance in the presence of its ligand *CXCL12* (Hunter *et al*, 2014, Treon, Xu *et al*, 2018, Treon, Cao *et al*, 2014).

Additional somatic mutations are *ARID1A* (17%) and *CD79A/CD79B* (8-12%, part of the BCR pathway) and are often part of *MYD88*-mutated disease (Hunter *et al*, 2017).

Wilde-type (wt)MYD88 diseases have a different genomic background, and overlap some mutations that are found in DLBCL (NF-kB activating mutations

that are downstream of BTK, chromatin-modifying genes and DNA damage repair genes) (Treon, Gustine *et al*, 2018).

Finally, genetic variation in the hyaluronan synthase 1 (*HAS1*) gene affects *HAS1* aberrant splicing in WM as well in other cancers. When studying the influence of inherited *HAS1* single nucleotide polymorphism (SNP), three linked SNP in *HAS1* intron3 were significantly associated with B-cell malignancies, but not for solid tumours. Furthermore, *HAS1* is up-regulated in diseases associated with inflammation (Kuppusamy *et al*, 2014, Adamia *et al*, 2008, Siiskonen *et al*, 2015)

Clinical implications

Diagnostic impact

The single point mutation *MYD88* ^{L265P} can be used as a diagnostic marker for WM and distinguishing WM from other sub types of lymphomas with lymphoplasmacytic differentiation (e.g., MZL) and IgM MM (Treon *et al*, 2012, Varettoni *et al*, 2013b, Jimenez *et al*, 2013, Xu *et al*, 2013). *MYD88* ^{L265P} is also a good diagnostic tool in extramedullary diseases, as it is present in cerebrospinal fluid (Bing Neel) as well as pleuritic fluid (Poulain *et al*, 2014).

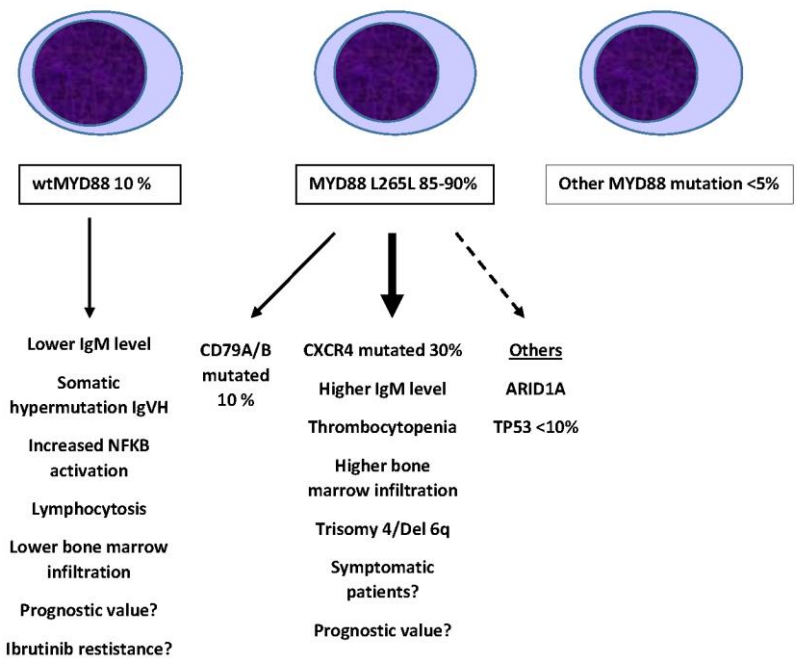
Clinical presentation and prognosis

The status of *MYD88* and *CXCR4* mutations partly influences the clinical presentation (Figure 4). Patients with *MYD88* ^{L265P} mutation have significantly increased bone marrow involvement, higher serum IgM levels, and lower IgA levels while *wtMYD88* have shorter overall survival (Treon, Cao *et al*, 2014). Patients with *CXCR4* mutations have a significantly lower frequency of adenopathy, but there is no difference in OS compared with *wtCXCR4*. There might be a difference between nonsense and frame shift mutated *CXCR4* as patients with *CXCR4* nonsense mutations have an increased bone marrow involvement, higher serum IgM levels, and increased risk of symptomatic hyperviscosity (Treon, Cao *et al*, 2014). See Figure 4.

Therapeutic implications

Patients with *MYD88* ^{L265P} showed more major responses and higher progression-free survival (PSF) to ibrutinib compared with patients with *wtMYD88.* Moreover, patients with mutated *MYD88* ^{L265P} and *CXCR4* mutations had fewer major responses to ibrutinib than patients with *wtCXCR4*. Furthermore, major response was delayed among patients with *CXCR4* mutations (Treon *et al*, 2015). A recently published systematic review study showed that WM patients with *CXCR4* mutation have lower response and PFS rates to BTK inhibitors (Castillo *et al*, 2019).

Figure 4. Clinical presentations according to *MYD88* status



Familial WM

Family studies

After Massari et al. (MASSARI *et al*, 1962) reported the occurrence of WM in two brothers, familial clusters of WM were described in many case reports or larger family studies, indicating a potential role for genetic factors in the disease development (Fine *et al*, 1986, Blattner *et al*, 1980, Renier *et al*, 1989, McMaster *et al*, 2007, Taleb *et al*, 1991). The inheritance pattern within the families was variable, and the most common relationships in the families were siblings. Often only two or three family members were affected. WM might cluster with other B-cell lymphoproliferative disorders, including CLL and other subtypes of B-cell lymphoma as well as with MGUS (Fraumeni *et al*, 1975, Bjornsson *et al*, 1978, Steingrimsdottir *et al*, 2011). Large population-based studies have confirmed the results and in a large study from Sweden Kristinsson et al. showed that first-degree relatives to patients with WM/LPL had a 20-fold risk of developing WM/LPL and an increased risk, although to lesser content, for other B-cell malignancies and MGUS (Kristinsson *et al*, 2008). The risks for developing MM, myeloid malignance, or solid tumours are more controversial. Case-control studies have provided further evidence supporting the coaggregations of WM and other haematological malignancies. One example is the earlier described study from the InterLymph Non-Hodgkin Lymphoma Subtypes Project that showed a 64% increased risk for developing WM/LPL in individuals with a first-degree relatives diagnosed with a haematological malignancy (Vajdic *et al*, 2014a). Furthermore, a single centre study including 257 consecutive and unrelated WM patients reported similar results: 18.7% patients had at least one first-degree relative with WM 5.1% or another B-cell disorder including non-Hodgkin's lymphoma (NHL) 3.5%, MM 3.1%, CLL 2.7%, MGUS 1.9%, acute lymphocytic leukaemia 1.2%, and Hodgkin's disease 1.2% (Treon *et al*, 2006). Family studies have showed that IgM MGUS are common in WM families, but the prevalence is unknown (McMaster *et al*, 2007, Ogmundsdottir *et al*, 1994). IgM MGUS is the strongest prognostic factor for developing WM and other B-cell malignancies, at a rate of 1.5% per year, but it is unknown whether the risk is higher in familial cases (Kyle *et al*, 2003). Other immunoglobulin abnormalities such as MGUS of IgG and IgA type, polyclonal IG or decreased levels of IG of IgM, IgG, and IgA are a frequent finding in relatives of WM patients, although the prevalence of subclinical immunoglobulin abnormalities in the general population is unknown (Seligmann *et al*, 1967, Kalff & Hijmans, 1969).

Family studies also show a correlation with autoimmune diseases and WM. Some studies only show elevated titres of autoantibodies as seen in autoimmune disorders and in other as a clinical diagnosis of a symptomatic

autoimmune disorder (Linet *et al*, 1993, Blattner *et al*, 1980, Renier *et al*, 1989). Altogether, the above studies might indicate an underlying immune dysfunction in familial WM. (Kristinsson & Landgren, 2011)

The clinical presentation of familial WM does not differ compared with sporadic cases. In descriptions of specific multiple-cased families, the WM patients were diagnosed at a younger age and they were more likely to be men (McMaster, 2003). In other studies, there was no difference in age at diagnosis (Royer *et al*, 2010, Kristinsson *et al*, 2008). Compared to other forms of hereditary cases, there are no clear associations with lower age at onset. Recent studies have shown that familial WM had worse outcomes. For example, a population-based study from Sweden showed that LPL/WM patients with a family history of any lymphoproliferative disorder had an increased risk of death compared with sporadic LPL/WM patients (HR = 1.34; 95% CI, 1.03-1.75) (Steingrimsson *et al*, 2015).

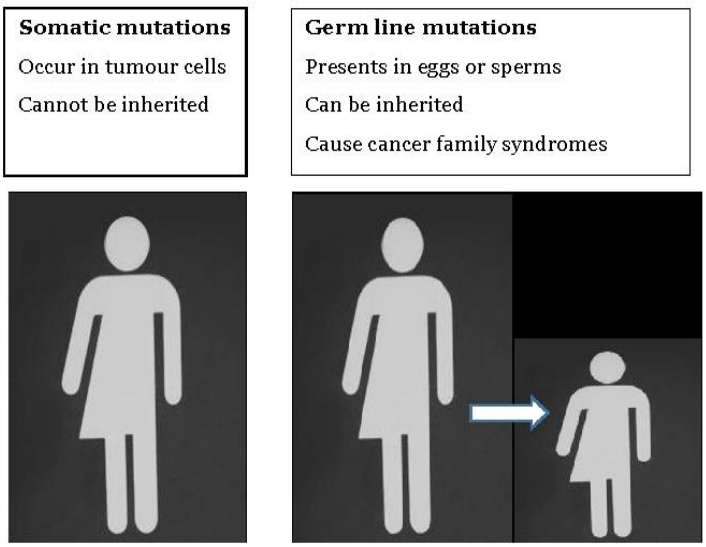
The variable inheritance pattern in the WM families and the co-aggregation of different B-cell disorders suggest that the pathogenesis is heterogeneous, more than one gene might be involved, and the causative genes might be a common oncogenic genes somewhere along the course of the B cell development.

Germ line mutations in familial WM

Genetic studies in familial WM are inconclusive.

In the effort to identify susceptible genes for WM, McMaster et al. performed a genome wide linkage analysis in 11 high-risk families with WM (McMaster *et al*, 2006). The strongest evidence of linkage was found on chromosomes 1q and 4q, but also on chromosome 3 and 6.

Figure 5. Differences between somatic and germ line mutation



As described earlier, *MYD88* ^{L265P} was detected as the most common somatic mutation in WM; however, the mutation is not present in the germ line in familial WM (Pertesi *et al*, 2015). In a study with exome sequencing on germ line DNA obtained from four families with WM, *LAPTM5* ^{C403T} and *HCLS1* ^{G496A} were the most recurrent mutations and more common in familial WM compared with non-familial cases. Previous studies have reported a *LAPTM5* overexpression in patients with B-cell lymphomas and associated with NF-κB activation (Roccaro *et al*, 2016). More studies will be needed to better characterise the relevance of *LAPTM5* in the pathogenesis of WM.

The genetic basis of WM is heterogeneous, and another way to approach the questions are candidate gene association studies. For example, Liang et al. performed a study with 152 SNPs based on data from different genome-wide association studies (GWAS) on NHL. The candidate genes are involved in cell cycle regulation, DNA cell repair, immune regulation, and NF-kB pathway and the following genes were significantly associated with WM: *BCL6, IL10, IL6, IL8RA, and TNFSF10* (Liang *et al*, 2009).

Recently, a two-stage genome-wide association study investigated WM/LPL in 530 unrelated cases and 4362 controls. Two high-risk loci that were associated with WM/LPL were identified at 6p25.3 and 14q32.13. Both risk alleles are observed at a low frequency among controls (2-3%) and occur in excess in

affected cases within families (McMaster *et al*, 2018). Further studies are needed that investigate the role of these alleles in the pathogenesis of WM.

Paratarget proteins

Antigenic targets of monoclonal immunoglobulins (MI) might play a role in the pathogenesis of WM, MM, and MGUS. Paratarg-7 (P-7), a protein of unknown function, is expressed in all human tissues and has been identified as a MI target of 11% of immunoglobulin M (IgM) MI in WM and MGUS of IgM type and of 15% of immunoglobulin A (IgA) and immunoglobulin G (IgG) MI in MGUS and MM (Grass *et al*, 2009, Preuss *et al*, 2009). In Germany, the frequency in healthy controls is 2%.
In patients with an anti-P-7-specific MI, the protein is hyperphosphorylated (pP-7). P-7 hyperphosphorylation can be induced in wild-type P-7 (wtP-7) carriers by PKCζ and reverted by protein phosphatase 2A (PP2A). In pP-7 carriers, dephosphorylated pP-7 is defective due to inactivation of the PP2A (Preuss *et al*, 2011). The carrier state of pP-7 is inherited in an autosomal dominant fashion and carrier of pP-7 has a higher risk for developing MM, MGUS, and WM (MM: odds ratio = 7.9, $P = 0.0001$; WM: odds ratio = 6.2, $P = 0.001$) (Grass *et al*, 2010, Grass, Preuss *et al*, 2011).

The frequency of the pP-7 carrier state is lower in Japan and higher in African-Americans in the US compared with patients from Germany (Zwick *et al*, 2014, Grass, Iida *et al*, 2011).

As described above, chronic antigenic stimulation might have a role in the pathogenesis of WM, in this case, mediated through chronic autoantigenic stimulation helped by CD4+ cells and a specific HLA-DR subtype (Neumann *et al*, 2015).

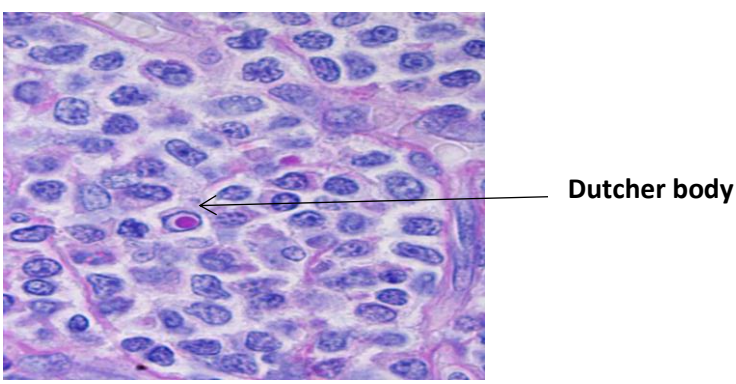
Diagnostic consideration

Lymphoplasmacytic Lymphoma and Waldenstrom's Macroglobulinemia

According to the WHO Classification (2017), LPL is 'a neoplasm of small B lymphocytes, plasmacytoid lymphocytes, and plasma cells, usually involving bone marrow and sometimes lymph nodes and spleen, which does not fulfil the criteria for any of the other small B-cell lymphoid neoplasm that can also have plasmacytic differentiation'. Accordingly, LPL is a diagnosis of exclusion, as plasmacytic differentiation might occur in almost all small B cell lymphomas. The WHO definition continues: 'because the distinction between LPL and one of these other lymphoma, especially some marginal zone lymphoma, is not always clear-cut, some of the cases may need to be diagnosed as a small B-cell lymphoma with plasmacytic differentiation'. WM is defined as 'LPL with bone marrow involvement and an IgM MI of any concentration' (Harris *et al*, 2008). Mastcells is usually increased in WM, and Dutcher bodies (PAS-positive intranuclear pseudoinclusions) are another common feature (Figure 6).

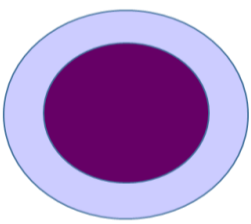
The majority of the WM patients has a characteristic immunophenotype. Most WM cells express the pan B-cell markers CD 19, CD20, and CD79 together with monoclonal expression of IgM surface immunoglobins (sIG) restricted to kappa or lambda light chain expression and heavy chain. Plasmacytic cells express the same immunoglobuline in the cytoplasm. In addition, a majority express CD22 (weak), CD 25, and CD27, but lack expression of CD5, CD10, CD103, and CD23. Typically, the immunophenotype shows markers for both B-cells and plasma cells. The plasma cells are positive for CD138 (Gascue *et al*, 2018) (Figure 7).

Figure 6. Dutcher body in LPL

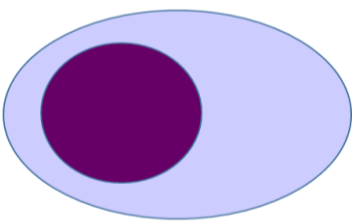


In the revised WHO classification from 2017, *MYD88* ^{L265P} mutation was added to the diagnostic criteria (Swerdlow, Campo *et al*, 2016). Most nodal or extramedullary lymphoplasmacytic lymphomas also harbour *MYD88* $^{L265P.}$ (Hamadeh *et al*, 2015) Non-IgM LPL (IgG or IgA MI) are rare (< 5% of LPL) and therefore only a few small studies have been conducted. These lymphomas can harbour *MYD88* ^{L265P} mutation, but at a lower rate than classic WM (King *et al*, 2016a).

Figure 7. Immunophenotype of WM; B-cell and plasma cell



IgM+, CD19+, Cd20+, CD79b+, CD22+ (low), CD25+, CD200+/-, CD27+/-, CD5-, CD10-, Cd11c-, CD103-, CD23-, CD305-



CD19+, CD81+, CD38+, CD138+, CD45+, CD27+

International workshop on WM (iwWM) uses the same diagnostic criteria as WHO (bone marrow infiltration of LPL and an IgM paraprotein in serum of any concentration) (Owen *et al*, 2003), whereas the Mayo Clinic criteria requires an IgM paraprotein > 3.0 g/dL and/or LPL infiltration in the bone marrow > 10% (Ansell *et al*, 2010).

IgM Monoclonal Gammopathy of Undetermined Significance

The prevalence of MGUS overall varies with age, gender, ethnic background, and geographical area. The largest population-based study was from Olmsted County, US, in 21 463 residents > 50 years old, MGUS was found in 4.0% in men and 2.7% in women, increasing with higher age (Kyle *et al*, 2006). Another recent published study from US of 12 482 adults > 50 years old found that the overall prevalence was 2.3%. IgM MGUS accounts for 17.2% and 15.4%, respectively in above mentioned studies, the estimated prevalences for IgM MGUS were 0.6% and 0.4%, respectively (Landgren *et al*, 2014).

The prevalence of IgM MGUS is higher in the white population compared with the Asian and Hispanic population. In addition, it seems that the prevalence of Ig MGUS also has regional differences; studies from Europe show the highest proportion of IgM MGUS (25%) from western France (Saleun *et al*, 1982, Cabrera *et al*, 2014) and the lower proportion of IgM MGUS (6-8%) from Greece and Sweden (Axelsson *et al*, 1966).

Prognostic factors

Prognostic clinical factors

Several clinical prognostic factors have been identified, mostly in small patient cohorts and in non-population-based retrospective analyses – these studies are summarised in Table 1. Most prognostic studies focus on overall survival and include both asymptomatic (smouldering WM) and symptomatic patients. The studies are difficult to compare because they use various diagnostic and inclusion criteria and prognostic factors with different cut-off values. The main adverse prognostic factor was older age and other prognostic factors indicate the presence of high tumour burden.

The most used prognostic index is the International Prognostic Scoring System for WM (IPSSWM) based on five disease parameters: age > 65 years; β2-microglobulin level > 3 mg/L; monoclonal protein level > 70g/L; haemoglobin concentration ≤ 115g/L; and platelet counts ≤ $100x10^9$/L (Morel *et al*, 2009). IPSSWM is validated for symptomatic patients at the time of first-line treatment.

Other proposed prognostic index include The Southwest Oncology Group (SWOG) indices based on the parameters β2-M < 3 mg/L, haemoglobin <120 g/L, and serum IgM < 40 and in follow-up 10 years later based on the parameters age, previous therapy, and LDH (Dhodapkar *et al*, 2009) and Mayo Clinic prognostic index based on the parameters age > 65 years and organomegaly (Ghobrial *et al*, 2006). The studies from the SWOG included patients both at their first-line therapy and at relapse and the Mayo clinic study only provides symptomatic patients receiving their first-line therapy.

Table 1: Studies on clinical prognostic factors

	N	Patients status	Adverse prognostic factors, Univariate	Adverse prognostic factors, multivariate	Survival
Facon T, 1993(Facon *et al*, 1993)	167	Asymptomatic and symptomatic patients	Age ≥60, male, haemoglobin <100 g/L, leukocytes <4 x 10^9, neutrophils < 1.7 x 10^9, platelets < 150 x 10^9, B-symptoms	Age ≥60, male gender, haemoglobin <100 g/L neutrophils <1.7 x 10^9	From diagnosis: Median OS 60 months
Gobbi PG, 1994(Gobbi *et al*, 1994)	144	Asymptomatic and symptomatic patients	Age ≥70, platelets <120 x 10^9 platelet presence of red blood cells in the urine, haemoglobin < 90 x 10^9 g/L, erythrocyte sedimentation rate >110 mm, cryoglobulinemia, weight loss.	Age ≥70, weight loss, haemoglobin < 90 x 10^9 g/L, cryoglobuline mia	From diagnosis: Median OS 72 months
Morel P, 2000(Morel *et al*, 2000)	232	Asymptomatic and symptomatic patients	Age ≥65, male, albumin <40, haemoglobin <120 g/L, platelets <150 x 10^9, leukocytes <4 x 10^9, high β2-M, hepatomegaly	Age ≥65 albumin <40, At least one cytopenia, At least two cytopenias	From diagnosis: Median OS 61 months
Dhodapkar V, 2001 (SWOG trial S9003)(Dhodapkar *et al*, 2001)	183	Symptomatic, first line therapy	High β2-M, haemoglobin <120, IgM < 40 g/L Age ≥ 70, previous therapy, disease duration > 1 year, Elevated CRP, albumin <35 g/L, β2M > 3 mg/L	β2M > 3 mg/L haemoglobin <120 IgM < 40 g/L	From treatment: 5 years OS 62 %
Garcia-Sanz R, 2001(Garcia-Sanz *et al*, 2001)	217	Asymptomatic and symptomatic patients	Age >65, haemoglobin ≤ 115 g/L, symptoms at diagnosis, high β2-M, hyperviscosity, Bence Jones proteinuria, IgM > 45 g/l, hepatomegaly	Age >65, haemoglobin ≤ 115 g/L, symptoms at diagnosis, high β2-M, hyperviscosity	From diagnosis: 10 year OS 55 %
Owen, 2001(Owen *et al*, 2001)	111	Asymptomatic and symptomatic patients	Age >60 years, performance status > 1, platelet count <100 × 10^9, pancytopenia, and diffuse bone marrow infiltration	Age >60 years, performance status > 1, platelet count <100 × 10^9,	From diagnosis: Median 60 months

Dimopoulos MA, 2003(Dimopoulos *et al*, 2003)	122	Symptomatic, first line therapy	Age ≥65, splenomegaly, B-symptoms, haemoglobin <100g/L, platelets < 100 x 10^9, albumin <35 g/L, bone marrow infiltration ≥50%	Age ≥65, haemoglobin <100g/L	From treatment: Median OS 106 months
Merlini G, 2003(Merlini *et al*, 2003)	215	Symptomatic		Age, haemoglobin level, low albumin level, high β2-M	From treatment: Median OS 77 months
Morel P, 2009 (IPSSWM)(Morel *et al*, 2009)	587	Symptomatic	Age > 65 years, β2-microglobulin level > 3 mg/L, monoclonal protein level > 70g/L, haemoglobin concentration ≤ 115g/L, and platelet counts ≤ 100x10^9/L. Neutrophils ≤1.5 x 10^9, albumin ≤35 g/L	Age > 65 years, β2-microglobulin level > 3 mg/L, monoclonal protein level > 70g/L, haemoglobin concentration ≤ 115g/L, and platelet counts ≤ 100 x 10^9/L.	From treatment: Median OS 87 months
Dhodapkar V, 10 year follow-up SWOG trial S9003(Dhodapkar *et al*, 2009)	183	Symtomatic	β2-M,> 3 ml/L haemoglobin <120, IgM < 40 g/L, M-component <22g/L, Age ≥ 70, previous therapy, disease duration > 1 year, β2M > 3 mg/L, Elevated LDH	Age ≥ 70, β2-M,> 3 ml/L, Elevated LDH, previous therapy	From treatment: Median OS 6.8 years
Ghobrial IM, 2006(Ghobrial *et al*, 2006)	337	Symptomatic	Age >65 years, organomegaly, elevated beta2-microglobulin, Haemoglobin < 100 g/L), leucocytes <4.0 x 10^9/l, platelets <150 x 10^9/l, albumin <40 g/l quantitative IgM < 0.4 g/l	age >65 years, organomegaly	From diagnosis: 6.4 years

IgM Monoclonal Gammopathy of undermined significance

The best well-known risk factor for developing WM is IgM MGUS. In addition to WM, IgM MGUS is also a risk factor for developing CLL and other B-cell lymphoma. The overall risk for progression is 1.5% per year, most often progression to WM, and the risk remained at the same level more than 20 years after diagnosis (Kyle *et al*, 2003). A risk factor for progression is the size of the M-protein. In a Swedish study including 728 cases of MGUS (both IgM MGUS and non-IgM MGUS) including a follow-up of up to 30 years, M-protein concentration ≥15 g/L, an abnormal free light-chain (FLC) ratio, and the reduction of one or two non-involved immunoglobulin isotype levels (immunoparesis) was significantly associated with progression (Turesson *et al*, 2014). Several studies have shown that MGUS patients appear to have a shorter life expectancy compared with the general population, both from malignant transformations and non-malignant causes (Kristinsson *et al*, 2009, Blade *et al*, 1992).

Survival

The OS in population based studies seems to have improved over the years. The population-based Surveillance, Epidemiology, and End Results study (SEER) from US of 5784 patients showed a median OS for the 1991-2000 and the 2001-2010 cohorts was six and eight years, respectively (Castillo *et al*, 2015). In another study also based on SEER data but focused on relative survival (RS) for 6231 patients with WM showed that five-year and ten-years RS was worse for patients diagnosed between 1980 and 2000 than for patients diagnosed between 2001 and 2010 – 66% vs. 49% and 78% vs. 67 %, respectively (Castillo *et al*, 2014a). A similar study based on data from the SCR in Sweden on 1555 patients with WM showed that the five-year RS rate for 1555 patients with WM had increased from 57% between 1980 and 1985 to 78% between 2001 and 2005 (Kristinsson *et al*, 2013). An encouraging study from 2018 showed that younger patients had a favourable prognosis with a ten-year OS of 86% (Babwah et al., 2018). Finally, an observational retrospective study based on 454 patients with WM, outside clinical trials, from ten European countries showed a ten-year OS of 69% (Buske *et al*, 2018).

WM is a disease of the elderly and unrelated WM mortality is significant and should be taken into account when calculating OS. A Greek non-population based study including 408 symptomatic patients with WM reports the five-year non-WM-related death rate in patients ≤75 years old and patients >75 years old

to be 5.1% and 17%, respectively. The corresponding figures for five-year WM-related were 21% and 22%, respectively (Kastritis et al., 2015). The SEER study of 5784 patients found the five-year cumulative incidence rates on non-WM related deaths for 1991-2000 and 2001-2010 to be 30% and 25%, respectively.

Because WM is a rare disease, very few randomised phase III trials have been conducted that compare survival outcomes with different treatments. Usually, treatments in WM are based on results in smaller non-randomised phase II studies or recommendations from expert groups.

Clinical presentation

WM progresses from a pre-malignant phase, IgM-MGUS, to smouldering WM and, finally, symptomatic WM. Common clinical manifestations of WM include symptoms due to tumour infiltration such as cytopenia (most commonly anaemia), lymphadenopathy and organomegaly (in 15-20% of the patients), or rarely other sites such as pulmonary or CNS infiltration (Bing-Neel syndrome). Other manifestations are related to the IgM MI as hyperviscosity, cryoglobulinemia, and antibody-mediated disorders such as haemolytic anaemia, ITP, and peripheral neuropathy, and coagulation disturbances. IgM deposition of the light chain of the IgM MI in the tissue can lead to AL amyloidosis. Furthermore, some patients might show B-symptoms including night sweats, recurrent fever, weight loss, and fatigue (Vitolo *et al*, 2008, Ghobrial, 2012).

Hyperviscosity (< 15% at diagnosis) is due to high IgM levels and symptoms for hyperviscosity often correlate with serum levels of the IgM paraproteins > 30-40 g/l. Common symptoms include headache, blurred vision, and oronasal bleeding. The increased blood viscosity and expanded plasma volume caused by increased osmotic pressure can aggravate congestive heart failure and contribute to worsen anaemia (Gustine *et al*, 2017).

Cryoglobulinemia is observed in approximately 10% of the patients with WM. Cryoglobulinemia type I precipitate immunoglobulins (in this case IgM) in temperature below normal body temperature and dissolve again when the temperature rises. The precipitate causes impaired blood flow in small vessels, resulting in Raynaud phenomenon, acrocyanosis, arthralgias, purpura, and skin ulcers.

In type II cryoglobulinemia, the monoclonal IgM have an autoantibody activity against polyclonal IgG, forming IgM-IgG immune complex, depositing on the walls of small vessels and activating the complement cascade. Type II cryoglobulinemia can be associated with Hepatitis C and might indicate a premalignant condition. Clinically, the patients develop a systemic vasculitis, with purpura, arthralgia, weakness, cryoglobulinemic glomerulonephritis, and peripheral neuropathy (Stone, 2009, Stone, 2011).

Cold agglutinin hemolytic anaemia or disease (CAD): In < 10% of WM patients, monoclonal IgM can act as an antibody with cold agglutinin activity against specific antigens of red blood cells, producing mild chronic immune haemolytic anaemia, which aggravates after cold exposure. The agglutination of RBCs in the cooler peripheral circulation might result in Raynaud syndrome, acrocyanosis, and livedo reticularis. Patients with CAD lack *MYD88* L265P mutation, but often have somatically-mutated clonal IGHV4-34 gene rearrangement, indicating a distinct type of IgM-producing lymphoproliferative disease. The symptoms is often mild and the prognosis is good (Berentsen, 2018).

Peripheral neuropathy has been reported in up to 40% of the patients with WM and caused by a variety of different mechanism. Antibodies against myelin-associated glycoprotein (MAG) is the most common antibody-causing peripheral neuropathy and has been described in 50% of the cases. It is a sensory ataxic neuropathy associated with tremor. Another antibody is anti-ganglioside M1, resulting in multifocal motor neuropathy. Other anti-gangliosides and anti-sulphatide antibodies can occur. Furthermore, other mechanisms can be cryoglobulinaemic vasculitis, AL-amyloidosis, intra-neural tumour cell invasion (compare with Bing-Neel syndrome), and rarely light chain deposition disease in peripheral nerves (D'Sa *et al*, 2017).

Treatment of Waldenstrom's macroglobulinemia

Because WM is a rare disease, only a few randomised studies on treatment have been published (Dimopoulos *et al*, 2018, Buske *et al*, 2009, Leblond *et al*, 2013, Rummel *et al*, 2013). Due to this lack of evidence, optimal treatment has yet to be determined and currently the first-line therapy should be based on the individual patient's characteristics and disease presentation (Leblond *et al*, 2016).

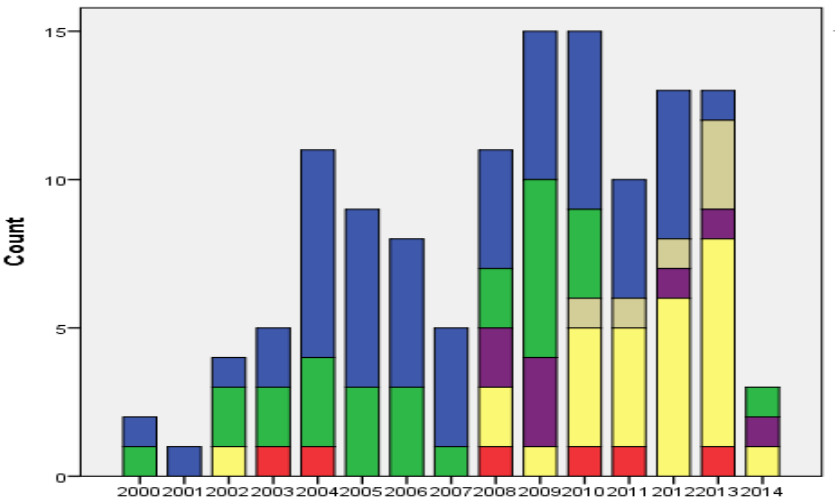
Historically, in patients with symptomatic disease, first-line therapy was often based on single agent therapies, often with an alkylating drug such as chlorambucil. The standard therapies today are immunochemotherapy combinations. For first-line treatments in Sweden see Figure 8.

One of the most used combinations are dexamethasone, rituximab, and cyclophosphamide (DRC). In a prospective study with 72 patients in first-line treatment, the overall response rate (ORR) was 83% and a two-year progression-free survival (PFS) rate was 67% for all treated patients and 80% for responders, with limited toxicities but slow response (median time for response 4.1 months) (Dimopoulos *et al*, 2007). The DRC combination can be used in a majority of patients with WM.

Recently, a non-randomised study investigated 160 consecutive patients with WM (first-line treatment and relapsed/refractory WM), treated with bendamustine and rituximab (BR) or DRC. The first-line treatment was ORR high, 93-95% and 96-87%, respectively. Two-year PFS was higher for BR compared with DRC both in first-line treatment and at relapses – 88%-66% and 61%-53%, respectively. The results for BR and DRC appear to be unaffected by patient's *MYD88* mutation status (Paludo *et al*, 2018). BR has also been compared with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in a randomised phase III study with 41 WM patients. The ORR was similar (95%) but the PSF was longer for BR (median 69.5 vs. 28. 1 months) along with less toxicity (Rummel *et al*, 2013). In Sweden, RB is often used in patients with high tumour burden and when a fast response is called for (Roccaro et al., 2016).

Single rituximab is an option in patients with low tumour burden (e.g., anaemia) or fragile patients. Two schedules for rituximab monotherapy have been studied in WM: the standard schedule (one once-per-week infusion for 4 weeks (Treon *et al*, 2001, Gertz *et al*, 2004)), and the extended schedule (four or more once-per-week infusions) (Treon *et al*, 2005). With the standard schedule of rituximab administration, ORR varies between 27-60%. The extended rituximab schedule leads to more major responses and longer durations of response.

Figure 8. Different first-line therapies for the years 2000 – 2014 in Sweden, data from Swedish Lymphoma Registry



= Chlorambucil

= Fludarabine

= Bendamustine

= CHOP-like

= Cyclophosphamide containing (DRC)

= Other, mostly CNS lymphoma treatments or traditional myeloma therapies

Transient increase in serum IgM levels (IgM flare) occurs in about 50% of patients. WM patients with high IgM levels should undergo plasmapheresis before rituximab treatment, or rituximab should be avoided during the first one or two courses of systemic therapy (Ghobrial *et al*, 2004).

Several studies have used bortezomib in different combinations (dexamethasone, cyclophosphamide, and rituximab) both in first-line treatment and in relapsed disease (Treon *et al*, 2007, Ghobrial *et al*, 2010, Dimopoulos *et al*, 2010). Generally, high ORR (up to 90%) has been shown. The main toxicity was peripheral neuropathy, which can be reduced with once-per-week and subcutaneous administration (Dimopoulos *et al*, 2013). Bortezomib is often used when a rapid response is required or in patients with renal failure. Carfilzomib, a second-generation proteasome inhibitor, is associated with a lower risk of neurotoxicity. The drug is evaluated in combination with rituximab

and dexamethasone and has an ORR in line with bortezomib (Treon, Tripsas *et al*, 2014).

Purine analogues such as fludarabin alone or in combination with rituximab and/or cyclophosphamide are effective therapies, but today these purine analogues are only used in select patients because of the risk of long-lasting cytopenias with increased risk of infections and secondary malignancies (Leblond *et al*, 2013).

New biological treatment regimens are emerging. Ibrutinib, a BTK-inhibitor, has shown high ORR (>90%) with limited toxicities (Dimopoulos *et al*, 2017, Treon *et al*, 2015). Recently, a phase III study comparing single rituximab with ibrutinib-rituximab found that the experimental arm had higher major responses (72% vs. 32%) and longer PFS (82% vs. 28% at 30 months) (Dimopoulos *et al*, 2018). Other new upcoming drugs are venetoclax, (BCL-2 inhibitor) (Castillo *et al*., 2018), ixazomib (oral proteosome inhibitor)(Castillo *et al*., 2018), and second generation of BTK-inhibitors such as acalabrutinib (Spinner *et al*, 2018).

Materials and Methods

Patients

In this thesis, we included patients with WM/LPL primarily identified through the Swedish Lymphoma Registry (SLR) and for Paper III and IV also WM/LPL patients reported to the Northern Lymphoma Registry from the counties Norrbotten and Västerbotten. For details se Figure 1. SLR was initiated by Swedish Lymphoma Group in 2000 to gather complementary clinical information for adult patients ≥18 years, such as clinical stage, prognostic factors, and therapies. All patients reported to the SLG are also reported to the Swedish Cancer Registry (SCR), a compulsory registry established in 1958. To achieve high coverage, all cancer subtypes are reported to SCR both by the responsible pathologist and physician, and with ability to cross-check. The coverage for SLG is high (>95%) (Swedish Lymphoma Study Group). Between 2000 and 2006 data were restricted to clinical characteristics and prognostic factors, but after 1 January 2007, when a web-based registry was introduced (INCA - a national IT platform for quality registries in cancer care), more detailed data about primary therapies, responses, and relapses were added.

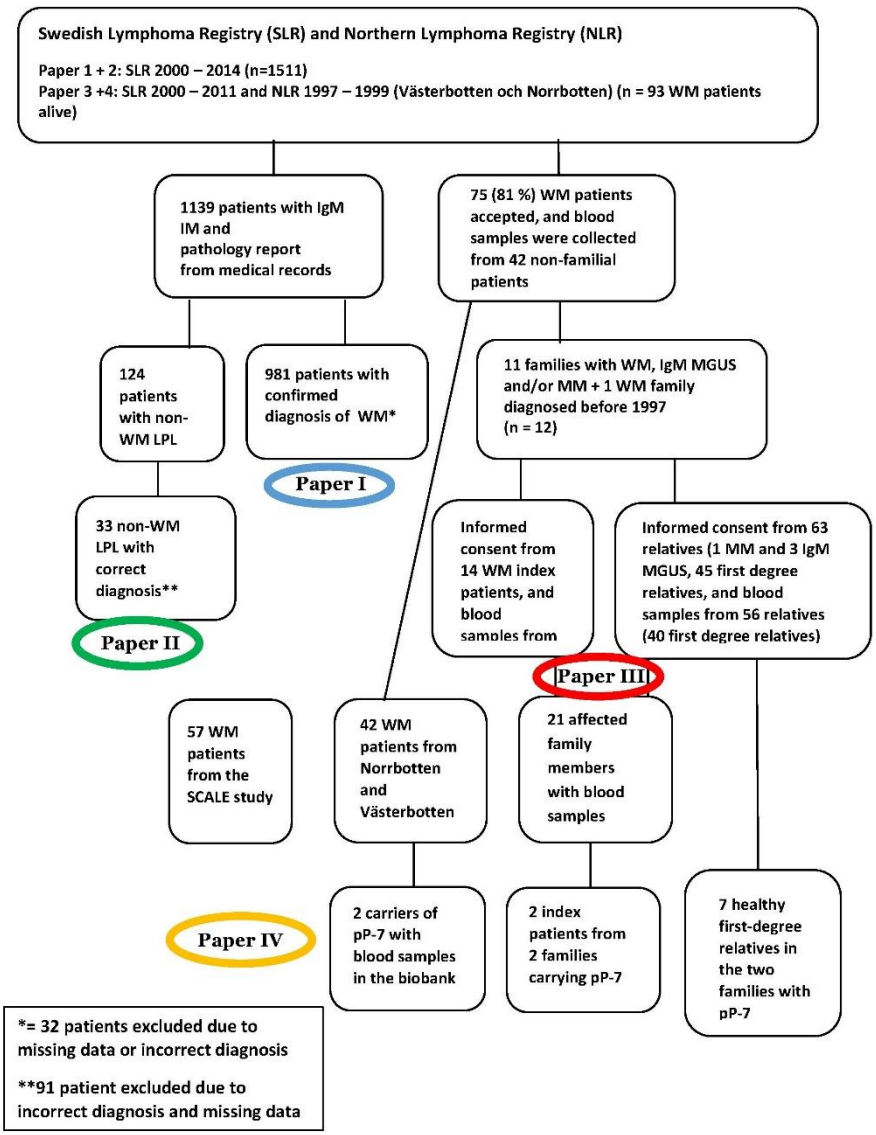
Paper I and II – Register studies

In Paper I and II, we identified 1511 patients with a diagnosis of WM/LPL between 1 January 2000 and 31 December 2014 from SLR. Because the paraprotein was not registered, we could, in many cases, not distinguish between WM and non-WM LPL. To confirm the diagnosis, available serum protein electrophoresis and the pathology report for diagnosis were extracted from the medical records in 1139 (75%) of all patients. For details, see Figure 9.

Paper II

In Paper II, we gathered patients that did not fulfil the criteria for WM and named them non-WM LPL (n = 124). Because of diagnostic challenges in the diagnosis of non-WM LPL, we conducted a review of an experienced haematopathologist and only patients with correct non-WM LPL diagnosis have been included to further analysis (n = 33).

Figure 9. Flowchart over patient selection process



Paper III and IV

In paper III and IV, we identified families with two or more family members with WM, IgM MGUS, and/or MM through the Northern and the Swedish Lymphoma Registry for the years 1997-2011 from the counties Norrbotten and Västerbotten. For details, see Figure 1. We contacted all living patients with WM/LPL (n = 93) by letter; the response rate was high (n = 75 or 81%). We identified 12 families with two or more family members with WM, IgM MGUS, and/or MM (one family with a family member diagnosed before 1997).

To verify the diagnosis and obtain additional information, we conducted telephone interviews and reviewed their medical records. In addition, we asked if they were willing to take part in blood sampling to be used in future research.

Paper IV

Additional blood and serum samples were also collected from 42 non-familial WM patents. Two of these non-familial WM patients positive for pP-7 had pre-diagnostic blood samples in the biobank at Umeå University for analysis of the pre-diagnostic status of pP-7. In addition, as a control group, blood samples from 57 Swedish patients with WM participating in the Scandinavian Lymphoma Aetiology study (SCALE) were analysed for pP-7.

All studies were approved from the Regional Ethical Review Board in Umeå (Dnr: 2011-44-31M, additional applications: 2014-245-32M, 2014-261-31M, 2015-103-32).

Statistics

Paper I and II

The incidence calculations were age-adjusted by using a standard population of the general population for Sweden and the two counties obtained from *Statistiska Centralbyrån* (paper I) and the *European Standard Population 2013* (paper II). To calculate the Hazard ratios for prognostic factors, we used Cox proportional hazards regression, univariate and multiple (multivariable)**.** The Kaplan-Meier method was used to estimate overall survival and the relative survival was estimated using the Pohar-Perme estimator. Significance testing used a log-rank or log-rank-like test. The Pearson Chi-square test was used for

frequency tabulation. The median follow-up time was estimated using reverse Kaplan-Meier.

Serum and blood samples analysis

Complete blood counts, serum protein electrophoresis (SPE) with immunofixation (IF), LDH, beta-2-microglobulin, and creatinine were analysed in the local clinical laboratory at Sunderby Hospital, Luleå and free light chains in serum (FLC/s) were analysed at the University Hospital in Umeå. SPE was performed using the agarose gel electrophoresis technique on Hydrasys 2 (Sebia, ILS Laboratories Scandinavia/Thermo Fisher). The serum proteins were quantified by immunological turbidimetric assay (Cobas 6000 c501, Roche).
The analyses of the pP-7 and the titres of the corresponding antibodies were analysed at José Carreras Centre for Immuno and Gene Therapy, Department of Internal Medicine I, Saarland University Medical School, Homburg/Saar, Germany.

Available formalin-fixed, paraffin-embedded blocks were used for immunohistochemically stainings and, if possible, for *MYD88* ^{L265P} mutation analysis. The analysis were performed at the Karolinska University laboratory, Department of Pathology.

Methodical considerations

Swedish Lymphoma Registry

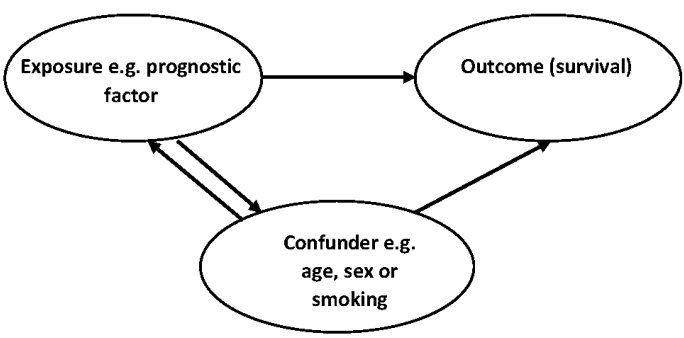
All four studies are based on SLR, a population-based registry of all subtypes of lymphomas diagnosed in Sweden since 2000. The strengths of the studies are that the registry has a high coverage (>95%). Bias is a systematic error and can distort the study result. In this case, selection bias could be avoided – i.e., the study population largely represents the target population. The registry contains clinical data such as exact lymphoma subtype, prognostic factors, and detailed treatment.
Furthermore, other strengths of this prospective population-based registry are real world (observational) data in a large number of unselected patients in a rare

disease. The registry can be used for surveillance of the incidence, possible prognostic factors, and outcome.

The limitations are that the IgM paraprotien were not reported to the registry and we could not differentiate patients with the diagnosis of WM from patients with non-WM LPL. Before 2007, reported data were restricted to clinical characteristics and about 50% of first-line treatments were missing. On 1 January 2007, the web-based registry INCA was introduced with detailed data on first-line treatments and outcome. The data (or the variables) reported to the registries have changed over the years and new variables have been introduced; for example, paraprotein was first reported last year. Furthermore, the lymphoma classification has changed; in the first year the REAL classification was used, but since 2002 the WHO classification has been used (revised 2008 and 2017).

Other limitations are varying quality of data, missing/incomplete data, and confounding factors. A confounding factor obscures the real effect of an exposure on outcome. Additionally, a potential confounder must have an association (risk factor) for the disease and be associated with the exposure, but not a consequence of it and might not be part of the causal pathway (Andersson, 2013)(Figure 10). In the registry studies, we have information on sex and age, but no information on other potential confounders.

Figure 10. The confounders impact for outcome



Diagnostic considerations

The pre-malignant stage of MGUS gradually develops to smouldering or asymptomatic WM and finally to symptomatic WM. Obviously, the timing of the patient's diagnosis (i.e., when a bone marrow biopsy identifies the disease) influences the time for survival. Lead-time bias is an increase in survival due to detecting a disease at an early stage, resulting that the patient lives a longer time with the diagnosis (or follow-up begins at earlier/different times). To minimise lead-time bias, we performed some of the analyses at time of start of first-line treatment. In our WM cohort, we had a large proportion of asymptomatic patients (about 75%). In other studies, the proportion of asymptomatic patients are lower (Pophali *et al*, 2018). This indicates that in Sweden WM patients included in the SLR are diagnosed at an earlier stage compared to registries in other countries.
The diagnosis of WM/LPL is not always distinct and can be misdiagnosed with other indolent B-cell lymphomas with plasmacytic differentiations, especially some MZL (Swerdlow, Kuzu *et al*, 2016). In paper II, we discuss the diagnostic difficulties of non-WM LPL. Bone marrow or other tissue samples were reviewed by an experienced haematopathologist. After full diagnostic work-up, half of the patients had diagnosis other than non-WM LPL, mostly MZL, and for six patients it was impossible to classify other than indolent B-cell lymphoma UNS. In a majority of the cases, the non-WM LPL had a paraprotein of IgG or IgA type, but other indolent B-cell lymphomas can also possess a MGUS of IgG, IgA, or IgM type.

Another tool for differential diagnosis is *MYD88* ^{L265P} mutation, as this mutation is found in more than 90% of patients with WM. In non-WM LPL, the frequency seems to be lower, 40-71% in some smaller studies. However, *MYD88* ^{L265P} is either specific or required for the WM diagnosis and can be positive, but in much lower frequencies, in other B-cell lymphomas. Some authors discuss *MYD88* ^{L265P} positive and negative WM/LPL as if they are two different diseases. There are some clinical differences in *MYD88* mutated WM (as increased bone marrow involvement, higher serum IgM levels, and longer overall survival) compared with *wtMYD88*, but today there is no data regarding non-WM LPL.
In the WM cohort from Paper I, serum electrophoresis and the bone marrow- and pathology reports have been reviewed from the medical records, but the bone marrow and tissue samples have not been reviewed by a haematopathologist. In most cases, *MYD88* ^{L265P} has not been performed.

Consequently, there might be some uncertainty about the correct diagnosis in these patients.

Lymphoma classifications have changed (REAL vs. WHO classification, presented in 2001). The corresponding diagnosis of LPL is in the REAL classification lymphoplasmacytoid lymphoma or “immunocytoma”. Some of the immunocytomas are in the WHO classifications considered MZL. In addition, the definition of WM varies between countries (e.g., Mayo vs. WHO classification). Generally, studies of survival or incidence are often not comparable because they often use different study populations (e.g., population-based, populations from clinical trials), criteria for diagnosis, inclusion criteria, or standard populations (used in age adjusting).

Main results

Paper I

The age-adjusted incidence of WM/LPL (European standard population) for the years 2000-2014 in Sweden was 11.5 per million persons, the corresponding figures for males and females were 15.5 and 8.5, respectively. The OS improved between 2000-2006 and 2007-2014 with a five-year OS of 61% and 70%, respectively. In patients receiving therapy 0-3 months after diagnosis, age, elevated lactate dehydrogenase level, and haemoglobin ≤115 g/l were significant prognostic factors for OS in multivariable analyses. In watch and wait patients, age, poor PS, haemoglobin ≤115 g/l, and female sex were significant prognostic factors for OS in multivariable analyses. The level of the IgM paraprotein at diagnosis had no significant prognostic value. Rituximab included in first-line therapy was associated with improved survival.

Paper II

We found diagnostic difficulties as 54/124 patients had a diagnosis other than non-WM LPL, mainly MZL (33 patients). Most of the patients with non-WM LPL secrete a paraprotein, mostly IgG (18/33 patients; 54.5%) or IgA MI (5/33 patients; 15.6%). Non-secretory LPL was seen in 4/33 (12.2%) of the cases. The non-WM LPL patients had more adverse prognostic factors as elevated LDH, anaemia, and lymphocytosis at diagnosis compared to the patients with WM. In addition, the non-WM LPL patients more often were symptomatic and more often received treatment at diagnosis (68.4% vs 26.6%). The OS did not significantly differ between the groups (P = 0.247), with a median OS for non-IgM LPL on 71 months. The three-year and five- year survival was 71 % and 55%, respectively.

Paper III

The age-adjusted incidence of WM/LPL (Swedish standard population) for the years 2000-2012 in Sweden was 10.5 per million persons per year; the corresponding figures for Norrbotten and Västerbotten were 17.5 and 14.8, respectively. We identified 12 families with two or more family members with WM, IgM MGUS, and/or MM. These families had a high frequency of autoimmune/inflammatory diseases (in 9/15 families) or other haematological malignancies (in 5/12 families) in their own or in their relatives' medical history. The relatives in these families had a high proportion of abnormal serum protein

electrophoresis: 12/56 (21%) MGUS and 13/56 (25%) abnormalities in the immunoglobulin levels (i.e., subnormal levels and poly/oligoclonality).

Paper IV

In patients with WM from Sweden and from the northern counties, the frequency of pP-7 (8.8% and 7.1%, respectivily) was about the same level or lower as what earlier studies from Europe found. The occurrences of pP-7 in the index patients from families with aggregation of WM, IgM MGUS, and/or MM had a tendency to be higher compared with the non-familial cases (16.7% vs. 7.1%) from the same area. The two families carrying pP-7 had several family members with autoimmune diseases, indicating an association with these diseases. Two patients with non-familial WM from the northern part of Sweden were positive for pP-7 before the diagnosis (6.5 and 10 years, respectively). One family was a carrier of paratarg-6 (P-6) (*LAPTM5*), and P-6 was hyperphosphorylated and inherited in the same way as described for pP-7 and other paratarg proteins.

Results, Analysis, and Discussion

Incidence - Paper I and III

The incidence was calculated and discussed in both Paper I and III. In Paper III, we used SLR for the years 2000-2012 and the age-adjusted incidence for VM and non-WM LPL for all of Sweden was 10.5 per 10^6 persons per year; the corresponding figures in Norrbotten and Västerbotten were 17.5 and 14.8 per 10^6 persons per year, respectively. Paper I also used SLG but for the years 2000 - 2014. The corresponding figure in Sweden was 11.5, Norrbotten 18.1, and Västerbotten 15.4. The incidence is higher for males compared with females. In Sweden, the incidence was 15.5 and 8.5 per 10^6 persons per year, respectively, and increased with age: <65 years, 3.4; 65–75 years, 32.7; >75 years, 45.5 per million persons per year, respectively. The incidence has not changed over the time.

The figures above show the incidence for WM and non-WM LPL together. The overall age-adjusted incidence for WM alone in Sweden could not be calculated because of incomplete data of the specific diagnosis in 372 (25%) patients. However, the estimated age-adjusted incidence for WM is 9.9 per million persons per year. In Norrbotten and Västerbotten, we had complete diagnostic data, and the true age-adjusted incidences for WM were 17.6 and 14.3 per million persons per year, respectively. In the calculations for age-adjusting we have used different standard populations: in Paper I the European Standard Population and in Paper III the Swedish Standard Population. This was done so we could compare incidence figures between different studies. That is also one reason for different incidences between Paper I and III.

The incidence for WM worldwide is estimated to 3-4 persons per million per year. Correct figures are challenging to calculate. The incidence seems to vary between different geographic areas and among different ethnic groups (Wang *et al*, 2012, Iwanaga *et al*, 2014) WM is a gradually developing disease with IgM MGUS as a precursor stage, smouldering or asymptomatic WM, and finally symptomatic WM. The differences in the incidence might partly reflect different organisations of healthcare, availability to haematologists and haematopathologists, and local practices regarding the time at which the bone marrow biopsies are performed. In SLR, many patients have an asymptomatic disease at diagnoses (75%). The corresponding figure in the literature is about 25%, a percentage that might reflect the fact that Sweden has a mandatory cancer registry. WM is a rare and rather “new” diagnosis and the diagnostic criteria have changed and vary between countries, resulting in parallel classifications. Cancer incidence is often calculated on population-based cancer

registries, and the registries can vary in quality and how the reporting is performed. SCR is considered to have a high accuracy. The registry was validated in 2007 for lymphomas reported between 1964 and 2003. Patients with lymphoproliferative diseases retrieved from the SCR and Swedish Inpatients Registry (hospital-based population) were compared, and the diagnostic accuracy for WM was high (92.5%), but the overall completeness (i.e., the number of individuals actually registered) was low (68.1%). Patients diagnosed at older ages, earlier in the period, and with low-stage disease were overrepresented in the cohort of patients who had not been registered (Turesson *et al*, 2007).

One can speculate that the higher incidence in Norrbotten compared with other areas probably is true, because all diagnoses are validated and Norrbotten has a rather high frequency of symptomatic patients at diagnosis compared with other counties. One possible explanation for the high incidence of WM in the northern Sweden is that this part of Sweden has a small but stable and, at least in earlier years, in some areas isolated populations that might have been influenced by both genetic and environmental factors.

Survival

Waldenstrom's macroglobulinemia

The median OS of all WM patients (2000–2014) was 96 months and the three-year and five-year survival rates were78% and 66%, respectively. The OS and the RS have improved between the years 2000-2006 and 2007-2014, with a five-year survival of 61% and 70%, respectively. Other studies have showed the same result, except one study from Greece (Kristinsson *et al*, 2013, Castillo *et al*, 2014b, Kastritis *et al*, 2011). One explanation for the better outcome might be more effective therapies. In Sweden, rituximab in routine care for WM was introduced rather late (about 2007) and was used alone or in combination with chemotherapies. This is probably one reason for the better outcome. Better outcome with rituximab containing therapies has also been shown in several studies (Buske *et al*, 2009, Kastritis *et al*, 2015). Changes in OS over the years have many explanations. Several factors can influence these changes: when in the course of the disease the patient is diagnosed, type of therapy, prophylactic care and follow-up, socioeconomical factors, differences in healthcare system, access to modern drugs, and biological factors such as biological sex. For example, SEER data from US show that there was an improvement in RS in

whites and other ethnic groups, but not in African-Americans (Castillo *et al*, 2014a)

Non-WM Lymphoplasmacytic Lymphoma

The non-WM LPL cohort had no significant difference in OS compared with the WM patients, median OS 71 months, and three- and five-years survival of 71% and 55%, respectively, despite more adverse prognostic factors. The outcome differs between the few studies that exist and because the rarity of non-WM LPL the studies are small and often single centre experiences.

Prognostic factors

Age

Age was the strongest prognostic factor for survival in WM and non-WM-LPL, both for OS and RS, and is a strong prognostic factor in most subtypes of lymphomas. In WM, the outcome has improved in all age groups, but the improvement is higher in the younger patients compared with the very old (≥76 years) (Figure 10). This improvement could be the result of different treatments for younger compared to older patients. In Sweden, older patients more often have been treated with chlorambucil and in most cases not in combination with rituximab (i.e., a palliative treatment). We have no data on comorbidity, but comorbidity increases with age. Diminishing organ functions and increasing comorbidities, perhaps complicated by polypharmacy, are key factors that worsen tolerance to lymphoma therapies and could explain why the elderly receive different treatments. In addition, the toxicity of the treatments in some cases prevents adequate dosage and decreases the number of treatment cycles and the intervals between the cycles and the SLR has incomplete data about dosage and cycles of the given drugs. Nevertheless, older patients have worse OS and RS and the best treatment options and supportive care have to be defined.

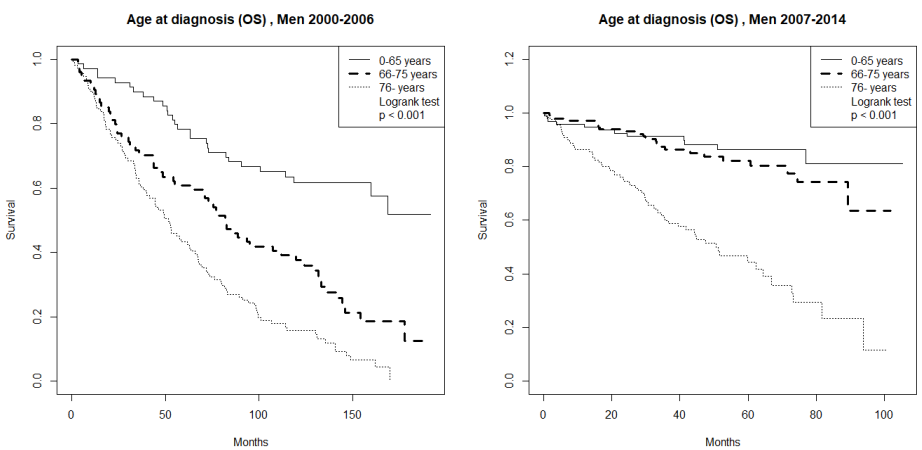
Biological factors responsible for the worse prognosis in elderly patients are poorly understood. Most age-related biological differences have been described for DLBCL. Elderly patients have a higher frequency of DLBCL and often subtypes associated with poorer prognosis. The prognostically inferior ABC subtype of

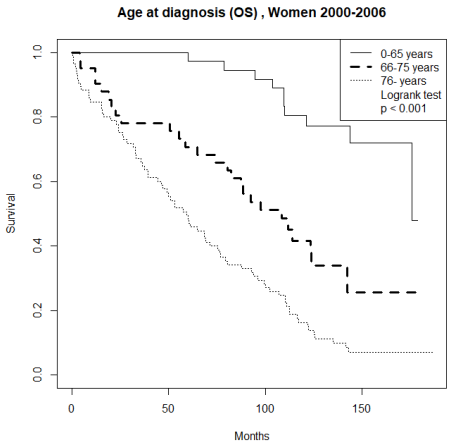
DLBCL is more prevalent in elderly, and Epstein-Barr virus (EBV)-positive DLBCL of the elderly occurs rarely in patients younger than age 50 and is usually associated with a poor prognosis. Moreover, *BCL2* expression and cytogenetic complexity increase with age. Various genetic alterations, such as gains in 1q21, 18q21, 7p22, and 7q21, change in 3q27 and gains and translocations affecting the BCL6 locus are significantly associated with older age (Klapper *et al*, 2012, Park *et al*, 2007). The differences in biological factors in WM tumour cells between younger and elderly patients are poorly understood and in the future more studies addressing this question must be done.

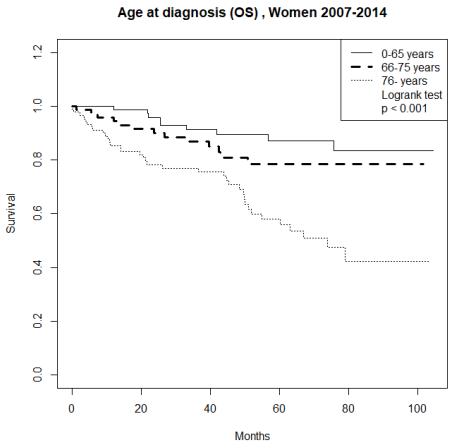
Gender

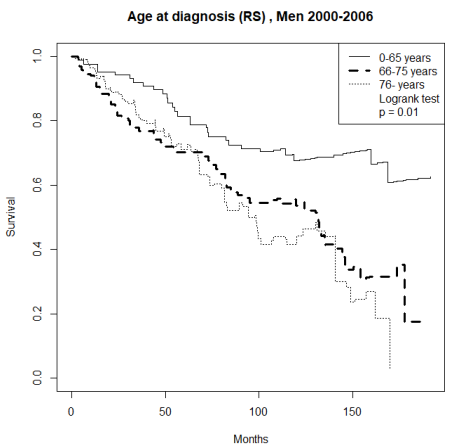
Many studies show that WM and other subtypes of lymphomas have better outcomes in females. In our study, we showed a significantly better survival in cox multivariable analysis adjusting for other prognostic factors in all WM patients and in "watch and wait" WM patients for females, but not in the WM patients cohort that received early treatment. This finding might be the result of a smaller cohort. There were no significant differences in OS and RS between

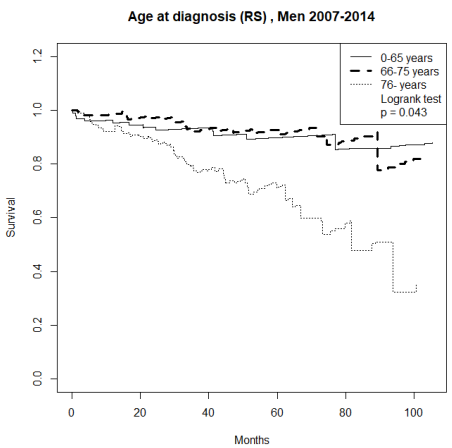
Figure 11. Kaplan-Meier curves for overall survival (OS) and relative survival (RS) for males and females ≤65 years, 66 – 75 years, and ≥76 years and for the two time periods 2000-2006 and 2007-2017 with WM (data from SLR).

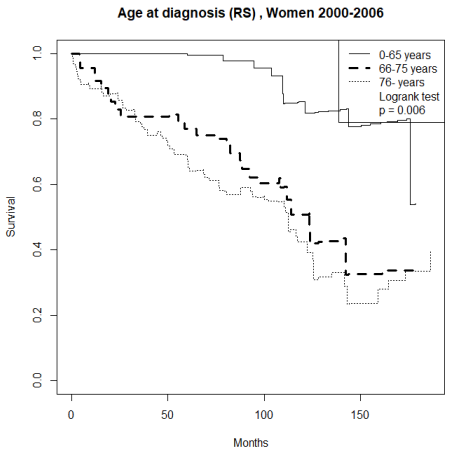


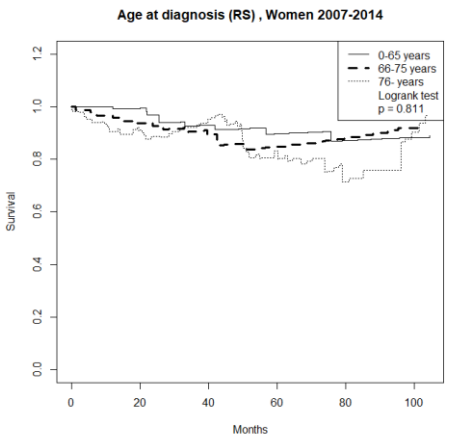

Age at diagnosis (OS) , Women 2000-2006
0-65 years
66-75 years
76- years
Logrank test
p < 0.001
Survival
Months


Age at diagnosis (OS) , Women 2007-2014
0-65 years
66-75 years
76- years
Logrank test
p < 0.001
Survival
Months


Age at diagnosis (RS) , Men 2000-2006
0-65 years
66-75 years
76- years
Logrank test
p = 0.01
Survival
Months


Age at diagnosis (RS) , Men 2007-2014
0-65 years
66-75 years
76- years
Logrank test
p = 0.043
Survival
Months





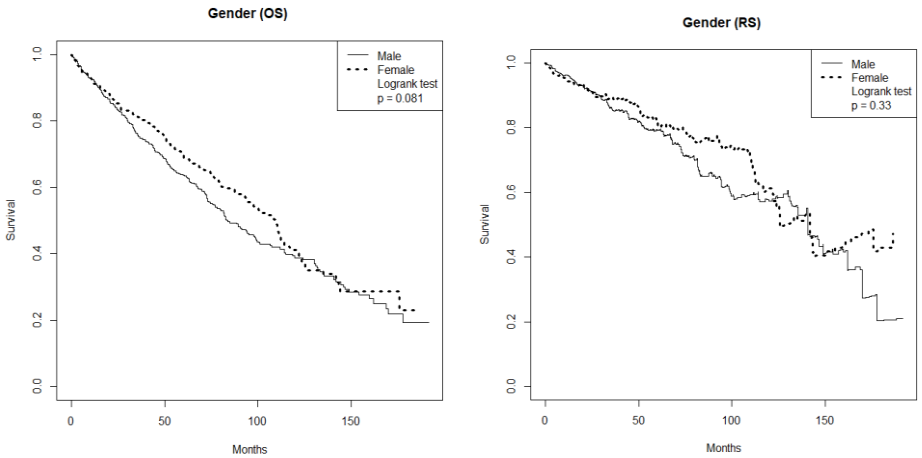
males and females in all WM patients (Figure 12). Comparing the OS and RS between males and females between different age groups for the period 2007-2014, we found that the females > 76 years had improved OS compared with males and the RS improved for females > 66 years (Figure 11).

Although our study was rather small, we found differences in treatments between males and females; specifically, treatment intensity differed. Males were often treated with more intense regimes (such as fludarabin and bendamustine) with more side effects compared to females, who received therapies considered less toxic (such as chlorambucil and rituximab). Males also received more modern therapies. There are no studies that address gender imbalance in therapies in lymphomas, but future registry studies should highlight this imbalance.

In many studies, it is unknown why females have better outcome. Perhaps sex might have an important role in responsiveness to standard treatment for B-cell lymphoma, most well-known in regimes including rituximab. Pfreundschuh et al. demonstrated that the addition of rituximab to therapy resulted in better outcomes for elderly women compared to elderly men and could be explained by the difference in clearance rate of rituximab, which was found to be significantly lower among elderly women (Pfreundschuh *et al*, 2014). This result might partly explained our finding that elderly females had a better outcome compared with elderly males (2007 – 2014), as rituximab was introduced in WM therapies about 2007.
Other factors that contribute to differences in outcome between males and females with lymphoma are less understood. Female hormonal status is one of the factors that has been discussed. A large Swedish registry study on DLBCL showed that premenopausal females had longer survival compared to males of the same age, but this difference was not found in the older patients (or disappeared at the time of menopause) (Hedstrom *et al*, 2015). However, environmental factors and the role female lifestyle and attitude to health might also influence survival. Other biological differences might also be present such as ones found in a study examining global transcriptome DLBCL data from The Cancer GenomeAtlas: female sex was associated with decreased interferon signalling, transcription, cell cycle, and PD-1 signalling (Beheshti *et al*, 2015). The knowledge is sparse of biological differences between male and female patients with WM.

To better understand the differences in outcomes for lymphomas between the sexes and the underlying mechanisms, pharmacokinetic data of the drugs involved, planned, and received doses must be incorporated in clinical trials. Furthermore, in future studies should investigate biologic differences (e.g., genetic or hormonal) between the males and females with lymphomas and correlate these findings with outcome results. This knowledge will enable us to adjust lymphoma treatment in a more personalised way.

Figure 12. a) Kaplan-Meier curves for OS and b) RS for males and females with WM (data from SLR)



Serum Lactate dehydrogenase level

LDH is a prognostic factor in other subtypes of lymphomas, but is not included in the IPSSWM. In our study, LDH was a significantly prognostic factor, also described in other studies, especially in symptomatic or advanced stage disease patients. Kastritis et al. suggest addition of LDH to IPSSWM to identify a subset of patients with very poor outcome (a median OS less than three years) (Kastritis *et al*, 2010). LDH is an established prognostic factor in most lymphoma subtypes and is a strong candidate for a prognostic factor in symptomatic patients with WM in need of treatment in future prognostic indices.

The paraprotein of M-type

The size of the IgM paraprotein (> 70g/L) is one of five prognostic factors in IPSSWM. In our study, IgM paraprotein was not a significantly prognostic factor in all WM patients or symptomatic or asymptomatic patients, not even when we change cut-off levels or used a continuous scale. The size of the IgM paraprotein as a prognostic factor is controversial, some studies have shown a correlation, others have not (Table 1). In addition, the cut-off of the IgM paraprotein is high, 70 g/L. In our data set, only 6.7% of the symptomatic patients had a paraprotein above this level.
The level of the IgM paraprotein seems not correlate to the tumour burden, although, at least in part, correlates with the degree of plasma cell differentiation seen in the tumour. (de Tute *et al*, 2013). The IgM paraprotein is

included in the response criteria; however, IgM clearance from the serum takes about three weeks and is not a sensitive marker for early response. The paraprotein might also increase following treatment with rituximab (flare) and persist for several months (Ghobrial *et al*, 2004). In some cases, it could be difficult to distinguish between disease progression and flare. Finally, some therapies such as monoclonal antibodies and purine analogue-based therapies seem to deplete the B cells and spare the plasma cell clone, which can delay the IgM paraprotein response up to one year (Treon *et al*, 2009). On the other hand, bortezomib-containing regimes can have a very rapid IgM response, while the response in the bone marrow is poorer (Treon *et al*, 2007). In summary, the IgM paraprotein is one of the diagnostic criteria, otherwise an unsafe marker for tumour burden and response assessments, and possible also for use as a prognostic factor.

Beta-2-microglobuline

Finally, beta-2-microglobuline (B2M) seems to be a robust prognostic factor. We have only used it as a prognostic factor in the univariate analysis due to many missing values. B2M seems to be a promising candidate for a prognostic index in the future.

Prognostic score

A prognostic score should be easy to use and include easily accessible variables used in routine health care. When a patient is diagnosed with WM, one of the first questions the patient asks is this: What is my prognosis? For symptomatic patients, the IPSSWM is validated, but often only used in clinical trials. For "watch and wait" patients, there are no prognostic scores in clinical routine care available, so it is important to design a prognostic index for this patient group. In our study, age, haemoglobin level, PS, and gender were significant prognostic factors in multivariate analysis. Studies are needed to create the best prognostic index, and future prognostic indices might include a biological marker.

Clinical presentation non-WM LPL

Non-WM LPL seems to have another clinical presentation compared with WM. In our cohort, the patients were younger, and had more adverse prognostic factors such as elevated LDH, anaemia, and lymphocytosis at diagnosis. Other authors described that the non-WM LPL patients more often had enlarged lymph nodes, splenomegaly, and extra nodal involvement (Varettoni *et al*, 2019) They are more often symptomatic and in need of treatment at diagnosis. Non-WM LPL is a heterogonous disease and due to its rarity not well described in the literature. In addition to our study, there are only five small studies and a small number of case reports published (Table 2).

Treatments

Treatments of Waldenstrom's macroglobulinemia

In general, the number of patients treated with different therapies was too small for statistical analyses regarding outcome. Treatment with rituximab-containing regimes was the only therapy with significantly better outcome. This correlation has been shown in many studies and in many subtypes of lymphoma. As WM is rare disease, WM has been investigated by only a few randomised clinical trials. In this circumstance, registry studies can provide important clinical information – i.e., outside clinical trials in a large unselected (real world data) patient population.

Table 2. Summary of studies and case reports on patients with non-WM-LPL

	N	Med age, years	Sex M-F, N	Paraprotein 1gG-IgA-other, N	MYD88 Yes-no, N	Survival
(Kang *et al*, 2018)	8	65.5	7-1	5-1-2	NA	Med OS 10 mon
(Cao *et al*, 2016)	17	63	9-8	9-8-0	6-15	5 yr OS 67% 10yr OS 56%

(King *et al*, 2016b)	23	70	17-6	21-2-0	10-13	NA
(Varettoni *et al*, 2019)	45	65.5	19-26	22-7-16	8-19	5 yr OS 81%
(Itchaki et al)	31	63	11-20	20-5-6	10-14	5 yr OS 90% 10 yr OS 81%
Brandefors et al, 2919	33	69	18-15	18-5-8	8-3	Med OS 71 mon 3 yr OS 71% 5 yr OS 55%
(Treon *et al*, 2012)	3	NA	NA	3-0-0	3-0	NA
(Anelli *et al*, 2014)	1	NA	1-0	1-0-0	1-0	NA
(Manasanch *et al*, 2014)	1	NA	0-1	NA	0-1	NA
(Bassarova *et al*, 2015)	2	NA	NA	2-0-0	2-0	NA
Total	163		82-70	101-28-32	48-65	

N=number, M=male, F=female

Treatments of non-WM lymphoplasmacytic Lymphoma

For the period 2007-2014, 13/19 (68.4%) of the non-WM LPL were treated at time of diagnosis; the corresponding figure for patients with WM was 26.6%. Most of the patients received rituximab-containing regimens (10/13 patients). The treatment choice did not seem to differ compared with patients with WM. Because of the rarity of non-WM LPL, there are no clinical trials in this group of patients and they are often included in trials for WM patients or for other indolent B-cell lymphomas. These patients are often treated using guidelines for WM.

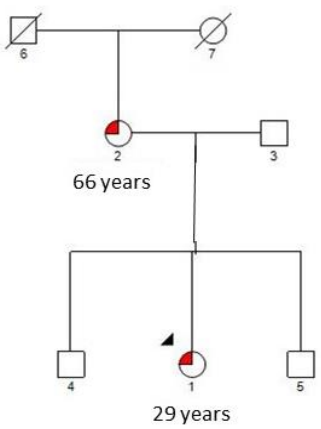
Familial Waldenstrom's macroglobulinemia – Paper III and V

Description of the families

The basis of paper III and VI is a description of 12 families with two or more family members with WM, IgM MGUS, and/or MM. The families were identified from the SLR (2000-2011) and the northern lymphoma registry (1997-1999) and

lived in the counties of Norrbotten and Västerbotten. They did not differ from other families described earlier in the literature, except that the distribution of the genders was equal (i.e., in other studies, there was a male dominance). There were only a few family members affected (usually 2-4 persons) and the two most common relationships between the relatives were siblings and parent-child. Compared to other forms of hereditary cancers, there are no clear associations with lower age of onset and the question of anticipation is unresolved. Our cohort had a median age at diagnosis of 70 years (median age at diagnosis in Sweden is 73 years). Because of the high age for diagnosis, the parent generation in many cases is not alive at the time point when the child generation received their diagnosis. Furthermore, the diagnosis of WM was not always recognised in earlier generations. In our study, two families had a parent-child relationship where the child generation was diagnosed at an earlier age.

Figure 13. Pedigree of a Family with a mother and a daughter with WM



In one of the families, the daughter was diagnosed at 29 years and was first treated 10 years later. Her mother was accidentally diagnosed at 66 years of age when hospitalised for other reasons and has not needed any treatment so far (Figure 13).

Immunological and genetic factors

Our families had a high frequency of autoimmune diseases (75%) and other haematological malignancies (42%) in their own or in their relatives' medical history. This brings up the discussion about whether the aetiology of WM is immunological, genetic, environmental, or some combination of these. The correlation between autoimmune disease, such as Sjögren's syndrome, or specific infections, such as hepatitis C, and WM is well-described in several studies (Vajdic *et al*, 2014b, Kristinsson *et al*, 2010, Giordano *et al*, 2007). One of the main hypotheses is that chronical antigen stimulation and lymphocyte activation play a role in the pathogenesis (Baecklund *et al*, 2014b, Smedby *et al*, 2008). Another hypothesis, supported by the fact that both a personal and a family history of autoimmune diseases and infections are associated with an increased risk for WM, is the presence of shared immune-related susceptibility gene (Kristinsson *et al*, 2010). The increased risk is not only for WM but also for other B-cell malignancies (Treon *et al*, 2006). This might indicate a shared common oncogenic genes somewhere along the development of the B-cell.
The increased risk for first-degree relatives of patients with WM to develop WM, other B-cell malignancies, and MGUS is another factor that supports shared susceptibility genes (Kristinsson *et al*, 2008). The genetic causes of familial WM are still not clear, and as for other cancer types, there are different types of inheritance in different families. In families with only two cases of WM, some are likely to have the disease due to a chance.

Monoclonal gammopathy of determent significance and other immunoglobulin disturbance

We found high frequencies of pathological serum protein electrophoresis (SPE) in the relatives in these families. A high proportion (12/56; 21%) of the family members had a MGUS. A majority (8/12) were of IgM type, but three MGUS were of IgG type and one was monoclonal FLC/s. In the same family, we observed MGUS both of kappa and lambda type, a finding that reflects the heterogeneousness of the disease. Many studies show that WM co-aggregates with B-cell lymphoid disorders, but the co-aggregations to MM have been more controversial. Our study shows that the same family might exhibit co-aggregation with IgG MGUS and MM with IgM MGUS and WM, and a large Swedish study (N = 24 137) of MM patients showed an increased risk of WM and non-WM LPL in offspring when a parent was diagnosed with MM (Frank *et al*, 2016).

Other IG abnormalities observed in one-quarter of the previous unaffected relatives were hypoglobulinemia, polyclonal hyperglobulinemia, oligoclonality, and acute-reaction protein pattern. Half of the patients with polyclonal hyperglobulinemia and hypoglobulinemia had a medical history of autoimmune diseases or allergy, and the patient with oligoclonality had a connective tissue disease. When we retrospectively reviewed the medical records of our index patients, we noticed that five patents, before they developed a MI, had a polyclonal hyperglobulinemia. In four of the cases, there was an underlying rheumatic disease. In one patient, there was no obvious explanation for this phenomenon. Perhaps these findings reflect a more generalised dysregulation in the immune system or an early step in prolonged immune stimulation.

One considerable limitation of our study is lack of controls. Since WM families are rare, a descriptive study could still be of high value.

Paratarg proteins

Paper IV included the same families with two or more family members with WM, IgM MGUS, and MM as in Paper I and they were analysed for pP-7. In addition, non-familial WM patients from the counties Norrbotten and Västerbotten were included. For two of these patients, prediagnostic blood samples bio-banked within the Västerbotten Intervention project (VIP) were included. In addition, blood samples for patients with WM from Sweden participating in the SCALE study were analysed for pP-7 (Figure 8).

The frequency of pP-7 in Sweden (8.8%) and in the northern counties (7.1%) was in line or lower with earlier studies from Europe. The frequencies of pP-7 in the index patients from our WM families had a tendency to be higher compared with the non-familial cases from the same area – 16.7% and 7.1%, respectively. In the literature, only a few families with aggregations of MM have been analysed, and no families with WM have been analysed. Most analysis on heredity of pP-7 are made on non-familial MM and WM, and in these families pP-7 are inherited in an autosomal dominant pattern. Our families had the same inheritance pattern and an association with autoimmune diseases. As described before, WM is associated with autoimmune disease and specific infections and one of the main hypothesis is that chronical antigen stimulation is part of the pathogenesis. In this case, the antigen is pP-7, which stimulates the development of a pP-7-specific paraprotein and the progression of the disease, builds up titres over time. For the first time, we also were able to describe two

patients who were positive for pP-7 before the diagnosis of WM (one 6.5 and one 10 years before diagnosis). One of these was positive for pP-7, but did not carry a paraprotein or antibodies against pP-7 when the blood sample was collected. In this case, we could show that pP-7 was the first event before antibodies against pP-7- and a pP-7-specific paraprotein was developed. We conclude that carrier state of pP-7 might be one of the causes of WM and contribute to family clustering. Today, we do not know if carriers of pP-7 will develop disease, but pP-7 can be used to identify family members at increased risk. If we learn more about the mechanisms responsible for the defective dephosphorylation maintaining the hyperphosphorylated state of pP-7, it might be a way to prevent development of WM.

The question regarding screening of the relatives to patients with familial WM or non-familial WM is controversial. Today, screening is not recommended because the risk of WM is very low in the general population and despite the observed increased risk among relatives in both familial and non-familial WM cases, the risk of developing WM still remains low. The average transformation rate is 1-1.5% per year for non-familial patients with IgM MGUS to develop WM. If the transformation risk is increased in familial cases is unknown. In addition, the main argument is that early detection of WM is not likely to affect the outcome, because today asymptomatic patients with WM are not treated and the disease is considered incurable.

Future perspectives

Familial studies

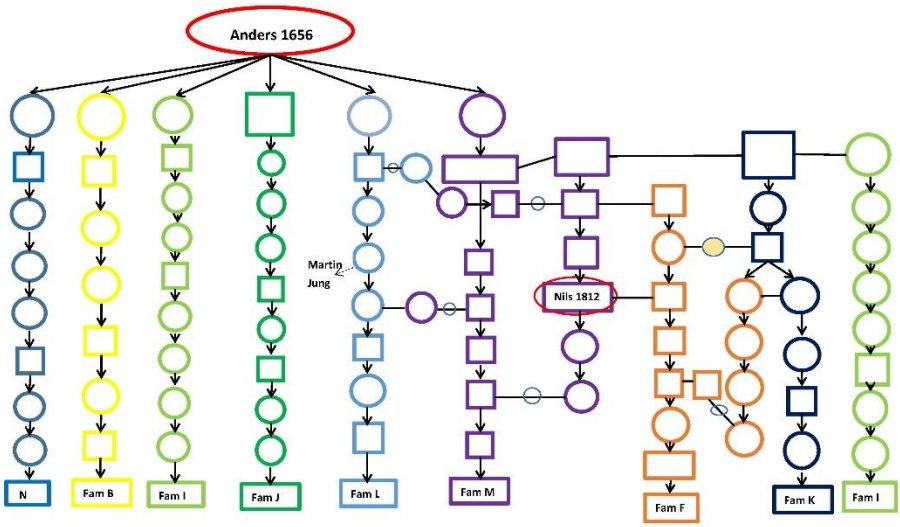
We noticed that many of our families originate from areas in southern part of Norrbotten County and northern part of Västerbotten County. In this area of the country, there is a high prevalence of some rare hereditary diseases, such as acute intermittent porphyria, hereditary transthyretin amyloidosis, and Gaucher's disease type III. These diseases have another genetic background, often autosomal dominant mutations with variant penetrance. Furthermore, we do not know to what extent environmental factors can influence disease development. One of our preliminary hypotheses was that individuals with distant relationships shared a proportion of genes and facilitate the detection of disease carrying genes/mutations. Using genealogy, we have managed to tie seven of the families' pedigrees together. We added a patient that had her diagnosis at an early age and lived in the same area; her pedigree also connected with these families. In most cases, we had to go back to the late 17th century to find relationships, but we also found relationships in the 19th century. We could identify several ancestors and the pedigrees weave together on several levels. This pattern is common in isolated areas and lead to a limited gene pool and an elevated level of homozygosity. The strongest ancestor was Anders, born in 1656 (Figures 14). The church book, where the birth and death of parishioners are recorded, the following entry is made about Anders:

Att han är såsom intet vid sitt förstånd utan förryckt i huvudet, understundom i synnerhet när han super litet bränvin och att han för några år sedan var så elak att om honom måste hållas vakt.

(At times, especially when he was drunk, he get so mad and mean that he had to be taken into custody. author's translation).

Eight index patients and seven first-line relatives from these families are whole genome sequenced, and efforts are in progress to search for germ-line mutations that involved in the development of WM. One challenge in this study (and other genetic studies) is to distinguish disease carrying mutations from other mutations, which requires knowledge about the genetic variation in the population. Earlier studies have shown a pronounced genetic difference and an

Figure 14. Pedigrees for eight familial WM patients and their ancestor Anders



elevated level of homozygosity between the northern parts of Sweden compared with the rest of Sweden and northern Europe. This part of Sweden has a small but stable population and a large proportion of the inhabitants lived their whole life in the same area as where they were born. Recently, a control population (n = 300), including healthy (=free of cancer) persons 80 years or older from Västerbotten (ACpop), has been identified from Västerbotten Intervention Programme (VIP) and whole-genome sequenced (Rentoft *et al*, 2019, Norberg *et al*, 2012). This population had an increased number of genetic variants reflecting the genetic variation in the population in this part of Sweden and in future studies we will use this population (as well as other established populations) to analyse our WM families.

Registries studies

The SLR are known to have high quality and coverage despite that they have some limitations as discussed earlier. SLR as well in other registries for malignant haematology have started to up-date the registries for missing data, and in some cases for new data. In the future, I hope that we will have the opportunity to up-date WM/LPL in SLR for missing or incomplete data, as type of treatment before 2007, indication for start treatment, and important prognostic factors such as platelets count and β2M, treatment outcome, number of relapses, start and

type of second-line treatments, and cause of death. With these data, we could conduct studies to answer questions that we cannot answer today such as PFS, treatment outcome, competing risk analyses for death, analysis according to IPSSWM, and treatments at relapses. We could also have the opportunity to study the use of new (and often expensive) drugs in population-based settings.

Concluding remarks

Future therapies should be developed that are more targeted and personalised. Efforts to understand the genetic background are important to enable the development of new, more effective, and tailored therapies. To further individualise the therapies, we have to learn more about the biology of the disease and how factors such as age, comorbidity, and sex influence the disease progression and treatments. New biological markers might help us choose the most effective treatment. Furthermore, understanding the early events in the pathogenesis might enable us to develop strategies to prevent the disease. Today, WM is considered to be incurable, but with the current rapid scientific development a cure might be within reach.

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