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PLASMA CELL MALIGNANCIES IN SWEDEN: SUBGROUP DESCRIPTIONS AND REGIONAL OUTCOMES FOR MULTIPLE MYELOMA

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PLASMA CELL MALIGNANCIES IN SWEDEN: SUBGROUPS DESCRIPTIONS AND REGIONAL OUTCOMES FOR MULTIPLE MYELOMA
THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

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Till min älskade familj, Pantea, Matilda, Ida och Leo

POPULAR SCIENCE SUMMARY OF THESIS IN SWEDISH

Plasmaceller mognar ut från lymfocyter och bildar antikroppar till kroppens immunförsvar. Utvecklingen startar i benmärgen där en receptor på B lymfocytens yta så småningom modifieras till en tidig typ av antikropp. Cellerna lämnar sen benmärgen för fortsatt utveckling i lymfknutor. Inuti lymfknutorna utvecklar B lymfocyterna med hjälp av T celler och dendritiska celler succesivt antikroppar som kan fastna på specifika typer av bakterier och virus. Därefter sker en fortsatt utmognad till plasmaceller och minnesceller som sen cirkulerar i kroppen.

Antikroppar består av två tunga och två lätta kedjor och de vanliga typerna hos vuxna är immunoglobulin G och A (IgG och IgA). Vid plasmacellssjukdom har plasmacellerna blivit tumöromvandlade och därmed förlorat sin vanliga reglering. Plasmacellerna kan då ansamlas i benmärgen såsom vid myelom eller i andra delar av kroppen såsom vid plasmacytom. Man delar in solitära plasmacytom (SP) beroende på om de sitter i skelettet (SBP) eller i andra vävnader och organ (EMP). Typiska sjukdomssymtom vid myelom är högt calcium, njursvikt, blodbrist och benbrott. Dessa symtom hänvisas till på engelska som CRAB (hypercalcemia, renal failure, anemia, bone lesions).

I stället för att bilda en mängd olika antikroppar så bildar sjuka plasmaceller bara en typ av antikropp som kallas for M (monoklonalt) protein eller M komponent. Vid låga nivåer av monoklonala antikroppar utan sjukdomssymtom och utan ökning av plasmaceller i benmärg eller påvisat plasmacytom betraktas inte antikroppen i sig som en sjukdom utan kallas för MGUS (monoclonal gammopathy of undetermined significance). MGUS har dock en ökad risk för myelomutveckling på sikt beroende på nivå och typ av M protein samt förekomst av fria lätta kedjor i serum (S-FLC).

En del ovanligare typer av myelom bildar bara en del eller inget M protein och kallas då för oligo eller icke sekretoriska myelom. Dessa tillstånd kan på grund av sin avsaknad av M protein vara svåra att upptäcka och följa upp eftersom effekten av behandlingen ofta bedöms utifrån M proteinets nivå. Myelom kallas för plasmacellsleukemi (PCL) när man hittar en viss mängd plasmaceller i blodet. Detta är den mest aggressiva och svårbehandlade typen av myelom och har ofta en dålig prognos.

Vanlig ålder i Sverige för att få myelom är ungefär 70 år. Myelom är den näst vanligaste hematologiska cancern efter lymfom. Till yngre patienter består behandlingen ofta av att man, efter att fått sjukdomen under kontroll, genomför en s.k. stamcellsskörd och efter en hög dos cellgift ger dessa stamceller tillbaka. Denna procedur kallas högdosbehandling med autologt stamcellstöd (HDT-ASCT). Ett fåtal patienter med svår sjukdom eller snabbt återfall kan också bli aktuella för allogen stamcellstransplantation (allo-SCT), dvs få benmärg från en annan person. Till äldre patienter ges ofta enbart läkemedel utan efterföljande ASCT. Dessa olika läkemedel kan ges var för sig eller i olika kombinationer och är av typen immunomodulerare (IMiDs), proteasominhibitorer (PIs) och monoklonala antikroppar. Ofta ges också kortison och ibland även cellgifter såsom exempelvis cyklofosfamid. CAR-T celler

och bispecifika antikroppar (BiTE: s) där man utnyttjar kroppens T celler för att angripa plasmacellerna utgör nya lovande läkemedel för myelom och är under prövning.

Det Svenska myelomregisteret insamlar uppgifter från sjukhus i Sverige att användas för nationell kvalitetsuppföljning och forskning. Vi har i tre olika studier, med data hämtade från det Svenska myelomregistret, undersökt hur det går för patienter med SP, PCL samt oligo och icke sekretoriska myelom. Vi har även jämfört överlevnaden för patienter med behandlingskrävande myelom i olika delar i Sverige.

I den första studien kunde vi konstatera att patienter med SBP oftare utvecklar myelom jämfört med patienter med EMP. Patienter med EMP hade dock inte bättre överlevnad än de med SBP. Vår studie visar också att patienter med PCL har en dålig prognos.

I den andra studien jämfördes patienter med oligo och icke sekretoriska myelom med sekretoriska behandlingskrävande myelom. Hela gruppen med oligo och icke sekretoriska myelom delades in i undergrupper baserat på nivå av M protein i serum och urin samt S-FLC. Vi noterade att äkta icke sekretoriska myelom, dvs de utan någon mätbar sekretion alls, är ovanligt (6% av de patienter som hade FLC tillgängligt). Vi kunde inte hitta någon väsentlig skillnad i överlevnad för oligo eller icke sekretoriska myelom vid jämförelser med sekretoriskt myelom.

I den tredje studien jämförde vi patienter som fått behandling för myelom i de sex sjukvårdsregionerna i Sverige. Vi kunde se en skillnad i överlevnad när vi jämförde hela gruppen i region A mot övriga regioner. I undergruppen som fått behandling med ASCT sågs också en skillnad som även kvarstod efter att man justerat för andra tänkbara förklaringar såsom ålder, stadium och tidpunkt för diagnos. Eftersom region A hade hög andel som fått modern initial behandling (definierat i studien såsom vissa särskilda mediciner) drar vi slutsatsen att denna behandling förmodligen kan antas haft betydelse för skillnaden i överlevnad. För patienter som inte erhöll ASCT sågs ingen skillnad i överlevnad mellan de olika regionerna för patienter under 75 år. För patienter 75 år och äldre sågs inte heller någon klar signifikant skillnad när man enbart tittade på patienter vid liv 6 månader efter diagnos. Vi spekulerar i studien om att långtidseffekter av läkemedel hos äldre troligen kan vara svårare att påvisa pga andra orsaker till död till följd av ålder eller samsjuklighet

Sammantaget så har vi med våra studier beskrivit olika plasmacellssjukomar och subgrupper utifrån uppgifter i det Svenska Myelomregistret. Vi har också använt information om nya markörer såsom S-FLC för att klassificera patienter utan mätbart myelom på ett strukturerat sätt. Vi har även beskrivit skillnader i överlevnad mellan olika sjukvårdsregioner i Sverige.

(Sammanfattningen är baserad på de studier och referenser som beskrivs i avhandlingen).

ABSTRACT

Plasma cell disorders appear in various forms such as aggressive plasma cell leukemia (PCL), typical symptomatic multiple myeloma (MM), non-symptomatic smouldering multiple myeloma (SMM) and solitary plasmacytoma (SP). The different entities all require specific considerations regarding diagnosis and treatment. MM can be further characterized by secretion of M protein, CRAB features (elevated calcium, renal impairment, anemia, bone lesions) and cytogenetic aberrations. New methods and markers, such as serum free light chains (S-FLC), cytogenetics and skeletal surveys with CT and MRI, now enable the clinicians to approach MM with a different set of diagnostic possibilities than before.

The aim of this thesis is to investigate the outcome in SP and PCL as well as in the oligo and non-secretory MM subgroups, referred to in study II as non- measurable MM. We also aim to describe outcome and treatment for MM patients in the six healthcare regions in Sweden. Data was retrospectively collected from the Swedish Myeloma register for all three studies.

In study I, data from patients with solitary bone plasmacytoma (SBP) and extramedullary plasmacytoma (EMP) were analyzed in comparison with MM. Progression to MM, described by cumulative incidence function (CIF), at two-years was more frequent for SBP than EMP (35% compared to 7%). However, relative survival was not better for EMP than for SBP. Patients in the study with primary PCL had a poor prognosis with no patients being alive 5 years after diagnosis.

In study II, data from patients with oligo and non-secretory MM were studied and presented in subgroups based on secretion of M protein in serum and urine as well as serum free light chains (S-FLC). True non secretory MM (with no elevated secretion of S-FLC and M protein) constituted only 6% of patients with available S-FLC. No differences in overall survival (OS) were seen between secretory and non-measurable MM disease, or between secretory MM and the non-measurable MM subgroups.

In study III, we saw differences in survival between healthcare regions in Sweden for MM patients, with a better survival in region A. For patients eligible for autologous stem cell transplantation (ASCT) there was also a better survival in region A in comparison with other regions. The difference persisted also in multivariate analysis including ISS stage, age, and time-period of diagnosis. The regions were also evaluated depending on use of modern initial treatment (specific types of drugs, as defined in the study). Region A had the highest use of modern initial treatment and the high usage appeared to correlate to better survival in the ASCT group. We therefore suggest that the superior survival seen in region A possibly depended on differences in treatment between the regions. For the two groups not treated with ASCT (below 75 years and 75 years and older) no clear significant differences in OS were seen after a time to treatment bias was adjusted for in both groups. We speculate that long term effects due to treatment may be harder to show in elderly patients with more comorbidity and death from other causes than myeloma.

In conclusion we have characterized plasma cell disease in Sweden by describing SP, PCL and non-measurable MM including subgroups. We also demonstrate differences in survival between the healthcare regions in Sweden for patients undergoing ASCT and suggest these differences may depend on the usage of initial modern treatment as defined in the study.

LIST OF SCIENTIFIC PAPERS

I. Nahi H, Genell A, **Wålinder G**, Uttervall K, Juliusson G, Karin F, Hansson M, Svensson R, Linder O, Carlson K, Björkstrand B, Kristinsson SY, Mellqvist UH, Blimark C, Turesson I. Incidence, characteristics, and outcome of solitary plasmacytoma and plasma cell leukemia. Population-based data from the Swedish Myeloma Register. Eur J Haematol. 2017 Sep;99(3):216-222. doi: 10.1111/ejh.12907. Epub 2017 Jun 28. PMID: 28544116.

II. **Wålinder G**, Samuelsson J, Näsman P, Hansson M, Juliusson G, Forsberg K, Svensson R, Linder O, Carlson K, Kristinsson SY, Mellqvist UH, Hveding Blimark C, Turesson I, Nahi H. Outcome and characteristics of non-measurable myeloma: A cohort study with population-based data from the Swedish Myeloma Registry. Eur J Haematol. 2020 May;104(5):376-382. doi: 10.1111/ejh.13351. Epub 2020 Feb 22. PMID: 31733147.

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ABSTRACT

I. **Wålinder G**, Genell A, Juliusson G, Svensson R, Santamaria A, Crafoord J, et al. P-184: Regional differences in treatment and outcome for myeloma patients in Sweden: a population based Swedish Myeloma Register study. Clinical Lymphoma Myeloma and Leukemia. 2021;21:S138. https://doi.org/10.1016/S2152-2650(21)02311-9

CONTENTS

LIST OF ABBREVIATIONS

ADC	Antibody-drug-conjugates
ASCT	Autologous stem cell transplantation
BAFF	B cell activating antigen
BCMA	B cell maturation antigen
BCR	B-cell receptor
BiTE	Bispecific T cell engager
BM	Bone marrow
BMPC%	Bone marrow plasma cell percentage
BMSC	Bone marrow stromal cell
CAR	Chimeric antigen receptor
CD	Cluster of differentiation
CIF	Cumulative incidence function
CR	Complete response
CRAB	Elevated calcium, renal impairment, anemia, bone lesions
CRBN	Cereblon
CSR	Class switch recombination
CT	Computerized tomography
DLI	Donor lymphocyte infusion
ECM	Extracellular matrix
EMP	Extramedullary plasmacytoma
FLC	Free light chains
GC	Germinal center
GDPR	General data protection regulation
GVHD	Graft versus host disease
HDT	High-dose treatment
Ig	Immunoglobulin
IL	interleukin
IGH	Immunoglobulin heavy chain gene
IGL	Immunoglobulin light chain gene
IMiD	Immunomodulatory drug

IMWG	International Myeloma Working Group
ISS	International staging system
LMWH	Low-molecular weight heparin
MGUS	Monoclonal gammopathy of undetermined significance
MHC	Major histocompatibility complex
MM	Multiple myeloma
MRI	Magnetic resonance imaging
ORR	Overall response rate
OS	Overall survival
PCL	Plasma cell leukemia
PI	Proteasome inhibitor
RAG	Recombination activation genes
RANKL	Receptor activator of NF-κB ligand
RIC	Reduced intensity condition
RRMM	Relapsed/refractory multiple myeloma
SCT	Stem cell transplantation
SHM	Somatic hyper mutation
SMM	Smouldering multiple myeloma
SP	Solitary plasmacytoma
SBP	Solitary bone plasmacytoma
VEGF	Vascular endothelial growth factor
VTD	Bortezomib-thalidomide-dexamethasone
VTE	Venous thromboembolism

1 INTRODUCTION

The plasma cell disorders described in this thesis present themselves in several forms. They include the very aggressive plasma cell leukemia (PCL), classical multiple myeloma (MM) and local manifestations of clonal plasma cells called solitary plasmacytoma (SP). The various forms of MM are further described as secretory, oligo-secretory and non-secretory depending on level of M protein and free light chains in serum (S-FLC).

Novel markers and methods including S-FLC and cytogenetics as well as skeletal surveys such as CT and MRI have the potential to change the ways plasma cell disease can be diagnosed and followed up. The development of drugs has also contributed to new prospects in MM with possibilities for long term disease control and hopefully a possible curative treatment in the future. Furthermore, the establishment of registries such as the Swedish Myeloma Registry can provide comprehensive long-term data regarding follow up, survival and treatment in these groups of patients.

2 LITERATURE REVIEW

2.1 HISTORY

MM was probably first characterized by Dr Solly who in 1844 published an article about a condition he referred to as “mollities ostium”. One of the cases had pain and bone fractures. The examination of bones showed that they were filled with a certain distinct red substance. The cells were examined by microscope and described as clear, with distinct edges, oval outline, and a central bright nucleus (1).

In 1850, another case with similar characteristics was described by Dr MacIntyre. This patient also had pain, in this case from the chest, back and loins. The autopsy later revealed a soft specific substance in the bones, also of red colour, similar to Dr Solly’s description. Since the patient had oedema, urine was also examined. This examination was done by Dr Bence Jones who thought that the substance in urine was an oxide of albumin and pointed out the importance of these specific findings in diagnosing the disease (2, 3).

MM as a name was first used in 1873 by von Rustizky (4). After a case report, MM was also early on referred to as “Kahler’s disease”. The name “plasma cell” appeared in a description 1875 by Waldeyer, although the cells he examined might have been mast cells. Plasma cells are considered to have been correctly described first by Ramon y Cajal. The name Bence-Jones protein was introduced in 1880, and later Wilson and Bayne-Jones in 1922 reported that there were two sorts of these proteins. (5). Korngold and Lipari could later also show that Bence-Jones protein and myeloma protein in blood reacted to the same antiserum (6).

High levels of proteins in MM was reported in 1928 by Perlzweig and during the 1930: s, electrophoresis for separation of serum globulins was accomplished. In 1961 Waldenström introduced a novel way of thinking regarding monoclonal and polyclonal gammopathies (5). Edelman and Gally showed that Bence- Jones protein in urine and light chains in serum were the same thing (7). Direct immunoelectrophoresis was demonstrated by Wilson in 1964 (5,8).

2.2 PATHOGENESIS OF MYELOMA

2.2.1 Development of plasma cells

Plasma cells develop during the last stages of B cell maturation. The process starts by rearranging the heavy immunoglobulin (Ig) gene (IGH), and then later the Immunoglobulin light chain gene (IGL). The IGH gene has four domains: the VH (variability) domain, the DH (diversity) domain, the JH (joining) domain and the constant domain. The VH domain

consists of over one hundred segments of DNA while the DH is made up of twenty-seven, the constant domain nine, and the JH domain six segments. First, the DH segment is attached to a JH segment through supposedly random deletions independent of antigen presence. If this is successful, the DH-JH segment is joined with a VH segment. The enzyme RAG (recombination activation genes) controls the process by identifying certain DNA sequences in the different DH, JH, and VH segments. If rearrangement is functional, the IGL kappa gene is rearranged. IgM kappa is then produced and expressed on the surface of the cell. If kappa rearrangement is not successful, the IGL lambda gene is rearranged instead resulting in the expression of IgM lambda (9). A basic schematic overview of clonality concepts is presented in Figure 1.

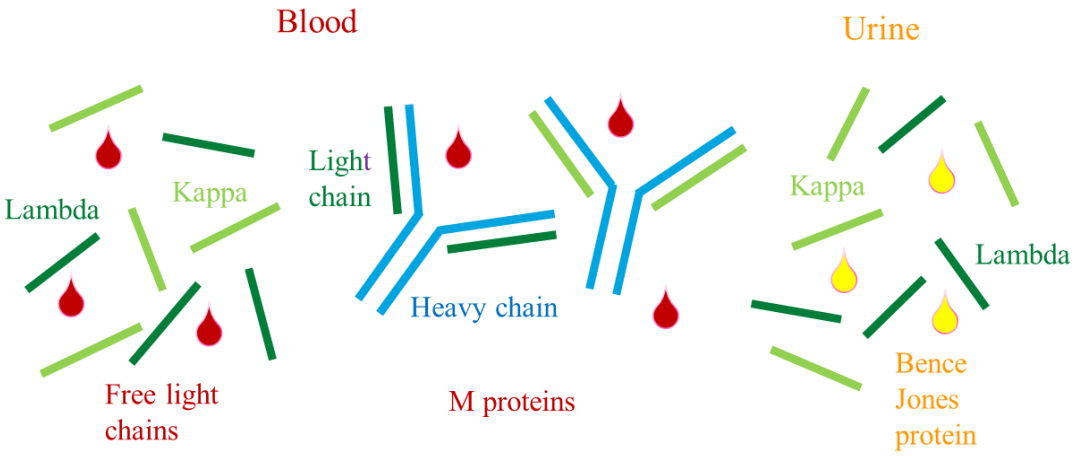


Figure 1. Schematic basic overview of clonality and where to measure it (picture by the author). M proteins (monoclonal antibodies), S-FLC (serum free light chains) and Bence Jones protein.

The rearranged IgM kappa or lambda specific B cells leave the bone marrow to further differentiate within germinal centres (GC: s). Interaction with dendritic cells and T cells then will trigger somatic hyper mutation (SHM), a process that is antigen dependent and results in improved specificity of the antigen or alternatively apoptosis for the B-cell. The T cells and B-cells interact through the B cell receptor (BCR), T cell receptor, and MHC II. The B cells are then selected due to their ability to bind, internalize, and present antigen on MHC II molecules for the T cells (9, 10, 11).

Finally through a process called class switch recombination (CSR), DNA segments referred to as the switch regions are recombined. This will result in cells with different types of Ig: s. CSR is mediated by an enzyme called activation-induced deaminase. The result will be cells with the capacity to produce highly specific antibodies. The cells are then presumed to develop further into both memory B-cells or plasma cells (9, 10, 12).

MM generally evolves from MGUS (monoclonal gammopathy of undetermined significance). This is a condition increasing in occurrence with age with a progression to MM in adults of about 1% each year (13).

Plasma cells can be produced from B cells before entering GC: s. However, MM cells are considered to be derived from post-germinal centre cells due to the fact that the IGH sequences are somatically hypermutated. Since plasma cells with CD 138 expression appear to lack ability for significant proliferation capacity, a subpopulation of clonal memory B cells termed myeloma cancer stem cells may possibly be the cause of relapse and progression (10).

2.2.2 Chromosomal alterations

Chromosomal abnormalities are common in MM and involve many different mechanisms such as translocations, deletions, gains, and mutations (14). The two main pathways and primary events for progression to MM are considered to be hyperdiploidy (55%) and IGH translocations (40-50%) (9).

Translocations at 14q32 is a common event in plasma cell disease and involves the IGH locus (15). IGH translocations leads to overexpression of proteins under control of IGH enhancers (9). Five known translocations are connected to the IGH locus. These are translocations (4;14), (6;14), (11;14), (14;16) and (14;20). The translocations lead to enhanced expression of the genes MMSET, FGFR3, CCND3, CCND1 as well as the MAF and MAFB genes, considered to give survival advantages to the MM cells. Regarding the timing of IGH rearrangements, possibly 100% of t (4;14) occurs during CSR while 21% of t (11;14) and 25% of t (14;20) may happen through DH-JH mechanisms in the early B-cell stages (16).

Hyperdiploidy is linked to additions of chromosomes with uneven numbers (3, 5, 7, 9, 11, 15, 19 and 21) (14). Patients with hyperdiploidy seems to have a better prognosis. This may possibly be associated with gain of 5q31(15).

Other chromosomal changes, such as monosomy 13 (45%), gain 1q (30-35%) and deletions in 1p, 8p, 12p, 17p, 20p, 6q, 14q and 16q regions are considered as secondary events since they are seen mostly in subclones. Subclonality may however be something that happens early and is considered important for disease progression, and a minor clone, hardly noticed at diagnosis, may be the main one causing relapse. Two separate types of subclonality have been proposed; one with a linear accumulation of clonal hits and the other with branched separate subclones with more individual mutations (9).

T (4; 14) is a translocation thought to be unique for MM, changing regulating for the oncogenes FGFR3 (a tyrosine kinase receptor) and MMSET (Multiple myeloma SET domain protein) involved in growth and adhesion of the plasma cells (15). T (4:14) can be

found in 15% of the patients with MM (10) and is linked to poorer outcome but does not appear to have an inferior survival after allogenic transplantation (17). A subgroup of the t (4; 14) patients who have high hemoglobin and low β 2 microglobulin appear to have a positive effect on survival when treated with tandem autologous stem cell transplantation (ASCT) (17, 18).

T (14; 20) is seen in 1-2% MM. It is associated with worse prognosis. It involves the MAFB oncogene (10, 15).

T (14; 16) is uncommon in MM but also linked to poor prognosis. It involves the MAF oncogene. (9, 10).

T (6;14) occurs in 4% of MM patients and can be seen in lymphoma as well (15).

T (11; 14) is seen in 15-17% of MM cases. It is the most frequently seen translocation in MM and is also seen in mantle cell lymphoma (MCL). The translocation is linked to upregulation of cyclin D1 and has also been connected with non-secretory disease. It is however not considered to be associated with worse prognosis (15, 19, 20).

Chromosome 1 changes are common in MM. Amp (1q21) is found in 40% at diagnosis and 70% at relapse and is linked to poor prognosis. It is seen together with elevated expression of the CKS1B gene which may favour cell proliferation. Gains/amplifications of 1q21 are seen in cells from patients with MM and smouldering MM but is possibly not a feature in MGUS (15, 21, 22). Deletion of 1p has been reported in 7-40% patients and reduces expression of the CDKN2C gene. This may contribute to the evolution from MGUS to MM. Del 1p is associated with a poor prognosis (15).

Deletions on chromosome 13 is seen in about 50% of patients with MM. Del 13q probably does not have an independent prognostic role since it is also associated with other chromosomal changes such as del (17p) and t (4;14) (15).

Deletion of 17p13 involves inactivation of the tumour suppressor gene p 53. Deletions of p 53 is seen in 9-34% in MM. The prognosis is poor with a shorter overall survival (OS) after ASCT than for patients without p53 deletions (23). Deletion 17p13 is also correlated to poor responses and shorter event free survival after allogenic stem cell transplantation (17).

8q24 is coding for the c-Myc-gene. A rearrangement is seen in 15% of MM patients. The MYC pathway is considered important in the transition from plasma cells to myeloma cells. However, no association to prognosis in MM has been seen, and the chromosomal breaks at the site may vary (15).

2.2.3 Heredity aspects of MM

Relatives to patients with MM have been estimated to have a 2 to 4-fold higher risk for MM. Some data have also demonstrated that MM in certain families can be seen in three generations (24). Metanalyses of studies have indicated support for at least seventeen risk loci in the genome for developing MM including at 8q24, possibly involving mechanisms for dysregulation of the MYC pathway (25).

2.2.4 Microenvironment in the bone marrow

The bone marrow microenvironment contains several cells and proteins that interact with plasma cells and mediate survival, growth, and migration. The cells include hematopoietic cells, endothelial cells, stromal cells (BMSCs), fat cells, osteoclasts, and osteoblasts. The microenvironment also consists of growth factors, cytokines, and extracellular matrix (ECM), including fibronectin and collagen (10, 26, 27).

SDF-1 alfa regulates the return of MM cells to the bone marrow through binding to the CXCR4 receptor on the MM cells. In vitro anti CXCR4 antibodies have also been shown to stop migration of MM cells. Adhesion is mediated by a variety of molecules such as VLA-4 (very late antigen), CD54, CD 56 and CD138. VLA-4 is found on MM cells and helps attach them to the ECM and BMSC: s by binding of fibronectin and VCAM-1. Fibronectin binding activates the nuclear factor NF-κB in MM cells. NF-κB also induces IL-6 secretion from BMCSC: s which promotes growth of the MM cells. CD138 binds to type I collagen of the ECM starting MMP-1 (metalloproteinase 1) expression, leading to further invasion of MM cells and bone resorption (10, 27).

The regulation of bone homeostasis is balanced by osteoclasts and osteoblasts. In MM, the plasma cells change this balance by adherence to the stroma, inducing expression of several factors such as the receptor of NF-κB ligand (RANKL) which stimulates osteoclasts. RANKL is normally balanced by osteoprotegerin (OPG). Bone formation activity of osteoblasts is also decreased by dysregulation of molecules such as IL-3, IL-7, and DKK-1(26).

Angiogenesis is also being altered in MM with a microvessel density that is higher in patients with MM compared to patients with smouldering multiple myeloma (SMM) and MGUS (26). Angiogenesis is induced through hypoxia. Vascular endothelial growth factor (VEGF) made by MM cells and BMCS: stimulate angiogenesis and favours growth of MM cells (26).

Other hematopoietic cells such as macrophages, can stimulate MM cells in vitro through action of vascular endothelial growth factors and IL-6. Dendritic cells can form giant bone resorption cells after interaction with plasma cells, and eosinophils are also known to be able to stimulate growth of MM cells. T cell balance is changed by MM cells as well, with a decrease of Th 1 cytokine IL2 and higher levels of Th 2 cytokines IL-10 and IL-4 (26).

2.3 EPIDEMIOLOGY

In Sweden, the age-adjusted incidence of MM is 6.8 myeloma cases per 100000 inhabitants and year. The incidence is higher in men than women. At diagnosis, median age in Sweden for MM is 71 years. In standards for European and world population the incidence is 4.8, and 3.2 (28). The incidence globally has increased since the 1990: s with aging as one probable explanation (29). In African Americans, MM is more common (30).

2.4 DIAGNOSIS

MGUS is a condition without symptoms that precedes MM and can be found in 3-4% of people above 50 years of age. Progression to MM is 0.5-1% a year (31). Risk factors include bone marrow plasma cell percentage (BMPC %), S-FLC ratio and type of M-protein (31). The relation between MGUS and MM is presented as an overview in Figure 2. Definitions of plasma cell disorders are presented in table 1.

Smouldering multiple myeloma (SMM) is the condition between MGUS and MM. It has been reported to constitute about 18.6% of all patients with newly diagnosed myeloma (28). Patients with SMM lack symptoms of active disease but the group includes patients with both low and high risk for progression to MM. SMM is defined by clonal BMPC% between 10-60%, and/or S-M protein at least 30g/L or U-M protein at least 500mg /day (31).

MM is defined by having at least ten percent of clonal bone marrow plasma cells, or by having a biopsy from plasmacytoma. Also there needs to be one or more defining events for myeloma demonstrated (31). Myeloma events are referred to as CRAB features and consists of hypercalcemia, renal insufficiency, anaemia, or one / several lytic bone lesions on skeletal survey (31). Additional criteria (clonal BMPC% at least 60%, or S-FLC with a ratio for involved and uninvolved chain of at least 100, or more than 1 focal lesion by MRI), have also been added recently since patients with these traits seem to have much higher risk to develop MM symptoms compared to typical SMM patients (31).

Bone lesions is the most common CRAB feature in MM and occurs in 77% of patients, according to the Swedish Myeloma Registry, followed by anaemia (49%), renal impairment(18%) and hypercalcemia (13%)(28).

In solitary plasmacytoma (SP) there is only one lesion to be found. In solitary bone plasmacytoma (SBP) the lesion is in the skeleton and in extramedullary plasmacytoma (EMP) it is situated in soft tissue. Skeletal survey in SP is normal except for the one solitary lesion, and the bone marrow does not contain clonal plasma cells. If clonal plasma cells are present in the marrow (<10%) the condition is referred to as solitary plasmacytoma with minimal marrow involvement (31).

PCL is a form of plasma cell disorder with poor prognosis where plasma cells characteristically is being present also in blood. It is defined by having at least 20% of the white blood cell count being plasma cells or having a total number of $2x10^{9}$/L (32).

AL amyloidosis and POEMS (defined in table 2) are both conditions related to underlying plasma cell disorder defined by the criteria for organ involvement. In AL amyloidosis, the presence of light chain amyloid deposits must be proven, in POEMS there are besides polyneuropathy and monoclonal plasma cell proliferation also three major criteria and six other minor criteria (table 1) that must be considered (31).

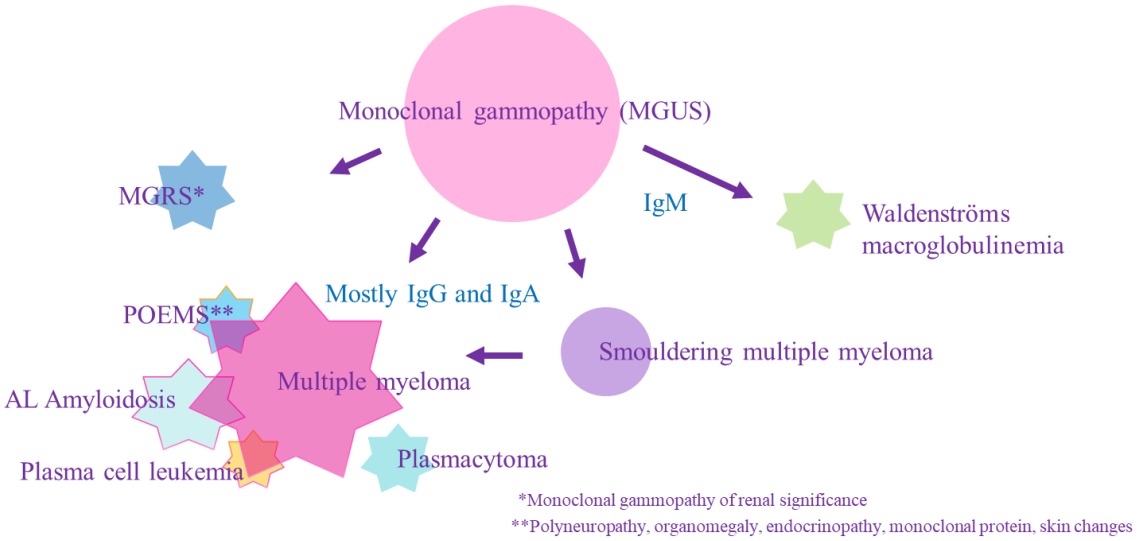


Figure 2. Schematic basic overview of plasma cell disorders (picture by the author). For criteria according to IMWG, see reference (31).

Table 1. Plasma cell disorders According to IMWG criteria, reference (31)
MGUS
Serum(S)- M protein less than 30 gram/liter
Urine (U) -M protein less than 500mg/24hours
Abnormal S-FLC ratio and increased level of involved light chain
(Or normal S-FLC ratio if M protein is present)
Clonal bone marrow plasma cells less than 10 percent
No damage to organs and no amyloidosis due to clonal plasma cells
SP
Biopsy of the bone (SBP) or of soft tissue (EMP) with clonal plasma cells
No clonal bone marrow plasma cells*
Skeletal survey with no lesions (except for the one solitary lesion)
No damage to organs due to clonal plasma cells
SMM
S-M protein (IgG or Ig A) at least 30g/L or U-M protein at least 500mg/24hours
And/or clonal plasma cells in bone marrow 10- <60 percent
No defining events for MM and no amyloidosis due to clonal plasma cells
MM
Clonal bone marrow plasma cells at least 10% or biopsy with plasmacytoma
And at least one MM defining event:
-Organ damage due to CRAB features
-Clonal bone marrow plasma cell percentage (BMPC%) at least 60%
-Ratio of Involved and uninvolved S-FLC chain at least 100
-At least one lesion on MRI (at least 5mm)
AL amyloidosis
An amyloid related syndrome
Amyloid staining in tissue by Congo red
Proof that the amyloidosis is caused by light chains
Monoclonal plasma cells
POEMS
Monoclonal plasma cells and polyneuropathy
A major criterion**
A minor criterion***
MGUS, monoclonal gammopathy of undetermined significance
SP, solitary plasmacytoma, SMM, smouldering multiple myeloma
MM, multiple myeloma
IMWG, International myeloma working group
*If clonal plasma cells less than 10% it is SP with minimal marrow involvement
**Sclerosis in the bone, Castleman's disease, elevated levels of VEGFA
***Organomegaly, endocrinopathy, extracellular volume overload,
skin changes, papilloedema, thrombocytosis and polycythemia

2.5 STAGING

In the year 1975, the DS system (Durie and Salmon) was introduced using M protein, hemoglobin, creatinine, calcium, and bone lesions to predict prognosis in MM. Later β2

microglobulin was found to also be a prognostic marker. In the international staging system (ISS), albumin was added and three different stages for prognosis defined (33).

In addition to ISS criteria, the international myeloma working group (IMWG) 2014 further classified patients in high, standard, and low risk based on ISS, cytogenetics abnormalities, and age (34). The R (revised) –ISS classification is based on ISS, cytogenetic abnormalities (CA) and LDH (lactate dehydrogenase), (Table 2). High risk cytogenetics is defined in R-ISS as having one or more of the chromosomal changes, t (14:16), t (4;14) and del17p. R-ISS has been suggested for use at diagnosis in all MM patients since it has demonstrated a greater differentiation in prognosis between groups after ASCT compared to ISS and IMWG 2014 (35, 36).

Table 2. R-ISS and ISS stage
According to criteria in reference (34, 35)

R-ISS	Definition
Stage I	ISS stage I and no high-risk CA and also normal LDH
Stage II	Not R-ISS stage I and not ISS stage III
Stage III	ISS stage III and high LDH or high-risk CA
ISS	**Definition**
Stage I	S-beta 2 microglobulin below 3.5mg/liter and s-albumin at least 35g/liter
Stage II	Not ISS stage I and not ISS stage IIII
Stage III	S-beta 2 microglobulin at least 5.5mg/liter

CA, chromosomal abnormalities
High risk: deletion17p, translocations (4;14) and (14:16)

2.6 TREATMENT

2.6.1 Early treatment and Melphalan

In 1947 Alwall described treatment for MM with urethane (37). This remained as a treatment in MM patients for 15 years (5). In 1966 however a randomized trial could show no benefit for urethane when compared to placebo (38). Effects of sarcolysin (melphalan) in MM were described by Blokhin in 1958 (5, 39). In 1962 Bergsagel, and then also Hoogstraten demonstrated improvements in patients with MM who were treated with melphalan. Steroids in MM was first tried in a study by Maas who could demonstrate that prednisone lowered serum globulin. However, no difference could be shown in survival compared to placebo (5).

Melphalan and prednisone (MP) as standard treatment was introduced after results in a

randomized study reported in 1969 by Alexanian. Median survival in the study was 6 months longer for MP than with melphalan as a single drug (40).

2.6.2 Stem cell transplantation (SCT)

Allogenic stem cell transplantation (allo-SCT) in myeloma was described in 1957 by Thomas et al where body irradiation was followed by intravenous bone marrow infusion. The patient however died on day 47 (41). Transplantation between identical twins was described in 1982 (42) and in 1986 (43). In 1987, Gahrton reported transplantations with HLA compatible sibling donors (44).

Allo-SCT is a treatment that has been associated with serious side effects such as GVHD (graft versus host disease) and infections due to immunosuppression. However, allo-SCT remains an alternative to consider in MM for selected subgroups of young patients with high-risk cytogenetics and patients with relapse. The use of DLI (donor lymphocyte infusions), maintenance treatment post transplantation and reduced intensity conditioning (RIC) for selected patients may possibly improve outcome further in the future (45).

High dose treatment (HDM) with melphalan in MM was reported by McElwain and Powles in 1983 in a patient with plasma cell leukaemia and eight patients with MM, with responses in all patients (46). Use of HDM and TBI (total body irradiation) in combination with ASCT in refractory patients was demonstrated by Barlogie as a new approach with convincing responses in 1987 (47).

In patients not eligible for SCT, MP continued as standard of choice since other combinations did not appear to result in benefits regarding survival (48).

2.6.3 Immunomodulatory drugs (IMiDs)

Thalidomide was initially introduced in 1957 when it was used as a sedative and for treatment of morning sickness (49). However, in 1961 it was confirmed that the drug was teratogenic and was therefore withdrawn (5).

Thalidomide as an inhibitor of angiogenesis was described by D'Amato in 1994 (50). In 1999 a study by Barlogie including eighty-four patients with relapsed/refractory MM (RRMM) saw a response rate of 32% using thalidomide as a single agent (51). The response rate was later shown to increase in combination with steroids (52). In combination with both steroids and cyclophosphamide a response rate of 67% was further reported (52).

Lenalidomide is an IMiD that in combination with dexamethasone was approved 2006 for RRMM and in 2015 also for newly diagnosed patients (53). The drug demonstrated effect

in patients earlier treated with thalidomide and did not show side effects such as somnolence and neuropathy seen with thalidomide. Instead, the most important side effects were cytopenia (53, 54). In newly diagnosed MM, Lenalidomide together with dexamethasone has demonstrated an overall response rate of 91% (55).

The next relevant IMID, Pomalidomide, was approved for RRMM in 2013 for patients earlier treated with lenalidomide and bortezomib. Side effects were mostly connected with cytopenia (53). Pomalidomide has proved to be effective in patients both refractory to thalidomide as well as lenalidomide with 37% and 47% reported overall response rates respectively (56, 57).

The IMiDs have similar structures but differ regarding the glutarimide part. They also differ in pharmacological traits such as clearance, metabolism, and interactions. For thalidomide, fifty separate metabolites have been demonstrated. Lenalidomide however is not much metabolized and is thought to be excreted unchanged in the urine. Pomalidomide may interact with other drugs since it acts as substrate for several CYP enzymes (53).

The antiangiogenic traits of thalidomide have been attributed to the inhibition of basic fibroblast growth factor (bFGF) (50). Effects of IMiDs have also been accredited to many other causes such as possible inhibition of NF-κB (53) and decrease of IL-6 and VEGF secretion (58). Lenalidomide is also known to decrease osteoclastic activity and lowers levels of osteoclast stimulators such as the receptor activator NF-kB ligand (RANKL) (59, 60). In addition, effects of IMiDs may increase CD4 and CD8 T cell priming and enhance antigen uptake by dendritic cells leading to improved antigen presentation (61).

The binding and inhibition of the protein cereblon (CRBN) and its ubiquitin ligase activity was recognized in 2010 as a factor responsible for the teratogenic effects of thalidomide (62). CRBN binding proteins have been found to decrease after lenalidomide treatment (63). Lack of CRBN is toxic for MM cells but for surviving cells without CRBN, it leads to resistance for Lenalidomide and Pomalidomide. The presence of CRBN is therefore required for adequate IMiD activity of these drugs in myeloma (64).

The incidence for venous thromboembolism (VTE) with thalidomide in monotherapy is lower than 5% and not considered significantly increased. However, in combination with dexamethasone, the incidence has been reported to increase to 8-26%. In combination with melphalan and prednisone the incidence has been reported to 17% and with anthracyclines, to 6-28% (65). Studies with lenalidomide and dexamethasone has shown VTE rates at 8-75% (53). When aspirin and low-molecular weight heparin (LMWH) were compared as thromboprophylaxis during lenalidomide treatment, the incidence for VTE was 2,27% and 1,20% respectively (66). A phase III study from 2011 with thalidomide treated patients, randomized to aspirin, to LMWH or to low dose warfarin, showed no significant difference in incidence for VTE or vascular event between the groups, although high risk patients for

thromboembolism were not included (67). Aspirin has been recommended for patients without risk factors for VTE, and LMWH for patients with increased risk for thrombosis and patients treated with high dose dexamethasone or doxorubicin (68).

2.6.4 Proteasome inhibitors (PIs)

The ubiquitin proteasome pathway involves enzymes that attach ubiquitin to proteins, targeting them for degradation in the proteasome. The system degrades about 80% of intracellular proteins. Bortezomib is a reversible proteasome inhibitor (PI) that makes the cells accumulate intracellular proteins. Bortezomib is also active by increasing osteoblast activity, reducing osteoclast activity, affecting bone marrow microenvironment, and can overcome resistance to chemotherapy (69).

Bortezomib was approved by FDA in 2003 for RRMM and for first line treatment in MM patients in 2008 (69) after phase I-II studies (70, 71). Bortezomib was also compared with high dose dexamethasone with favourable results in relapsed MM patients (72, 73). An important dose limiting problem with bortezomib is the risk for peripheral neuropathy (PN) (74). The risk for PN might be reduced if the drug is given less frequent (once instead of twice weekly) and subcutaneously (instead of intravenously) (75).

Carfilzomib is an irreversible PI resulting in continuous inhibition of the proteasome. The risk for PN is considered less in comparison with bortezomib (69). Lasting responses were shown in phase II studies with RRMM patients with prior and no prior treatment with bortezomib (76, 77). Carfilzomib was further investigated in the phase III studies ASPIRE (comparing carfilzomib and lenalidomide with lenalidomide), and ENDEVOUR (comparing carfilzomib with bortezomib) (78, 79).

Ixazomib is a proteasome inhibitor that can be administered orally. In a randomized phase III trial for RRMM, ixazomib, lenalidomide, dexamethasone was associated with longer progression free survival (PFS) than lenalidomide and dexamethasone (80).

2.6.5 Monoclonal antibodies

CD38 is a transmembrane protein expressed by plasma cells but also by epithelial, pancreatic, lymphoid, myeloid and NK- cells as well as in blood platelets (81). CD38 works as a receptor and binds CD31 leading to intracellular signalling. It also has enzymatic activity.

Daratumumab is an IgG kappa monoclonal antibody that binds and inhibits CD38 activity. In addition, the Fc dependent activity include antibody and complement dependent

cytotoxicity and causes apoptosis due to crosslinking. Daratumumab has immunomodulatory activity through enhancing cytotoxic and helper T-cells while reducing CD38 expressing T regulatory cells as well as myeloid derived suppressor cells (82).

Daratumumab has demonstrated effect in RRMM patients in a phase I-II trial in 2015 (83). It was approved as monotherapy for MM, with reports of overall response of 29-36% (82-84). Combinations with bortezomib and lenalidomide was further investigated in the CASTOR and POLLUX studies (85, 86). Later, the combination with pomalidomide was described in the EQUULEUS trial (87). The MAIA and ALCYONE trials combining daratumumab with lenalidomide and melphalan-bortezomib respectively, led to approval of daratumumab in untreated patients not eligible for ASCT (82, 88, 89). The CASSIOPEIA trial compared Bortezomib-thalidomide-dexamethasone (VTD) with daratumumab-VTD in transplant eligible patients, showing significantly more stringent complete responses for patients treated with VTD and daratumumab compared to patients treated only with VTD (90).

Isatuximab is another Ig G kappa antibody binding CD38. In phase 1 b studies, isatuximab in combination with dexamethasone and lenalidomide had an overall response rate (ORR) of 52% in patients refractory to lenalidomide, and a 62% ORR in combination with pomalidomide and dexamethasone in RRMM patients (91).

2.6.6 CAR-T and T-cells engagers

BCMA (B cell maturation antigen) is expressed by MM cells but is only expressed to a limited extent in normal plasma cells and B cell lymphocytes. It is also supposedly not expressed in other tissues (91). BCMA binds to factors BAFF (B cell activating antigen) and the proliferation inducing ligand APRIL (92).

T cells can be modified to express a chimeric antigen receptor (CAR) (92). CAR-T-cell therapy was shown to have anti myeloma activity in 2016 by using BCMA as the target (93).

BiTEs (bispecific T cell engagers) are made from two linked monoclonal antibodies with different targets. One arm binds to a target on the tumour cell and the other to a target on the T cells (such as CD3). Cross-linking to the tumour cells induces release of perforin and granzyme B from the T cells resulting in cell death. Cytokines also activate T cells further against the myeloma cells (91). BiTEs targeting BCMA has demonstrated efficacy in depletion of myeloma cells and is thus a promising novel concept for treatment in MM (94,95).

ADCs (Antibody-drug-conjugates) are monoclonal antibodies connected to cytotoxic agents that can enter the tumour cells after the antibody binds to it. ADS: s has shown effect with 60% response rate in a phase I study in RRMM patients (91).

Further studies are under way regarding both CAR-T cell therapy and T cell engagers since initial trials have shown promising results (91, 95). The role of possible CAR-NK therapy in the future is also currently being investigated (96).

3 RESEARCH AIMS

Plasma cell disorders are conditions that have various clinical characteristics. This affects approaches to treatment and follow-up. There is a gap of knowledge regarding population-based data for these various groups of patients in Sweden and abroad.

SP and PCL have, due to their rarity been studied mainly in smaller study settings.

The oligo and non-secretory subgroups within MM are heterogenous disease groups. Frameworks for classification including S-FLC can be used to improve our understanding of the clinical relevance of M protein in MM.

In Sweden, treatment and care for MM patients is handled by the six different health care regions. Differences between the regions regarding treatment in relation to survival has not been studied thoroughly in MM.

The aim of the thesis is a better understanding of plasma cell disease by the specific study of:

- Survival and progression to multiple myeloma in solitary plasmacytoma (SP), and survival in plasma cell leukemia (PCL).
- Survival and characteristics in oligo and non-secretory disease in comparison to secretory multiple myeloma.
- Survival and treatment for patients with MM in different health care regions of Sweden.

4 MATERIALS AND METHODS

4.1 STUDY POPULATION

The national quality registries of Sweden today constitute important tools for research in cancer patients. The Swedish Cancer Registry for reporting dates all the way back to 1958. Since 2008, The Swedish Myeloma Registry has been collecting data on patients diagnosed with plasma cell disorders. Compared to the Swedish Cancer Registry, the Swedish Myeloma Registry has had a reported coverage of (97%) (28).

In study I, II and III, data were retrospectively collected from the Swedish Myeloma Register since 2008. Diagnosis and response criteria used in the register were assumed to adhere to the IMWG criteria at the time of diagnosis (31, 97, 98).

For study I, data was retrieved from patients diagnosed between 2008 to 2014. We collected data for patients with SBP, EMP and PCL. Data on MM was collected for comparison. Data included gender, age, and laboratory markers. In connection with the study, The Swedish myeloma register was validated regarding plasmacytomas with a questionnaire including information if patients had received radiotherapy, chemotherapy, or surgery. Information regarding how many of the plasma cells below 10% were monoclonal was not included. For this reason, the number of patients with solitary plasmacytoma and minimal bone marrow involvement was not assessed. Patients with plasmacytoma who developed MM in 3 months after diagnosis were classified as MM instead of as plasmacytoma.

For study II, data from MM patients diagnosed 2008 -2016 was used. Only patients with symptomatic MM at first diagnosis were considered in the study. The collected data included age, ISS stage, gender, M protein type, use of drugs (initial drugs and consolidation), SCT, response, complications, amyloid, BMPC%, bone involvement as well as laboratory values. Secretory MM disease was defined by S-M protein at least 10grams/liter or U-M protein at least 200 (by mg/day or mg/L or mg /mmol creatinine). The entity of non- measurable MM was defined as either oligo or non-secretory MM disease. For oligo-secretory MM, S-M protein less than 10grams/liter and U-M protein less than 200 (by mg/day or mg/liter or mg/mmol creatinine) was required. Non secretory MM was defined by having no S- M protein and no U-M protein. Patients with non-measurable disease was further classified with S-FLC level and ratio, when available. A schematic overview is presented in Figure 3.

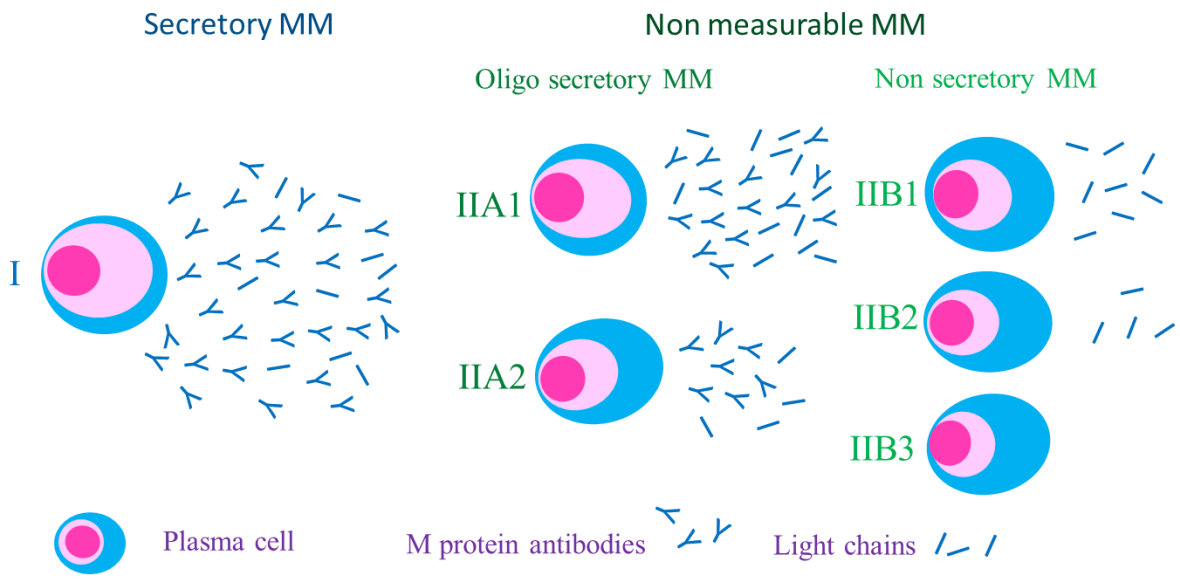


Figure 3. Schematic overview of secretory and non-measurable MM, study II, with oligo and non-secretory subgroups, (picture by the author). Secretory MM (I), measurable oligo secretory, (IIA1), non-measurable oligo secretory, (IIA2), measurable S-FLC only, (IIB1), non-measurable S-FLC only, (IIB2), true non secretory (IIB3). Definitions adapted from a proposal in reference (99).

For study III, data from patients with MM diagnosed between 2008-2017 was used, and patients also needed to have a one year follow up report for evaluation of treatment. Patients in the six health care regions of Sweden were compared. Variables in the analyses included gender, age, ISS stage, period of diagnosis and initial treatment. Patients were divided in subgroups based on if they had been treated with ASCT or not. Patients not treated with ASCT were further categorized in two groups depending on age. Analyses were also done with patients alive 6 months after diagnosis, adjusting for the possibility of a time to treatment bias. The regions were evaluated by usage of initial modern treatment (highest, low and intermediate) and compared regarding survival. Region A was the region with most extensive use of the novel drugs, as defined in the study, in the whole group of treated patients as well as in all investigated subgroups. It was thus the only region included in the highest usage group and used as the reference for comparisons. In the low and intermediate usage groups, the regions included differ and were assembled due to similar levels regarding usage percentage. The specific level between high, intermediate, and low usage therefore differs and depend on each subgroup. Modern initial treatment was specified for this study as treatment with pomalidomide, carfilzomib, lenalidomide, and daratumumab, or as bortezomib together with melphalan, cyclophosphamide or thalidomide. Initial and consolidation/maintenance treatment were restricted to what was given during the first year from diagnosis to correct for variations of delay in the reporting from the regions. Separate multivariate analyses were done regarding treatment since these variables were not present at baseline.

4.2 ETHICAL CONSIDERATIONS

For each of the three studies an application was sent to the ethics committee, and all three studies were approved separately. Study I, dnr 2014/525-31/3, study II, dnr 2016/1756-31, (amendments dnr 2016/2519(5?)-32 and dnr 2017/683-32), study III, dnr 2018/60-31/2 with amendment dnr 2020-00394. The studies were considered conducted in accordance with the declaration of Helsinki.

For study II, two amendments were approved, the first to clarify that the study design was a cohort study and the other to ensure that all data in the registry could be used in the study. A third amendment (dnr 2019-00778) was returned because of a failed payment and was after reconsideration not considered necessary to resubmit. For study III, an amendment (dnr 2020-00394) was made to clarify the extent of the data extract from the register. A minor revision regarding the definition of oligo secretory MM was also made in study II between the first and second publication since this group included some patients with mg/mmol creatinine as measurement for secretory disease, which was not the initial intention. However, since mg/mmol creatinine can serve as a measurement for secretion as well, and the distinctions between the cohorts are approximations, we considered this acceptable. The revision was approved by the research principal, communicated to the editor, and clearly stated as a modification in the methods part of the article.

Approved consent was not requested from participants for each of the specific studies since the studies were all register studies based on the Swedish Myeloma Register, a national quality register with research as a stated purpose. For study III, the ethics committee pointed out that the research was approved assuming that the Swedish Myeloma Register adhered to the principles of information and consent through the "opt-out principle" for national quality registers in the law regarding data from patients. The Swedish myeloma register´s routine for information and consent is available and explained at their website online. Informed consent relies on the responsibility of the hospital reporting to the register. This means that before registration, the health care provider is responsible for giving adequate information to the patients that the data in the register can be used for research. We therefore considered the studies to be in accordance with the Swedish law of ethics for consent (lag om etikprövning § 3, §17-19 and §20-22), the Swedish law of personal data (patientdatalag chapter 7 §1-10 that refers to national quality registers) and the law on personal information (personuppgiftslag § 10 and §19). We also considered the studies to be in line with GDPR article 6.1, 9.2 and 89 regarding consent and the exemptions and precautions stated for handling of registry-based historical data. All studies were however initially approved prior to implementation of GDPR in 2018.

The data extracted from the Swedish Myeloma Registry were handled either as pseudonymized or deidentified data during the statistical work and presented in aggregated form so that no individual subject could be identified in the results.

4.3 DEFINITION OF ENDPOINTS

For all the papers we used the response criteria given in the Swedish Myeloma Registry which we assumed corresponded to IMWG criteria for each patient at that point in time. Complete response (CR) and stringent CR were not separated in the dataset in study II. For patients with non-measurable disease, we point out that only stringent CR earlier has been recommended for assessment of these patients (98), although this could not be considered in the study.

For **study I** these terms were used

- Survival by CIF (Cumulative incidence function) for competing risk analyses.
- OS (Overall survival): Survival from diagnosis to the time of death. In the study both observed OS and relative OS are used.

For **study II** these terms were used

- OS (Overall survival) : Survival from diagnosis to the time of death. In the study observed overall survival is used.

For **study III** these terms were used

- OS (Observed survival) : Survival from diagnosis to the time of death. In the study, observed OS is used.

4.4 STATISTICAL METHODS

Study I.

Test of significance for categorical variables was done using chi square test. For small table cell counts Fischer's exact test was used, and p value simulated, based on replications. Kruskal Wallis rank sum test was used for continuous variables. Survival curves were analyzed according to the Kaplan-Meier method. Relative survival was estimated by the Ederer II method for expected survival in comparison with the Swedish population. Hazard ratios (HR) were estimated by Cox´s proportional hazard regression. P values <0.05 were considered significant. Competing risk analyses was done by calculating Cumulative incidence functions (CIF) for the competing events MM and death. Age standardization was done with weights for standard populations. Patients were censored at loss to follow up and at the end of follow up.

Study II

Test of significance for categorical variables was done using the chi square test. Fischer's exact test was given for small table cell counts. The survival curves were analyzed with the Kaplan Meier method and log rank test. For exploratory variables in relationship to survival

we used Cox´s proportional hazard regression with 95% confidence interval (CI). P values <0.05 were regarded as being significant although they were not adjusted for multiple testing. Missing data was not part of analyses, the variables with largest amount missing data were noted. Patients were censored only at end of follow up with the assumption that data for all included patients were up to date with the Swedish death register.

Study III

Test of significance for categorical variables was done with chi square test and Fischer´s exact test. Survival curves were analyzed with the Kaplan-Meier method and log rank test. We used Cox´s proportional hazards model for multivariate analyses with hazard ratios (HR) and 95% CI. P values <0.05 were considered as being significant but were not adjusted for multiple testing. Schoenfeld residuals was used as a test of proportionality for hazard ratios. Patients were censored at loss to follow up and end of follow up. The study was handled as a complete case analysis regarding missing data in multivariate analysis. Patients with the variable stage missing were however included as a separate entity in the analysis.

5 RESULTS

5.1 STUDY I

From 4518 patients with plasma cell disorders, 735 patients with SMM were excluded. Out of the rest, 3549 patients had MM (94%), 124 patients had SBP (3%), 67 patients had EMP (2%), and 43 patients had PCL (1%).

Distribution, median age and incidence is presented in table 3. Median age was 68, 71, 69 and 71 years for SBP, EMP, PCL and MM respectively. Patients that had PCL also had a lower level of albumin and hemoglobin as well as higher β 2 microglobulin, calcium and creatinine compared to plasmacytoma and other MM patients. Radiotherapy as only treatment was more often done in SBP while surgery as the only treatment was most performed in EMP. Information on treatment for PCL patients was not further assessed.

Table 3. Distribution of MM, SBP, EMP and PCL, **study I**

Group	Number	Percentage	Age median	Incidence	
Total	3783	100%		Male	female
MM	3549	94%	71	6.074	4.613
SBP	124	3%	68	0.239	0.135
EMP	67	2%	71	0.109	0.093
PCL	43	1%	69	0.066	0.063

Progression from SBP and EMP to MM by CIF, and relative survival is presented in table 4. At two years, relative survival was 90% for SBP, 77% for EMP, 27% for PCL and 71% for MM. No patients with PCL were alive 5 years after diagnosis.

Table 4. Progression to MM for EMP and SBP by CIF, and relative survival for EMP, SBP, MM and PCL, **study I**

	Group	2 years	4 years	8 years
Progress	EMP	7%	9%	14%
	SBP	35%	51%	53%
Survival	EMP	77%	74%	62%
	SBP	90%	80%	68%
	MM	71%	52%	30%
	PCL	27%	6%	0%

CIF, Cumulative incidence function

Progression to MM at two years by CIF was 35% for SBP and 7% for EMP. The combined event of progression to MM or death by CIF appeared as a more frequent event for SBP than EMP over time. Death for EMP by CIF was more frequent over time compared to SBP.

5.2 STUDY II

Using data from 4918 patients with symptomatic MM, 4235 patients could be further analyzed for secretory and non-measurable MM disease. Patients who had SMM and not MM at first diagnosis were not included in analyses. Classification of non-measurable MM is presented in table 5. Of all the patients investigated, 3936 patients (91%) were found to have secretory MM while 389 patients (9%) were classified as non-measurable MM. From the group of 389 patients with non-measurable MM, 253 patients (6%) were classified as oligo secretory while 136 patients (3%) were classified as non-secretory.

Table 5. Secretory and non- measurable MM.
As being defined in **study II**

Group	S-M protein	U-M protein	Number	%
Secretory	At least10g/L	At least 200*	3936	91%
Non-measurable	< 10g/L	< 200*	389	9%
Oligo-secretory	< 10g/L	< 200*	253	6%
Non-secretory	Not present**	Not present**	136	3%

g/L= grams/ liter
** Either mg/day, mg/L or mg/mmol creatinine
*** Immunofixation not assessed since data for this was not available

A total of 202 patients with non-measurable MM had information registered regarding S-FLC. Subgroups based on S-FLC is presented in table 6. In the group of patients with S-FLC available there were twelve patients to be found (6%) with true non-secretory MM.

Table 6. Definitions of non-measurable MM subgroups
Subgroups based on ref (99) as defined and adapted in **study II**

Group	S-FLC ratio	S- FLC level	Number
Oligo secretory			253
Measurable OS	Abn	>=100mg/L	83
Non-measurable OS	Normal or Abn	<100mg/L	35
Non- secretory MM			136
Measurable SFLC only	Abn	>=100mg/L	48
Non measurable SFLC only	Abn	<100mg/L	24
True non secretory MM	Normal	Normal	12

Abn, abnormal, OS, oligo secretory

Median age was 72, 70, 70 and 69 years for secretory, non-measurable, oligo-secretory, and non-secretory MM. In oligo-secretory MM, amyloidosis appeared to be more frequent than in secretory MM while IgG M protein appeared to be less. For non-secretory MM, bone involvement seemed to occur more often than in secretory MM. Data on type of light chain was lacking to a great extent for non-secretory MM but had approximately the same distribution for oligo and secretory MM, with kappa chains being more common.

Median survival was 42.7, 40.2, 38.6 and 44.6 months in secretory, non-measurable, oligo-secretory, and non-secretory MM patients. When patients with amyloidosis were not included, the results were similar (43.3, 41.1, 39.9 and 44.6 months).

No significant differences could be proven in survival between non-measurable and secretory MM as a whole or for any of the subgroups.

In univariate analyses low age, stage I vs III, treatment with novel drugs in first line (as defined in the study), SCT, low BMPC% and CR were superior for survival in the oligo as well as in the non-secretory MM subgroups. In oligo secretory MM, IgG M protein and absence of light chains in urine were both indicators for better survival while amyloidosis and hypercalcemia were indicators for worse. In non-secretory MM, normal creatinine predicted a better survival while anemia and hypercalcemia predicted the opposite.

5.3 STUDY III

Out of 5576 patients diagnosed with MM in the six health care regions of Sweden, 5326 patients were included in analysis. 250 patients who did not receive treatment were not further investigated. SMM constituted 18% of all myeloma patients. For all patients with MM, incidence per 100 000 (age adjusted) was 6.3, 6.1, 6.0, 5.9, 6.4 and 7.3, in each of the regions (A, B, C, D, E and F).

Treatment, age and stage for all treated MM patients by health care region is presented in table 7. The mean age was lowest in region A (68,7). This region also had the highest percentage of patients 0-49 years of age (6.5%). ISS stage varied between the regions. Region B had highest percentage with stage I (24%) and region A had highest percentage with stage II (53%). Initial modern treatment also varied with most extensive use in region A (66%). ASCT was also most widely used in region A (37%).

Table 7. Age, stage and modern initial treatment
All treated patients with MM, by region.
Parts of data presented in table I, **study III**

Health care region		A	B	C	D	E	F
Number (n), (5326)		(892)	(551)	(1175)	(968)	(1041)	(699)
Age %	0-49	6.5	3.3	3.3	3.6	4.6	5.6
	50-59	13.8	13.1	11.4	11.4	9.8	11.6
	60-69	30.7	27.8	29.1	28.1	26.5	27.6
	70-79	29.6	32.5	35.1	32.5	36.2	32.2
	80 +	19.4	23.4	21.1	24.4	22.9	23.0
ISS Stage	I	20.0	24.0	23.8	17.9	19.0	23.7
% (n)	II	52.5	38.2	45.0	40.3	40.9	45.3
	III	27.5	37.8	31.2	41.8	40.1	31.0
	NA*	(153)	(67)	(473)	(274)	(357)	(193)
Modern initial treat**	No***	34.0	48.1	53.5	55.3	51.2	51.2
%	Yes	66.0	51.9	46.5	44.7	48.8	48.8
ASCT	No	62.6	67.2	70.1	73.6	73.6	68.8
% (n)	Yes	37.4	32.8	29.9	26.4	26.4	31.2
	NA*	(12)	(2)	(12)	(10)	(11)	(9)

*NA, means not assessed in number of patients, n, number
** Modern initial treatment
*** No, means no modern initial treatment or missing data.

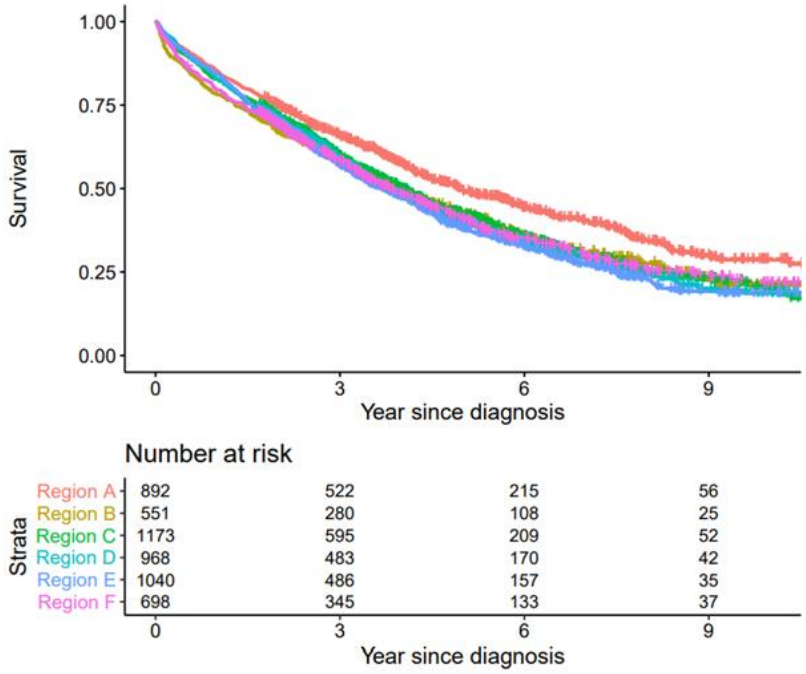
Survival for all treated patients with MM is presented in Figure 4 A. In this group we observed a significantly better survival in region A in relation to the other regions ($p<0.01$ for each). If only patients alive after six months were included the differences also remained significant.

In the subgroup with patients treated with ASCT a significant difference was seen in survival between region A and region C ($p=0.01$), D ($p<0.01$), E ($p<0.01$) and F ($p= 0.04$). Between region A and B, the difference however was not significant ($p=0.08$). The results after time to treatment bias adjustment are shown in Figure 4 B (A compared to region B ($p=0.08$), C ($p<0.01$), D, ($p<0.01$), E, ($p<0.01$) and F, $p=0.05$)).

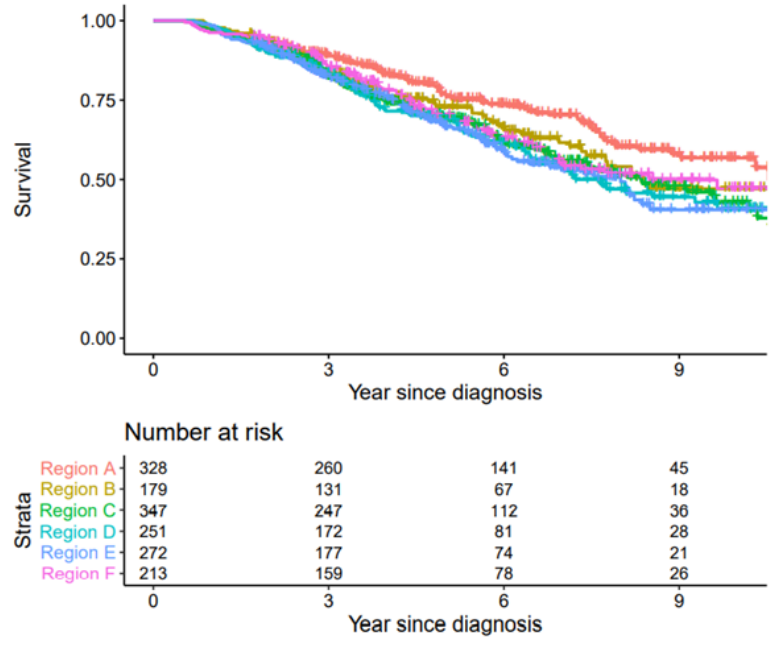
For patients not undergoing treatment with ASCT below 75 years of age, survival did not differ significantly neither before nor after having adjusted for the six-month time to treatment bias.

In the group not being treated with ASCT and at least 75 years of age, differences in survival were not clearly visible after adjusting for the time to treatment bias (of note only A in relation to E, ($p=0.04$ (log rank), HR 1.2, CI 1.00-1.44, $p=0.06$), Figure 4 C.

A


1.00
0.75
0.50
0.25
0.00
Survival
0
3
6
9
Year since diagnosis
Number at risk
Strata
Region A 892 522 215 56
Region B 551 280 108 25
Region C 1173 595 209 52
Region D 968 483 170 42
Region E 1040 486 157 35
Region F 698 345 133 37
0
3
6
9
Year since diagnosis

B


1.00
0.75
0.50
0.25
0.00
Survival
0
3
6
9
Year since diagnosis
Number at risk
Strata
Region A 328 260 141 45
Region B 179 131 67 18
Region C 347 247 112 36
Region D 251 172 81 28
Region E 272 177 74 21
Region F 213 159 78 26
0
3
6
9
Year since diagnosis

C

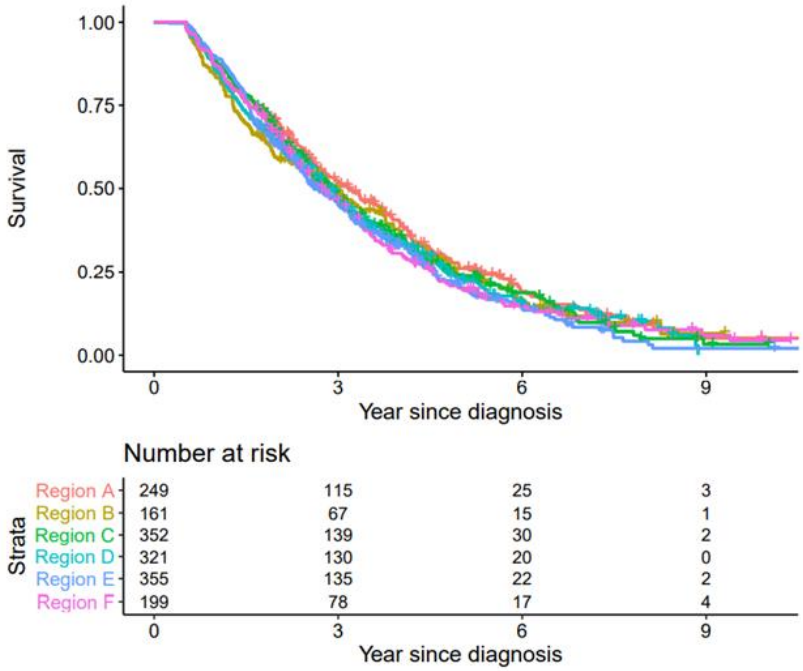
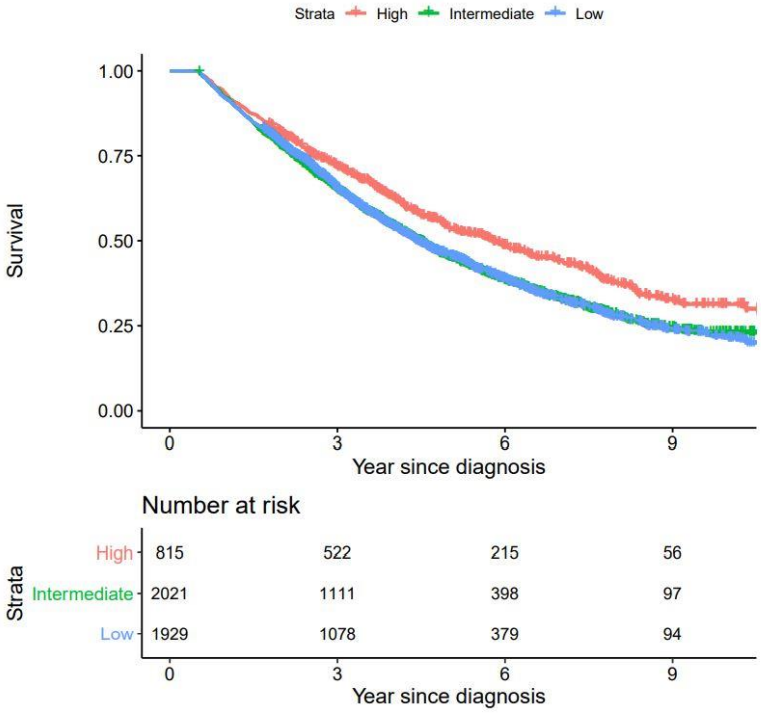


Figure 4. (A) Overall observed survival by region and with number at risk. (A) MM patients (all treated patients), (B) MM patients receiving ASCT, with the time to treatment bias adjusted for (alive six months after diagnosis), (C), No ASCT, at least 75 years of age, with time to treatment bias adjusted for. Adapted from: Regional differences in treatment and outcome for myeloma patients in Sweden: A population based Swedish myeloma register study. Göran Wålinder, Anna Genell and Hareth Nahi, et al. Cancer Rep (Hoboken). 2022 Mar 3:e1614. doi: 10.1002/cnr2.1614. Epub ahead of print. PMID: 35243814.

After all treated MM patients alive after six months (the time to treatment bias) were split into three groups based on region and usage of initial modern treatment, a better survival was seen for the group with highest usage (region A) in comparison with groups that had low and intermediate usage ($p<0.01$ for each), as shown in figure 5 A. For patients treated with ASCT the results were similar. A better survival for the group with highest usage (region A) was seen in comparison with the other two groups ($p<0.01$ for each), as shown in figure 5 B. The proportion of patients receiving initial modern treatment seemed to increase with time and differences in usage between the regions appeared to be less pronounced during later years (Figure 6).

A



B

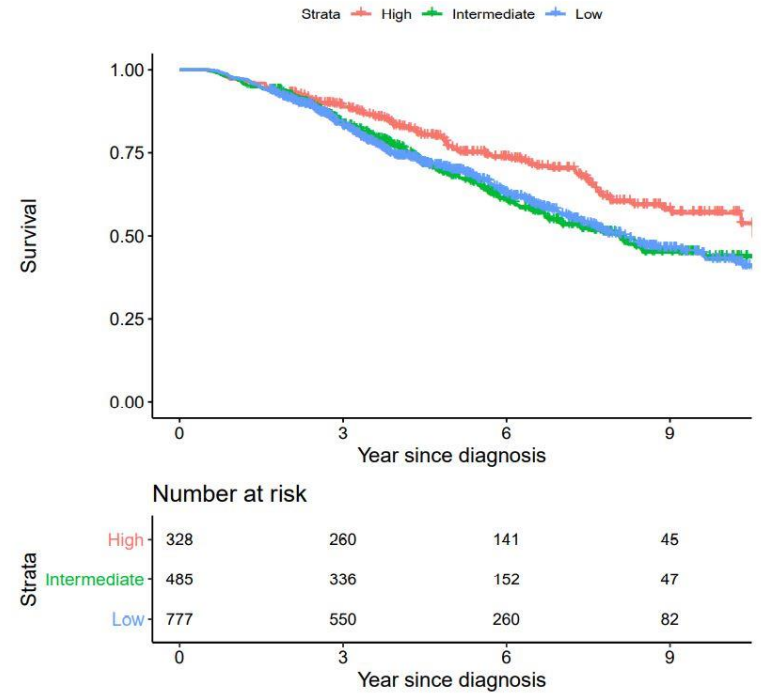


Figure 5. Overall survival, by region and use of initial modern treatment, time to treatment bias of six months accounted for and with number at risk. (A) All treated MM patients, region A (high), region B, E, F (intermediate), region C, D (low). (B) MM patients receiving ASCT, region A (high), region E, F (intermediate), region B, C, D (low). Adapted from: Regional differences in treatment and outcome for myeloma patients in Sweden: A population based Swedish myeloma register study. Göran Wålinder, Anna Genell and Hareth Nahi et al. Cancer Rep (Hoboken). 2022 Mar 3:e1614. doi: 10.1002/cnr2.1614. Epub ahead of print. PMID: 35243814.

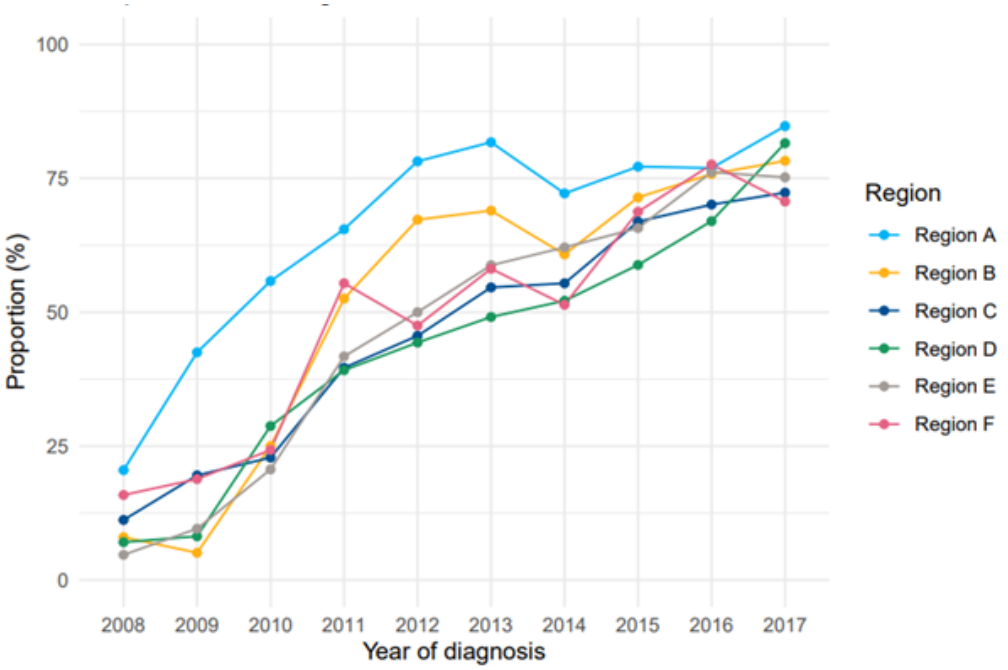


Figure 6. Initial modern treatment by year and region. Adapted from: Regional differences in treatment and outcome for myeloma patients in Sweden: A population based Swedish myeloma register study. Göran Wålinder, Anna Genell and Hareth Nahi, et al. Cancer Rep (Hoboken). 2022 Mar 3:e1614. doi: 10.1002/cnr2.1614. Epub ahead of print. PMID: 35243814.

In univariate analyses (with time to treatment bias adjusted for) modern initial treatment, consolidation / maintenance treatment, ISS stage I (vs stage III and stage missing), and later time-period were significant factors for survival (by log rank test) for all subgroups. Age was also associated with better survival in the subgroups treated with ASCT (age 0-49 vs 50-59, 60-69 and 70-79) and in the group who was not being treated with ASCT, 75 years and older (age 70-79 vs 80 years and older).

In multivariate analysis for patients treated with ASCT, survival differences persisted after adjusting for the time to treatment bias, ISS stage, age, and time-period of diagnosis.

In multivariate analyses (with time to treatment bias adjusted for) with modern initial drugs (as stated in the study) and consolidation/ maintenance treatment, these variables were significant in the two subgroups who did not receive ASCT.

6 DISCUSSION

6.1 Methodological aspects of the study design

The studies in this thesis are all cohort studies where patients were divided by exposure and compared regarding the survival outcome, measured as time to event analysis or specific years of survival. Since the studies were started after completed follow up, they are retrospective in design (100,101). The cohort design was chosen since we in study I and study II wanted to investigate small groups and had access to a complete data set that also could be managed statistically. Alternatives for study I and II could have been making a case control study or a case cohort study (102, 103). However, since the study base was clearly defined, and the data amount was relatively small for a register study there was no need for either of those approaches. A population-based approach strengthens the external validity of the results since the studies can be generalized to the whole population. However, it also assumes an adequate internal validity with correct data and that the models used in the studies reflects the results in an adequate way. For the plasmacytoma study a limited validation of data was carried out in connection with that project.

One aspect of the cohort design includes loss to follow up which is handled by censoring. In study I and III censoring was done at loss to follow up and end of follow up. In study II we assumed that there was no loss of follow up since the extract from the register was assumed to be updated for date of death. It was noted after analysis that three patients were deregistered (two with symptomatic MM and one with oligo secretory MM). These patients had no death dates and were therefore presumed to be alive in our study. Censoring at last follow up would have been more appropriate if this had been known since these patients possibly were not updated for death.

Cox´s proportional hazards regression model was used in all three studies for exploratory variables in the multivariate analyses. In study II and III, treatments were included in some of these analyses although they were not predictors for survival at base line/diagnosis. The treatment variables are therefore probably best considered as markers of intent for treatment at baseline under these circumstances. In study II we acknowledge the shortcomings of a retrospective investigative approach in the text and in study III separate multivariate analyses were made with and without treatment variables for this reason.

A time dependent bias may be introduced with treatment as a variable since patients who did not live to the point where treatment is possible always will have a worse prognosis. Possible ways to avoid this selection bias such as a landmark analysis or time dependent Cox´s regression have been suggested to overcome this (104-106). In paper III we therefore include an analysis with the condition that patients must be alive 6 months after diagnosis. Also, considering that there may be different delays for reporting between the regions, initial and consolidation/maintenance treatment had to be started within one year from diagnosis.

A disadvantage with cohort studies is that you cannot claim a casual effect since the exposure is not random and other variables as confounders could change and alter the relation between exposure and outcome (100). The confounder may not be known (such as co morbidity in our studies) or known and adjusted for in a multivariate analysis. In the multivariate setting it is however also possible that two variables /confounders may overlap to large extents. This may be the case in study II for variables “age>75” and “no SCT” and may explain inconsistent and divergent results in the multivariate analyses comparing results in the oligo-secretory and non-secretory groups. In the study we comment on the issue with interrelated variables and point out that results in the multivariate analyses should therefore be interpreted with caution in this specific study setting.

For register studies another general concern is multiple testing of exploratory variables. This may lead to false positive associations by chance (101). Although results in a retrospective cohort study may be better suited for generalization than results in a randomized control study (RCT), the associations found in this setting need to be interpreted with care and in the context of hypothesis generating purposes (100). To avoid publication and reporting bias of only positive finding we therefore state which variables were investigated and for what clinical reasons. We also reason in the studies about the consistency of the results, the limitations of register-based studies and the importance for confirmation of results, if possible, in other studies.

6.2 Main findings in the studies

In **study I** we present data that demonstrates that SBP and EMP are rare in comparison with MM, which is in line with earlier findings (107). The relative survival for SBP and EMP appeared superior in our study when compared to MM. Divergent from earlier reports (108,109) was our finding that EMP seemed to have a (statistically non-significant) trend towards worse survival when compared to SBP. A speculative explanation for a worse prognosis in EMP patients could perhaps be more inaccessible locations of the disease, although this does not explain why the result in our study varies in this respect in survival when compared to other studies.

Earlier studies report various findings regarding progression from plasmacytoma to MM (108-111). In our study, progression to MM, as described by CIF, was higher at 2 years for SBP with 35%, than for EMP with 7%. The higher turnover to MM for SBP may indicate that this group holds a larger number of patients that would perhaps be classified as MM initially with modern skeletal surveys compared to patients in the EMP group. The high progression to MM in SBP patients may therefore indicate a need for better prognostic and diagnostic tools such as CT and MRI in the future.

Regarding PCL we could demonstrate that this entity is uncommon and remains a plasma cell disease with poor outcome (112). No patients were alive after 5 years despite the introduction of novel MM treatments the last decade, thus indicating the need for the development of other treatment options for this group of patients.

In **study II** we could show that the occurrence of non-measurable MM was probably approximately in line with earlier results for oligo/ non-secretory MM, although our definition differed in not requiring immunofixation for M protein and regarding measurement of M protein concentration in urine (113,114). We could also present patients with non-measurable disease using M protein and S-FLC in a detailed framework based on earlier proposals (99). The number of cases with true non secretory MM was small, constituting only 6% (n=12) of the patients in the non-measurable group where S-FLC was available. This is in line with a later study using a similar way of classification as our study (115).

We could not demonstrate any differences in survival between non-measurable MM and secretory MM as a whole group or for any of the subgroups. Sample sizes however limited the assessments in the smaller sub-groups.

Patients treated with SCT appeared in the univariate analyses to have superior survival in non-secretory patients. However, these results must be interpreted with reservations, since time to treatment bias and overlapping variables may be an issue not further addressed in the study.

Regarding oligo secretory MM we found that amyloidosis was a more frequent feature than in secretory MM. This may be because this trait possibly is more common within the group that has oligo-secretory MM. However, it may also reflect that amyloidosis is more often investigated in these patients. There was no difference to be seen in survival compared to secretory MM also when patients with amyloidosis were excluded from analyses.

IgG M protein appeared to be more unusual in oligo secretory than in secretory MM disease. A thought to further investigate is if IgG predisposed cells with a block in secretion perhaps, if compared, would make up a big part of the non-secretory cell population. For non-secretory MM, bone involvement appeared as more frequent than in secretory MM. The different characteristics of the non-measurable MM subgroups may point at fundamental differences between the groups, regarding how MM disease is triggered.

In **study III** we observed that there are differences for patients with MM when comparing survival between health care regions in Sweden. For all treated MM patients there was a significantly better survival for region A in comparison to the other regions. In patients receiving ASCT a significant better survival was also seen for region A when compared to the other regions, except for comparison with region B. After adjustments for time to treatment bias, ISS stage, time of diagnosis and age, differences favoring region A in the ASCT subgroup persisted. In patients not undergoing ASCT, no difference in survival could be observed in the group of patients less than 75 years of age. For patients at least 75 years of age, differences in survival favoring region A were not clearly apparent after the time to treatment bias was accounted for.

We found that usage of initial modern treatment differed for the regions, being most extensive in region A. High use also translated into better survival for region A in all treated

MM patients and in patients receiving ASCT. Differences in use of modern initial treatment between the regions seemed to diminish over time. Noting that later years for diagnosis also correlated to better survival, it seems probable that superior survival, high use of modern initial treatment and a later time-period correlated. In the two subgroups not receiving ASCT the correlation between use of modern initial treatment and survival was however not evident. It is possible that the reason for this was that death from other causes due to comorbidity in these groups made it harder to link treatment and differences in survival over time. Residual confounding factors such as social circumstances, access to health care, personal economy and education or varying cycles of pretreatment could also have additional relevancy but could not be further addressed in the study.

ISS stage I (vs III and vs stage not assessed), time of diagnosis, modern initial treatment and consolidation/maintenance treatment were all associated with better survival in both patients undergoing and not undergoing ASCT. However, missing data for ISS varied considerably between the regions. Survival for patients with missing data for ISS stage seemed similar to patients with ISS stage III in the MM group as a whole.

Overall, survival in the study appeared superior in region A for patients treated with ASCT. Survival appeared to correlate with high use of modern initial treatment in that region. In the other two subgroups not receiving ASCT, survival differences between the regions were not evident despite highest use of modern treatment in region A also in these groups.

7 CONCLUSIONS

By use of population-based register data from the Swedish Myeloma Registry, this thesis concludes that:

- EMP, SBP and PCL are uncommon entities of plasma cell diasese in Sweden. EMP does not appear to have a better prognosis than SBP although SBP progresses to MM more often. Also, PCL has a poor prognostic outcome, showing the need for new treatment regarding these patients.

- Non measurable MM can be defined in subgroups by use of serum and urine electrophoresesis and S-FLC as a marker for secretory disease. Only 6% of the patients with non-measurable MM and S-FLC available had true non- secretory MM. No differences in survival were evident between secretory MM and non measurable MM for the group as a whole or for any of the subgroups in separate comparisons.

- There are differences in survival for MM patients when comparing the six health care regions in Sweden, using region A as the region of reference. Differences appear to be mostly confined to patients receiving ASCT. In the group not undergoing ASCT, below 75 years of age, survival did not appear to differ. For patients not treated with ASCT and at least 75 years of age, differences in survival were not clearly apparent when a time to treatment bias was also considered.

8 POINTS OF PERSPECTIVE

Plasma cell disorders require different approaches depending on the character of the disorder present in each patient. Although MM still is considered a chronic condition, novel drugs can now often provide disease control, perhaps providing steps towards a possible cure in the future.

As demonstrated in our studies, the Swedish Myeloma Registry can contribute useful data for investigating characteristics in subgroups of plasma cell disorder and for comparisons of survival between the Swedish health care regions. The register will probably continue to constitute an essential source of information for MM care in Sweden provided that the internal validation of the registry can be affirmed continuously. In the longer perspective however, it would seem desirable to structure data needed for quality control and studies within the patients' electronical medical records, the primary source of information.

The classification of plasma cell disorders remains complex. With the use of cytogenetics, gene sequencing, CT and MRI, the definition and distribution of SP and SMM may be redefined in the future. In MM, the use of S-FLC as a marker has already to a large extent replaced the role of urine electrophoresis as a tool in both diagnosis and management. Although urine sampling may still play an important role in patients with renal impairment or amyloidosis, S-FLC now provides an easy and possibly more consistent marker for classification of disease and for follow up of treatment.

I believe in the future the diagnostic possibilities of non and oligo secretory MM as well as amyloidosis and POEMS needs to be further addressed. Also, the still unclear transition from MGUS and SMM to MM should be investigated in depth. Studies with S-FLC in non-secretory disease may further clarify this entity and could possibly reveal more specific characteristics and genomic changes if larger groups of patients would be investigated.

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