Monoclonal gammopathy of what significance?

Overcoming the methodological limitations of studying an asymptomatic precursor disorder

Sæmundur Rögnvaldsson

Thesis for the degree of Philosophiae Doctor

October 2022

School of Health Sciences

FACULTY OF MEDICINE

UNIVERSITY OF ICELAND

Monoclonal gammopathy of what significance?

Overcoming the methodological limitations of studying an asymptomatic precursor disorder

Sæmundur Rögnvaldsson

Thesis for the degree of Philosophiae Doctor

Supervisor

Sigurður Yngvi Kristinsson

Doctoral committee

Thor Aspelund, Gyða Björnsdóttir, Elías Ólafsson, Ola Landgren

October 2022

School of Health Sciences

FACULTY OF MEDICINE

UNIVERSITY OF ICELAND

Klínísk þýðing góðkynja einstofna mótefnahækkunar

Aðferðafræðilegar lausnir til að rannsaka einkennalaust forstig

Sæmundur Rögnvaldsson

Ritgerð til doktorsgráðu

Leiðbeinandi

Sigurður Yngvi Kristinsson

Doktorsnefnd

Thor Aspelund, Gyða Björnsdóttir, Elías Ólafsson, Ola Landgren

Október 2022

Heilbrigðisvísindasvið

LÆKNADEILD

HÁSKÓLI ÍSLANDS

ISBN 978-9935-9200-9-6

ORCID: 0000-0003-2162-8414

Reykjavik, Iceland 2022

Ágrip

Á Góðkynja einstofna mótefnahækkun (e. monoclonal gammopathy of undetermined significance, MGUS) er einkennalaust ástand orsakað af uppsöfnun einstofna mótefnaframleiðandi frumna í beinmerg. Klínískt mikilvægi MGUS felst fyrst og fremst í því að það er forstig mergæxlis og skyldra sjúkdóma. Núgildandi leiðbeininagar ráðleggja eftirfylgd með einstaklingum með MGUS til að greina þróun þess yfir í illkynja sjúkdóma. Gagnsemi þessarar eftirfylgdar og þess að greina MGUS hefur þó ekki verið að fullu sönnuð en talsverðar vísbendingar eru um að með því að greina þróun MGUS yfir í illkynja sjúkdóma snemma megi grípa fyrr inn í og þannig bæta horfur í þeim sjúkdómum talsvert. Kerfisbundin skimun fyrir MGUS gæti verið leið til að auka aðgengi að slíkri snemmbúinni meðferð en ekki liggja fyrir neinar rannsóknir á slíkri skimun. Til viðbótar við að vera forstig illkynja sjúkdóma hefur MGUS verið tengt fjölda annarra sjúkdóma. Fyrri rannsóknir eru þó líklega nokkuð bjagaðar og enn er því óljóst hvaða sjúkdóma MGUS tengist raunverulega og hvaða klínísku þýðingu þær sjúkdómstengingar hafa.

Markmið þessarar ritgerðar er að skýra klínískt mikilvægi MGUS, einkum með tilliti til sjúkdóma sem ekki eru illkynja. Ætlunin er að nota nýja aðferðarfræðilega nálgun sem getur stórbætt skilning okkar á MGUS og sjúkdómum sem því tengjast.

Rigerðin byggir á fjórum greinum. Í grein I og II eru tengsl MGUS við úttaugamein og beinbrot skoðuð í sænskum gagnagrunni og öðruvísi aðferðarfræðilegri nálgun beitt á það gagnasett en áður hefur verið gert. Seinni tvær greinarnar byggja á rannsókninni Blóðskimun til bjargar. Rannsóknin er lýðgrunduð skimunarrannsókn fyrir MGUS og slembiröðuð rannsókn á eftirfylgd með það að markmiði að kanna gagnsemi og skaðsemi þess að skima fyrir MGUS. Rannsókninni og hönnun hennar er ítarlega lýst í grein III. Í grein IV eru gögn rannsóknarinnar notuð til að rannsaka tengsl MGUS og COVID-19 í því óbjagaða þýði einstaklinga með MGUS sem greinast með skimun í Blóðskimun til bjargar.

Í grein I og II sýna niðurstöðurnar að MGUS tengist úttaugameini og beinbrotum. Auk þess benda niðurstöðurnar til þess að úttaugamein sé ein af a.m.k. þremur orsökum beinbrota í MGUS samhliða ógreindum mergæxlis beinasjúkdómi og MGUS beinasjúkdómi. Í grein III er öflun þátttakenda í Blóðskimun til bjargar lýst en alls skráðu 80,759 Íslendingar sig í rannsóknina. Í grein IV sáust nokkuð óvænt engin tengsl milli MGUS og tíðni eða alvarleika COVID-19.

Niðurstöðurnar benda til að MGUS hafi klínískt mikilvægi. Það er bæði forstig illkynja sjúkdóma og tengist líklega öðrum sjúkdómum líka. Einnig staðfesta niðurstöðurnar fyrri

grun um að eldri rannsóknir hafi byggt á bjöguðum þýðum sem hafa gefið okkur skakka mynd af umfangi tengsla MGUS við sjúkdóma og sýna glögglega mikilvægi þessi að rannsaka þessi tengsl innan skimaðra þýða eins og í Blóðskimun til bjargar. Slíkar rannsóknir eru nauðsynlegar til að skýra raunverulegt klínískt mikilvægi MGUS og með því betrumbæta eftirfylgd einstaklinga með MGUS og þannig vonandi horfur þeirra.

Lykilorð:

Góðkynja einstofna móefnahækkun, Faraldsfræði, Tengslarannsóknir sjúkdóma, Skimun, Lýðgrundaðar rannsóknir.

Abstract

Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic disorder caused by the accumulation of monoclonal immunoglobulin secreting cells in the bone marrow. The main clinical implication of MGUS is that it's the precursor of multiple myeloma (MM) and related disorders. Current guidelines recommend indefinite follow-up of individuals with MGUS in order to detect MGUS progression. The benefits of this follow-up and of detecting MGUS have not been sufficiently studied but recent evidence has suggested that the detection of MGUS provides opportunities of early treatment in MM and related disorder at an asymptomatic stage and that such early treatment can significantly improve outcomes. Systematic screening may provide a means to vastly expand the availability of early treatment but has not been sufficiently studied. MGUS may also have other clinical significance with multiple studies associating the asymptomatic disorder with a wide range of non-malignant disorders. However, previous studies have been heavily afflicted by bias and the extent of these associations and their clinical relevance is not clear.

The aim of this thesis is to further clarify the clinical significance of MGUS with an emphasis on the association of MGUS and non-malignant disease and to demonstrate methodologies that can significantly improve our understanding of this asymptomatic precursor disorder and its clinical significance.

Four papers are presented. The first two papers apply alternative study designs and statistical methods to registry-based data on MGUS from Sweden to study the relationship between MGUS and peripheral neuropathy (PN) and fractures. The latter two papers pertain to the Iceland screens, treats, or prevents multiple myeloma study (iStopMM), a population-based screening study with the aim of gathering a population-based cohort of individuals screened for MGUS and to assess the benefits and harms of such screening in a clinical trial. The study is described in detail in paper three and the fourth paper demonstrates the opportunities to found in studying MGUS disease associations within the screened cohort of iStopMM by assessing the relationship of MGUS and coronavirus disease 2019 (COVID-19).

In paper I and II, MGUS was found to be associated with PN and fractures. Based on the findings we hypothesized that PN is one of, at least, three causes of fractures in MGUS alongside undetected MM bone disease and MGUS inherent bone disease. In paper III, the recruitment of iStopMM is described with around 54% of eligible Icelanders (n=80,759) signing up to participate in the study. In paper IV, MGUS was unexpectedly not found to be associated with increased incidence or severity of COVID-19.

In conclusion, MGUS has clinical significance by being the precursor of MM and related disorders and by leading to non-malignant complications. The findings of this thesis confirm that previous studies have been afflicted by significant bias. Further studies, particularly within screened cohorts like that of iStopMM, are needed to further clarify the clinical significance of MGUS and to focus and improve care of individuals with MGUS.

Keywords:

Monoclonal gammopathy of undetermined significance (MGUS), Epidemiology, Disease association studies, Screening, Population-based studies

Acknowledgements

There are many that I want to thank for their part in this project and their support while on the PhD journey.

First, I would like to thank my supervisor, Sigurður Yngvi Kristinsson. It is through him that I have gotten to know research and the world of multiple myeloma. I'm hooked. Sigurður has given me countless opportunities and shown me immense confidence and trust throughout my PhD journey, not least by allowing me inside the design and implementation of the iStopMM study when I was still just a medical student. I feel that I have grown immensely under his supervision, which somehow has always found a perfect balance between holding my hand and letting me fend for myself. His lessons of research excellence and rigor coupled with optimistic and positive ambition as well as healthy salesmanship will stay with me for the rest of my career.

I would also like to thank my doctoral committee for their support. Thor Aspelund for his willingness to take time from his busy schedule to share his vast statistical knowledge with me. Ola Landgren for bringing new and interesting perspectives to every paper. Gyða Björnsdóttir for sharing her knowledge of neuropathies and her keen interest in the project. Elías Ólafsson for teaching me about neurology and neuropathies.

I'm very grateful for the financial support I've received from the Landspítali Research Fund and the funding the research group has received from The Icelandic Center for Research (Rannís), The European Research Council, and the International Myeloma Foundation. Special thanks go to my employer, the Department of Internal Medicine at Landspítali.

My fellow doctoral students and medical friends in research have been an invaluable source of joy and inspiration throughout this period. Particular thanks go to Vilhjálmur, Elías, Ingigerður, Kristrún, Jón Þórir, Kristján Godsk, Þórir, and Hrafnhildur for the many discussions about science, medicine, and how to change the world. Special thanks go to Sigrún, who has not only been my favorite conference travel companion and co-"bubblor" drinker, but almost like an extra sibling, the older sister I never had, a great friend and role model. I look forward to a lifetime of working together with these amazing people. Additional thanks go to my friends in the public health department for their support and friendship, they really are some of the friendliest and open people I've met.

The great coworkers I've had in the iStopMM team have been an invaluable part of my PhD journey. Thank you for all the fun moments, all the excellent solutions to impossible

problems, and all of your hard work and dedication to the project. Particular thanks go to Ásdís Rósa, the project manager of iStopMM who has been with me through all of it. She's been supportive, helpful, and a friend through thick and thin. I would also like to thank the thousands of Icelanders who have generously provided their informed consent to participate in the iStopMM study, making it a reality.

I would like to thank my parents Rögnvaldur and Birna for their unwavering belief in me, endless encouragement, boundless love, and wise guidance through life. I would like to particularly thank my dad for reading and commenting on this thesis. My younger siblings are a constant source of inspiration. I am immensely proud and lucky to get to enjoy their company and be associated with these incredibly smart and fun people who excel and are admired wherever they go. I specifically want to thank Sölvi for always being interested and engaged, willing to talk about science, and basically teaching me statistics, Kári for being such a kind, interested and humorous person always excited about new ideas, especially ridiculously complicated and time-consuming recipes, and Auður Halla for always bringing joy and making sure that I know my age and use terms like "goon" and "lowkey" correctly. I would also like to thank my gradparents Bjarni, Ingibjörg, Kristín, and Sæmundur as well as my late grandparents Sigríður and Helgi. They have all been such a big part of my upbringing and of who I am.

My own little family, my fiancé Iðunn and our daughter Kristín Birna are the most meaningful people in my life. The endless love and support I receive from them light up my existence and makes it all worth it. This work is dedicated to them.

Contents

List of Abbreviations

95% CI – 95% confidence interval

AGES-Reykjavík – Age, Gene/Environment Susceptibility – Reykjavik study

AL – Amyloid light-chain amyloidosis

BMPC – Bone marrow plasma cells

CIDP – Chronic idiopathic demyelinating peripheral neuropathy

CLL – Chronic lymphocytic leukemia

COVID-19 – Coronavirus disease 2019

CT – Computerized tomography

CZE – Capillary zone electrophoresis

DAG – Directed acyclic graph

DM – Diabetes mellitus

FLC – Free light chains

HC-MGUS – Heavy chain MGUS

HR – Hazard ratio

ICD – International classification of disease

IFE – Immunofixation electrophoresis

IgG/A/M – Immunoglobulin G/A/M

IMWG – International myeloma working group

iStopMM – Iceland screens, treats, or prevents multiple myeloma

LC-MGUS – Light chain MGUS

LUH – Landspítali, the National University Hospital of Iceland

MAG – Myelin associated glycopeptides

MGCS – Monoclonal gammopathy of clinical significance

MGOS – Monoclonal gammopathy of ocular significance

MGRS – Monoclonal gammopathy of renal significance

MGUS – Monoclonal gammopathy of undetermined significance

MM – Multiple myeloma

MRI – Magnetic resonance imaging

NHL – Non-Hodgkin's lymphoma

OR – Odds ratio

PN – Peripheral neuropathy

POEMS - Polyneuropathy, organomegaly, endocrinopathy/edema, monoclonal protein, and skin changes

Pro-BNP – Pro-brain natriuretic peptide

qPCR – Quantitative polymerase chain reaction

SARS-CoV-2 – Severe acute respiratory syndrome coronavirus 2

SMM – Smoldering multiple myeloma

SPEP – Serum protein electrophoresis

SWM – Smoldering Waldenströms macroglobulinemia

TnT – Troponin T

WBLDCT – Whole-body low-dose CT

WHO – World Health Organization

WM – Waldenströms macroglobulinemia

List of Figures

List of Tables

List of Original Papers

This thesis is based on four published papers listed below:

I. Rögnvaldsson S, Steingrímsson V, Turesson I, Björkholm M, Landgren O, Kristinsson SY (2020) Peripheral neuropathy and monoclonal gammopathy of undetermined significance: A population-based study including 15,351 cases and 58,619 matched controls; *Haematologica*; 105(6); p2679-2681

II. Rögnvaldsson S, Aspelund T, Thorsteinsdottir S, Turesson I, Björkholm M, Landgren O, Kristinsson SY (2021) Untangling fracture risk in monoclonal gammopathy of undetermined significance: A population-based cohort study; *European Journal of Hematology*; 107(1); p137-144

III. Rögnvaldsson S, Love TJ, Thorsteinsdottir S, Reed ER, Óskarsson JÞ, Pétursdóttir Í, Sigurðardóttir GÁ, Viðarsson B, Önundarson PT, Agnarsson BA, Sigurðardóttir M, Þorsteinsdóttir I, Ólafsson Í, Þórðardóttir ÁR, Eyþórsson E, Jónsson Á, Björnsson AS, Gunnarsson GÞ, Pálsson R, Indriðason ÓS, Gíslason GK, Ólafsson A, Hákonardóttir GK, Brinkhuis M, Halldórsdóttir SL, Ásgeirsdóttir TL, Steingrímsdóttir H, Danielsen R, Wessman ID, Kampanis P, Hultcrantz M, Durie BGM, Harding S, Landgren O, Kristinsson SY (2021) Iceland screens, treats, or prevents multiple myeloma (iStopMM): a population-based screening study for monoclonal gammopathy of undetermined significance and randomized controlled trial of follow-up strategies; *Blood Cancer Journal*; 11(94)

IV. Rögnvaldsson S*, Eythorsson E*, Thorsteinsdottir S, Viðarsson B, Önundarson PT, Agnarsson BA, Sigurðardóttir M, Þorsteinsdóttir I, Ólafsson Í, Runolfsdottir HL, Helgason D, Emilsdottir AR, Agustsson AS, Bjornsson AH, Kristjansdottir G, Indriðason ÓS, Jónsson Á, Gíslason GK, Ólafsson A, Steingrímsdóttir H, Kampanis P, Hultcrantz M, Durie BGM, Harding S, Landgren O, Pálsson R, Love TJ, Kristinsson SY (2021) Monoclonal gammopathy of undetermined significance and COVID-19: a population-based cohort study; *Blood Cancer Journal*; 11(191)*Equal contribution

In addition, some unpublished data is presented.

All papers are reprinted by kind permission of the publishers.

Declaration of Contribution

The doctoral candidate designed the studies of paper I and paper II, crosslinked datasets and performed statistical analysis, and wrote the manuscripts with input and supervision from Sigurður Yngvi Kristinsson. Statistical advice was received, particularly for paper II, from Thor Aspelund.

The doctoral candidate has been a member of the iStopMM core team since early in the study design period in 2016 and has participated in the design of the study, including writing up the main study-protocol for iStopMM with Sigurður Yngvi Kristinsson, the principal investigator of the study. Furthermore, the doctoral candidate has taken an active part in the implementation of the study, particularly regarding the recruitment of participants and study locations. Paper III was authored by the doctoral candidate with input from multiple co-authors and the data analysis performed by the doctoral candidate. The study in paper IV was written by the doctoral candidate and designed in collaboration with Sigurður Yngvi Kristinsson, and Elías Eyþórsson, who performed the statistical analysis and was co-first author.

1 Introduction

Monoclonal gammopathy of undetermined significance (MGUS) is the asymptomatic precursor of multiple myeloma and related disorders. The benefits of detecting and following individuals with MGUS have not been fully elucidated with the current, limited, evidence indicating that detection of MGUS can lead to early intervention and improved outcomes in multiple myeloma. The promise of such early detection and early intervention might be better realized by utilizing systematic screening for MGUS. Although asymptomatic, MGUS has been associated with various both malignant and non-malignant disorders. However, because of the asymptomatic nature of MGUS, disease association studies are usually heavily afflicted by bias and the extent of these disease associations and their clinical relevance is therefore unclear. This thesis is based on four papers seeking to better clarify the significance of MGUS, particularly its association with non-malignant disease.

The introduction of the thesis is in four chapters. The first chapter describes the definition of monoclonal gammopathies, their detection, and some important symptomatic monoclonal gammopathies. The second chapter describes MGUS, its epidemiology and clinical follow-up in detail, including a discussion on smoldering multiple myeloma. In the third chapter, the rationale for population-based screening for MGUS is discussed and the key limitations of our current knowledge needed to be bridged in order to apply such screening in the real world. In the fourth chapter, the association of MGUS and a number of non-malignant disorders will be discussed and some of the key limitations of previous studies detailed.

1.1 Monoclonal gammopathies

The distinction between monoclonal and polyclonal gammopathy was first determined by Jan Gösta Waldenström who presented his findings in 1961 in the Harvey lecture series. He described patients with narrow bands of hypergammaglobulinemia on serum protein electrophoresis (SPEP) as having monoclonal gammopathy and those with a broad band of hypergammaglobulinemia as having polyclonal gammopathy. [1] These two entities represent the presence of elevated levels of immunoglobulins in the serum. These Y-shaped proteins are normally produced in response to the activation of the humoral immune system, usually during infection.[2]

Figure 1.1 Jan Gösta Waldenström. Image source: Wikimedia commons

Immunoglobulins are made up of heavy chains of 5 types, A, D, E, G, and M, and light chains of either kappa or lambda types. These heavy- and light chains give rise to the classification of immunoglobulins into IgA, IgD, IgE, IgG, and IgM of either kappa or lambda type. Normally, the tips of the Y, termed the variable region, can have multiple permutations and can have affinity to virtually any epitope.[3] This immense repertoire of immunoglobulins is produced by B-cells and mainly one of their terminal offspring, plasma cells, that through various mechanisms undergo scrambling of the DNA coding for the variable region of the immunoglobulin. This gives rise to multiple B cell clones that each produced their own version of immunoglobulins. Proliferation of these B cells and maturation to plasma cells due to infection or other strong antigen stimulation, for example autoimmunity, therefore gives rise to a wide array of different immunoglobulins that are seen as wide bands on SPEP and because they arise from multiple slightly different clones, this is termed *polyclonal gammopathy*.[2]

In contrast, *monoclonal gammopathy* is caused by the production of identical immunoglobulins by a population of immunoglobulin-producing cells originating from the same clone. Because these immunoglobulins are identical, they have the same molecular weight and charge giving rise to the narrow band on SPEP described by Dr. Waldenström.[1] These monoclonal cell populations underlying the monoclonal gammopathy are virtually always malignant cells of the humoral immune system that have stepped outside their normal function and either present as overt malignancy or are precursors to malignancies.ref These malignancies rise from the humoral immune system and include multiple myeloma, Waldenström's macroglobulinemia, amyloidosis, and precursors to those diseases as well as a multitude of rare syndromes.[4] Rarely, monoclonal gammopathy can be caused by autoimmunity and some chronic infections including hepatitis C.[5]

1.1.1 Detection of monoclonal gammopathies

Jan Gösta Waldenström described monoclonal gammopathy on SPEP in 1961.[1] Although SPEP technology has improved since then, the principles of SPEP remain the same. The serum sample is stained for proteins and pulled through a gel using an nelectric current resulting in smaller and more negatively charged proteins being pulled further into the gel. The resulting pattern is a waveform representing different serum proteins (Figure 1.3.A). In the setting of monoclonal gammopathy, a discrete narrow waveform will appear on this pattern and can be detected by inspection representing monoclonal immunoglobulins often termed M proteins or paraproteins (Figure 1.3.B). In cases where monoclonal gammopathy is seen on SPEP, it can be confirmed and typed into immunoglobulin subtypes using immunofixation electrophoresis (IFE). In IFE, multiple serum samples are stained for the different immunoglobulins and drawn through a gel and the monoclonal peak can then be confirmed as monoclonal immunoglobulins and typed.[6]

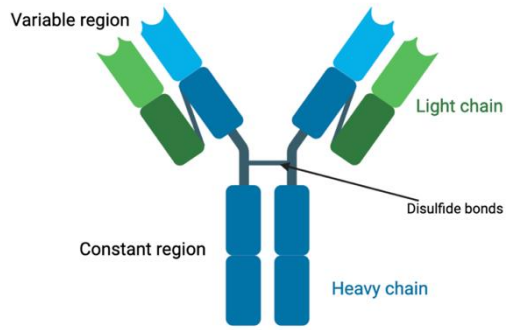


Figure 1.2 The molecular structure of immunoglobulins.

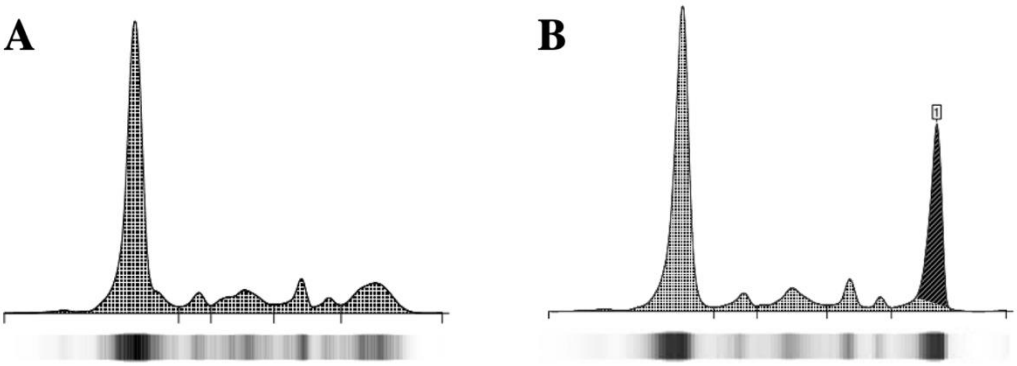


Figure 1.3: Examples of a normal serum protein electrophoresis (SPEP) pattern (A) and an abnormal SPEP pattern with a monoclonal peak in the gamma zone representing an IgG monoclonal immunoglobulin (B).

Free light chains (FLC) are light chains not connected with heavy chains and are normally present in the serum. They are detectable in the serum using a serum FLC assay.[7] Although first believed to be waste products of immunoglobulin production, recent evidence has shown that FLC have various physiologic functions in the normal immune response.[8] Monoclonal FLC are sometimes present alongside whole monoclonal immunoglobulins but can also be the only monoclonal protein present in a monoclonal gammopathy. These monoclonal FLC are rarely identified on SPEP or IFE but are usually identified by a skewed ratio of kappa and lambda FLC in the serum with the monoclonal FLC of either kappa or lambda type skewing the FLC ratio.[9]

Recently, mass spectrometry-based technologies have emerged as a novel method of detecting M proteins and monoclonal FLC directly. The technique is based on the principle that immunoglobulins have differing amino acid sequences and molecular mass. By measuring individual immunolglobulins molecular mass, much smaller M protein peaks can be detected.[10] The clinical utility of this technology and its place in routine care of individuals with monoclonal gammopathies is still unclear. Current recommendations recommend the use of mass spectrometry essentially as a replacement for IFE.[11] But it may have an even greater role in assessing treatment response in monoclonal gammopathies.[12, 13] Even further, the technology may present a new way to detect and diagnose monoclonal gammopathies. However, mass spectrometry is significantly more sensitive for monoclonal immunoglobulins, leading to the detection of very low levels of M proteins that have unknown clinical significance and are transient in most cases.[14]

1.1.2 Multiple myeloma

Multiple myeloma (MM) is the prototypal monoclonal gammopathy. It is a cancer of bone marrow plasma cells characterized by the presence of M proteins and its clinical hallmarks of hypercalcemia, renal disease, anemia, and bone disease (CRAB) or MM defining events (Figure 1.4).[15] The underlying malignant cells are plasma cells that develop from less mature B-cells producing IgM, who undergo *class switching* and the M protein most commonly observed is of IgG and IgA and/or monoclonal FLCs in serum.[15, 16] MM is the second most common hematologic malignancy with MM is the second most common hematological malignancy with an estimated 140,000 new cases worldwide and an age-standardized incidence rate of 2.1 per 100,000 and the incidence has been increasing in recent years due to increasing age in the population. The current incidence of MM has been estimated to be ~5-7 per 100,000 person years in the Western world[17] MM is caused by the proliferation of malignant, monoclonal plasma cells in the bone marrow that have accrued genetic aberrations, including chromosomal abnormalities. No specific genetic abnormalities have however been identified as driver mutations in MM.[18]

Historically, MM has carried a very poor prognosis with a median survival of 1-2 years after diagnosis. Prognosis improved somewhat in the latter half of the 20th century due to the advent of melphalan and steroids. However, with the advent of novel therapies around the turn of the 21st century, survival has improved dramatically.[19]

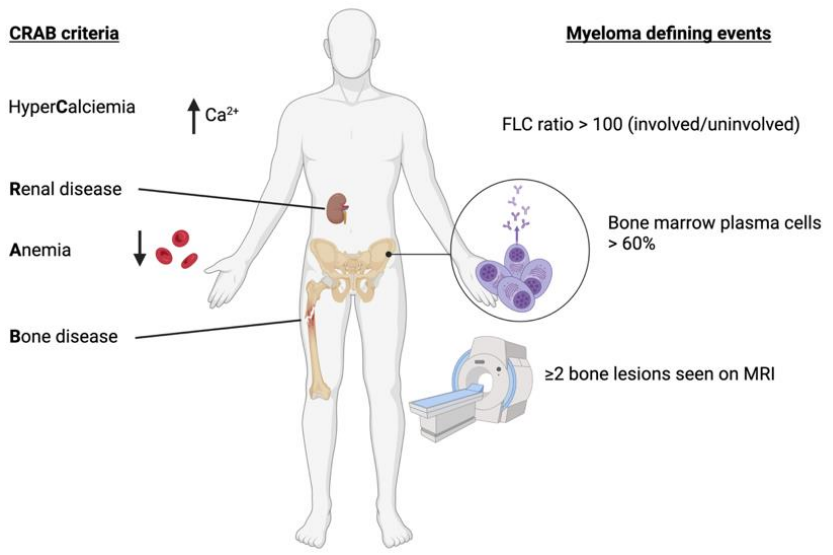


Figure 1.4: Diagnostic criteria for active multiple myeloma. FLC: Free light chain; MRI: Magnetic resonance imaging.

1.1.3 Waldenströms Macroglobulinemia

Waldenströms Macroglobulinemia (WM) is a related disorder of MM caused by the accumulation of malignant lymphoplasmacytic lymphocytes in the bone marrow. Although the cell of origin for WM is not fully known it is believed to derive from B memory cells in most cases.[20] These cells do not undergo class switching and WM is therefore defined by the presence of IgM M proteins in serum and infiltration of lymphoplasmacytic lymphocytes in the bone marrow alongside symptoms of WM which include lymphadenopathy and splenomegaly, anemia, neuropathy, hyperviscosity syndrome, cryoglobulinemia, and common symptoms of lymphomas and other malignancies including weight loss, fevers, and night sweats.[21] The disease is commonly associated with a mutation in the gene MYD88 and usually has a relatively indolent course and often does not always require therapy at the time of diagnosis.[22]

1.1.4 Amyloid light chain amyloidosis

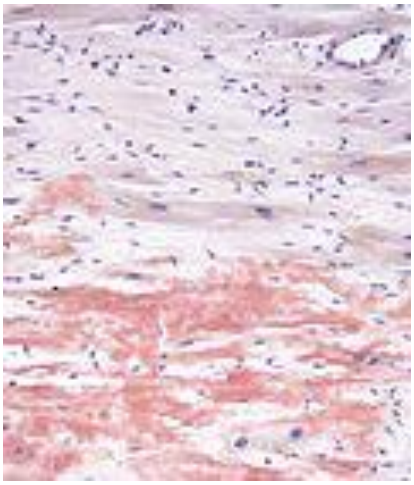


Figure 1.5 Amyloid depositions in cardiac muscle, stained using Congo red. Image source: Wikimedia commons, Nephron

Amyloid light-chain amyloidosis (AL) is caused by abnormal folding of normally soluble light chains and their aggregation in organs leading to organ dysfunction.[23] Most frequently this is due to the production of monoclonal lambda light chains and can be present alongside other monoclonal gammopathies including MM and WM but can also present in the absence of MM or WM symptoms. The symptoms of AL include cardiomyopathy, nephropathy, neuropathy, skin disease and more, depending on the organs involved. AL is a tissue diagnosis requiring a biopsy showing amyloid aggregates on congo red staining.[24] This and the wide clinical spectrum of AL often leads to late diagnosis of AL and despite advances in therapy, the prognosis of the disease remains poor.[25]

1.2 Monoclonal gammopathy of undetermined significance (MGUS)

Monoclonal gammopathy of undetermined significance (MGUS) is the asymptomatic precursor condition of other advanced monoclonal gammopathies, preceding all cases of MM,[26, 27] AL,[28] and WM as well as preceding some cases of chronic lymphocytic leukemia (CLL) and non-Hodgkins lymphomas (NHL).[29] MGUS is defined by the presence of M proteins in the serum or an abnormal FLC ratio in the absence of symptoms or biomarkers consistent with other more advanced monoclonal gammopathies described above.[30]

It was Jan Gösta Waldenström, who first described MGUS when in 1961 he described a series of patients with a narrow band of hypergammaglobulinemia as having monoclonal proteins. Many of these patients had MM but others had no signs of overt malignancy and had what he called essential hypergammaglobulinemia or benign monoclonal gammopathy.[1] The term MGUS was later coined and further defined by Robert A. Kyle at the Mayo Clinic in the United States who in 1978 described 241 cases of MGUS and showed that a significant proportion of these individuals progressed to more advanced disease.[31]

MGUS can be further subdivided by the type of monoclonal antibody present. Non-IgM MGUS, the most common subtype of MGUS, precedes MM and amyloid light chain

(AL) amyloidosis.[26-28] IgM MGUS is a less common and distinct disease entity from non-IgM MGUS caused by the presence of malignant mature B lymphocytes rather than malignant plasma cells. As a result, IgM MGUS precedes WM, chronic lymphocytic leukemia (CLL), and non-Hodgkin's lymphoma (NHL) and does not precede MM.[29] Light chain(LC) MGUS, is the most recently described subtype of MGUS. LC MGUS is characterized by the presence of an abnormal ratio of free kappa and lambda light chains without the presence of monoclonal heavy chains. Similar to non-IgM MGUS, LC MGUS is caused by the presence of malignant plasma cells and precedes LC MM and AL amyloidosis.[9, 28]

1.2.1 Prevalence of MGUS

MGUS is common in the general population. In 2006, the prevalence of MGUS was found to be 3.2% in a screened cohort in Olmstead County in Minnesota in the United States. [32] An Additional 1% was later detected with the addition of LC-MGUS in 2010,[9] bringing the total prevalence of MGUS to 4.2% over the age of 50. Importantly, the prevalence of MGUS was found to be highly dependent on age and its prevalence increases significantly with age ranging from 2.2% in those aged 50-59 years and 8.7% in those aged 80-89 years. Additionally, males had a close to 50% higher age-adjusted incidence of MGUS (Figure 1.6).[32]

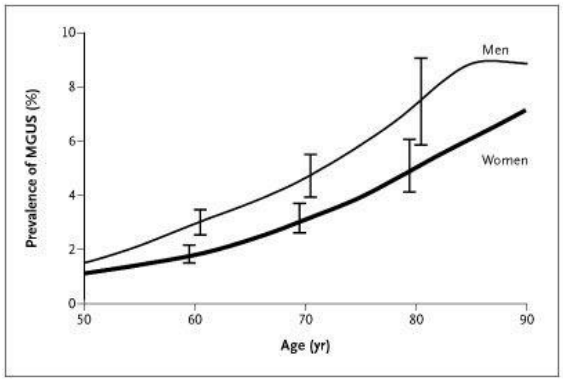


Figure 1.6: Figure from Kyle et al. (2006) depicting the rate of monoclonal immunoglobulins in the residents of Olmestead county, MI in the US by age and sex.[32]

The prevalence of MGUS also varies considerably by ethnicity. The previously cited prevalence figures from Olmstead County were acquired in a population primarily of Scandinavian descent. However, MGUS has been estimated to be three times more common in black individuals than white individuals in the US[33] and the prevalence of MGUS in men in Accra, Ghana over the age of 50 was found to be 5.84% compared to the age-adjusted prevalence of 2.97% in Olmstead county.[34] Conversely, the prevalence of MGUS is significantly lower in Asian populations with 1.11% of residents in Beijing, China over the age of 50[35] and 1.7% in an outpatient hospital-based cohort in India over the age of 50 having MGUS.[36]

Despite being common, due to its asymptomatic nature, MGUS usually goes undetected. In fact, less than 10% of those who develop MM have known MGUS before

being diagnosed with MM.[37-39] This makes population-based screening necessary to accurately estimate the prevalence of MGUS.

1.2.2 MGUS progression

The main clinical implication of MGUS is the risk of it developing into overt malignant disease, including MM, WM, AL, and NHL. Overall, the risk of progression is 0.5-1.5% per year.[9, 29, 40, 41] However, subsets of the MGUS population have been identified that are at a higher and lower risk of progression. Current guidelines for MGUS follow-up are based on the risk criteria developed at the Mayo Clinic in the US.[30] The risk score gives equal measure to an M protein ≥15 g/L, an abnormal FLC ratio, and non-IgG isotype. Individuals are classified into low, low-intermediate, high-intermediate, and high risk MGUS by whether they have 0-3 of these risk factors. The risk of progression within 20 years in these subgroups is 2, 10, 18, and 27% respectively after accounting for death as a competing risk.[42]

	MGUS	**SMM**	**Active MM**
Criteria	M protein or abnormal FLC ratio **and** No symptoms or markers of active MM	BMPCs ≥ 10% **or** M protein ≥ 3g/dL **and** No symptoms or markers of active MM	Hypercalcemia Anemia Renal disease Lytic bone lesions on CT or X-ray **Or** 1 or more myeloma defining event*
Progr-ession	0.5-1.5% per year	10% per year for 5 years 3% per year for 5 years 1% per year after that	NA

**: BMPCs ≥60%, FLC ratio ≥100, or ≥2 bone lesions or MRI. MGUS: Monoclonal gammopathy of undetermined significance; SMM: Smoldering multiple myeloma; MM: Multiple myeloma; FLC: Free light chain; BMPCs: Bone marrow plasma cells; CT: Computerized tomography.*

Table 1.1: The diagnostic criteria for MGUS, SMM, and MM with MM[30,15] as well as the associated risk of progression for MGUS and SMM.[9,29,40,41,44]

A more advanced disease state on the MGUS to MM spectrum was defined in 1980 by Kyle and Greipp as smoldering multiple myeloma (SMM). They described 6 patients with bone marrow plasma cells (BMPCs) ≥10% who remained asymptomatic and stable for ≥5 years without therapy.[43] This "disease", which in truth is more like a risk category of MGUS, has since been further characterized and is currently defined as ≥10% BMPCs or ≥30g/L of M-proteins in the absence of the clinical signs of active MM.[30] SMM carries a significantly higher risk of progression to active MM than MGUS with 10% progressing every year for the first 5 years, then 3% per year the 5 years after that, and then 1% per year after that.[44] Two separate risk stratification criteria have been developed to further stratify SMM patients by risk of progression. The Mayo 2/20/20 criteria is based on measurements of M proteins, FLCs, and BMPCs while the Spanish criteria is based on flow cytometry results and immunoparesis.

The underlying causes of MGUS progression have not been fully elucidated. The genetic changes observed in clonal cells in individuals with MGUS are similar to those in patients with MM, including chromosomal aberrations like IgH translocations and hyperdiploidy.[45] Although the number of mutations increases from precursor to active disease and there are some specific mutations that are more common in MM than in MGUS, including those in the RAS and MYC genes,[46, 47] the main differences seem to be the population size and dominance of the bone marrow.[48] It is clear that cell level alterations and DNA mutations are not sufficient to achieve this and it has become clear that changes in the bone marrow microenvironment and particularly the cells of the immune system that reside there are one of the main drivers of MGUS progression or stabilization.[49]

1.2.3 MGUS follow-up and treatment

Currently, treatment is not recommended in individuals with MGUS but rather indefinite follow-up to detect progression, at which time treatment can be initiated.[30] Although there are no randomized trials evaluating the benefits of this follow-up, at least three observational studies from Sweden and the United States have shown that a diagnosis of MGUS prior to MM diagnosis is associated with 13-15% improved survival with MM.[37-39] These repeated observations may indicate that MGUS follow-up and early detection of MGUS progression to MM improves survival. Particularly because MM has been associated with a considerable diagnostic delay of 3-6 months at which time end-organ damage may develop that is not reversible and may limit the application of effective therapies.[50, 51] However, these findings may also, at least in part, be explained by lead-time bias and there is still considerable uncertainty on the benefits of MGUS follow-up.

Current guidelines for the assessment and follow-up of individuals with MGUS were published in 2010 by the International Myeloma Working Group (IMWG).[30] These recommendations are mostly based on expert opinion rather than clinical studies. The

initial assessment of individuals with MGUS is focused on ruling out active MM or related disorders by clinical assessment and exam as well as by basic blood testing, including a complete blood count, serum calcium and creatinine measurements as well as testing for proteinuria. Repeat SPEP and FLC assay is then recommended 3-6 months after the initial diagnosis of MGUS to detect those who are on a fast trajectory towards active disease. In those who have lowrisk MGUS, no further testing is recommended unless there are signs of active disease and follow-up is recommended after 2-3 years. In those with non-low risk MGUS bone marrow sampling and imaging of the bones is recommended at baseline and annual follow-up thereafter (Table 1.2).[30]

	Low risk MGUS	**Non-Low risk MGUS**	**IgM MGUS**	
Criteria	IgG isotype **and** M-protein <15g/L **and** Normal FLC ratio		Non-IgG isotype **or** M-protein >15/L **or** Abnormal FLC ratio	IgM isotype
Initial assessment	Clinical evaluation and blood testing. Repeat SPEP and FLC.		Clinical evaluation and blood testing. Repeat SPEP and FLC	Clinical evaluation and blood testing. Repeat SPEP and FLC
Bone marrow sampling	If clinically indicated		Yes	Yes
Imaging	If clinically indicated		WBLDCT	CT of the abdomen
Follow-up	In 2-3 years		Annual	Annual

Table 1.2: Current guidelines for the initial assessment and follow-up for individuals with MGUS. MGUS: Monoclonal gammopathy of undetermined significance; FLC: Free light chain; SPEP: Serum protein electrophoresis; WBLDCT: Whole-body low-dose computeried tomography; CT: Computerized tomography.

Imaging has historically been mostly with plain radiographs of the bones to detect lytic bone lesions although guidelines do recommend computerized tomography (CT) of the abdomen in individuals with IgM MGUS. In 2020, new guidelines for imaging in monoclonal gammopathies were published that recommend whole body low dose CT (WBLDCT) in those with non-IgM MGUS. The method is a more sensitive method to detect lytic bone lesions and it has rendered plain radiographs obsolete in the follow-up of individuals with MGUS. In cases of equivocal results on WBLDCT, whole-body MRI is recommended to detect myelomatous lesions with even greater sensitivity.[52]

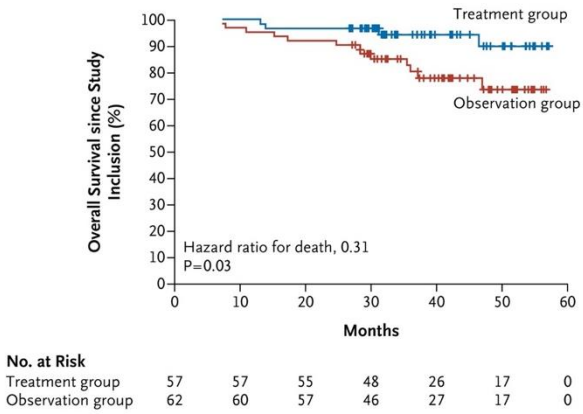


Figure 1.7: Figure from Mateos et al. (2013) comparing overall survival in patients with SMM who receive and dont receive lenalidomide and dexatmethasone in a randomized trial.[56]

In those who meet the criteria for SMM, a more aggressive follow-up strategy is recommended.[30] This includes bone marrow sampling and imaging at baseline and much closer follow-up every 4-6 months. Furthermore, an annual MRI is recommended for those with negative or inconclusive WBLDCT.[52] Whether individuals with SMM should receive treatment has been the subject of debate but has been gaining favor in the last few years. The idea of treating MM early at the stage of SMM has been an attractive idea for some time but multiple early studies have failed to show a survival benefit.[53-55] In 2013, Mateos and colleagues published a landmark trial comparing therapy with lenalidomide and dexamethasone to observation in patients with high-risk SMM. They found that therapy markedly improved progression free survival, but more importantly also found an improved overall survival benefit (Figure 1.7) in those who received treatment.[56] These findings were further supported by Lonial and colleagues, who in 2020 published the results of a randomized trial of lenalidomide monotherapy and observation in high risk SMM. They found an improved progression-free survival but the trial was stopped early due to efficacy and an overall survival benefit was not observed during the resulting shorter study period.[57] Taken together the findings strongly indicate that early treatment for MM at the stage of SMM can improve outcomes in MM and although there are some ongoing studies on the issue, many in the MM scientific community believe that it is time to routinely treat patients with high risk SMM.[30, 58, 59] In fact, in 2014 an entity previously known as "ultra-high risk" SMM defined by BMPCs ≥60%, FLC ratio ≥100, or ≥2 bone lesions on MRI, were included into the definition of active MM and affected patients are considered eligible for treatment.[15]

1.3 Screening for MGUS

Although all cases of MM are preceded by MGUS,[26, 27] it is usually not recognized before progressing into active disease. Instead, MM patients have symptomatic end-organ damage for a median of 3-6 months before diagnosis.[50, 51] This end-organ damage is often irreversible and may limit the application of effective therapies at diagnosis of MM. Intervening at an earlier stage could, at least theoretically, prevent this end-organ damage and significantly improve outcomes in MM. Observational studies have indicated that prior knowledge of MGUS improves outcomes in MM, possibly due to early intervention.[37-39] Further evidence has emerged from randomized clinical trials showing that treating asymptomatic individuals with SMM improved progression-free and overall survival.[56, 57] However, less than 10% of MM patients have previously known MGUS at diagnosis, severely limiting the availability of early treatment in MM.[37-39] This poses the question whether it may be appropriate to utilize population-based screening for MGUS to identify those who may benefit from early treatment. No studies have been conducted on population-based screening for MGUS and clinical relevance of MGUS detected by population-based screening is not known and the benefits and potential harms of such screening have not been assessed. Here we'll discuss cancer screening and when it is appropriate and then discuss the possibility of population-based screening for MGUS.

1.3.1 Cancer screening

Figure 1.8: US president Richard Nixon signing The National Cancer Act of 1971 into law in the US which introduced screening as one of the cornerstones of cancer care. Image source: National Cancer Institute

Cancer screening has been one of the cornerstones of cancer care since the introduction of the "War on Cancer" National Cancer Act into law in the United States in 1971.[60] Cancer screening can be classified into two different categories based on the aims of screening.[61]

Preventative screening aims to detect precursor conditions that may be treated, thereby preventing the development of overt malignancy. Examples of preventative cancer screening include screening for cervical and colorectal cancer. For these cancers, screening

leads to the detection of early malignant changes like cervical in situ neoplasia and colorectal polyps that can be removed, preventing their development into invasive malignancy. *Early detection screening* aims to detect cancer that has already developed at an early, most likely local, stage. Examples of early detection cancer screening are screening for breast, lung, and prostate cancer. In these diseases. The idea in these cases is that early-stage cancer is easier to treat, particularly in these solid tumors and that removal of a local tumor, for example by breast lumpectomy, may prevent its development into metastatic disease.

Current guidelines recommend early detection screening for breast cancer in adult women and lung cancer in adults with a significant history of smoking as well as preventative cancer screening for colorectal cancer in all adults and cervical cancer in adult women. Additionally, early detection prostate cancer screening is recommended in men who have been informed about the potential benefits and harms of screening and prefer such screening.[62]

1.3.2 When is screening appropriate?

For cancer screening to be implemented, the benefits of screening need to outweigh the harms, both on an individual level and on the societal level. Although in principle, the benefits of cancer screening are obvious they may not always materialize, and screening may also carry significant harm. That is why cancer screening, particullary early detection cancer screening, is becoming increasingly controversial.[63]

The potential harms of cancer screening can be considerable and warrant discussion. Firstly, cancer screening, particularly early detection cancer screening can lead to overdiagnosis and overtreatment through false positives and treatment of disease that would never have become clinically evident. For example, it has been estimated that 15-30% of invasive ductal carcinoma in situ diagnoses in women offered screening represent overdiagnosis.[64, 65] In the UK it has been estimated that breast cancer screening leads to 129 in 10,000 women (~1%) experience overdiagnosis and harm in order to prevent 42 deaths per 10,000 women.[66] There is also considerable evidence of overdiagnosis in relation to prostate cancer screening with up to 50% of cases representing overdiagnosis.[67] However, by limiting screening to younger age groups and by initially reacting with "watchful waiting", that is, moving more towards preventative screening, the rate and associated harms of overdiagnosis can be significantly attenuated.[68, 69]

Secondly, knowledge of early cancer or precursor conditions may lead to anxiety, a decreased sense of well-being, and lower quality of life. Considerable evidence has shown that a cancer diagnosis lowers quality of life and increases the incidence of psychiatric illness.[70] Patients with MM for example have been found to have higher rates of self-harm and suicide.[71] However, it may be difficult to discern the effects of the underlying cancer and treatments and the psychological trauma of the cancer

diagnosis. One way to better isolate this psychological effect is to study those that have a false-positive result and never undergo treatment. In the short term, false positive cervical cancer screening results have been associated with a decreased quality of life.[72] However, longer term studies have shown that this effect is likely transient and will normalize in the longer term.[73-75]

Thirdly, population-based cancer screening can lead to increased costs for society and increased utilization of already strained healthcare systems. For example, in one assessment, CT scans were estimated to be increased by 25% in England if lung cancer screening were to be implemented from 2 million to 2.5 million scans per year.[76] Similarly, 6.3 million screening colonoscopies were estimated to have been performed in 2012 in the US, of which an estimated 17-25.7% were not even indicated according to guidelines at the time.[77] There is however, an extensive literature showing that cancer screening for breast, colorectal, and cervical cancer is highly cost-effective by decreasing the need for more extensive treatment and preventing death.[78-80] However, many of these studies are likely to have overestimated the cost-savings of cancer screening by not accounting for quality of life losses and the cost of competing risks incurred due to screening, particularly due to prolonging life leading to more aging-related disorders (for example dementia, cardiovascular disease, and other cancers) that increase healthcare costs. Despite this, most current cancer screening programs are either cost-saving or have acceptable costs.[81]

In 1968, Wilson and Jungner, on behalf of the World Health Organization (WHO), developed and published a 10-item criteria for when screening for disease is appropriate (Table 1.3).[82] The Wilson and Jungner criteria has since become an accepted framework to assess screening programs, including cancer screening programs. Although it has stood the test of time, with the advent of novel technologies, changing societal norms, and new healthcare standards, ten additional criteria, termed "emerging screening criteria" were set forth by Anderman and colleagues and published by the WHO (Table 1.4).[83] These 20 items make up a useful framework for determining whether cancer screening is appropriate.

1.3.3 Is screening appropriate in MGUS?

Screening could detect MM and related disorders at its asymptomatic precursor state of MGUS vastly expanding the availability of early treatment in MM which may prevent the development of active MM. MGUS screening could therefore be considered a form of preventative screening. Most of the criteria of both the original and emerging criteria from the WHO on disease screening are already fulfilled in MGUS screening while further studies are needed to answer critical questions regarding the appropriateness of MGUS screening (Table 1.3).

MGUS is a recognizable early stage in MM and related disorders that is rarely detected and there is an emerging accepted treatment for recognized disease, that is, high-risk SMM, fulfilling original criteria 2,4, and 8 as well as emerging criteria 1 and 2. Furthermore, the natural history of MGUS, SMM, and MM is relatively well understood due to previous long-term studies for example, in Olmstead county in the US.[40]

Original criteria	**Fulfilled in MGUS**
1. The condition sought should be an important health problem	Yes
2. There should be an accepted treatment with recognized disease	Yes
3. Facilities for diagnosis and treatment should be available	Yes
4. There should be a recognizable or early symptomatic stage	Yes
5. There should be a suitable test or examination	Yes
6. The test should be acceptable to the population	Yes
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood	Yes
8. There should be an agreed policy on whom to treat as patients	Yes
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditures on medical care as a whole	Probably
10. Case finding should be a continuing process and not a "once and for all" project	Yes
Emerging screening criteria[83]	
1. The screening program should respond to a recognized need	Yes
2. The objectives of screening should be defined at the outset	Yes
3. There should be a defined target population	No
4. There should be scientific evidence of screening program effectiveness	No
5. The program should integrate education, testing, clinical services, and program management	Practical issue
6. There should be quality assurance, with mechanisms to minimize potential risks of screening	Practical issue
7. The program should ensure informed choice, confidentiality, and respect for autonomy	Practical issue
8. The program should promote equity and access to screening for the entire population	Practical issue
9. Program evaluation should be planned from the outset	Practical issue
10. The overall benefits of screening should outweigh the harm	Unknown

Table 1.3 : The original Wilson and Jungner- and the emerging screening criteria set forth by the World Health Organization (WHO) and whether the crietria are fulfilled in MGUS.

Although MM is relatively rare with an incidence of ~5-7 per 100,000 person-years in the Western world[17] it still remains a significant cause of mortality and morbidity. MM often leads to significant symptoms leading to decreased physical functioning and a considerable burden of disease for patients and their families.[84] The costs of MM care are also significant with the average lifetime cost of myeloma therapy per patient recently estimated at 184,495$ in the US.[85] Therefore, MM is an important health problem (original criterion 1) and due to the high cost of care, screening is likely to carry economic benefits although there have been no studies on the subject to date (original criteria 9).

The screening test is a simple blood test which is acceptable to the population and highly repeatable (original criteria 5 and 10). SPEP and IFE are sensitive to the detection of M proteins that are clinically relevant and precede MM.[26, 27] The FLC assay is also sensitive to light-chain MGUS but is an indirect measure of monoclonal FLC and is affected by age and kidney function.[86] Furthermore, an increasing FLC ratio in the general, termed "kappa-drift" has been observed in some laboratories.[87] The FLC assay may therefore not be as specific as the SPEP and IFE. However, these tests can be repeated with ease causing limited discomfort and very low risk of complications. Furthermore, treatment is not indicated in those who are not found to have SMM or MM limiting the harms of overdiagnosis in those who have a false-positive test.

Some of the other criteria including emerging criteria 5, 6, 7, 8, and 9 are more practical and organizational and do not require scientific debate. However, important questions remain unanswered. Most importantly, there is currently no scientific evidence on the benefits and harms of screening for MGUS and the disorders it precedes (emerging criteria 4). It is therefore not possible to assess whether the benefits of screening outweigh its potential harms (emerging criteria 10). Although there is considerable evidence that early intervention can delay the development of active MM and improve survival (see chapter 1.2.3), it is not clear how these benefits would translate into improved outcomes for individuals diagnosed with MGUS duing screening. Furthermore, the harms of screening have not been sufficiently studied. Potential harms include overtreatment, exposure to diagnostic tests including radiation, and the effects of knowing about a precursor condition on psychiatric health and sense of wellbeing. In some healthcare systems, a diagnosis of MGUS could lead to significant "financial toxicity" since the costs of follow-up, diagnostic testing, and treament can be significant and long-term. Extensive studies are needed to answer these critical questions.

Finally, no target population for screening has been defined (emerging criteria 3). This is of particular importance since the prevalence of MGUS is highly dependent on age and ethnicity. The median age at MM diagnosis is around 70 and any cancer screening program will therefore have to target a population that is younger than that to capture the majority of cases before they become overt MM. However, only 1% of MM cases

are diagnosed before the age of 40[88, 89] and the yield of screening is likely to be very low. In those who are older, diagnosing a precursor disease that is unlikely to progress during their shorter remaining lifespan or will progress to an advanced precursor which treatment they may not be fit for, is likely to lead to more benefit than harm. Patients with MM who are of African descent have a lower median age of 66 at the time of MM diagnosis[90] and the prevalence of MGUS is higher.[34] The target population for screening may therefore be different in this population. Even further, a family history of MM is associated with a more than 2-fold risk of MM[91] may therefore be needed to be taken into account. An integrated risk assessment including age, ethnicity, and family history of MM and related disorders will probably be required to identify individuals for whom MM screening is appropriate. Crucially, the target population should not be defined solely based on the prevalence of MGUS but rather based on the clinical significance of the MGUS that may be discovered. Screening is of no more use in a population with a high or low prevalence of MGUS if the utility of knowing about MGUS is the same for both groups, the population simply has different numbers of individuals with the same ratio of benefits and harms from screening. This is because screening itself does not carry significant risks or costs but rather the clinical testing, follow-up, and interventions that may follow. Furthermore, it will be important to identify and take into account individual preferences with regard to wanting to know about a precursor condition and in some cases, having the financial means to stay in long-term follow-up for that precursor, these priorities will differ across individuals, cultures, and healthcare systems.

Taken together, there are many signs that MGUS screening could provide real benefits and might lead to a paradigm shift in MM care to early detection and intervention, preventing active disease and improving survival and quality of life. However, there are critical questions that remain unanswered and there is not sufficient evidence to start systematic population-based or targeted screening for MGUS. Current evidence is mostly made up of observation studies and studies of MGUS, SMM, and early MM that are detected clinically and their disease burden may not always represent the disease burder found in a screened population. The evidence is therefore lacking to impliment such a consequential population-based measure as screening and large, randomized studies are required to answer important questions that remain unanswered.

1.4 Disease associations of MGUS

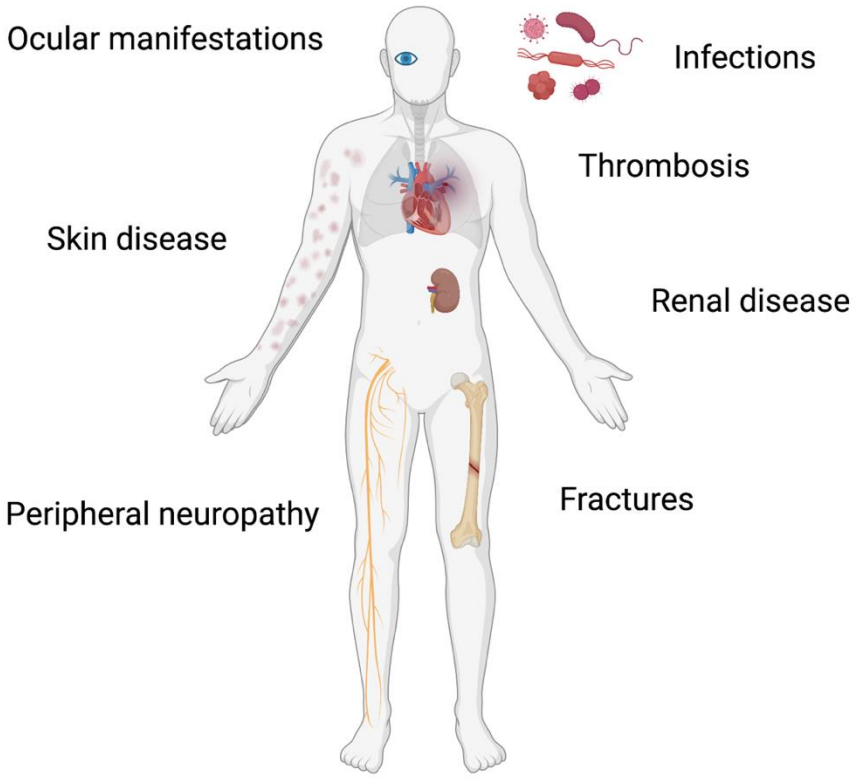


Figure 1.9: An illustration of some of the main non-malignant disorders that have been associated with MGUS.

The main clinical implication of MGUS is the risk of progression to MM or related disorders. However, multiple studies have implicated MGUS as a possible cause of multiple other medical problems. In some of these cases, histopathological evidence has shown a role of monoclonal immunoglobulins or light chains in the pathological mechanisms of the disease. But in other cases, these associations are based on observations of co-occurence of MGUS and these disorders. When associated with disease, MGUS has been termed monoclonal gammopathy of clinical significance (MGCS).[92] Importantly, MGCS and similar terms referring to specific disease associations of MGUS, are not diseases, but rather a concept where MGUS is viewed as having actual significance by causing non-malignant complications that are the actual underlying diagnosis. The term MGCS has particularly been applied to disorders of the kidney, skin, nerves, and certain rheumatological disorders with relatively clear associations with MGUS. However, MGUS has also been found to have more vague

associations with diseases like fractures,[93] infections,[94] and thrombosis.[95] Some of these assocations will now be briefly discussed and the limitations of our current knowledge emphasized.

1.4.1 Peripheral neuropathy

Peripheral neuropathy (PN) is characterized by pain and/or sensory and motor deficits brought on by impairment of peripheral nerve function. Sub-clinical PN is common in the general population, especially in the elderly, where the estimated prevalence of PN is around 7%.[96] Although there are many known causes of PN, ranging from nutritional deficiencies or side-effects of medications to various systemic diseases, PN is most commonly caused by diabetes mellitus (DM) where it is a significant contributing factor in DM morbidity. As a result, patients with DM are routinely screened for symptoms of PN.[97]

A considerable portion of individuals with MGUS have been reported to develop PN. The prevalence of PN among individuals with MGUS varies greatly in the literature and has been reported to be from 3% to 70%.[98-105] However, the studies to date have mostly been small, retrospective, or include outdated criteria for the diagnosis of MGUS. Most studies on the association of MGUS and PN have been performed on clinical cohorts where MGUS is diagnosed incidentally. In one study, including a screened MGUS population, using registry-based data on 605 MGUS cases in Olmsted County in Minnesota, the authors did not find an association between MGUS and PN.[104]

Due to the high prevalence of PN and MGUS, it can be difficult in practice to ascertain whether MGUS is the cause of a patient's PN. Neuropathy is more common in IgM MGUS than in non-IgM MGUS.[106] Over 50% of PN in IgM MGUS is associated with the production of anti myelin-associated glycopeptide antibodies (anti-MAG). Anti-MAG binds to myelin on peripheral nerves leading to a relatively mild demyelinating neuropathy typically characterized by distal sensory and sensorimotor symptoms with unsteadiness and tremor.[107] Other IgM MGUS PN has been associated with some other auto-antibodies, for example, antiganglioside GM1 and GD1b antibodies leading to multifocal motor neuropathy, IgM deposition in peripheral nerves, and in some cases no specific cause is found.[107] In non-IgM MGUS PN, causality has not been as clearly defined. A common presentation is with symptoms and neurophysiological findings of chronic idiopathic demyelinating peripheral neuropathy (CIDP) with detectable M proteins in the serum. This may in many cases be CIDP in an individual with an incidental M protein but in some cases, these develop into amyloidosis, POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy/edema, monoclonal protein, skin changes), or other plasma cell disorder which proved to be the underlying cause.[108, 109] Treatment is limited to treating the underlying disorder and intravenous immunoglobulins or plasmapheresis in some cases.[110] Importantly, PN can also be a feature of active advanced disease rather than MGUS. This includes Waldenströms

macroglobulinemia and amyloidosis as well as radiculopathy in relation to pathologic fractures in MM.[111]

The effects of PN on clinical outcomes in MGUS have not been sufficiently studied. In one study from Austria, the authors found a non-significant association between PN and MGUS progression to active disease.[102] However, this was a single-center, retrospective study including only 223 individuals with MGUS and 36 with PN. There are multiple studies linking MGUS PN to a decreased quality of life that decreases even further as symptoms deteriorate.[112-114] PN has been associated with falls and fractures when associated with other disorders, this is logical since the loss of sensation, particularly the loss of proprioception, often leads to sensory ataxia.[115] MGUS PN specifically has, however, not to our knowledge, previously been evaluated as a cause of falls and fractures.

1.4.2 Fractures

Bone disease is a clinical hallmark of MM but MM patients have an increased risk of fractures in the years preceding the diagnosis.[116] Some of these fractures are likely due to MM-related bone disease but it is unlikely that an increased fracture risk more than one year before MM diagnosis is due to undetected MM bone disease. Two studies from the US and Sweden based on clinical population-based MGUS cohorts found that individuals with MGUS had an increased risk of fractures, particularly axial fractures, similar to MM.[117, 118] These findings were further corroborated in a population-based study in Iceland including individuals screened for MGUS from the Age, Gene/Environment Susceptibility – Reykjavik study (AGES-Reykjavik). In the study, which was smaller than the previous studies, fracture risk was increased for males with MGUS and not for females, and the overall fracture risk did not reach statistical significance.[93]

The cause of this fracture risk in MGUS has not been fully elucidated. Early studies showed high rates of MGUS among individuals with osteoporosis indicating that perhaps MGUS led to systemic bone density reduction, paving the way for local bone erosions, that is, lytic lesions in MM.[119] However, bone density was compared between individuals with MGUS and controls without MGUS, as determined by screening, in the previously mentioned AGES-Reykjavik cohort, bone density was not found to be different between the two groups. Instead, bone volume was found to be greater in those with MGUS suggesting abnormal bone structure and architecture rather than decreased density as the cause.[93] Furthermore, studies using high-resolution CT scans of the bones of individuals with MGUS have shown increased cortical porosity, decreased cortical and trabecular thickness, increased endocortical area, and decreased bone strength despite the bones being slightly larger.[120, 121] Taken together these findings indicate that MGUS is not associated with osteoporosis but rather an abnormal bone architecture leading to increased fracture risk.

1.4.3 Infections

Several studies have assessed infection risk in individuals with MGUS. In a paper from 1998, Gregersen et al observed 40 cases of bacteremia among 1,237 individuals with MGUS in Denmark between 1981 and 1993 and found a 2.2-fold increased risk of infections in this group.[122] These findings were corroborated in a cohort of 605 individuals with MGUS where an increased risk of spontaneous bacterial peritonitis, mycobacterium infection, and upper respiratory infection.[104] In the largest population-based study to date in Sweden, including 5,326 individuals with MGUS, these findings were even further validated. The authors observed a 2.2-fold risk of infections in general with a 2.2-fold risk of bacterial infections and 2.7 fold risk of viral infections. Several specific infections were observed to be associated particularly associated with MGUS including osteomyelitis and meningitis, but overall infection risk was higher for all observed infection sources in the study.[94]

This increased risk of infections is likely due to the abnormal humoral and cellular immunity previously reported in MGUS. Measurements of normal immunoglobulins show hypogammaglobulinemia in up to 25% rate of individuals with MGUS[32, 122] with the rate going being even higher in SMM.[123] Additionally, differences in the T cell compartment have also been observed in those with MGUS compared to healthy controls. This immunodeficiency observed in individuals with MGUS are, however, milder than those observed in MM and data on immune dysfunction in MGUS are not as robust as that for MM.[124] Furthermore, the association of infections and MGUS has mostly been studied in clinical cohorts with MGUS where other disorders, including infections, autoimmune disease, and more are likely to be selected for. The pathologic mechanisms of these disorders or their treatment may have affected the observed immune dysfunction and increased infection risk.

In late 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was detected for the first time. The virus and coronavirus disease 2019 (COVID-19) has since become a global pandemic leading to at least 476 million cases and 6.1 million deaths worldwide as of late March 2022.[125] The spectrum of disease is highly variable from asymptomatic to severe disease with multi-organ failure. Various risk factors for severe COVID-19 have been identified, including comorbidities like cancer.[126] MM has been shown to be associated with a particularly high risk

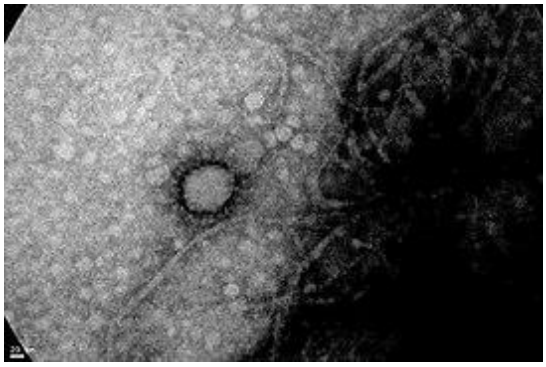


Figure 1.10: An image of the SARS-CoV-2 virurs that causes COVID-19 taken by electron microscopy. Image source: Wikimedia commons, ANLIS - Malbrán

of severe COVID-19 with disease factors being drivers of severe COVID-19 risk including immune dysfunction, rather than treatment-related factors.[127-130] Because similar immune dysfunction has been observed in MGUS, it is plausible that those with MGUS also have higher risks during the COVID-19 pandemic.[131] Earlier studies are sparse with one cases series presenting seven COVID-19 cases in individuals with MGUS with one fatality.[132] However, prior to the work in this thesis, no systematic data had been published on the matter.

1.4.4 Renal disease

Renal disease is one of the clinical hallmarks of MM but can also present alongside MGUS leading to a clinical phenomenon termed monoclonal gammopathy of renal significance (MGRS).[133] According to the current definition by the international kidney and monoclonal gammopathy research group, MGRS applies to any B cell or plasma cell clonal lymphoproliferation with both one or more kidney lesions that are related to the monoclonal immunoglobulin and the underlying process not causing complications meeting criteria for therapy (for example MM).[134] MGRS includes a very broad group of renal diseases with some having observable monoclonal immunoglobulins, usually light chains, in renal biopsies for example monoclonal fibrillary glomerulonephritis and light chain proximal tubulopathy. Some of the disorders included as MGRS lesions do not include monoclonal immunoglobulins but have been strongly linked to MGUS like C3 glomerulopathy, where MGUS is present in up to 80% of cases over 50 years old. Other nephropathies have a less clear association with MGUS but have been reported alongside MGUS.[135]

1.4.5 Other disease associations

Various disease associations with MGUS have been reported. Thrombosis, both venous and arterial have been reported in cohorts from Sweden and the US.[95, 136] Ocular manifestations of MGUS have been reported, particularly keratopathy. Although rare and not based on population-based data, this has given rise to the term monoclonal gammopathy of ocular significance.[137] Cutaneous manifestations have been reported alongside monoclonal gammopathies, particularly cryoglobulinemia, POEMS syndrome, and amyloidosis, but other less clear associations have also been reported.[138] Mortality has also been observed to be higher in those with MGUS.[139, 140]

1.4.6 Methodological limiations of studying MGUS disease associations

Several methodological limitations have affected studies on the disease associations of MGUS:

Firstly, virtually all previous studies have been performed in clinical cohorts with MGUS where only a small portion of individuals with MGUS in the population have been

identified during the workup of other symptomatic medical problems. This will invariably lead to biased selection of individuals with other disorders into the MGUS group. In fact, individuals with clinically diagnosed MGUS have higher rates of heart failure, endocrine disorders, rheumatological disease, and neurological disease.[141] These disorders, their pathological mechanisms, or warranted treatments may instead be the reason for the observed disease association rather than a causal link with MGUS. Furthermore, physicians who are aware of the previous studies associating MGUS with various diseases may be likely to look for and diagnose MGUS in patients with disorders that have already been shown, possibly in biased studies, to be associated with MGUS. This can perpetuate a "self-fulfilling prophecy" where more recent data could be even more affected by this selection bias.

Secondly, individuals with MGUS are under regular clinical observation and testing and may themselves be more aware of their health due to their diagnosis. Cohorts with MGUS may therefore be more likely to seek medical care for their symptoms and disease may come to light during clinical follow-up and testing, including imaging and blood testing, that would otherwise have gone undiagnosed leading to biased detection of various diseases. This is most likely to apply to asymptomatic or subclinical disorders and disorders that have previously been associated with MGUS. For example, this includes PN which is often present subclinically in the general population.[96] However, this is not likely to apply to severe diseases like femur fractures or life-threatening infections.

Thirdly, MGUS will in some cases progress to more advanced diseases whose symptoms may represent some of the disease associations previously reported. Although individuals with MGUS are under clinical observation, it is unavoidable that there remains some diagnostic delay between the development of these advanced disorders and their detection. Complications of these disorders, for example, fractures due to MM bone disease, infections due to immunoparesis, and PN due to WM-related cryoglobulinemia, may be observed in the leadup to the diagnosis of MGUS progression and be misclassified as a disease associated with MGUS rather than advanced disease. This is not always accounted for, particularly in registry studies. For example, in studies on fractures in MGUS, MM related fractures have not been excluded and may therefore have impacted the estimates for fracture risk in MGUS.[117, 118]

These limitations make it likely that previous studies on the association of MGUS and other disorders have provided some false associations and incorrectly estimated the risk of disease associated with MGUS. Even in MGCS disorders, where histopathological testing has proven monoclonal immunoglobulins to cause disease,[92] these biases have probably led to overestimation of the prevalence and thereby the importance of MGCS in the clinical setting. They also underscore the importance of studying MGUS disease associations in screened cohorts with MGUS. One such disease association study was

performed in the screened Olmstead County cohort.[104] The study included 605 individuals with MGUS and the incidence of a multitude of diseases were evaluated. The authors could confirm some of the previous disease associations including MM and WM as well as fractures, kidney transplant, and urticaria. Interestingly, multiple previously reported disease associations could not be confirmed, including PN, various skin diseases, and thromboembolism suggesting that these previously observed associations were artifacts found in clinical MGUS cohorts. However, the study was highly explorative utilizing specific diagnostic codes rather than relevant disease categories and the authors adjusted for multiple testing which may have rendered the study underpowered to detect more subtle disease associations. Furthermore, some of the diagnoses associated with MGUS including uterus retroversion, inhalation of fumes, and "open wound, buttock" are not likely to be causally related to MGUS and suggest that some of the associations found may have been spurious.[104]

1.4.7 MGUS screening and non-malignant disease

As discussed in section 1.3. MGUS is associated with a multitude of clinically significant complications including infections, fractures, PN, renal disease, and more. Besides offering the opportunity to treat MM at an early stage, MGUS screening may also provide an opportunity to detect and treat or mitigate these complications. For example, balance training and physical therapy can improve symptoms and the risk of falls in patients with PN.[142-144] Renal disease due to MGRS is one of the few underlying causes of chronic kidney disease where it is possible to treat the underlying cause and MGUS screening may also identify MGRS patients who will benefit from treatment and may have a lower risk of progressing to end-stage renal disease and requiring dialysis.[145]

MGUS screening will also generate a novel population of individuals with screened MGUS who are seen in the clinic and evaluated for these reported complications of MGUS. By gathering such a cohort, these disease associations can be further studied and the true burden of these disorders assessed, leading to the optimization of MGUS follow-up and possibly to improved outcomes in those with MGUS.

1.5 Summary

MGUS is asymptomatic but carries some clinical significance. This significance is twofold.

Firstly, MGUS is the precursor of MM and related disorders and by having individuals with MGUS under observation progression may be detected earlier and therapies applied to improve outcomes in these overt malignancies. Because MGUS is asymptomatic, most cases go undetected, severely limiting the availability of such early interventions. Screening for MGUS could be a simple and low-cost method of detecting these individuals and dramatically expand the use of early treatment in MM and related disorders. However, this may carry significant harms and the benefits on an individual

level may be limited. It is imperative to move from observational studies and studies including only clinically detected MGUS, SMM, and MM and related disorders to examine the real effect of such a consequential population-wide intervention before it is implemented.

Secondly, MGUS has been associated with a multitude of non-malignant disorders including PN, fractures, infections, renal disease, and more. Most previous studies on these disease associations have severe methodological limitations and there is a considerable lack of clarity on which disease associations are true and which disease associations are strong enough to have real clinical significance in the care of individuals with MGUS. By clarifying these points, clinicians can focus their efforts on these non-malignant complications of MGUS that are of significance and apply therapies to treat or mitigate these complications.

There remains a lack of clarity on the extent of the clinical significance of MGUS. This is mostly due to methodological issues that arise from studying an asymptomatic disorder like MGUS. Alternative approaches to evaluating available data or large-scale screening studies are needed to further elucidate the significance of MGUS and estimate the benefits of detecting and following individuals with MGUS.

2 Aims

The overall aim of this thesis is to further clarify the clinical significance of MGUS with an emphasis on the association of MGUS and non-malignant disease and to demonstrate methodologies that can significantly improve our understanding of this asymptomatic precursor disorder and its clinical significance. This is done in two parts:

In the first section, population-based registry data from Sweden is used to evaluate the association of MGUS and PN and fractures in paper I and paper II respectively, with additional emphasis on the effect of PN on MGUS progression risk and fractures. Alternative study designs and statistical methods are applied to an iteration of this dataset in which MGUS has been extensively studied previously[94, 95, 118, 139, 146, 147] in order to gain new insight and shed further light on the clinical significance of MGUS.

In the second section, we introduce the Iceland screens, treats, or prevents multiple myeloma study (iStopMM). The primary aim of the study is to assess the potential benefits and harms of population-based screening through performing a large-scale population-based screening study and a randomized controlled trial of follow-up strategies, including no follow-up. The extensive study design of iStopMM and the result of the recruitment phase of the study is described in paper III. One of the secondary aims of iStopMM is to generate an unbiased population-based screened cohort with MGUS in which disease associations can be studied with far greater confidence than possible previously. This methodology is demonstrated in paper IV where data from iStopMM is used to evaluate the risk of COVID-19 and particularly, of severe COVID-19, in individuals with MGUS.

3 Registry studies

3.1 Swedish registry data

3.1.1 Central registries

Like in most Nordic countries, all residents of Sweden are assigned a unique national identification number at birth or immigration. Swedish residents use the number in all their interactions with government institutions and public services, including the nationwide universal healthcare system. In addition to the population registry which includes the national identity number, date of birth, sex, and legal residence of all Swedish residents, the Swedish authorities have multiple registries on health, causes of death, tax data, education, and more. Using the national identification number, data from these registries can be cross-linked with virtually complete accuracy and used for scientific studies. This data cross-linkage has been the foundation of many research studies and is the foundation of papers I and II of this thesis. The following registries were included in this thesis:

1)The Swedish cancer registry: Founded in 1958, the Swedish cancer registry was one of the first nationwide cancer registries in the world. Since its foundation, registration of cancer diagnoses has been compulsory for Swedish physicians. These laws and the constant efforts by the staff of the registry, make the data quality of the Swedish cancer registry very high and diagnostic accuracy has, for example, been reported to be as high as 95% for multiple myeloma.[148]

2)The Swedish cause of death registry: Causes of death have been registered in Sweden by decree of the Swedish parliament since the 18th century, but the Swedish cause of death registry, in its current form, was established in 1952. Registration of deaths and their underlying causes, as estimated by their treating physician or during autopsy, is mandated by law and is required before the release of the body to loved ones for interment. Therefore, the registry includes virtually complete data on all deaths in Sweden since its inception.[149]

3)The Swedish National patient registry: In the 1960s, data on inpatient care started to be collected in Sweden in the National patient registry. In the 80s reporting became mandatory for all institutions in the Swedish universal healthcare system to report admissions to the registry and data has been considered complete since 1987. The registry includes diagnoses as International classification of diseases codes (ICD codes). Since 2001, outpatient encounters in specialty and primary care have also been included in the registry.[150] The data quality of the Swedish national patient registry has been shown to be high with positive predictive values of disease ranging from 85-95%.[151]

3.1.2 MGUS registry

The universal Swedish healthcare system has a geographically defined specialist referral structure and patients with hematological disorders are typically diagnosed, treated, and followed at regional hematology or oncology centers that usually are affiliated with a university hospital. Usually, MGUS is diagnosed during workup for other medical problems and when the diagnosis is made, that individual is most often referred to one of these regional centers. Since the early 80s, the criteria for MGUS diagnosis in these centers in Sweden have essentially been the same as those used internationally. During the relevant study period of the studies included in this thesis, this has included the presence of M proteins <30 g/L in serum and bone marrow plasma cells <10% (if bone marrow examination was performed).

From a network of these regional hematology and oncology centers in Sweden, all recorded cases of MGUS were registered in a central MGUS registry. Furthermore, cases of MGUS reported in the National patient registry and the Swedish cancer registry were included even though not recorded by any of the regional centers. For each case of MGUS, 4 controls were randomly selected from the general population who were alive at the date of MGUS diagnosis and matched by age, sex, and county of residence. In some cases, 4 controls were not available at the time of matching, reducing the number of matched controls to those available. The generated dataset constitutes the MGUS registry of individuals with MGUS and their controls used for papers I and II of this thesis. The registry has been described in greater detail elsewhere.[91]

3.2 MGUS and peripheral neuropathy (paper I) - Methods

3.2.1 Study cohort

The aim of paper I was to evaluate the relationship between MGUS and PN in population-based data and compare it to the relationship between DM and PN. To do this we included MGUS cases and controls between 1986 and 2013 from the MGUS registry described above. Participants who had a diagnosis of MM or related disorders as recorded in the Swedish cancer registry were excluded since they did not, by definition, have MGUS but more advanced disease. Participants who were under 18 at the time of MGUS diagnosis were also excluded. Participants were enrolled and followed until the 31st of December 2013.

3.2.2 Diagnoses of peripheral neuropathy and diabetes mellitus

Diagnoses of PN and DM were acquired from the National patient registry as ICD 9 and 10 codes (Table 3.1). Because the precise cause of PN is often not verified in clinical practice, we included PN regardless of registered cause. However, we excluded acute inflammatory neuropathies like Guillain-Barré neuropathy. In order to capture more cases of PN, which often goes undetected, we also included codes for symptoms of neuropathy like the ICD-10 code R20.0, anesthesia of skin. However, these symptomatic were excluded in a sensitivity analysis. Codes for DM were chosen to capture as many cases of DM as possible. Furthermore, because ICD-9 only includes a single code for DM we chose to include all codes for DM regardless of classification or complications from ICD-10.

In practice, both DM and PN develop over time, usually months or years. However, it is impossible to ascertain the precise date of onset of the disorders using the current data. Therefore, we only consider participants to have DM or PN after the date of the first recorded diagnosis.

Disorder	**ICD-10**	**ICD-9**
Diabetes mellitus	E10, E11, E12, E13, E14	250
Peripheral neuropathy	E10.4, E11.4, E12.4, E13.4, E14.4, G13.0, G60.3, G61.8, G61.9, G62, G63, *R20.0, R20.1, R20.2, R20.8*	250F, 356E, 357C, 357D, 357F, 357G, 357H,357W, 357X, *782A*

Table 3.1: The ICD-10 and ICD-9 codes used to identify DM and PN in the study. Note that all sub-codes were include (for example G62 includes G62.0, G62.1 etc.). Italicized codes are symptomatic codes.

3.2.3 Primary analysis

In the primary analysis, we assessed whether MGUS was associated with PN development. Participants who did not have PN at diagnosis were followed from the date of MGUS diagnosis or, of controls, the date of MGUS for the corresponding case. We considered the diagnosis of PN as the endpoint and censored at death as recorded in the Swedish cause of death registry or at the end of follow-up. Because treatments for MM and related disorders often cause PN, we also censored participants at the diagnosis of MM and related disorders as recoded in the Swedish cancer registry or National patient registry. In the main analysis, we considered the whole included matched cohort and estimated the hazard ratio (HR) of PN between cases with MGUS and controls using Cox regression adjusting for age, sex, and year of inclusion.

Because individuals with MGUS are under regular medical surveillance, and physicians caring for individuals with MGUS should in general be aware of their previously observed increased risk of PN, there is a considerable risk of detection bias for PN between cases with MGUS and controls. Therefore, we further stratified the cohort by whether participants had a diagnosis of DM before inclusion. Patients with DM are also under regular medical surveillance and have a known increased risk of PN when compared to the general population. Therefore, physicians taking care of DM patients should be aware of their PN risk and many are regularly screened for PN.[97] The DM population, therefore, serves as a more appropriate comparison than the general population. This stratification generated four study groups: An *MGUS group* that included those with MGUS who did not have DM, an *MGUS and DM group* that had both MGUS and DM, a *DM group* that had DM and not MGUS, and a *Control group* that had neither MGUS nor DM. HRs were then estimated for the four study groups with the control group and the DM group as the reference in two separate models using Cox regression, adjusting for sex, age, and year of inclusion.

3.2.4 Secondary analysis

In a secondary analysis, we evaluated the association of PN and MGUS progression to more advanced disease and death. We followed participants with MGUS from the time of MGUS diagnosis to progression to MM, WM, AL, or other LP both together and in separate analyses and to death in a separate analysis. Participants were censored at death, end of follow-up, or diagnosis of MGUS progression to advanced disease not included in the outcome of that analysis (for example diagnosis of WM in the analysis for MM). In the analysis assessing the association of PN and death the only censoring event was end of follow-up.

We performed Cox regression to estimate the HR of MM, WM, AL, LP, any progression, and death while adjusting for age, sex, and year of MGUS diagnosis. Some participants who had PN were diagnosed after MGUS diagnosis. In these participants, the period leading up to PN diagnosis would be misclassified as time with PN. Furthermore, participants will inevitably not progress or die during this period since that would lead to censoring leading to *immortal time bias*. Therefore, we included PN as a time-dependent covariate where the PN binary covariate could be different for the same participants at different time points and their person-time with and without PN can be correctly accounted for.

3.2.5 Sensitivity analysis

Outpatient data in the National patient registry was not available until after 2001. Since diagnoses of PN are often made in the outpatient setting this different availability of data over the study period might affect the results. Therefore, we performed a sensitivity analysis restricting the time period to 2001-2013. In a second sensitivity analysis, the DM stratified primary analysis was performed with the starting date as

either the original date of inclusion or the date of DM diagnosis, whichever came last. Thirdly, we performed a sensitivity analysis where ICD-10 codes for symptoms of PN were excluded from the diagnosis of PN. Finally, we performed the secondary analysis after excluding those who developed PN after MGUS diagnosis negating the need for including PN as a time-dependent covariate.

3.3 MGUS and fractures (paper II) - Methods

3.3.1 Study cohort

The aim of paper II was to evaluate the risk of fractures in MGUS with and untangling the potentially multifactorial causes of fractures in MGUS, including PN and imminent progression to MM and related disorders. For the study, we included participants with MGUS and controls matched by sex, age, and county of residence in the same manner as for paper I. We restricted the study period from 1999-2013. This was done because coding of diseases changed in 1997 to using the ICD-10 coding system and by restricting to this time period the coding of PN and fractures would be the same throughout the study period. By starting in 1999, participants had a 2-year "wash-out" period where PN diagnoses would most likely be recorded for all those who had PN before that time.

3.3.2 Peripheral neuropathy and fracture diagnoses

Diagnoses of PN were acquired from the National patient registry in the same manner as for paper I but only including ICD-10 codes. Fractures were also acquired from the National patient registry and were included as three types of outcomes: *any fracture*, *peripheral fracture*, and *axial fracture*. The ICD-10 codes for these outcomes are detailed in table 3.2. We speculated that peripheral fractures were more associated with falls compared to axial fractures that are more associated with MM and related bone disease. Therefore, we speculated that PN would have a stronger association with peripheral fractures and that imminent MGUS progression would be more associated with axial fractures.

Outcome	**ICD-10 codes**
Any fracture	S02, S12, S22, S32, S42, S52, S62, S72, S82, S92
Peripheral fracture	S02, S52, S62, S82, S92
Axial fracture	S12, S22, S32, S42, S72

Table 3.2: The ICD-10 codes used to define the fracture outcomes of paper II. Note that all subcodes were included (S02 included S02.1, S02.2 etc.).

In some cases, the same fracture will be coded repeatedly in the months after the fracture due to transfers, readmissions, or rehabilitation. For example, if someone may be diagnosed with a hip fracture at a rural hospital, that patient would then be transferred to an orthopedic center elsewhere for an operation. That patient may then be transferred back to the rural hospital and then be discharged to a rehabilitation hospital. That same fracture would then be coded repeatedly at each of these centers. Furthermore, the patient may experience a complication or require further treatment leading to repeated registration of that same fracture. The same applies to less serious fractures like wrist fractures that will require repeat visits for follow-up after the fracture. To mitigate this issue and to correctly identify true fractures, fractures of the same body part as a previous fracture within 6 months were excluded.

3.3.3 Primary analysis

In the primary analysis, we evaluated the fracture risk associated with MGUS after adjustment for PN and controlling for MGUS progression. We also estimated the fracture risk associated with PN and the interaction of PN and MGUS. Participants were followed from the time of inclusion until any fracture, peripheral fracture, and axial fracture in three separate analyse and were censored at death, end of follow-up at the end of 2013, and the time of a fracture not included as an endpoint. To control for MGUS progression we also censored participants who progressed to MM or related disorders one year before the time of progression. This was done because fracture risk is increased considerably in the months preceding MM diagnosis and MM patients have been shown to have had symptoms for a median of 3-6 months before diagnosis. Furthermore, fractures are a common event leading to MM diagnosis.[116] Therefore, it is not reasonable to consider the months leading up to MM or related disorders as time with MGUS since many already have undiagnosed advanced disease. Fractures during this time period are therefore likely to actually be MM related fractures.

We hypothesized that there would be multiple important confounders that would need to be adjusted for in the final model. When choosing adjustment variables, we used a directed acyclic graph (DAG) to visualize the potential causal network of MGUS and fractures (Figure 3.1). We identified age, sex, year of inclusion, previous fractures, alcohol use, and comorbidities as potential confounders. Comorbidities can be difficult to capture and we are not aware of any comorbidity scores that predict fracture although there are scores that predict death, for example, the Charlson comorbidity index.[152] Therefore, we did not include comorbidities in the main analysis but did include them in two sensitivity analyses described below. To adjust for these confounders, we included age, sex, year of inclusion, previous fractures in the preceding 2 years, and having alcohol use related ICD-10 diagnosis in a propensity score for MGUS and PN using multinomial logistic regression that essentially divided the cohort into four groups: individuals with MGUS, individuals with MGUS and PN (MGUS+PN), individuals with PN but without MGUS (Control+PN), and individuals with

neither PN nor MGUS (Control). We then included the generated propensity score in an inverse probability-weighted Cox regression model with PN as a time-dependent covariate and MGUS as a regular covariate as well as an interaction term for MGUS and PN. Schoenfeld's global tests were used to check for proportional hazards.

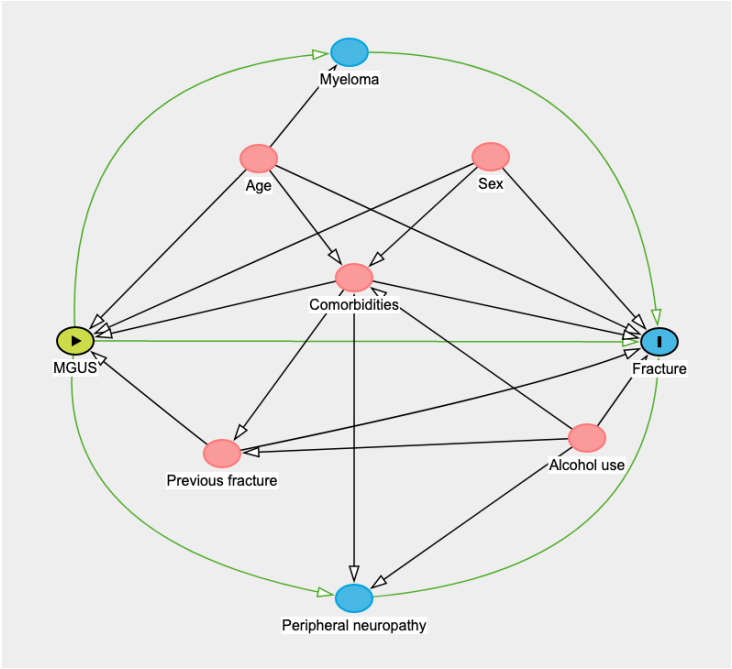


Figure 3.1: A directed acyclic graph (DAG) illustrating the hypothesized causal structure of fractures in monoclonal gammopathy of undetermined significance (MGUS).

We included comorbidities, which we had identified as potential confounders, in two sensitivity analyses. In the first sensitivity analysis. We included all the comorbidities included in the Charlson comorbidity index as binary covariates in the propensity score. In the second sensitivity analysis, we included in the propensity score the binary variable of having a hospital admission lasting four days or longer in the preceding 2 years as a general marker of frailty, comorbidity, and need of medical care.

3.3.4 Additional analyses

In order to further characterize the underlying causes of fractures in MGUS, we performed two additional analyses. First, we evaluated the risk of fractures in the year prior to MGUS progression, that is, the period when fracture risk may be increased due to undiagnosed progression to more advanced disease. We identified participants with MGUS who progressed to MM or related disease and then matched them by sex, age, and year of MGUS diagnosis (±1 year) at a ratio of 1:3 to individuals with MGUS that

were alive and free of progression at the same time in follow-up that progression happened. Logistic regression was then performed estimating odds ratios (ORs) for having any, peripheral, or axial fracture during the year before MGUS progression or the corresponding year in follow-up for those who did not progress.

In the second additional analysis, we evaluated the long-term risk of MGUS progression to MM or related disorders. We hypothesized that if fractures in MGUS were associated with long-term progression MGUS bone disease could be a precursor to MM bone disease. Conversely, we hypothesized that if no increased risk of MGUS progression would be observed, MGUS bone disease is likely to be unrelated to MM bone disease. For this analysis, we identified all participants with MGUS who experienced a fracture and were alive, free of progression, and in follow-up one year after that fracture. We considered that time, one year after the fracture, as the start of follow-up. We then matched each of these participants to three other individuals with MGUS who were alive, free of progression and fracture, and in follow-up at that time by sex, age, and year of MGUS diagnosis. This cohort was then followed until progression of MGUS to MM or related disorders with censoring at death or end of follow-up. Cox proportional hazard regression was then used to estimate the HR of progression for those who had a fracture compared to those who had not had a fracture. Cases where three matched controls could not be identified were excluded from both analyses.

3.4 Results and discussion

3.4.1 MGUS and peripheral neuropathy (paper I)

A total of 15,351 individuals with MGUS and 58,619 matched controls were identified. The proportion of male and female participants was relatively equal (51% vs 49%) and the median age of the cohort was 72 (range: 18-104). In total, 2,617 participants were diagnosed with PN at any time point, with half of those (n=1,301) being diagnosed with PN before the time of inclusion. Among individuals with MGUS, 6.5% were diagnosed with PN during the study period as compared to 2.8% among controls ($p<0.001$). (Table 3.3) These results confirm that a significant proportion of individuals with MGUS have clinically evident PN. However, since this is a registry-based study, the data represents recorded diagnoses (that is, the recognition) of PN in clinical practice rather than the true rate of PN and although the reported range of PN prevalence in MGUS is very wide) more recent studies, including modern MGUS definitions and clinical observational data rather than registry-based data, have reported PN rates of 15-20%.[102] Therefore, the rate of PN found in the study suggests under-recognition of PN in the real-world care of individuals with MGUS.

In the primary analysis, after exclusion of participants with a previous diagnosis of PN, participants with MGUS had an increased risk of developing PN when compared to matched controls (HR 2.7, 95% CI: 2.4-3.1; $p<0.001$) (Table 3.3). Next, we stratified the study population into the four study groups by MGUS and DM, providing a "fairer"

comparison by introducing patients with DM who are also under clinical supervision and at risk of PN. Rates of PN differed significantly between all study groups (MGUS alone: 5.3%, DM alone: 7.8%, MGUS and DM: 12.4%, Controls: 2.0%, p<0.001). Compared to controls, participants with MGUS alone and DM alone had an increased risk of PN (MGUS alone: HR 3.0, 95% CI: 2.6-3.4; p<0.001 and DM alone: HR 3.6, 95% CI: 3.2-4.2; p < 0.001). Participants with MGUS alone had a significantly lower risk of PN than individuals with DM alone (MGUS alone: HR 0.8, 95% CI: 0.7-0.9; p=0.02). Participants with both MGUS and DM had the highest risk of PN when compared to controls (MGUS and DM: HR 7.5, 95% CI: 6.2-9.0; p<0.001) and had more than a two-fold risk of those with DM alone (MGUS and DM: HR 2.1, 95% CI: 1.7-2.5; p<0.001) (Table 3.3 and Figure 3.2). The sensitivity analyses performed showed essentially the same results (data not shown). After this additional analysis we found that although individuals with MGUS had a lower risk of PN than individuals with DM, a well-recognized risk factor of PN, we found that individuals with both MGUS and DM had more than double the risk of PN than those with DM alone. This might suggest a synergistic effect of the two conditions. However, it is more likely that MGUS-associated PN is detected during screening for DM-associated PN in individuals with both MGUS and DM. From these findings, we conclude that MGUS is truly associated with excess risk of developing PN, contradicting previous findings that have put this into question.[25] In this non-screened population it is possible that this is due to a higher burden of comorbidities in those with MGUS. However, considering the patient group with DM, who are also more likely to have various other comorbidities including obesity,[153] cardiovascular disease,[154] autoimmune disease,[155] and more, the comparison of the DM and DM with MGUS groups is certainly better than in previous studies and is probably less prone to the same biases. It is therefore our conclusion that a causal relationship between MGUS and PN is likely.

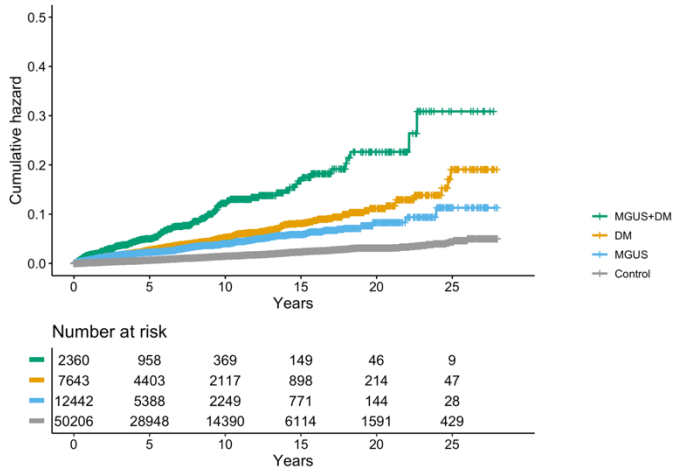


Figure 3.2: A graph illustrating the cumulative hazard of peripheral neuropathy (PN) throughout the study period by assigned study group. The figure is figure 1 in paper I, see attached manuscript.

	Original cohort		After DM stratification			
	MGUS	**Control**	**MGUS alone**	**DM alone**	**MGUS+DM**	**Control**
n	15,351	58,619	12,818	7,953	2,533	50,666
Median age (years)	73	72	72	74	74	72
Age range (years)	18-104	18-101	18-104	26-97	26-95	18-101
% male	51%	51%	50%	58%	58.1	50%
n by year of inclusion						
1986-1995	22%	22%	22%	23%	22%	21%
1996-2005	33%	33%	33%	35%	31%	33%
2006-2013	45%	45%	45%	42%	47%	46%
PN	996 (6.5%)	1,644 (2.8%)	681 (5.3%)	620 (7.8%)	315 (12.4%)	1024 (2.0%)
-before MGUS/ matching (% of PN)	549 (55%)	770 (47%)	376 (55%)	310 (50%)	173 (55%)	460 (45%)
Median follow-up (years)	4.0	6.1	4.1	6.1	3.7	6.1
Risk of PN in HR (95% CI)[1]						
	2.7 (2.4-3.1)***	Reference	-	-	-	-
	-	-	3.0 (2.6-3.4)***	3.6 (3.2-4.2)***	7.5 (6.3-9.1)***	Reference
	-	-	0.8 (0.7-1.0)*	Reference	2.1 (1.7-2.5)***	0.3 (0.2-0.3)***

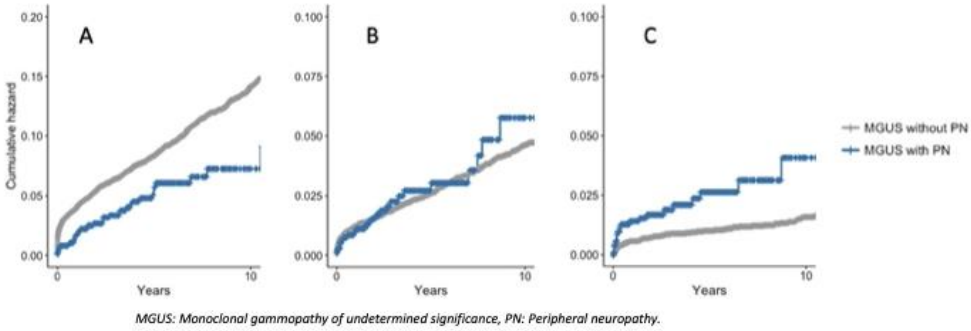
*1: Adjusted for sex, age, and year of inclusion. *: p<0.05, ***: p:<0.001, MGUS: Monoclonal gammopathy of undetermined significance, PN: Peripheral neuropathy, DM: Diabetes mellitus, HR: Hazard Ratio, 95% CI: 95% confidence interval.*

Table 3.3: Baseline characteristics of study participants in paper I in the original cohort and after additional stratification for DM as well as results of a Cox proportional hazard regression model of risk of PN for each group. This is table 1 in paper I, see attached manuscript.

	MGUS without PN	MGUS and PN
n	14,355	996
Male	51%	63%
Median age	73	69
Median follow-up (years)	4.0	3.0
Progressed to MM (incidence[1])	1328 (1.6)	40 (1.0)
- HR (95% CI)[2]	-	0.7 (0.5-0.9)*
Progressed to WM (incidence[1])	422 (0.5)	27 (0.7)
- HR (95% CI)[2]	-	1.2 (0.8-1.8)
Progressed to AL (incidence[1])	153 (0.2)	21 (0.5)
- HR (95% CI)[2]	-	2.3(1.5-3.7)***
Death (mortality rate[1])	5522 (6.8)	315 (8.0)
-HR (95% CI)[3]	-	1.3 (1.2-1.5)***

*1: Incidence per 100 person-years. 2: Adjusted for sex, age, and year of inclusion.3: Adjusted for sex, age, year of inclusion, and diabetes mellitus (DM) ***: p:<0.001, MGUS: Monoclonal gammopathy of undetermined significance, PN: Peripheral neuropathy, MM: Multiple myeloma, WM: Waldenström's Macroglobulinema, AL: Amyloid light-chain amyloidosis, HR: Hazard Ratio, 95%CI: 95% confidence interval,*

Table 3.4: Baseline characteristics, rates and incidence of progression, and risk of progression to MM, WM, and AL as well as death for study participants in paper I with MGUS with and without PN as assessed by Cox proportional hazard regression with PN as a time dependent covariate. The is table 2 in paper I, see attached manuscript.



MGUS: Monoclonal gammopathy of undetermined significance, PN: Peripheral neuropathy.

Figure 3.3: A graph illustrating the cumulative hazard of MGUS progression to MM (A), WM (B) and AL (C) over the first 10 years after inclusion by whether participants with MGUS had PN or not. Note that the y axis of graph A is longer since MM is more common than WM and AL.

The secondary analysis included all 15,351 participants with MGUS. Those diagnosed with PN were more likely to be male (63% vs 51%; p<0.001) and were younger at MGUS diagnosis than those who did not have PN (median age of 69 years vs 73 years; p<0.001). A total of 1,368 participants progressed to MM, 449 progressed to WM, and 173 progressed to AL. PN was associated with lower risk of MM (HR=0.7; 95%CI: 0.5-0.9; P=0.02) but was not associated with WM progression (HR=1.3; 95%CI: 0.9-1.9; P=0.2) (Table 3.4 and Figure 3.3). In previous studies, PN has been observed to be more common in those with IgM MGUS[106] who rarely progress to MM, but rather to WM and other lymphoproliferative disorders.[29, 153]In this study, isotype data was not available for the study cohort limiting the interpretation of these results. However, we believe that these results indicate that MGUS is unlikely to be associated with MGUS progression to MM or WM. This is in contrast to the only other study assessing MGUS progression risk associated with PN, that we are aware of, a small retrospective single-center study including 223 people with MGUS, where a non-significant trend of increased progression among those with MGUS-associated PN was observed.[23] Although the mechanism of MGUS-associated PN is not fully known, in many cases anti-myelin-associated glycopeptide (anti-MAG) or related autoantibodies are present.[32,33] Additionally, depositions of monoclonal antibodies in the myelin have also been observed[34] possibly indicating that some affected individuals actually have amyloid light-chain amyloidosis (AL amyloidosis), rather than MGUS. These findings indicate that the mechanism of MGUS-associated PN is unrelated to progression of the underlying plasma cell disorder. Therefore, potential therapies for MGUS-associated PN might need to be directed at the underlying mechanisms of the PN rather than at the underlying plasma cell disorder.

Interestingly, PN was associated with a 2.9-fold risk of MGUS progression to AL (HR=2.9; 95%CI: 1.8-4.6; *P*<0.001). Furthermore, nine out of the 11 individuals with PN who progressed to AL did so within a year of MGUS diagnosis. We believe that this represents a diagnostic delay rather than a biological process and that most of these cases had AL at the time of MGUS diagnosis. Under-recognition and diagnostic delay is common in AL and is mostly caused by the complicated clinical picture of AL and the need for tissue diagnosis.[156] Moreover, virtually all cases of AL are preceded by MGUS.[28] The findings, therefore, emphasize the need for a thorough clinical evaluation of individuals with MGUS who have PN, especially when present at diagnosis.

In total, 5,522 and 315 participants with MGUS with and without PN died during the study period translating to a mortality rate of 6.8 and 8.0 respectively. PN was associated with a 1.3-fold increased risk of death (HR: 1.3; 95% CI: 1.2-1.5; p<0.001). This could be due to excess risk of falls and fractures that is associated with PN in general[157] or because PN can decrease mobility and lead to increased frailty and mortality. However, PN can be caused by a multitude of disorders that increase the risk of death, including chronic alcohol use, autoimmune disease, drug therapy for various disorders including cancers, and more.[158] Therefore, we cannot conclude with confidence that PN is truly associated with the risk of death in individuals with MGUS.

In conclusion, clinically evident PN was found in a significant proportion of individuals with MGUS (6.5%) and we concluded that PN is truly associated with MGUS. Additionally, our findings suggest that PN is under-recognized in the real-world clinical care of individuals with MGUS. PN was not associated with an increased risk of MGUS progression to MM and WM but was associated with a 2.9-fold risk of AL. PN has been shown to lead to poorer quality of life in previous studies[112-114] and may lead to increased mortality as observed in this paper. Based on these findings, increased vigilance for symptoms of PN is warranted for physicians caring for individuals with MGUS.

3.4.2 MGUS and fractures (paper II)

In total, 8,395 individuals with MGUS and 30,851 matched controls were identified in the study period. Of those 597 and 849 individuals had PN in the MGUS and control groups respectively. These 39,921 participants were followed for a total of 219,834 person-years. Participant acquisition increased over the study period. PN was associated with previous alcohol use (p<0.001) and a recorded fracture in the preceding two years (p=0.02).

MGUS was associated with a 1.3-fold increased risk of fractures, independent of PN (HR: 1.29; 95% confidence interval [95% CI]: 1.21-1.37; p < 0.001). An increased risk was observed for both axial and peripheral fractures with slightly higher estimates found for axial fractures (HR 1.18 vs 1.37 for peripheral and axial fractures respectively) (Table 3.6). In this analysis, we excluded fractures one year before progression of

MGUS to MM or related disorders and thereby than MGUS, for example, MM bone disease. Previous studies that have not controlled for these progression-related fractures have estimated the risk of fractures to be higher. In one study in a similar cohort in Sweden, the risk increase for fractures in MGUS was estimated at 1.61 fold for all fractures and 2.37 fold for vertebral/pelvic fractures.[118] We speculate that this difference is most likely due to the inclusion of fractures that are actually related to MM or related disorders in these previous studies. Therefore, the risk estimates in this study are more likely to reflect the true fracture risk increase associated with MGUS

	MGUS	MGUS+PN	Control	Control+PN
n person years	39,559	2,263	174,844	3,168
n individuals	8,052	597	30,423	849
PN after inclusion (%)	-	254 (43%)	-	419 (49%)
Age (median)	71	68	70	71
Age (range)	18-97	28-89	18-97	23-96
Male	52%	63%	53%	62%
Year of diagnosis				
-1999-2003	29%	20%	28%	24%
-2004-2007	29%	29%	29%	29%
-2008-2012	43%	51%	43%	48%
Median follow-up[1]	4.1 years	3.7 years	5.1 years	3.6 years
Median potential follow-up[2]	4.8 years	4.1 years	5.8 years	4.1 years
Previous alcohol use (%)	242 (3%)	31 (5%)	720 (2%)	47 (6%)
Previous fracture (%)	428 (5%)	33 (6%)	1138 (4%)	44 (5%)
All participants				
All fractures (rate[3]*)*	1,402 (3.5)	89 (3.9)	4,535 (2.6)	120 (3.8)
Peripheral fractures (rate[3]*)*	545 (1.4)	38 (1.7)	1,978 (1.1)	46 (1.5)
Axial fractures (rate[3]*)*	887 (2.2)	54 (2.4)	2,647 (1.5)	76 (2.4)
Males				
All fractures (rate[3]*)*	560 (2.9)	48 (3.4)	1,718 (1.9)	60 (3.0)
Peripheral fractures (rate[3]*)*	188 (1.0)	17 (1.2)	667 (0.7)	20 (1.0)
Axial fractures (rate[3]*)*	383 (2.0)	32 (2.3)	1,078 (1.2)	40 (2.0)
Females				
All fractures (rate[3]*)*	842 (4.2)	41 (4.8)	2,817 (3.3)	60 (5.1)
Peripheral fractures (rate[3]*)*	357 (1.8)	21 (2.5)	1,311 (1.5)	26 (2.2)
Axial fractures (rate[3]*)*	504 (2.5)	22 (2.6)	1,569 (1.8)	36 (3.0)

1: As evaluated by the Kaplan-Meier estimator for any outcome. 2: As evaluated by the Kaplan-Meier estimator for censoring events. 3: rate per 100 person years. PN: Peripheral neuropathy. MGUS: Monoclonal gammopathy of undetermined significance. Study participants who develop PN after inclusion are included in the non-PN groups until the point of PN diagnosis and are counted there as well.

Table 3.5: Baseline characteristics and number of fractures in paper II. This is table 1 in paper II, see attached manuscript.

	Any fracture		**Peripheral fracture**		**Axial fracture**	
	HR (95%CI)	p	HR (95%CI)	p	HR (95%CI)	p
	All participants					
MGUS	1.29 [1.21-1.37]	<0.001	1.18 [1.08-1.30]	<0.001	1.37 [1.27-1.48]	<0.001
PN	1.34 [1.16-1.55]	<0.001	1.36 [1.09-1.71]	0.007	1.34 [1.12-1.61]	0.001
	Males					
MGUS	1.39 [1.27-1.53]	<0.001	1.23 [1.05-1.44]	0.009	1.51 [1.34-1.69]	<0.001
PN	1.39 [1.14-1.69]	0.001	1.38 [0.99-1.93]	0.057	1.37 [1.07-1.75]	0.012
	Females					
MGUS	1.22 [1.13-1.31]	<0.001	1.14 [1.02-1.28]	0.022	1.28 [1.16-1.41]	<0.001
PN	1.35 [1.10-1.65]	0.004	1.40 [1.04-1.88]	0.027	1.36 [1.04-1.77]	0.025

Table 3.6: Calculated hazard ratios (HR) for the different fracture types in MGUS and PN after before and after stratification by sex in paper II. This is table 2 in paper II, see attached manuscript.

We then evaluated if these "MGUS-fractures" were associated with MGUS progression in the long-term. In total, 1041 individuals with fractures in MGUS and 3123 matched MGUS controls were included. Among these participants, 171 (4.1%) progressed to MM or related disorders. We found that fractures were not associated with progression (HR: 0.95; 95% CI: 0.66-1.36; P = 0.77). In an additional analysis, we included all 2,068 participants with MGUS who progressed to MM or related disorders and 6,204 matched controls with MGUS. Those who progressed had a higher rate of fractures (4.2% vs 2.6%) with an estimated 1.66-fold increased risk of fractures in general (OR: 1.66; 95% CI: 1.27-2.16; p < 0.001) which was limited to only axial fractures where the risk was almost double in those who progressed to MM or related disorders (OR: 1.94; 95% CI: 1.44-2.62; P < 0.001) but an increased risk of peripheral fractures was not observed. These fractures are most likely due to bone disease in MM and related disorders which commonly affects the axial skeleton.[116]

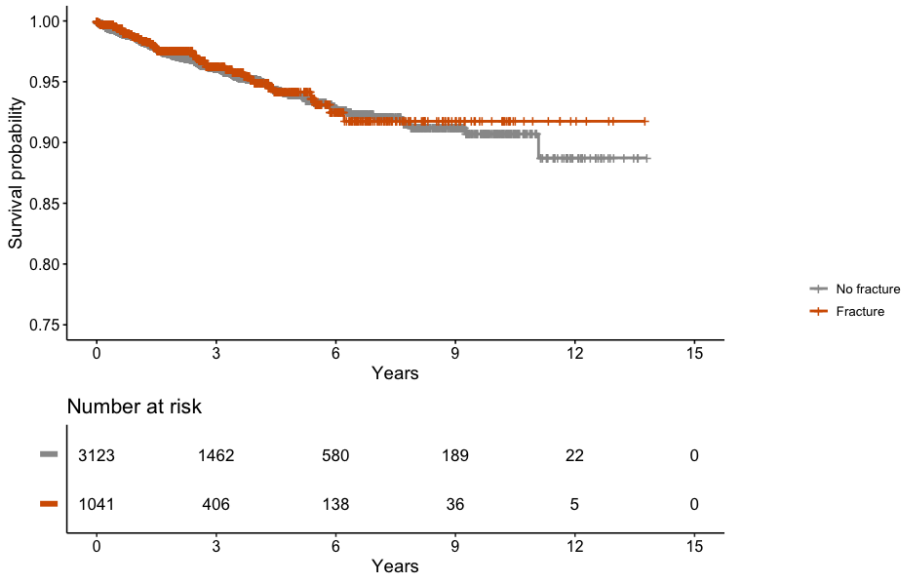


Figure 3.4: A Kaplan-Meier graph illustrating the risk of MGUS progression in those who had a fracture. This is figure 1 in paper II, see attached manuscript.

Therefore, we show that MGUS is associated with fractures after controlling for PN and for fractures associated with MGUS progression. Furthermore, we show that these MGUS-fractures were not associated with progression of MGUS. These findings indicate that fracture risk in MGUS is independent of more advanced disease and that the underlying processes may be unrelated to the processes advancing the disease toward MM or other related disorders. This division between MGUS-fractures and fractures relating to more advanced disease is further emphasized by the anatomical distribution of the fractures with MGUS-fractures happening both in the peripheral and axial skeleton but the risk increase being exclusively in the axial skeleton for more advanced disease. This is supported by previous studies which have shown MGUS to be associated with an abnormal bone architecture.[93, 120, 121] The findings of this study provide an epidemiological underpinning for the previously hypothesized MGUS bone disease which is unrelated to MM bone disease.

PN was associated with a 1.34-fold increased risk of fractures independently of MGUS (HR: 1.34; 95% CI: 1.16-1.55; $p < 0.001$). This association was found for both peripheral and axial fractures with a similar risk increase being observed for both fracture types (1.36 and 1.34 for peripheral and axial fractures respectively). We did not find a significant interaction between PN and MGUS (HR: 0.90; 95% CI: 0.68-1.2; p=0.49). A symptom of PN is loss of balance and a tendency to fall. Furthermore, PN can in some cases lead to autonomic dysfunction which further increases the risk of falls due to orthostatic hypotension. This might lead to falls and fractures in patients with PN.[115] This is the first study that we are aware of assessing the risk of fractures associated with PN in MGUS and the findings indicate that PN is a contributing factor

to fractures in MGUS in the same way that PN would increase the risk of fractures in other disorders. We conclude that PN is one of the causes of fractures in MGUS.

Both MGUS and PN were independent risk factors for fractures in both males and females. In a study from Iceland by Thorsteinsdottir et al. a screened cohort of individuals with MGUS was found to have an increased risk of fractures with the risk being limited to males.[93] Similarly, we found males to have a higher hazard ratio for fractures due to MGUS than females but an increased risk was also observed in females. The underlying risk of fractures is considerably higher in post-menopausal women[159] and because the hazard ratio is a relative measure a considerably higher absolute risk increase is needed to observe a similar relative risk increase. It is likely that previous findings, that were based on smaller cohorts, were underpowered to detect this smaller increase in relative fracture risk which we observe in this larger cohort. Interestingly, the hazard ratio for fractures was similar for males and females with PN. Because of the higher baseline risk of fractures, these findings likely indicate that PN leads to higher absolute fracture risk increases in females. This could be caused by a higher rate of osteoporosis in post-menopausal women[159] making falls, which can be caused by PN, more likely to result in fractures.

The most important limitation of this study is the potential confounding of comorbidities that may lead to both MGUS and PN development or diagnosis. We, therefore, included alcohol use and previous fractures as part of the propensity score in all analyses, but we also included comorbidities in two sensitivity analyses. In the first sensitivity analysis, we included the comorbidity classes of the Charlson comorbidity index and in the second sensitivity analysis, we included having one or more hospital admissions lasting four days or longer in the preceding two years as a marker of general frailty. Both markers of comorbidities and frailty were more common in those with MGUS and PN (Supplemental Table 1). The results of these sensitivity analyses were in the same direction and very similar to the results of the primary analysis of the study further strengthening the results of the study (Supplemental Table 2).

In conclusion, we found that the causes of the increased fracture risk in MGUS to be multifactorial. We hypothesized that there were at least three independent causes of fractures driving this increased fracture risk: undetected MM bone disease, MGUS-specific fracture due to MGUS bone disease, and PN leading to increased falls. Fractures are a major cause of morbidity and mortality, particularly in the elderly who are commonly affected by MGUS.[9, 32] Based on these findings we further speculate that fracture risk in MGUS may be mitigated by several measures. Firstly, by detecting MGUS progression early and initiating treatment, MM bone disease may possibly be prevented. Secondly, treatment may in some cases improve PN[105, 160] and physiotherapeutic interventions can improve balance and decrease the risk of falls in those who have PN.[161] The findings also highlight the need for further studies on MGUS

inherent bone disease, its study in screened MGUS cohorts, and the uncovering of its causes and potential therapeutic interventions.

3.4.3 Strengths of paper I and paper II

The registry-based studies presented in this thesis have some strengths. Firstly, they include thousands of participants and matched controls. As far as we are aware, these are the largest studies to date on neuropathy and fractures in MGUS. Secondly, these registries are population-based and include whole-nation cohorts of individuals with MGUS with all residents of Sweden available as possible controls, making generalization of the study results possible in similar populations. Thirdly, data from participants can be linked to nationwide registries of cancer- and other medical diagnoses with virtually complete accuracy using a national identifying number assigned to all residents of Sweden at birth or immigration. Furthermore, multiple studies have shown that these registries have high validity.[148, 149, 151] Finally, the study design and statistical analysis of both studies improves upon and bypasses some of the limitations of previous studies. In paper I, stratification of the study cohort by DM provides a fairer comparator than usual population controls and provides greater opportunities for causal speculation based on the study results. In paper II, fracture risk is viewed from multiple vantage points with MGUS and PN evaluated as independent risk factors and potential MM fractures excluded. Furthermore, the long-term prognostic value of fractures in MGUS and fracture risk in the period preceding MGUS progression were evaluated separately. This multi-faceted assessment provides greater validity to the study results and provides an opportunity to untangle the causes of fractures in MGUS. Additionally, comorbidity data was included in the analyses in paper II which mitigates some of the differential effects of comorbidities in individuals with MGUS and PN and controls.

3.4.4 Limitations of paper I and paper II

Both studies also have important limitations. Firstly, diagnoses of PN, DM, and fractures, as well as data on confounders used as covariates in statistical models, are acquired from registries of physician recorded diagnostic codes and there is likely considerable residual confounding. There are at least three different sources of this residual confounding: 1) The coding relies on the diagnostic skill and correct registration of the treating physician and the diagnosis may not always be correct. 2) The coded diagnoses do not express differences in disease severity which may be different between study groups. 3) The diagnostic codes sometimes classify diseases into categories that are not relevant to the study and in some cases, sub-disorders are not accounted for or the classification overlaps into disorders that are not relevant.

Secondly, similarly to most other MGUS cohorts, MGUS cases were acquired from a registry of MGUS that is diagnosed clinically, usually in relation to workup for other illnesses, leading to biased selection of individuals with other medical problems into

the MGUS group. This could predispose the group to PN and fractures. We did include comorbidities in some sensitivity analyses, but it is likely that some residual confounding remains.

Thirdly, individuals with MGUS are under clinical observation leading to differential detection of disease, including detection of subclinical PN that would not have come to light in controls.

Fourthly, MGUS isotype data was not available for this cohort and some isotypes, especially IgM, are likely to have a higher risk of PN and the results of the study may not be representative of each isotype. Importantly, IgM and non-IgM MGUS represent related but distinct pathogenic entities with IgM MGUS most often being caused by the accumulation of monoclonal lymphoplasmacytic lymphocytes rather than plasma cells like in non-IgM MGUS. This may skew the results on the association of PN and MGUS progression and fracture risk may be significantly different in IgM and non-IgM MGUS.

Finally, the Swedish population is relatively genetically and ethnically homogenous with most Swedes being white, limiting the generalization of these results in other non-white populations.

4 Iceland screens, treats, or prevents multiple myeloma (iStopMM)

4.1 Design of iStopMM (paper III)

The design and recruitment of the Iceland screens, treats, or prevents multiple myeloma (iStopMM) was described in paper III. The iStopMM study is a population-based screening study for MGUS and randomized controlled trial of follow-up strategies. The main aim of the study is to evaluate the potential benefits and harms of population-based screening for MGUS and the disorders it precedes. Secondary aims include assessing diagnostic modalities and strategies of MGUS follow-up to optimize the clinical care of individuals with MGUS, establishing a large biobank with longitudinal sample collection, and to generate a large dataset with clinical, patient-reported, socioeconomic, and demographic data for large scale epidemiological studies.

4.1.1 Recruitment

All residents of Iceland born in 1975 and before who were alive on September 9th, 2016, as registered in the Icelandic National Registry, were offered participation in the study. The only exclusion criteria was having a previous history of MM or related lymphoproliferative disorders, as registered in the Icelandic Cancer Registry. In total 148,704 eligible individuals were contacted by mail including an information brochure on the study and a consent form. These letters were followed by an extensive marketing campaign on social and conventional media, including appearances on major television shows in Iceland. Through this effort, the study was introduced and explained to the Icelandic public. Participants could then sign up for the study by providing informed consent in one of three different ways. Firstly, the consent form included in the letter could be signed and sent to the study team. Secondly, participants could use a unique participation code accompanying the letter to sign up online. Finally, participants could use a secure internet gateway run by the Icelandic government (island.is) which is accessible to all Icelandic residents using electronic identification also verified and made secure by the Icelandic government.

4.1.2 Screening

To screen for MGUS and the disorders it precedes, serum samples had to be collected from participants. This was mainly done passively alongside other sampling in the Icelandic healthcare system, including blood banks. The study team collaborated with the health informatics department of Landspítali – The National University Hospital of Iceland (LUH) to create an electronic solution linking the id numbers of participants to the central laboratory system in Iceland which is used by healthcare institutions in all urban or semi-urban centers in Iceland covering at least 92% of the residents of Iceland. In these centers, it is standard practice to print stickers from the laboratory system for blood test vials before sampling. When stickers were printed for individuals during routine clinical care, blood donation, or emergency care, an extra sticker for a serum sample intended for the iStopMM study was also printed. For smaller institutions and private clinics, a manual system was developed whereby staff performing blood draws asked their patients whether they were participants and cross-linked serum samples that were due for destruction to id numbers in the study and rerouting them to screening within the study. Lastly, to collect more samples and to capture the population who did not require blood sampling as part of regular clinical care, an

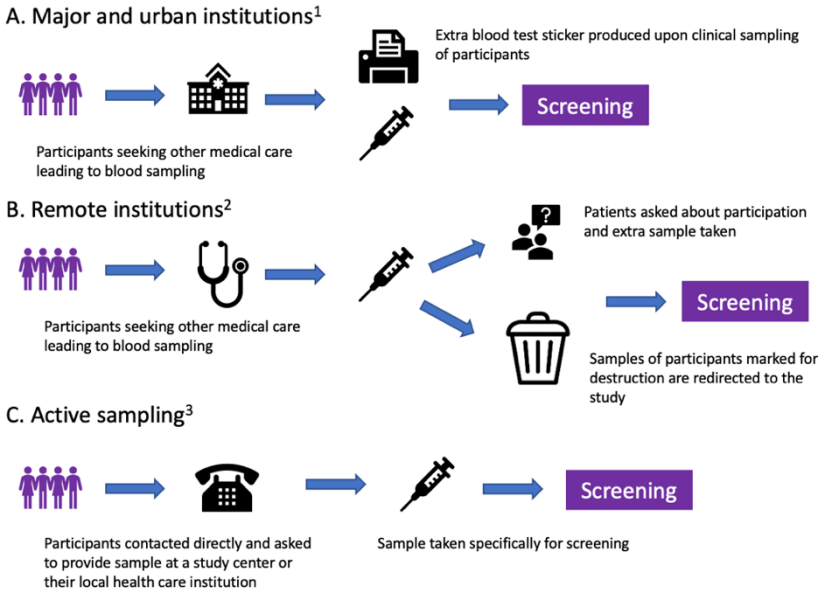


Figure 4.1: Methods of blood sample acquisition. A and B describe passive sampling starting during the fall of 2016 and C describes active sampling beginning during the fall of 2019. 1: Reykjavik Capital Area, Akureyri, Ísafjörður, Reykjanes Peninsula, Akranes, Healthcare Institution of Northern Iceland, Healthcare Institution of South Iceland, blood banks 2: Neskaupsstaður, Healthcare institution of West Iceland, Healthcare Institution of East Iceland. 3: Available for all Icelandic residents. This is figure 1 in paper III, see attached manuscript.

active sampling drive was initiated in late 2019 whereby participants who had not provided a serum sample were contacted directly and asked to come in for blood sampling (Figure 4.1). All samples collected were sent to the laboratory at LUH in Reykjavik, Iceland where the samples are aliquoted into identical sample tubes marked with anonymous study numbers using TC automation and aliquoter (Thermo Scientific®, MA, USA). These de-identifiable sample tubes are then shipped to The BindingSite laboratory in Birmingham, UK, for screening. SPEP is performed using capillary zone electrophoresis (CZE; Helena Laboratories, Texas, USA) and assays for FLC, immunoglobulins (IgG, IgA, IgM), and total proteins performed using Freelite® and Heavylite® assays are performed on an Optilite® turbidimeter (The Binding Site Group Ltd, Birmingham, UK). IFE is performed on samples where M-proteins are identified or suspected on CZE or when abnormal FLC results are found. All CZE and IFE gels are assessed by at least two independent experienced observers.

4.1.3 Randomization

Those participants who have an M protein on CZE and IFE or abnormal FLC results were eligible for randomization into one of three study arms. Randomization was done in a dynamic, non-predetermined manner, and by blocks of having an M protein >1.5g/dL and having LC-MGUS. Arm 1 was not informed about having MGUS but

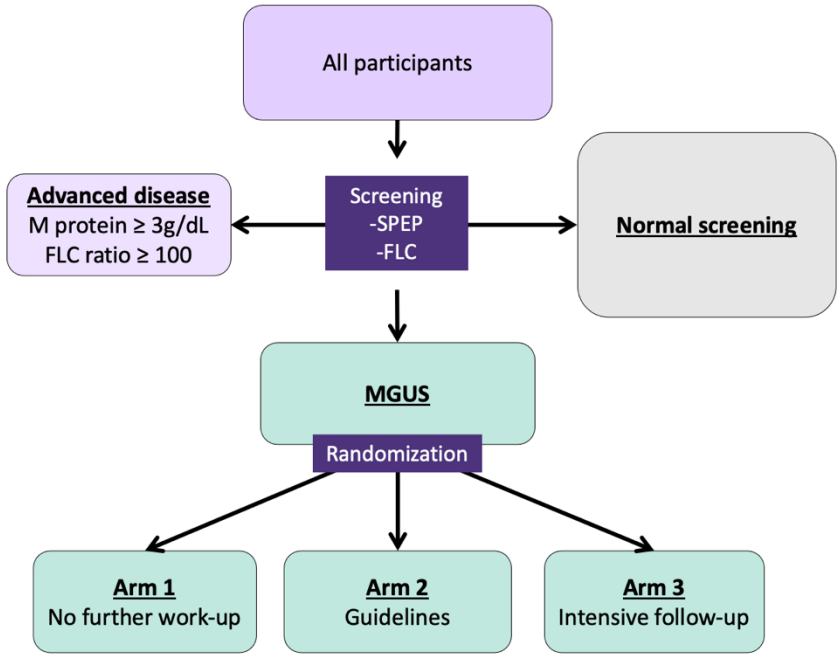


Figure 4.2: A flowchart outlining the study design for screening and randomization of individuals with MGUS. This is figure 2 in paper III, see attached manuscript. MGUS: Monoclonal gammopathy of undetermined significance. SPEP: Serum protein electrophoresis. FLC: Free light chain assay.

continued to receive healthcare in the Iceland healthcare system as if they had never participated in the study. Arm 2 was called into the study clinic and are assessed and followed according to guidelines at the outset of the study based on having low or non-low risk MGUS. Arm 3 followed a more intensive and uniform strategy of follow-up (Figure 4.2).

Participants who had an M protein >3.0g/dL had SMM and FLC ratio (involved/uninvolved) ≥100 had MM before randomization according to current diagnostic criteria and were therefore excluded from the randomized study and were all called in for initial assessment and were referred to treatment if eligible. Furthermore, because those who had a previous diagnosis of MGUS could not be blinded to their MGUS status, they were randomized to either arm 2 or 3.

4.1.4 Initial assessment and follow-up

Study participants randomized to arm 2 and 3 and those with advanced disease at diagnosis were seen at the iStopMM study clinic which has been established in Reykjavík, Iceland. Temporary clinics are also regularly established in Akureyri, Ísafjörður, Húsavík, Vestmannaeyjar, Egilsstaðir to improve the geographical coverage of the study. Individuals with MGUS are seen by specially trained study nurses and those with more advanced disease are seen by a physician. At each visit, participants undergo a clinical interview and exam and are given oral and written information on their diagnosis and prognosis.

Figure 4.3: A map of Iceland with the geographical location of blood sampling sites (cyan colored icons), temporary clinics (purple icons), and the main study clinic (red icon).

Test	Arm 2 – Low risk and LC-MGUS	Arm 2 – Non-low risk	Arm 3 – All	SMM and SWM	MM and WM
Physical exam[1]	First visit	First visit	Each visit	Each visit	At diagnosis
Blood sampling					
-SPEP *-FLC assay*	Each visit	Each visit	Each visit	Each visit	At diagnosis
-CBC	First visit	Each visit	Each visit	Each visit	At diagnosis
-Total calcium *-Albumin* *-Creatinine*	First visit	First visit	Each visit	Each visit	At diagnosis
-CRP *-LDH* *-ß2M*	-	-	Each visit	Each visit	At diagnosis
-TnT *-pro-BNP*	-	-	Annually	Annually	At diagnosis
Bone marrow					
-Smear *-Biopsy*	As clinically indicated	0 months Except if LC	0 and 60 months	Annually	At diagnosis
Urine					
-Protein dipstick	First visit	First visit	-	-	-
-UPEP	If positive dipstick	If positive dipstick	-	-	-
-Albumin/creatinine ratio	-	-	Annually	Annually	At diagnosis
ECG	-	-	Annually	Annually	At diagnosis
Imaging					
-WB-LDCT	-	-	0 and 60 months in LC- and non-IgM	Annually in LC- and non-IgM	At diagnosis of MM
-Plain X-ray of bones	As clinically indicated	First visit in LC- and non-IgM	-	-	-
-CT abdomen	-	First visit in IgM	0 and 60 months in IgM	Annually in IgM	At diagnosis of WM
-MRI of bones	-	-	-	If WB-LDCT is normal	-
Follow-up	Every 2-3 years	Annual	Annual	Every 4-6 months	Single visit

SMM: Smoldering Multiple Myeloma. SWM: Smoldering Waldenströms Macroglobulinemia. MM: Multiple myeloma. WM: Waldenströms macroglobulinemia. SPEP: Serum protein electrophoresis. FLC: Free light-chains. CBC: Complete blood count. CRP: C-reactive protein. LDH: Lactate dehydrogenase. ß2M: ß-2-microglobulin. TnT: Troponin T. pro-BPN: pro-Brain natriuretic peptide. UPEP: Urine protein electrophoresis. ECG: Electrocardiogram. WB-LDCT: Whole body low-dose computerized tomography. CT: Computerized tomography. MRI: Magnetic resonance imaging. LC: Light chain.

Table 4.1: Clinical assessment, imaging, and laboratory studies included for participants in the different study arms of the iStopMM study as per protocol. Note that additional sampling and imaging was permitted as clinically indicated and decided at regularly scheduled clinical decision meetings. This is table 1 in paper III, see attached manuscript.

Participants in arm 2 are assessed and followed according to current guidelines. They are stratified by whether they have low-risk MGUS (IgG M protein <1.5g/dL and normal FLC ratio) or not. Repeat SPEP and FLC assay as well as basic clinical chemistry is performed including measurements of calcium, hemoglobin, and creatinine in all in arm 2 but imaging and bone marrow sampling and imaging by plain skeletal survey is only performed in individuals with non-low risk MGUS (non-IgG isotype, normal FLC, M-protein <15g/L) or when clinically indicated. Follow-up frequency is then dictated by risk.[30] Those in arm 3 all undergo the same and more intensive assessment. This includes bone marrow biopsies and imaging by whole body low dose computerized tomography (WBLDCT) to identify lytic bone lesions. Follow-up in arm 3 is more uniform an all participants are seen annually. Furthermore, additional testing of blood cardiac markers (Troponin T (TnT) and pro-Brain natriuretic peptide (BNP)) are performed in arm 3 to identify AL amyloidosis. Congo red staining of bone marrow is performed in all cases where there is suspicion of AL amyloidosis. Computerized tomography (CT) of the abdomen is performed instead of other imaging in those with IgM MGUS in both arm 2 and arm 3. The initial assessment and follow-up of arms 2 and 3 are further described in table 4.1.

After each visit to the study clinic, each participant and their test results were reviewed at weekly clinical decision meetings attended by the principal investigator and clinic staff with regard to disease progression. Each case is reviewed to identify progression to SMM, smoldering WM (SWM), and MM and related disorders as determined by current diagnostic criteria.[15, 30, 162] If further testing, including repeat bone marrow sampling and imaging, further imaging (for example magnetic resonance imaging (MRI) or positron emission tomography (PET)) or closer evaluation is clinically indicated, clinical decision meetings can order this testing. Incidental findings are referred to the primary care physician of the participant in question.

Those who are diagnosed with SMM or SWM enter more intensive follow-up. This includes blood testing and clinical follow-up at 4-6-month intervals and annual bone marrow sampling and imaging. Those who develop MM or SMM that is deemed intermediate- or high risk according to the 2018 Mayo risk criteria ("2/20/20") or the Spanish risk classification for SMM, are offered participation in a phase 2 clinical trial of lenalidomide and dexamethasone for intermediate-risk SMM and carfilzomib, lenalidomide and dexamethasone for high-risk SMM and MM. Participants with MM and other related more advanced disorders who are not eligible or do not want to participate in the clinical trial are referred to the department of hematology at LUH for further assessment, treatment, and follow-up.

4.1.5 Assessment of imaging studies and bone marrow samples

Imaging studies are independently reviewed by an experienced physician in specialty training and an experienced senior radiologist at LUH. The detection of lytic lesions is

recorded and other incidental findings are relayed to the study team who refer them to primary care physicians.

Bone marrow is sampled as smears and trephine biopsies. Bone marrow smears are stained using hematoxylin and eosin and evaluated by two experienced senior hematologists at LUH and the percentage of plasma cells or lymphoplasmacytic lymphocytes is recorded. Trephine biopsies are stained using hematoxylin and eosin and stained for CD138 before being assessed by two senior hematopathologists at LUH. The sample with the higher percentage of plasma cells or lymphocytic lymphocyte infiltration at each sampling is used to guide staging, diagnosis, and follow-up.

4.1.6 Questionnaires

When participants registered on the study website participants were immediately asked to answer baseline questionnaires on depression, anxiety, and overall satisfaction with life (PHQ-9, GAD7, and SWLS).[163-165] Those who registered by mail were asked to provide an e-mail address that is used to mail these same questionnaires to these participants at baseline. Multiple other questionnaires on various aspects of psychological and physical health as well as baseline information on demographics and socio-economic factors were also sent to participants. These questionnaires are further detailed in table 4.2.

4.1.7 Registry crosslinking

All residents of Iceland are assigned a national identification number at birth or immigration. This number is used to identify the person in their interaction with government institutions and businesses including the universal national healthcare system.

Multiple national registries exist in Iceland that include healthcare-related data on Icelanders that can be crosslinked with virtually complete accuracy using the national identifier number. Some of these registries are crosslinked to all participants in the iStopMM study. They include: (1) The Icelandic Cancer Registry includes information on all cancers diagnosed in Iceland. It has been mandatory for all physicians and pathologists to register diagnoses of cancer since 1955 and it is virtually complete with high diagnostic accuracy and timeliness31; (2) The Icelandic Causes of Death Registry includes all deaths in Iceland including the date and the presumed causes of death. Registration has been mandatory since 1971; (3) The Icelandic Prescription Medicines Registry includes all prescriptions, in Iceland since 2002, including whether the prescriptions were filled or not; (4) The Icelandic Hospital Discharge Registry includes all inpatient admissions in Iceland from 1999 with the dates of admission and discharge, as well as international classification of diseases (ICD) codes for the diagnoses made by treating physicians. The registry also includes outpatient visits at hospitals, including emergency rooms since 2010; (5) The Icelandic Registry of Primary

Health Care Contacts includes all primary care visits and registered ICD-coded diagnoses for all primary care encounters in Iceland since 2004; (6) The Icelandic Central Laboratory Database comprises laboratory test results from all major clinical laboratories in Iceland stored in a central database since 1999, including all blood tests for participants prior to participation and during follow- up in the study; (7) All medical records at LUH, the only tertiary care medical center in Iceland and the general acute care hospital for the vast majority of Icelandic residents. This includes clinical notes, anthropometric data, written radiology and pathology reports, microbiology and virology test results, and all other documented clinical data.

Questionnaire	Subject	All	Arm 1 and normal screening		Arm 2 and 3 and advanced disease[1]	
		At registration	One time	Annually	One time	Each visit
Background						
Anthropomorphic data	Weight, height etc.		x		x	
Social history[2]	Socioeconomic status		x		x	
Medical history[3]	Medical history		x		x	
Habits[4]	Environment		x		x	
Industrial exposure	Environment		x		x	
Quality of life						
PHQ9	Depression	x		x		x
GAD-7	Anxiety	x		x		x
SWLS	Quality of life	x		x		x
Other questions	Quality of life	x		x		x
SF-36	Health related quality of life			x		x
PSS-10	Stress and anxiety			x		x
PCL-5 (MGUS specific)	PTSD from MGUS diagnosis					x
PCL-5 (nonspecific)	PTSD other			x		
Symptoms						
BPI	Pain			x		x
NSS	Neuropathy			x		x
DN4	Neuropathy			x		x
Symptoms of PMR	PMR			x		x
Social background						
MSPSS	Social support		x		x	
CD-RISC-10ICE	Resilience		x		x	
ACE	Childhood traumatic events		x		x	
LEC	Lifetime traumatic events		x		x	

Questionnaires were not sent to participants who did not provide an email address and were not called into the study. 1: Including MM, WM, SMM, and SWM. 2: Employment, marital status, education, income, and residence. 3: Including obstetric history for women. 4: Including smoking and alcohol intake. PHQ9: Patient health questionnaire. GAD-7: General anxiety disorder. SWLS: Satisfaction with life scale. SF-36: 36-item short form survey. PSS-10: Perceived stress scale. PCL-5: Post traumatic stress disorder checklist for DSM-5. BPI: Brief pain inventory. NSS: Neuropathy symptom scale. DN4: Douleur neuropathique. PMR: Polymyalgia rheumatica. MSPSS: Multidimensional scale of social support. CD-RISC-10ICE: Connor-Davidson resilience scale. ACE: Adverse childhood events. LEC: Lifetime events checklist.

Table 4.2 (Previous page): Questionnaires sent to participants by email or answered at the study clinic. Note that all participants were asked to answer four questionnaires when providing informed consent electronically or if they provided an email address in their written consent form. This is table 2 of paper III, see attached manuscript.

4.1.8 Biobanking

Blood, bone marrow, and urine samples collected during clinical follow-up in iStopMM are collected and stored in the study biobank. All cell fractions are cryopreserved, and other samples are frozen. All samples are stored in a secure state-of-the-art robotic biobanking facility in Reykjavík, Iceland. The samples collected are further detailed in table 4.3.

4.1.9 Study endpoints

The primary endpoint of the study is the overall survival of individuals with MGUS receiving follow-up (arms 2 and 3) compared to those not receiving any follow-up within the study (arm 1) after 5 years of follow-up. Secondary endpoints are cause-specific survival due to MM or other LPs, psychiatric health and well-being, and cost-effectiveness of screening. In addition, study data will be crosslinked to registries and samples in the biobank providing a large dataset for future studies. Assuming that 3360 individuals with MGUS are identified and the HR for the primary outcome is 0.81 as previously described[139] the study has 77.2% power to reject the null hypothesis of HR = 1 at 5 years of follow-up and 89.3% power at 7 years of follow-up at an alpha level of 0.05.

4.2 MGUS and COVID-19 (paper IV) - Methods

4.2.1 Study cohort

All participants in iStopMM who had been screened for MGUS before December 31st, 2020, and were alive and free from MM and related disorders before the first diagnosis of COVID-19 in Iceland on February 28th, 2020 were included in the study. Having MGUS was determined by a detectable M protein on SPEP and/or an abnormal FLC ratio (kappa/lambda ratio <0.26 and lambda >26.3 g/L or a kappa/lambda ratio >1.65 and kappa >19.4 g/L). Sub-analyses were performed for LC-MGUS and non-LC MGUS (here referred to as heavy chain (HC) MGUS).

Sample	Arm 2 – Low risk	Arm 2 – Non-low risk	Arm 3 – All
Bone marrow			
-Sorted and unsorted cells[1]	None	0 and 60 months	0 and 60 months
-Plasma	None	0 and 60 months	0 and 60 months
Blood			
-Cell-free plasma (EDTA tube)	0 months	Annually	Annually
-Plasma (Li-Hep tubes)	0 months	Annually	Annually
-Serum (SST tubes)	0 months	Annually	Annually
-Blood RNA (PaxGene® tube)	0 months	0 months	0 months
-Lymphocytes (CPT tube)	0 months	0 and 60 months	0 and 60 months
Urine	0 months	0 months	0 months
	SMM and SWM– 4-month follow-up	**SMM and SWM – 6-month follow-up**	**MM and WM**
Bone marrow			
-Sorted and unsorted cells[1]	Annually	Annually	At diagnosis
-Plasma	Annually	Annually	At diagnosis
Blood			
-Cell-free plasma (EDTA tube)	Every 4 months	Every 6 months	At diagnosis
-Plasma (Li-Hep tubes)	Every 4 months	Every 6 months	At diagnosis
-Serum (SST tubes)	Every 4 months	Every 6 months	At diagnosis
-Blood RNA (PaxGene® tube)	0 months	0 months	At diagnosis
-Lymphocytes (CPT tube)	0 and 60 months	0 and 60 months	At diagnosis
Urine	Annually	Annually	At diagnosis

MM: Smoldering Multiple Myeloma. SWM: Smoldering Walderströms Macroglobulinemia. MM: Multiple Myeloma. WM: Waldenströms Macroglobulinemia 1: Buffy coat from the bone marrow samples. Unsorted in IgM MGUS but stored as CD138+ and CD138- fractions using magnetic-activated cell sorting (MACS) in Non-IgM MGUS and LC-MGUS.

Table 4.3 (Previous page): Timing of sampling for biosamples collected to the study biobank. This is table 3 of paper III, see attached manuscript.

4.2.2 COVID-19 in Iceland

The first case of COVID-19 was diagnosed in Iceland on February 28th, 2020. Early on, the Icelandic authorities implemented an aggressive strategy of testing and tracing with targeted testing in clinical practice and open invitation population-based screening, random screening for SARS-CoV-2 in asymptomatic persons, and screening of traced contacts of those who tested positive for SARS-CoV-2. As the pandemic continued, random screening was discontinued as self-ordered testing with same-day results became available to the public. Double-screening was also performed at the border. This has led to Iceland having some of the highest case-capture rates found worldwide, especially early on.[166]

All SARS-CoV-2 testing during 2020 was done by real-time quantitative polymerase chain reaction (qPCR) of simultaneously acquired naso- and oropharyngeal swabs. All those who tested positive during this period were immediately contacted by the COVID-19 outpatient clinic at LUH and the individual enrolled into telehealth monitoring. In asymptomatic cases, a repeat qPCR test was performed and serum SARS-CoV-2 antibodies were measured. Those who had COVID-19 were followed with daily phone calls by nurses and physicians and when clinical deterioration was detected during interviews, patients were called in for further in-person assessment at the COVID-19 clinic at LUH. This comprehensive strategy of combined community and clinical care of patients with COVID-19 has been described in greater detail elsewhere.[167]

4.2.3 Statistical analysis

The primary exposure was MGUS as determined by M protein detectable on CZE and confirmed by IFE or an abnormal FLC ratio (kappa/lambda ratio <0.26 and lambda >26.3 g/L or a kappa/lambda ratio >1.65 and kappa >19.4 g/L). Those with MGUS were further subdivided into heavy chain-MGUS (HC-MGUS) and light-chain MGUS (LC-MGUS) subgroups.

In the first analysis, we evaluated whether there was an association between MGUS and testing positive for SARS-CoV-2 using a test-negative study design. Participants from the study cohort who had been tested at least once for SARS-CoV-2 between February 28 and December 31, 2020, were included. Those who had at least one positive qPCR test for SARS-CoV-2 were considered to be infected. The association between MGUS and SARS-CoV-2 infection was evaluated using logistic regression adjusting for sex and age.

In the second analysis, we evaluated the association between MGUS and severe COVID-19. Participants from the previous analysis who tested positive for SARS-CoV-2 were included. Those who were hospitalized for other medical problems or were living in a nursing home at the time of testing were excluded. Participants were followed until discharge from telehealth monitoring or until they had developed severe COVID-19.

Severe COVID-19 was defined as the composite outcome of requiring an emergency outpatient visit, requiring hospital admission, or death (emergency outpatient visit or worse). Additionally, we conducted an analysis where severe COVID-19 was defined as hospital admission or death (hospital admission or worse). The association of MGUS with severe COVID-19 was assessed using logistic regression, adjusting for sex and age.

Two sensitivity analyses were performed. In the first sensitivity analysis, we evaluated the association of MGUS and SARS-CoV-2 testing in the whole study cohort using logistic regression, adjusting for sex and age. In the second sensitivity analysis, we repeated the first analysis and included the entire study cohort regardless of whether the participants had been tested for SARS-CoV-2 or not.

All analyses were carried out separately for MGUS, HC-MGUS, and LC-MGUS. When included as a covariate, age was modeled with a four-knot restricted cubic spline. Analyses were carried out in R, version 3.6.3,[168] using the *rms* package.[169]

4.3 Results and discussion

4.3.1 Recruitment of iStopMM (paper III)

At the time when enrollment started into the iStopMM study, 148,704 individuals were in the target population. During the 15 months of recruitment, 80,759 (54.3%) provided informed consent for participation in the study (Figure 4.4.). This high participation rate can be attributed to the extensive promotional efforts of the study team which had a considerable presence in conventional and social media during the enrollment period. More importantly, participation in scientific studies in Iceland has historically been high. Furthermore, the study design whereby participants could easily register for the study and did not need to schedule a blood draw for the study, may also have led to a higher participation rate.

Slightly more women than men signed up for the study (54% vs 46%). There was an "inverted-u" relationship between age and participation with the highest participation rate among those aged 60-79 (64%). Participation was lower in the younger age groups for example 46% of those between 40-49 years old but lowest in those above 90 years old (18%) (Figure 4.5). Although a majority of participants resided in the Capital area of Iceland (59%). The participation rate was higher in the Capital area than outside it (51% vs 60%) (Table 4.4). Although we find this differential participation by sex, age, and residence, we believe the cohort of participants in the iStopMM study to be representative of the Icelandic population and many other populations that would be eligible and willing to undergo screening for MGUS.

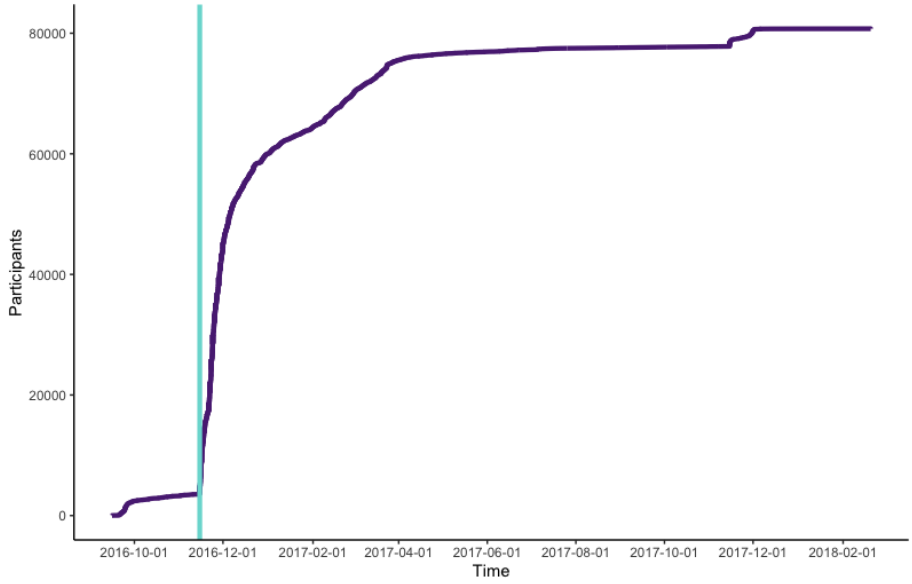


Figure 4.4: Participant enrollment over the recruitment period. The light green line represents the end of the pilot period and the initiation of nationwide recruitment. This is figure 3 from paper III, see attached manuscript.

In total, 548 (0.7%) participants had previously known lymphoproliferative disorders and were excluded and 246 (0.3%) had previously known MGUS. When enrollment for the study stopped on February 20th, 2018, 190,382 hospital admissions, 8,187,805 primary healthcare visits, 10,328 cancer diagnoses, and 15,839,376 medication prescriptions were registered in the national healthcare-related registries in Iceland.

With this high participation rate and representative population sample acquired during study enrollment, the number of participants that will be randomized is very likely to exceed the 3,360 assumed in the power calculations for the study. Furthermore, the extensive data acquired will make it possible to generate the high-quality dataset on MGUS or future studies on MGUS development, progression, and relationships to other disorders.

The primary aim of the iStopMM study is to evaluate the benefits and harms of population-based screening for MGUS. In evaluating the benefits, the most important outcome will be overall survival but other secondary endpoints, including MM-specific survival and the rate of symptomatic MM and MM-related end-organ damage will also serve as key indicators of benefit. When assessing harms, the most important hypothesized harm is psychological harm from knowing about a precancerous condition that may never lead to disease. This may take the form of increased anxiety and depression, post-traumatic stress, lowered sense of well-being, and a lowered general quality of life. These important factors are all frequently assessed in order to

identify these harms and what subgroups of individuals may be particularly vulnerable to these psychological harms. Other potential harms include radiation from imaging and potential complications of bone marrow and bone marrow sampling which are rare. It is now clear, based on this high participation rate, that the iStopMM study will be able to answer this key question whose answer is the missing link between the present and a future where population-based screening for MGUS is a reality. Such screening has the potential to lead to a paradigm shift in the care of patients with MM from the treatment of individuals with end-organ disease to preemptive treatment, preventing such end-organ disease, with the potential to improve survival, economic costs of care, and most importantly, the quality of life of patients with MM

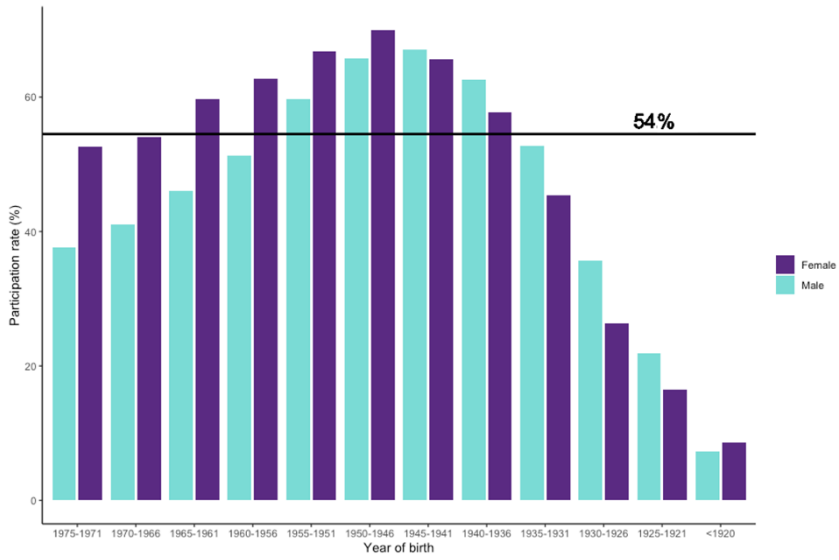


Figure 4.5: A figure showing the rate of participation in the iStopMM study by year of birth and gender. The black line represents the overall participation rate in the study.

	Registered participants	Target population
n	80 759	148 704
% females	54%	51%
median age[1]	59	57
Age range[1]	40-104	40-107
Participation rate		
All	54%	-
Males	51%	-
Females	58%	-
Age group (male/female)[1]		
40-49 (%)	21.2% / 23.7%	27.4% / 26.0%
50-59 (%)	27.7% / 29.9%	29.4% / 28.7%
60-69 (%)	28.4% / 26.1%	23.4% / 22.4%
70-79 (%)	16.6% / 14.4%	12.9% / 13.3%
80-89 (%)	5.7% / 5.3%	6.0% / 7.8%
>90 (%)	0.4% / 0.5%	0.9% / 1.8%
Place of residence		
Reykjavik Capital Area	58.7%	62.9%
Other urban centers[2]	17.5%	15.6%
Rural	23.3%	21.1%
Missing	0.6%	0.4%
Known MGUS[3]	246 (0.3%)	-
Previous LP[4]	548 (0.7%)	-
Data from registries[5]		
n hospital admissions	190 382	-
n primary care visits	8 187 805	-
n cancers diagnoses	10 328	-
n prescriptions	15 839 376	-

1: Age at the time of study initiation on September 9th 2016. 2: Urban centers with >5000 inhabitants outside the Capital area. 3: As registered before study enrollment in the Icelandic Cancer Registry since 1955, Icelandic Central Laboratory Database since 1999, and a registry of MGUS cases at Icelandic Private Clinics 4: As recorded before study enrollment in the Icelandic Cancer Registry since 1955 5: As recorded in national registries at the close of study enrollment on February 20th 2018.

Table 4.4: The age, sex, and geographical distribution of participants and the target population as well as available national registry data at the close of study recruitment. This is table 4 from paper III, see attached manuscript.

4.3.2 MGUS and COVID-19 (paper IV)

Out of the 80,759 participants who registered for the iStopMM study, 75,422 were screened for MGUS. At the start of the COVID-19 pandemic in Iceland on February 28th, 2020, 1,854 participants had died and 693 had been diagnosed with MM or a related disorder. In total, 32,047 of those screened were tested for SARS-CoV-2 during the year 2020, of whom 1,754 (5.5%) had MGUS. Those who had MGUS were more likely to be male (50% vs 41%, p<0.001) and were older (66.3 vs 59.1 years, p<0.001) than those who did not have MGUS. A total of 1,100 (3.4%) participants tested positive for SARS- CoV-2 during the study period, including 65 individuals with MGUS (3.7%) (Figure 4.6).

	No MGUS	**MGUS**	**HC-MGUS**	**LC-MGUS**
n	30,293	1,754	1140	614
Mean age (SD)	59 (10)	66 (11)	65.9 (11)	66.9 (11)
Men	12,283 (41%)	880 (50%)	571 (50%)	309 (50%)
Person-years	24,882	1,431	-	-
SARS-CoV-2 positive	1,035 (3.4%)	65 (3.7%)	41 (3.6%)	24 (3.9%)
OR of SARS-CoV-2 positivity (95% CI)[1]	Reference	1.05 (0.80-1.36)	1.02 (0.74-1.40)	1.11 (0.73-1.69)

1: Adjusted for age and sex; MGUS: Monoclonal gammopathy of undetermined significance HC-MGUS: Heavy chain MGUS; LC-MGUS: Light chain MGUS; SD: Standard deviation; COVID-19: Coronavirus disease 2019; OR: Odds ratio; CI: Confidence interval.

Table 4.5: Baseline characteristics of study participants in paper IV that were tested for SARS-CoV-2 and the association between MGUS and a positive test result. This is table 1 in paper IV, see attached manuscript.

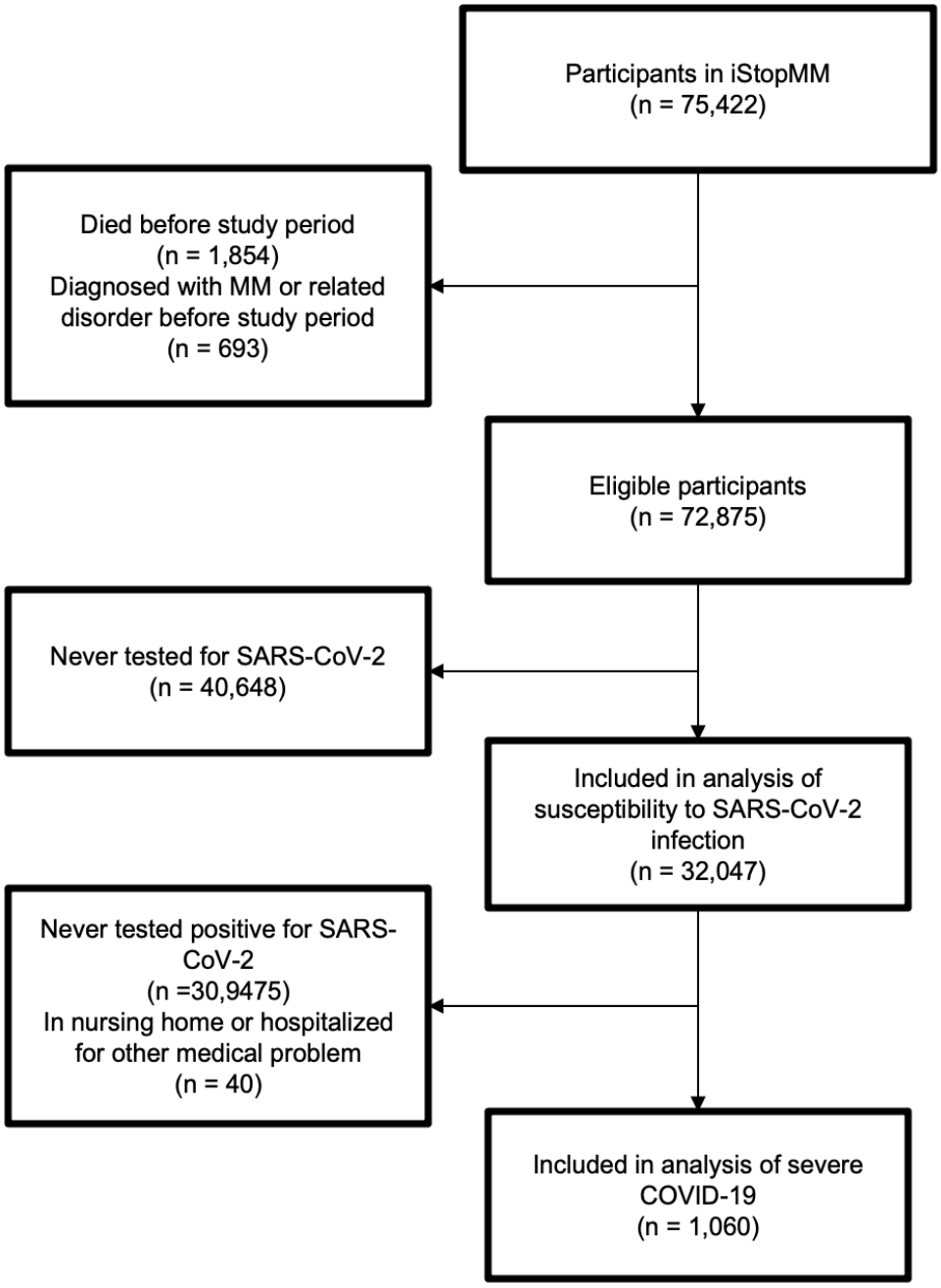


Figure 4.6: Flowchart demonstrating the inclusion and exclusion of participants in paper IV. This is figure 1 in paper IV, see attached manuscript.

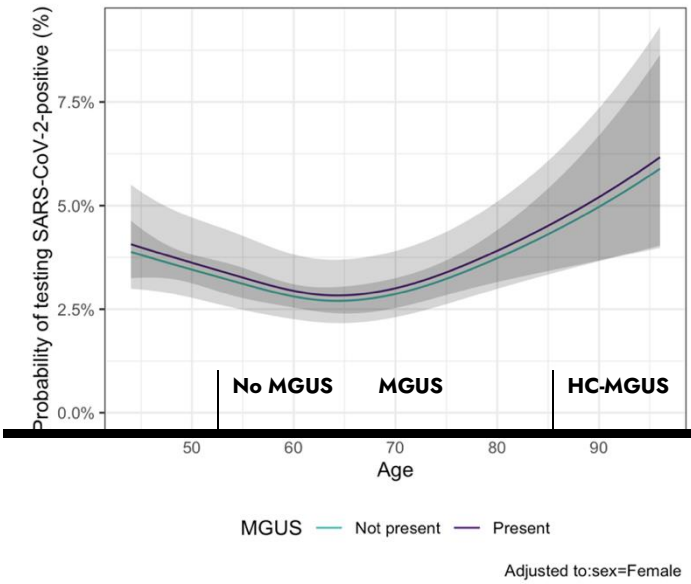


Figure 4.7: A graph illustrating the probability of individuals with and without MGUS of testing positive for SARS-CoV-2. This is figure 2 in paper 4, see attached manuscript.

After adjusting for age and sex we did not find MGUS to be associated with risk of testing positive for SARS-CoV-2 (HR: 1.05; 95% CI: 81-1.36; p=0.72). The findings were similar for LC and HC MGUS (Table 4.5. and Figure 4.8.).

These findings contradict previous studies that have shown an increased risk of infections, including viral infections, in MGUS.[94] These results might indicate that the previously observed risk for infections in MGUS does not apply specifically to COVID-19. However, a more likely explanation is to be found in the limitations of previous studies that included clinical cohorts with MGUS, where MGUS is diagnosed during workup for unrelated medical problems that may predispose to infections and to seeking medical care which results in the registration infections. Furthermore, the control groups in previous studies have not been tested for MGUS and a proportion of them may actually have MGUS. In contrast, this study includes a cohort that has been tested for MGUS and has a high rate of SARS-CoV-2 testing (42%). Importantly, we did not find an association between MGUS and the rate of SARS-CoV-2 testing (Table 4.5 and Figure 4.9) and a sensitivity analysis including the whole cohort regardless of SARS-CoV-2 testing showed essentially the same results as the primary analysis. These sensitivity analyses provide further confidence in the study results and that detection bias did not play a significant role in the results of the study. It is possible that the real risk of infections in individuals with MGUS is much lower than previously reported and future studies using the iStopMM data will be able to validate or displace previous dogma about infection risk in MGUS.

Out of the 1,100 participants who tested positive for SARS-CoV-2, 40 were hospitalized or residing in nursing homes at the time of testing and were excluded (Figure 4.7) from later analysis. Of the remaining 1,060, 56 had MGUS. During follow-up, 16 individuals with MGUS (29%) and 214 (21%) of those without MGUS needed emergency care at the COVID-19 outpatient clinic at LUH, were admitted, or died while they had COVID-19. After adjusting for age and sex, MGUS was not found to be associated with severe COVID-19 when defined as emergency outpatient visit or worse (OR: 0.99; 95%CI:

n	1004	56	35	21
Mean age (SD)	59 (10)	65 (11)	65 (12)	65 (10)
Men	448 (45%)	30 (54%)	22 (63%)	8 (38%)
Person-days	16,589	962	-	-
Emergency outpatient visit	176 (18%)	12 (20%)	7 (20%)	5 (24%)
Hospital admission	105 (11%)	11 (20%)	8 (23%)	3 (14%)
Intensive care unit admission	20 (2%)	3 (5%)	2 (6%)	1 (5%)
Death	5 (1%)	0 (0%)	0 (0%)	0 (0%)
Emergency outpatient visit or worse	214 (21%)	16 (29%)	10 (29%)	6 (29%)
OR (95% CI)[1]	Reference	0.99 (0.52-1.91)	0.90 (0.39-2.08)	1.10 (0.39-3.10)
Hospital admission or worse	106 (11%)	11 (20%)	8 (23%)	3 (14%)
OR (95% CI)[1]	Reference	1.13 (0.52-2.46)	1.25 (0.49-3.19)	0.83 (0.21-3.29)

1: Adjusted for age and sex; MGUS: Monoclonal gammopathy of undetermined significance HC-MGUS: Heavy chain MGUS; LC-MGUS: Light chain MGUS; SD: Standard deviation; COVID-19: Coronavirus disease 2019; OR: Odds ratio; CI: Confidence interval.

Table 4.6: The baseline characteristics and outcomes of participants in paper 4 who tested positive for SARS-CoV-2 and the association of MGUS and COVID-19 severity.

0.52–1.91; $p = 0.99$) or as hospital admission or worse, (OR: 1.13; 95%CI: 0.52–2.46; $p = 0.76$). The findings were similar for HC- and LC-MGUS (Table 4.6 and Figure 4.9). These findings indicate that individuals with MGUS are not at increased risk of severe COVID-19. This is contrary to previous studies that have found MM to be associated with severe COVID-19, with disease-related factors being the most likely cause of the increased risk rather than treatment-related factors.[127-130] Disease-related immune dysfunction in MM is believed to be the main driver of severe COVID-19 risk and previous studies have shown similar immune dysfunction in MGUS, albeit milder than in MM patients. This finding is therefore somewhat unexpected and highlights that

there are stark differences between MGUS and MM and that immune dysfunction issignificantly less pronounced in MGUS. Alternatively, these findings could indicate that treatment-related factors in MM have been underestimated as risk factors for severe COVID-19 in previous studies and that the lack of treatment in those with MGUS serves as the main reason for this discordance between MGUS and MM.

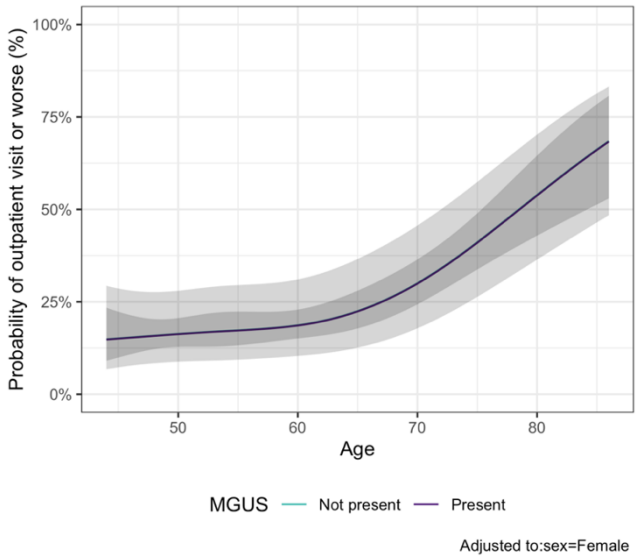


Figure 4.8: A graph illustrating the probability of individuals with and without MGUS developing severe COVID-19 after testing positive for SARS-CoV-2. This is figure 3 in paper 4, see attached manuscript.

In conclusion, we found MGUS not to be associated with contracting SARS-CoV-2 or with severe COVID-19 once infected. MGUS is one of the most common hematological disorders and the results are reassuring for this patient group. It is however important to note that the spectrum of disease in MGUS is quite wide and those at the extreme of this spectrum are likely to resemble those with MM more closely. In this study, subpopulations with MGUS who are at increased risk of severe COVID-19 may have been overshadowed by those at the lower end of the MGUS spectrum. It is therefore reasonable to assume that there are individuals with MGUS who are more like those with MM who are at increased risk of COVID-19, particularly those with high-risk disease and detectable immune dysfunction, for example decreased uninvolved immunoglobulins. However, in general, individuals with MGUS appear to have the same risks during the COVID-19 pandemic as other individuals in society. These findings can inform how treating physicians counsel their patients during the COVID-19 pandemic.

4.3.3 Strengths of paper III and IV

The iStopMM study has many strengths. Firstly, the iStopMM study is the largest clinical trial in the field of MM to date with over 80,000 participants who have provided their informed consent for participation. This high participation rate has been followed by a very high acquisition rate of samples through a passive sampling process with samples from 93% (n=75,422) of participants being collected for screening. The size and population-based nature of the study enable the study to answer the critical questions regarding screening for MGUS that may lead to a paradigm shift in MM care. Secondly, the screened cohort that is gathered during the study is unique in that selection bias that may have affected previous studies on MGUS is far less likely to affect this cohort. Furthermore, a majority of those with MGUS are further evaluated providing increased granularity in data and a clearer view of the cohort and how the participants are distributed across the disease spectrum of MM and its precursors. Thirdly, data from the study is crosslinked to national registries and blood and bone marrow samples from participants are collected repeatedly over time and stored in a study biobank. This extensive healthcare-related data and biobank will generate an extensive dataset for epidemiological, translational, and clinical studies that will lead to new discoveries and validation of previous findings or clinical practice that has lacked high-quality data.

These strengths are demonstrated in paper IV where this extensive dataset is crosslinked to high-quality data from LUH and an important and pressing question in the field is answered. There, the screened cohort sets the study out from previous studies and limits potential selection bias that is present in studies including clinical cohorts with MGUS. Secondly, due to the high testing rate and central registration and close follow-up of patients with COVID-19 in Iceland, the data on SARS-CoV-2 testing and COVID-19 outcomes are very robust. Finally, the study includes a large proportion of a whole nation's population making the findings more generalizable to other similar populations.

4.3.4 Limitations of paper III and IV

There are important limitations to the iStopMM study. Firstly, the Icelandic population is highly genetically and ethnically homogenous with almost all native Icelanders being white. This limits the generalization of the study findings to non-white populations. This is particularly important in Black Americans and Africans who are at an increased risk of MGUS and MM. Secondly, those who are diagnosed with asymptomatic MM, intermediate-risk SMM, and high-risk SMM are offered early treatment usual hard endpoints of MGUS progression are not reliable and because SMM is a clinical, rather than a biological entity, some important distinctions between those who will progress to active disease and those who will remain with stable SMM will not be captured. Finally, the primary outcome of overall survival is a very ambitious outcome for a disease like

MGUS where only 1% progress annually to overt malignancy whose prognosis is rapidly improving. However, there are other clinically important endpoints, particularly symptomatic MM, that can be seen as meaningful improvements that may justify screening in lieu of observing improved overall survival with screening.

Some specific limitations of paper IV are most notably the low number of events in the MGUS group, particularly mortality and admissions. This limits the choice of outcomes to composite measures including seeking emergency outpatient care which is not always in relation to the actual biological severity of COVID-19. Furthermore, this might leave the study underpowered to detect a modestly increased risk in those with MGUS.

5 Summary and conclusions

MGUS is common in the general population and although asymptomatic, it may have significant clinical consequences for affected individuals. Throughout the work of this thesis, the aim has been to uncover the clinical significance of MGUS and the results have shed some light on this issue. Furthermore, the studies have pointed in some directions that may provide a way forward to further studies to expand on our understanding of the clinical significance of this asymptomatic precursor disorder.

In the first section of the thesis, large-scale registry data on individuals diagnosed with MGUS in Sweden was used to gain insights into the relationship between MGUS and PN and fractures. One of the main limitations of this approach is the selection and information bias associated with the clinical diagnosis of MGUS which invariably occurs alongside the diagnosis of other medical problems and leads to clinical observation. In the first paper on the relationship between PN and MGUS, stratification by DM diagnosis, a disorder that leads to clinical observation, particularly for PN, was utilized to mitigate some of this bias leading to more confident conclusions on the association of MGUS and PN that would have been possible otherwise. In the second paper on the relationship between MGUS and fractures, a multi-faceted study design and inclusion of PN and important comorbidities into statistical models enabled a more nuanced analysis of the potential causes of fractures in MGUS. Taken together the results indicate that MGUS has clinical significance in that it is associated with PN and fractures. Based on the findings we then hypothesized that PN is one of, at least, three causes of fractures in MGUS alongside undetected MM bone disease and MGUS inherent bone disease. However, methodological limitations of studying MGUS in clinical cohorts and registries remain, including selection bias and residual confounding, which cast some doubt on the findings.

In the second section of the thesis, iStopMM, a population-based screening study and randomized controlled trial of follow-up strategies, was described. The study design, which was described in the third paper, aims to uncover the clinical significance of MGUS, primarily by evaluating the benefits and harms of population-based screening for MGUS and clinical follow-up with the aim of detecting MGUS progression and applying early treatment in MM. Over 80,000 participants signed up for the study and 75,422 samples have been collected for screening. This high participation rate and sampling rate will enable iStopMM to reach its aims. A secondary aim of the study is to generate a high-quality dataset including individuals with MGUS detected by screening that is not affected by the selection bias of previous studies and where MGUS-related information bias can be attenuated improving our understanding of the clinical

significance of MGUS. This was demonstrated in the fourth paper where the relationship between MGUS and COVID-19 was studied within the iStopMM cohort. MGUS was not found to be associated with contracting SARS-CoV-2 or developing severe COVID-19. These findings are in contrasts with previous studies that have found MGUS to be associated with an increased risk of infections, including viral infections. However, previous studies were performed within clinical cohorts and these unexpected findings, therefore, highlight the need to study MGUS disease associations in screened cohorts. Furthermore, this indicates that future studies on the iStopMM MGUS cohort will disprove some of the previously reported disease associations or find significantly attenuated risk increases associated with MGUS than previously reported. This would mean that the clinical significance of MGUS is less than previously believed.

In conclusion, MGUS has clinical significance. It is the precursor of MM and related disorders and seems to lead to multiple non-malignant complications. Methodological limitations of previous studies, which are mostly caused by the asymptomatic nature of MGUS, are likely to have led to some false associations and the actual risk of many non-malignant complications has probably been overestimated. One of the key results of iStopMM and other studies like it will be whether detection of MGUS is benifical and how follow-up of MGUS should be designed in order to detect MGUS progression effectively. Future studies should also seek to validate or disprove previously detected disease associations of MGUS within screened MGUS cohorts like that of iStopMM. This will clarify the clinical significance of MGUS and enable focused and effective care of those with MGUS. Hopefully, this can lead to improved outcomes in this common yet elusive precursor condition of still "undetermined" significance.

References

1. Waldenström J. Studies on conditions associated with disturbed gamma globulin formation (gammopathies). *Harvey Lect* 1960; 56: 211-231.

2. Murphy K and Weaver C. *Janeway's immunobiology*. 9th edition. ed. New York, NY: Garland Science/Taylor & Francis Group, LLC, 2016, p.xx, 904 pages.

3. Schroeder HW, Jr. and Cavacini L. Structure and function of immunoglobulins. *J Allergy Clin Immunol* 2010; 125: S41-52. DOI: 10.1016/j.jaci.2009.09.046.

4. Attaelmannan M and Levinson SS. Understanding and Identifying Monoclonal Gammopathies. *Clinical Chemistry* 2000; 46: 1230-1238. DOI: 10.1093/clinchem/46.8.1230.

5. Trendelenburg M and Schifferli JA. Cryoglobulins in chronic hepatitis C virus infection. *Clinical and Experimental Immunology* 2003; 133: 153-155. DOI: 10.1046/j.1365-2249.2003.02198.x.

6. Keren DF. *High-resolution electrophoresis and immunofixation: Techniques and interpretation*. Elsevier, 2017.

7. Harding SJ, Mead GP, Bradwell AR, et al. Serum free light chain immunoassay as an adjunct to serum protein electrophoresis and immunofixation electrophoresis in the detection of multiple myeloma and other B-cell malignancies. *Clin Chem Lab Med* 2009; 47: 302-304. DOI: 10.1515/cclm.2009.084.

8. Basile U, Gulli F, Gragnani L, et al. Free light chains: Eclectic multipurpose biomarker. *Journal of Immunological Methods* 2017; 451: 11-19. DOI: https://doi.org/10.1016/j.jim.2017.09.005.

9. Dispenzieri A, Katzmann JA, Kyle RA, et al. Prevalence and risk of progression of light-chain monoclonal gammopathy of undetermined significance: a retrospective population-based cohort study. *Lancet* 2010; 375: 1721-1728. 2010/05/18. DOI: 10.1016/s0140-6736(10)60482-5.

10. Mills JR, Barnidge DR and Murray DL. Detecting monoclonal immunoglobulins in human serum using mass spectrometry. *Methods* 2015; 81: 56-65. DOI: https://doi.org/10.1016/j.ymeth.2015.04.020.

11. Murray DL, Puig N, Kristinsson S, et al. Mass spectrometry for the evaluation of monoclonal proteins in multiple myeloma and related disorders: an International Myeloma Working Group Mass Spectrometry Committee Report. *Blood Cancer Journal* 2021; 11: 24. DOI: 10.1038/s41408-021-00408-4.

12. Mills JR, Barnidge DR, Dispenzieri A, et al. High sensitivity blood-based M-protein detection in sCR patients with multiple myeloma. *Blood Cancer J* 2017; 7: e590. 20170825. DOI: 10.1038/bcj.2017.75.

13. Santockyte R, Jin C, Pratt J, et al. Sensitive multiple myeloma disease monitoring by mass spectrometry. *Blood Cancer Journal* 2021; 11: 78. DOI: 10.1038/s41408-021-00473-9.

14. El-Khoury H, Lee DJ, Alberge J-B, et al. Prevalence of monoclonal gammopathies and clinical outcomes in a high-risk US population screened by mass spectrometry: a multicentre cohort study. *The Lancet Haematology* 2022. DOI: https://doi.org/10.1016/S2352-3026(22)00069-2.

15. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *The Lancet Oncology* 2014; 15: e538-e548. DOI: 10.1016/s1470-2045(14)70442-5.

16. Siegel RL, Miller KD and Jemal A. Cancer statistics, 2018. *CA: A Cancer Journal for Clinicians* 2018; 68: 7-30. DOI: https://doi.org/10.3322/caac.21442.

17. Turesson I, Bjorkholm M, Blimark CH, et al. Rapidly changing myeloma epidemiology in the general population: Increased incidence, older patients, and longer survival. *European Journal of Haematology* 2018; 101: 237-244. DOI: https://doi.org/10.1111/ejh.13083.

18. Morgan GJ, Walker BA and Davies FE. The genetic architecture of multiple myeloma. *Nature Reviews Cancer* 2012; 12: 335-348. DOI: 10.1038/nrc3257.

19. Thorsteinsdottir S, Dickman PW, Landgren O, et al. Dramatically improved survival in multiple myeloma patients in the recent decade: results from a Swedish population-based study. *Haematologica* 2018; 103: e412-e415. DOI: 10.3324/haematol.2017.183475.

20. García-Sanz R, Jiménez C, Puig N, et al. Origin of Waldenstrom's macroglobulinaemia. *Best Pract Res Clin Haematol* 2016; 29: 136-147. 20161006. DOI: 10.1016/j.beha.2016.08.024.

21. Owen RG, Treon SP, Al-Katib A, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: Consensus Panel Recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. *Seminars in Oncology* 2003; 30: 110-115. DOI: https://doi.org/10.1053/sonc.2003.50082.

22. Castillo JJ, Advani RH, Branagan AR, et al. Consensus treatment recommendations from the tenth International Workshop for Waldenström Macroglobulinaemia. *The Lancet Haematology* 2020; 7: e827-e837. DOI: https://doi.org/10.1016/S2352-3026(20)30224-6.

23. Merlini G, Seldin DC and Gertz MA. Amyloidosis: pathogenesis and new therapeutic options. *Journal of Clinical Oncology* 2011; 29: 1924.

24. Hasib Sidiqi M and Gertz MA. Immunoglobulin light chain amyloidosis diagnosis and treatment algorithm 2021. *Blood Cancer Journal* 2021; 11: 90. DOI: 10.1038/s41408-021-00483-7.

25. Schulman A, Connors LH, Weinberg J, et al. Patient outcomes in light chain (AL) amyloidosis: The clock is ticking from symptoms to diagnosis. *European Journal of Haematology* 2020; 105: 495-501. DOI: https://doi.org/10.1111/ejh.13472.

26. Landgren O, Kyle RA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. *Blood* 2009; 113: 5412-5417. DOI: 10.1182/blood-2008-12-194241.

27. Weiss BM, Abadie J, Verma P, et al. A monoclonal gammopathy precedes multiple myeloma in most patients. *Blood* 2009; 113: 5418-5422. DOI: 10.1182/blood-2008-12-195008.

28. Weiss BM, Hebreo J, Cordaro D, et al. Monoclonal gammopathy of undetermined significance (MGUS) precedes the diagnosis of AL amyloidosis by up to 14 years. American Society of Hematology, 2011.

29. Kyle RA, Therneau TM, Rajkumar SV, et al. Long-term follow-up of IgM monoclonal gammopathy of undetermined significance. *Blood* 2003; 102: 3759-3764.

30. Kyle RA, Durie BGM, Rajkumar SV, et al. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. *Leukemia* 2010; 24: 1121-1127. DOI: 10.1038/leu.2010.60.

31. Kyle RA. Monoclonal gammopathy of undetermined significance: Natural history in 241 cases. *The American Journal of Medicine* 1978; 64: 814-826. DOI: https://doi.org/10.1016/0002-9343(78)90522-3.

32. Kyle RA, Therneau TM, Rajkumar SV, et al. Prevalence of Monoclonal Gammopathy of Undetermined Significance. *New England Journal of Medicine* 2006; 354: 1362-1369. DOI: 10.1056/nejmoa054494.

33. Landgren O, Gridley G, Turesson I, et al. Risk of monoclonal gammopathy of undetermined significance (MGUS) and subsequent multiple myeloma among African American and white veterans in the United States. *Blood* 2006; 107: 904-906. DOI: 10.1182/blood-2005-08-3449.

34. Landgren O, Katzmann JA, Hsing AW, et al. Prevalence of monoclonal gammopathy of undetermined significance among men in Ghana. *Mayo Clin Proc* 2007; 82: 1468-1473. DOI: 10.1016/s0025-6196(11)61089-6.

35. Han J-h, Wang J-n, Zhang Y-l, et al. Prevalence of monoclonal gammopathy of undetermined significance in a large population with annual medical check-ups in China. *Blood Cancer Journal* 2020; 10: 34. DOI: 10.1038/s41408-020-0303-8.

36. Gupta R, Dahiya M, Kumar L, et al. Prevalence of Monoclonal Gammopathy of Undetermined Significance in India-A Hospital-based Study. *Clin Lymphoma Myeloma Leuk* 2018; 18: e345-e350. 20180612. DOI: 10.1016/j.clml.2018.06.005.

37. Sigurdardottir EE, Turesson I, Lund SH, et al. The Role of Diagnosis and Clinical Follow-up of Monoclonal Gammopathy of Undetermined Significance on Survival in Multiple Myeloma. *JAMA Oncol* 2015; 1: 168-174. 2015/07/17. DOI: 10.1001/jamaoncol.2015.23.

38. Goyal G, Rajkumar SV, Lacy MQ, et al. Impact of prior diagnosis of monoclonal gammopathy on outcomes in newly diagnosed multiple myeloma. *Leukemia* 2019; 33: 1273-1277. 2019/02/23. DOI: 10.1038/s41375-019-0419-7.

39. Go RS, Gundrum JD and Neuner JM. Determining the clinical significance of monoclonal gammopathy of undetermined significance: a SEER-Medicare population analysis. *Clin Lymphoma Myeloma Leuk* 2015; 15: 177-186.e174. 2014/12/03. DOI: 10.1016/j.clml.2014.09.004.

40. Kyle RA, Larson DR, Therneau TM, et al. Long-Term Follow-up of Monoclonal Gammopathy of Undetermined Significance. *New England Journal of Medicine* 2018; 378: 241-249. DOI: 10.1056/NEJMoa1709974.

41. Turesson I, Kovalchik SA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance and risk of lymphoid and myeloid malignancies: 728 cases followed up to 30 years in Sweden. *Blood* 2014; 123: 338-345. 20131112. DOI: 10.1182/blood-2013-05-505487.

42. Rajkumar SV, Kyle RA, Therneau TM, et al. Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. *Blood* 2005; 106: 812-817. DOI: 10.1182/blood-2005-03-1038.

43. Kyle RA and Greipp PR. Smoldering multiple myeloma. *N Engl J Med* 1980; 302: 1347-1349. DOI: 10.1056/nejm198006123022405.

44. Kyle RA, Remstein ED, Therneau TM, et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. *N Engl J Med* 2007; 356: 2582-2590. DOI: 10.1056/NEJMoa070389.

45. Fonseca R, Debes-Marun CS, Picken EB, et al. The recurrent IgH translocations are highly associated with nonhyperdiploid variant multiple myeloma. *Blood* 2003; 102: 2562-2567. DOI: 10.1182/blood-2003-02-0493.

46. Rasmussen T, Kuehl M, Lodahl M, et al. Possible roles for activating RAS mutations in the MGUS to MM transition and in the intramedullary to extramedullary transition in some plasma cell tumors. *Blood* 2005; 105: 317-323. 20040831. DOI: 10.1182/blood-2004-03-0833.

47. Chng WJ, Huang GF, Chung TH, et al. Clinical and biological implications of MYC activation: a common difference between MGUS and newly diagnosed multiple myeloma. *Leukemia* 2011; 25: 1026-1035. DOI: 10.1038/leu.2011.53.

48. López-Corral L, Gutiérrez NC, Vidriales MB, et al. The Progression from MGUS to Smoldering Myeloma and Eventually to Multiple Myeloma Involves a Clonal Expansion of Genetically Abnormal Plasma Cells. *Clinical Cancer Research* 2011; 17: 1692-1700. DOI: 10.1158/1078-0432.Ccr-10-1066.

49. van Nieuwenhuijzen N, Spaan I, Raymakers R, et al. From MGUS to Multiple Myeloma, a Paradigm for Clonal Evolution of Premalignant Cells. *Cancer Research* 2018; 78: 2449-2456. DOI: 10.1158/0008-5472.Can-17-3115.

50. Graziani G, Herget GW, Ihorst G, et al. Time from first symptom onset to the final diagnosis of multiple myeloma (MM) - possible risks and future solutions: retrospective and prospective 'Deutsche Studiengruppe MM' (DSMM) and 'European Myeloma Network' (EMN) analysis(). *Leuk Lymphoma* 2020; 61: 875-886. 20191128. DOI: 10.1080/10428194.2019.1695051.

51. Koshiaris C, Oke J, Abel L, et al. Quantifying intervals to diagnosis in myeloma: a systematic review and meta-analysis. *BMJ open* 2018; 8: e019758-e019758. DOI: 10.1136/bmjopen-2017-019758.

52. Jamet B, Bailly C, Carlier T, et al. Imaging of Monoclonal Gammapathy of Undetermined Significance and Smoldering Multiple Myeloma. *Cancers (Basel)* 2020; 12: 486. DOI: 10.3390/cancers12020486.

53. Hjorth M, Hellquist L, Holmberg E, et al. Initial versus deferred melphalan-prednisone therapy for asymptomatic multiple myeloma stage I–a randomized study. Myeloma Group of Western Sweden. *Eur J Haematol* 1993; 50: 95-102. DOI: 10.1111/j.1600-0609.1993.tb00148.x.

54. Musto P, Petrucci MT, Bringhen S, et al. A multicenter, randomized clinical trial comparing zoledronic acid versus observation in patients with asymptomatic myeloma. *Cancer* 2008; 113: 1588-1595. DOI: 10.1002/cncr.23783.

55. Witzig TE, Laumann KM, Lacy MQ, et al. A phase III randomized trial of thalidomide plus zoledronic acid versus zoledronic acid alone in patients with asymptomatic multiple myeloma. *Leukemia* 2013; 27: 220-225. 20120820. DOI: 10.1038/leu.2012.236.

56. Mateos M-V, Hernández M-T, Giraldo P, et al. Lenalidomide plus Dexamethasone for High-Risk Smoldering Multiple Myeloma. *New England Journal of Medicine* 2013; 369: 438-447. DOI: 10.1056/NEJMoa1300439.

57. Lonial S, Jacobus S, Fonseca R, et al. Randomized Trial of Lenalidomide Versus Observation in Smoldering Multiple Myeloma. *Journal of Clinical Oncology* 2020; 38: 1126-1137. DOI: 10.1200/jco.19.01740.

58. Mann H, Katiyar V, Varga C, et al. Smoldering multiple myeloma – Past, present, and future. *Blood Reviews* 2022; 52: 100869. DOI: https://doi.org/10.1016/j.blre.2021.100869.

59. Landgren O. Shall we treat smoldering multiple myeloma in the near future? *Hematology* 2017; 2017: 194-204. DOI: 10.1182/asheducation-2017.1.194.

60. Price DE. The Politics of the War on Cancer: Cancer Crusade. The Story of the National Cancer Act of 1971. Richard A. Rettig. Princeton University Press, Princeton, NJ, 1977. xxii, 382 pp. $15. *Science* 1978; 199: 966-967.

61. Bretthauer M and Kalager M. Principles, effectiveness and caveats in screening for cancer. *Br J Surg* 2013; 100: 55-65. DOI: 10.1002/bjs.8995.

62. Smith RA, Andrews KS, Brooks D, et al. Cancer screening in the United States, 2019: A review of current American Cancer Society guidelines and current issues

in cancer screening. *CA Cancer J Clin* 2019; 69: 184-210. 20190315. DOI: 10.3322/caac.21557.

63. Adami H-O, Kalager M, Valdimarsdottir U, et al. Time to abandon early detection cancer screening. *European Journal of Clinical Investigation* 2019; 49: e13062. DOI: https://doi.org/10.1111/eci.13062.

64. Jørgensen KJ, Gøtzsche PC, Kalager M, et al. Breast cancer screening in Denmark: a cohort study of tumor size and overdiagnosis. *Annals of internal medicine* 2017; 166: 313-323.

65. Kalager M, Adami H-O, Bretthauer M, et al. Overdiagnosis of invasive breast cancer due to mammography screening: results from the Norwegian screening program. *Annals of internal medicine* 2012; 156: 491-499.

66. Screening IUPoBC. The benefits and harms of breast cancer screening: an independent review. *The Lancet* 2012; 380: 1778-1786. DOI: 10.1016/s0140-6736(12)61611-0.

67. Draisma G, Boer R, Otto SJ, et al. Lead times and overdetection due to prostate-specific antigen screening: estimates from the European Randomized Study of Screening for Prostate Cancer. *J Natl Cancer Inst* 2003; 95: 868-878. DOI: 10.1093/jnci/95.12.868.

68. Wilt TJ, Jones KM, Barry MJ, et al. Follow-up of Prostatectomy versus Observation for Early Prostate Cancer. *New England Journal of Medicine* 2017; 377: 132-142. DOI: 10.1056/NEJMoa1615869.

69. Vickers AJ, Sjoberg DD, Ulmert D, et al. Empirical estimates of prostate cancer overdiagnosis by age and prostate-specific antigen. *BMC medicine* 2014; 12: 1-7.

70. Mitchell AJ, Chan M, Bhatti H, et al. Prevalence of depression, anxiety, and adjustment disorder in oncological, haematological, and palliative-care settings: a meta-analysis of 94 interview-based studies. *Lancet Oncol* 2011; 12: 160-174. 20110119. DOI: 10.1016/s1470-2045(11)70002-x.

71. Hultcrantz M, Svensson T, Derolf ÅR, et al. Incidence and risk factors for suicide and attempted suicide following a diagnosis of hematological malignancy. *Cancer Medicine* 2015; 4: 147-154. DOI: https://doi.org/10.1002/cam4.316.

72. Lerman C, Miller SM, Scarborough R, et al. Adverse psychologic consequences of positive cytologic cervical screening. *American journal of obstetrics and gynecology* 1991; 165: 658-662.

73. Lampic C, Thurfjell E, Bergh J, et al. Short-and long-term anxiety and depression in women recalled after breast cancer screening. *European Journal of Cancer* 2001; 37: 463-469.

74. Vasarainen H, Malmi H, Määttänen L, et al. Effects of prostate cancer screening on health-related quality of life: results of the Finnish arm of the European randomized screening trial (ERSPC). *Acta Oncol* 2013; 52: 1615-1621. 2013/06/22. DOI: 10.3109/0284186x.2013.802837.

75. Aro AR, Absetz SP, van Elderen TM, et al. False-positive findings in mammography screening induces short-term distress—breast cancer-specific concern prevails longer. *European Journal of Cancer* 2000; 36: 1089-1097.

76. Snowsill T, Yang H, Griffin E, et al. Low-dose computed tomography for lung cancer screening in high-risk populations: a systematic review and economic evaluation. *Health Technol Assess* 2018; 22: 1-276. DOI: 10.3310/hta22690.

77. Fraiman J, Brownlee S, Stoto MA, et al. An Estimate of the US Rate of Overuse of Screening Colonoscopy: a Systematic Review. *Journal of General Internal Medicine* 2022. DOI: 10.1007/s11606-021-07263-w.

78. Pignone M, Saha S, Hoerger T, et al. Cost-effectiveness analyses of colorectal cancer screening: a systematic review for the US Preventive Services Task Force. *Annals of internal medicine* 2002; 137: 96-104.

79. Goffin JR, Flanagan WM, Miller AB, et al. Cost-effectiveness of lung cancer screening in Canada. *JAMA oncology* 2015; 1: 807-813.

80. Lowry KP, Trentham-Dietz A, Schechter CB, et al. Long-Term Outcomes and Cost-Effectiveness of Breast Cancer Screening With Digital Breast Tomosynthesis in the United States. *JNCI: Journal of the National Cancer Institute* 2019; 112: 582-589. DOI: 10.1093/jnci/djz184.

81. Ratushnyak S, Hoogendoorn M and van Baal PHM. Cost-Effectiveness of Cancer Screening: Health and Costs in Life Years Gained. *American Journal of Preventive Medicine* 2019; 57: 792-799. DOI: 10.1016/j.amepre.2019.07.027.

82. Wilson J and Jungner G. *Principles and practices of screening for disease*. 1968. Geneva, Switzerland: World Health Organization.

83. Andermann A, Blancquaert I, Beauchamp S, et al. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. *Bull World Health Organ* 2008; 86: 317-319. DOI: 10.2471/blt.07.050112.

84. Baz R, Lin HM, Hui AM, et al. Development of a conceptual model to illustrate the impact of multiple myeloma and its treatment on health-related quality of life. *Support Care Cancer* 2015; 23: 2789-2797. 20150224. DOI: 10.1007/s00520-015-2644-6.

85. Bhattacharya K, Bentley JP, Ramachandran S, et al. Phase-Specific and Lifetime Costs of Multiple Myeloma Among Older Adults in the US. *JAMA Network Open* 2021; 4: e2116357-e2116357. DOI: 10.1001/jamanetworkopen.2021.16357.

86. Yadav P, Cockwell P, Cook M, et al. Serum free light chain levels and renal function at diagnosis in patients with multiple myeloma. *BMC Nephrol* 2018; 19: 178-178. DOI: 10.1186/s12882-018-0962-x.

87. Murray D, Dispenzieri A, Kumar S, et al. Free Light Chain Assay Drift: Potential for Misdiagnosis? *J Appl Lab Med* 2020; 5: 1411-1413. DOI: 10.1093/jalm/jfaa093.

88. Kyle RA, Therneau TM, Rajkumar SV, et al. Incidence of multiple myeloma in Olmsted County, Minnesota. *Cancer* 2004; 101: 2667-2674. DOI: https://doi.org/10.1002/cncr.20652.

89. Turesson I, Velez R, Kristinsson SY, et al. Patterns of multiple myeloma during the past 5 decades: stable incidence rates for all age groups in the population but rapidly changing age distribution in the clinic. In: *Mayo Clinic Proceedings* 2010, pp.225-230. Elsevier.

90. Waxman AJ, Mink PJ, Devesa SS, et al. Racial disparities in incidence and outcome in multiple myeloma: a population-based study. *Blood* 2010; 116: 5501-5506. 20100907. DOI: 10.1182/blood-2010-07-298760.

91. Landgren O, Kristinsson SY, Goldin LR, et al. Risk of plasma cell and lymphoproliferative disorders among 14621 first-degree relatives of 4458 patients with monoclonal gammopathy of undetermined significance in Sweden. *Blood* 2009; 114: 791-795. DOI: 10.1182/blood-2008-12-191676.

92. Fermand J-P, Bridoux F, Dispenzieri A, et al. Monoclonal gammopathy of clinical significance: a novel concept with therapeutic implications. *Blood* 2018; 132: 1478-1485. DOI: 10.1182/blood-2018-04-839480.

93. Thorsteinsdottir S, Lund SH, Lindqvist EK, et al. Bone disease in monoclonal gammopathy of undetermined significance: results from a screened population-based study. *Blood Adv* 2017; 1: 2790-2798. DOI: 10.1182/bloodadvances.2017010454.

94. Kristinsson SY, Tang M, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance and risk of infections: a population-based study. *Haematologica* 2012; 97: 854-858. DOI: 10.3324/haematol.2011.054015.

95. Kristinsson SY, Pfeiffer RM, Björkholm M, et al. Arterial and venous thrombosis in monoclonal gammopathy of undetermined significance and multiple myeloma: a population-based study. *Blood* 2010; 115: 4991-4998. DOI: 10.1182/blood-2009-11-252072.

96. Hanewinckel R, van Oijen M, Ikram MA, et al. The epidemiology and risk factors of chronic polyneuropathy. *European journal of epidemiology* 2016; 31: 5-20. 2015/12/23. DOI: 10.1007/s10654-015-0094-6.

97. Pop-Busui R, Boulton AJ, Feldman EL, et al. Diabetic Neuropathy: A Position Statement by the American Diabetes Association. *Diabetes Care* 2017; 40: 136-154. DOI: 10.2337/dc16-2042.

98. Gosselin S, Kyle RA and Dyck PJ. Neuropathy associated with monoclonal gammopathies of undetermined significance. *Ann Neurol* 1991; 30: 54-61. DOI: 10.1002/ana.410300111.

99. Isobe T and Osserman EF. Pathologic conditions associated with plasma cell dyscrasias: a study of 806 cases. *Ann N Y Acad Sci* 1971; 190: 507-518. DOI: 10.1111/j.1749-6632.1971.tb13560.x.

100. Nobile-Orazio E, Barbieri S, Baldini L, et al. Peripheral neuropathy in monoclonal gammopathy of undetermined significance: prevalence and immunopathogenetic studies. *Acta Neurol Scand* 1992; 85: 383-390. DOI: 10.1111/j.1600-0404.1992.tb06033.x.

101. Ösby E, Noring L, Hast R, et al. Benign monoclonal gammopathy and peripheral neuropathy. *Br J Haematol* 1982; 51: 531-539. DOI: 10.1111/j.1365-2141.1982.tb02816.x.

102. Steiner N, Schwärzler A, Göbel G, et al. Are neurological complications of monoclonal gammopathy of undetermined significance underestimated? *Oncotarget* 2017; 8: 5081-5091. DOI: 10.18632/oncotarget.13861.

103. Vrethem M, Cruz M, Wen-Xin H, et al. Clinical, neurophysiological and immunological evidence of polyneuropathy in patients with monoclonal gammopathies. *J Neurol Sci* 1993; 114: 193-199. DOI: 10.1016/0022-510x(93)90297-c.

104. Bida JP, Kyle RA, Therneau TM, et al. Disease Associations With Monoclonal Gammopathy of Undetermined Significance: A Population-Based Study of 17,398 Patients. *Mayo Clinic Proceedings* 2009; 84: 685-693. DOI: 10.4065/84.8.685.

105. Chaudhry HM, Mauermann ML and Rajkumar SV. Monoclonal Gammopathy-Associated Peripheral Neuropathy: Diagnosis and Management. *Mayo Clinic proceedings* 2017; 92: 838-850. DOI: 10.1016/j.mayocp.2017.02.003.

106. Yeung KB, Thomas PK, King RH, et al. The clinical spectrum of peripheral neuropathies associated with benign monoclonal IgM, IgG and IgA paraproteinaemia. Comparative clinical, immunological and nerve biopsy findings. *J Neurol* 1991; 238: 383-391. DOI: 10.1007/bf00319857.

107. Nobile-Orazio E, Meucci N, Baldini L, et al. Long-term prognosis of neuropathy associated with anti-MAG IgM M-proteins and its relationship to immune therapies. *Brain* 2000; 123 (Pt 4): 710-717. DOI: 10.1093/brain/123.4.710.

108. Keddie S, Foldes D, Caimari F, et al. Clinical characteristics, risk factors, and outcomes of POEMS syndrome: A longitudinal cohort study. *Neurology* 2020; 95: e268-e279. 20200630. DOI: 10.1212/wnl.0000000000009940.

109. Notermans NC, Franssen H, Eurelings M, et al. Diagnostic criteria for demyelinating polyneuropathy associated with monoclonal gammopathy. *Muscle Nerve* 2000; 23: 73-79. DOI: 10.1002/(sici)1097-4598(200001)23:1<73::aid-mus9>3.0.co;2-5.

110. Nobile-Orazio E, Bianco M and Nozza A. Advances in the Treatment of Paraproteinemic Neuropathy. *Curr Treat Options Neurol* 2017; 19: 43. 20171016. DOI: 10.1007/s11940-017-0479-9.

111. Rison RA and Beydoun SR. Paraproteinemic neuropathy: a practical review. *BMC Neurology* 2016; 16: 13. DOI: 10.1186/s12883-016-0532-4.

112. Falzone YM, Campagnolo M, Bianco M, et al. Functioning and quality of life in patients with neuropathy associated with anti-MAG antibodies. *Journal of Neurology* 2018; 265: 2927-2933. DOI: 10.1007/s00415-018-9081-7.

113. Delmont E, Hiew FL, Cassereau J, et al. Determinants of health-related quality of life in anti-MAG neuropathy: a cross-sectional multicentre European study. *J Peripher Nerv Syst* 2017; 22: 27-33. DOI: 10.1111/jns.12197.

114. Opalic M, Peric S, Palibrk A, et al. Quality of life in patients with polyneuropathy associated with different types of monoclonal gammopathy of undetermined significance. *Acta Neurologica Belgica* 2020; 120: 1133-1138. DOI: 10.1007/s13760-019-01155-x.

115. Sudarsky L, Bronstein A, Brandt T, et al. Cerebellar gait and sensory ataxia. *Clinical Disorders of Balance, Posture and Gait* 2004: 163.

116. Melton III LJ, Kyle RA, Achenbach SJ, et al. Fracture Risk With Multiple Myeloma: A Population-Based Study. *Journal of Bone and Mineral Research* 2005; 20: 487-493. DOI: https://doi.org/10.1359/JBMR.041131.

117. Melton III LJ, Rajkumar SV, Khosla S, et al. Fracture risk in monoclonal gammopathy of undetermined significance. *J Bone Miner Res* 2004; 19: 25-30. DOI: 10.1359/jbmr.0301212.

118. Kristinsson SY, Tang M, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance and risk of skeletal fractures: a population-based study. *Blood* 2010; 116: 2651-2655. 20100707. DOI: 10.1182/blood-2010-04-282848.

119. Abrahamsen B, Andersen I, Christensen SS, et al. Utility of testing for monoclonal bands in serum of patients with suspected osteoporosis: retrospective, cross sectional study. *Bmj* 2005; 330: 818. 20050314. DOI: 10.1136/bmj.38376.401701.8F.

120. Farr JN, Zhang W, Kumar SK, et al. Altered cortical microarchitecture in patients with monoclonal gammopathy of undetermined significance. *Blood* 2014; 123: 647-649. 20131113. DOI: 10.1182/blood-2013-05-505776.

121. Ng AC, Khosla S, Charatcharoenwitthaya N, et al. Bone microstructural changes revealed by high-resolution peripheral quantitative computed tomography imaging and elevated DKK1 and MIP-1α levels in patients with MGUS. *Blood* 2011; 118: 6529-6534. 20111031. DOI: 10.1182/blood-2011-04-351437.

122. Gregersen H, Madsen KM, Sørensen HT, et al. The risk of bacteremia in patients with monoclonal gammopathy of undetermined significance. *European Journal of Haematology* 2009; 61: 140-144. DOI: 10.1111/j.1600-0609.1998.tb01075.x.

123. Kyle RA, Remstein ED, Therneau TM, et al. Clinical Course and Prognosis of Smoldering (Asymptomatic) Multiple Myeloma. *New England Journal of Medicine* 2007; 356: 2582-2590. DOI: 10.1056/NEJMoa070389.

124. Tete SM, Bijl M, Sahota SS, et al. Immune Defects in the Risk of Infection and Response to Vaccination in Monoclonal Gammopathy of Undetermined

Significance and Multiple Myeloma. *Frontiers in Immunology* 2014; 5. DOI: 10.3389/fimmu.2014.00257.

125. Roser M. Coronavirus Pandemic (COVID-19). *Our World in Data* 2020.

126. Chen R, Liang W, Jiang M, et al. Risk factors of fatal outcome in hospitalized subjects with coronavirus disease 2019 from a nationwide analysis in China. *Chest* 2020; 158: 97-105.

127. Hultcrantz M, Richter J, Rosenbaum CA, et al. COVID-19 Infections and Clinical Outcomes in Patients with Multiple Myeloma in New York City: A Cohort Study from Five Academic Centers. *Blood Cancer Discovery* 2020; 1: 234-243. DOI: 10.1158/2643-3230.bcd-20-0102.

128. Wang B, Van Oekelen O, Mouhieddine TH, et al. A tertiary center experience of multiple myeloma patients with COVID-19: lessons learned and the path forward. *Journal of Hematology & Oncology* 2020; 13. DOI: 10.1186/s13045-020-00934-x.

129. Chari A, Samur MK, Martinez-Lopez J, et al. Clinical features associated with COVID-19 outcome in multiple myeloma: first results from the International Myeloma Society data set. *Blood* 2020; 136: 3033-3040. DOI: 10.1182/blood.2020008150.

130. Martínez-López J, Mateos M-V, Encinas C, et al. Multiple myeloma and SARS-CoV-2 infection: clinical characteristics and prognostic factors of inpatient mortality. *Blood Cancer Journal* 2020; 10. DOI: 10.1038/s41408-020-00372-5.

131. Jain A and Ramasamy K. Potential 'significance' of monoclonal gammopathy of 'undetermined significance' during COVID-19 pandemic. *Blood Cells Mol Dis* 2020; 85: 102481-102481. 2020/07/24. DOI: 10.1016/j.bcmd.2020.102481.

132. Gonzalez-Lugo JD, Bachier-Rodriguez L, Goldfinger M, et al. A case series of Monoclonal Gammopathy of Undetermined Significance and COVID-19. *British Journal of Haematology* 2020; 190. DOI: 10.1111/bjh.16906.

133. Leung N, Bridoux F, Hutchison CA, et al. Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant. *Blood* 2012; 120: 4292-4295. DOI: 10.1182/blood-2012-07-445304.

134. Leung N, Bridoux F, Batuman V, et al. The evaluation of monoclonal gammopathy of renal significance: a consensus report of the International Kidney and Monoclonal Gammopathy Research Group. *Nature Reviews Nephrology* 2019; 15: 45-59. DOI: 10.1038/s41581-018-0077-4.

135. Bridoux F, Leung N, Hutchison CA, et al. Diagnosis of monoclonal gammopathy of renal significance. *Kidney Int* 2015; 87: 698-711. 20150121. DOI: 10.1038/ki.2014.408.

136. Muslimani AA, Spiro TP, Chaudhry AA, et al. Venous thromboembolism in patients with monoclonal gammopathy of undetermined significance. *Clin Adv Hematol Oncol* 2009; 7: 827-832.

137. Karakus S, Gottsch JD, Caturegli P, et al. Monoclonal gammopathy of "ocular" significance. *Am J Ophthalmol Case Rep* 2019; 15: 100471-100471. DOI: 10.1016/j.ajoc.2019.100471.

138. Lipsker D. Monoclonal gammopathy of cutaneous significance: review of a relevant concept. *J Eur Acad Dermatol Venereol* 2017; 31: 45-52. 20160808. DOI: 10.1111/jdv.13847.

139. Kristinsson SY, Björkholm M, Andersson TML, et al. Patterns of survival and causes of death following a diagnosis of monoclonal gammopathy of undetermined significance: a population-based study. *Haematologica* 2009; 94: 1714-1720. 2009/07/16. DOI: 10.3324/haematol.2009.010066.

140. Gregersen H, Ibsen J, Mellemkjoer L, et al. Mortality and causes of death in patients with monoclonal gammopathy of undetermined significance. *Br J Haematol* 2001; 112: 353-357. DOI: 10.1046/j.1365-2141.2001.02533.x.

141. Sigurbergsdóttir AÝ, Rögnvaldsson S, Thorsteinsdottir S, et al. Estimating Selection Bias in Previous Monoclonal Gammopathy of Undetermined Significance Research – the Importance of Screening: Results from the Population-Based Screening Study Iceland Screens, Treats or Prevents Multiple Myeloma (iStopMM) *63rd American Society of Hematology Meeting and Exposition*. Atlanta, GA, USA2021.

142. Missaoui B and Thoumie P. Balance training in ataxic neuropathies. Effects on balance and gait parameters. *Gait Posture* 2013; 38: 471-476. 20130305. DOI: 10.1016/j.gaitpost.2013.01.017.

143. Ites KI, Anderson EJ, Cahill ML, et al. Balance interventions for diabetic peripheral neuropathy: a systematic review. *Journal of geriatric physical therapy* 2011; 34: 109-116.

144. Pignataro RM and Swisher AK. Chemotherapy induced peripheral neuropathy: risk factors, pathophysiology, assessment, and potential physical therapy interventions. *Rehabilitation Oncology* 2010; 28: 10.

145. Fermand J-P, Bridoux F, Kyle RA, et al. How I treat monoclonal gammopathy of renal significance (MGRS). *Blood* 2013; 122: 3583-3590. DOI: 10.1182/blood-2013-05-495929.

146. Kristinsson SY, Björkholm M, Goldin LR, et al. Risk of lymphoproliferative disorders among first-degree relatives of lymphoplasmacytic lymphoma/Waldenström macroglobulinemia patients: a population-based study in Sweden. *Blood* 2008; 112: 3052-3056. DOI: 10.1182/blood-2008-06-162768.

147. Kristinsson SY, Rögnvaldsson S, Thorsteinsdottir S, et al. Screening for monoclonal gammopathy of undetermined significance: A population-based randomized clinical trial. First results from the Iceland Screens, Treats, or Prevents Multiple Myeloma (iStopMM) study. *Blood* 2021; 138: 156.

148. Turesson I, Linet MS, Björkholm M, et al. Ascertainment and diagnostic accuracy for hematopoietic lymphoproliferative malignancies in Sweden 1964-2003. *Int J Cancer* 2007; 121: 2260-2266. DOI: 10.1002/ijc.22912.

149. Brooke H, Talbäck M, Hörnblad J, et al. The Swedish cause of death register. *European Journal of Epidemiology* 2017; 32. DOI: 10.1007/s10654-017-0316-1.

150. Anonymous. *Kvalitet och innehåll i patientregistret - Utskrivningar från slutenvården 1964–2007 och besök i specialiserad öppenvård (exklusive primärvårdsbesök) 1997–2007*. 2009. Stockholm, Sweden: Socialstyrelsen.

151. Ludvigsson JF, Andersson E, Ekbom A, et al. External review and validation of the Swedish national inpatient register. *BMC Public Health* 2011; 11: 450. DOI: 10.1186/1471-2458-11-450.

152. Charlson M, Szatrowski TP, Peterson J, et al. Validation of a combined comorbidity index. *Journal of Clinical Epidemiology* 1994; 47: 1245-1251. DOI: https://doi.org/10.1016/0895-4356(94)90129-5.

153. Verma S and Hussain ME. Obesity and diabetes: an update. *Diabetes & Metabolic Syndrome: Clinical Research & Reviews* 2017; 11: 73-79.

154. Chamberlain JJ, Johnson EL, Leal S, et al. Cardiovascular disease and risk management: review of the American diabetes association standards of medical care in diabetes 2018. *Annals of internal medicine* 2018; 168: 640-650.

155. Ozen G, Zanfardino A, Confetto S, et al. The Association of Autoimmune Diseases with Type 1 Diabetes Mellitus in Children Depends Also by the Length of Partial Clinical Remission Phase (Honeymoon). *International Journal of Endocrinology* 2020; 2020: 2630827. DOI: 10.1155/2020/2630827.

156. Lousada I, Comenzo RL, Landau H, et al. Light chain amyloidosis: patient experience survey from the Amyloidosis Research Consortium. *Advances in therapy* 2015; 32: 920-928.

157. DeMott TK, Richardson JK, Thies SB, et al. Falls and gait characteristics among older persons with peripheral neuropathy. *American journal of physical medicine & rehabilitation* 2007; 86: 125-132.

158. Azhary H, Farooq MU, Bhanushali M, et al. Peripheral neuropathy: differential diagnosis and management. *American family physician* 2010; 81: 887-892.

159. Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. Study of Osteoporotic Fractures Research Group. *N Engl J Med* 1995; 332: 767-773. DOI: 10.1056/nejm199503233321202.

160. Stork AC, Lunn MP, Nobile-Orazio E, et al. Treatment for IgG and IgA paraproteinaemic neuropathy. *Cochrane Database Syst Rev* 2015; 2015: Cd005376. 20150324. DOI: 10.1002/14651858.CD005376.pub3.

161. Streckmann F, Zopf EM, Lehmann HC, et al. Exercise intervention studies in patients with peripheral neuropathy: a systematic review. *Sports Med* 2014; 44: 1289-1304. DOI: 10.1007/s40279-014-0207-5.

162. Castillo JJ, Garcia-Sanz R, Hatjiharissi E, et al. Recommendations for the diagnosis and initial evaluation of patients with Waldenström Macroglobulinaemia: A Task Force from the 8th International Workshop on Waldenström

Macroglobulinaemia. *Br J Haematol* 2016; 175: 77-86. 20160705. DOI: 10.1111/bjh.14196.

163. Spitzer RL, Kroenke K, Williams JB, et al. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* 2006; 166: 1092-1097. DOI: 10.1001/archinte.166.10.1092.

164. Martin A, Rief W, Klaiberg A, et al. Validity of the brief patient health questionnaire mood scale (PHQ-9) in the general population. *General hospital psychiatry* 2006; 28: 71-77.

165. Diener E, Emmons RA, Larsen RJ, et al. The Satisfaction With Life Scale. *J Pers Assess* 1985; 49: 71-75. DOI: 10.1207/s15327752jpa4901_13.

166. Gudbjartsson DF, Norddahl GL, Melsted P, et al. Humoral Immune Response to SARS-CoV-2 in Iceland. *New England Journal of Medicine* 2020; 383: 1724-1734. DOI: 10.1056/nejmoa2026116.

167. Helgason D, Eythorsson E, Olafsdottir LB, et al. Beating the odds with systematic individualized care: Nationwide prospective follow-up of all patients with COVID-19 in Iceland. *Journal of Internal Medicine* 2020. DOI: 10.1111/joim.13135.

168. R Core Team. R: A language and environment for statistical computing. 2013.

169. Harrell Jr FE. *Regression modeling strategies: with applications to linear models, logistic and ordinal regression, and survival analysis*. Springer, 2015.

Original Publications

Paper I

Peripheral neuropathy and monoclonal gammopathy of undetermined significance: a population-based study including 15,351 cases and 58,619 matched controls

Monoclonal gammopathy of undetermined significance (MGUS) is a common benign precursor condition of multiple myeloma (MM) and related disorders.[1,2] MGUS is considered asymptomatic but has been shown to be associated with peripheral neuropathy (PN).[3] However, the literature is unclear regarding the prevalence, clinical implications, and even the existence of MGUS-associated PN.[4] We therefore conducted a large population-based study of MGUS and PN. We found PN to be truly associated with MGUS and under-recognized in clinical practice. Furthermore, PN was associated with a 2.9-fold risk of a light-chain amyloidosis (AL).

We included individuals with MGUS diagnosed in Sweden between 1986-2013, as has been described previously.[5] Four controls that were alive and free of lymphoproliferative disease were matched to each case on the day of MGUS diagnosis by sex, year of birth, and county of residence. Data from Swedish national registries were cross-linked to participants using a unique identification number.

The primary endpoint was PN as recorded in the Swedish Patient Registry using International Classification of Diseases (ICD) codes by Swedish physicians recording their clinical diagnoses. However, underlying symptoms or diagnostic testing leading to PN diagnosis were not available. Acute inflammatory neuropathies and critical care neuropathy were excluded. Symptomatic codes were included but were excluded in a sensitivity analysis. We assessed the prevalence of PN in the full cohort and followed those who did not have PN at inclusion until PN or censoring at death as recorded in the Swedish Cause of Death Registry, lymphoproliferative disease as recorded in the Swedish Cancer Registry, or end of follow-up. We then estimated hazard ratios (HR) using Cox proportional hazard regression adjusting for age, sex, and year of inclusion. PN is common in the general population but is often undetected.[6] Individuals with MGUS, who are under medical surveillance, might therefore have more diagnoses of a PN that might otherwise have stayed subclinical in the control population. To mitigate this bias, we also stratified the cohort by diabetes mellitus (DM) and repeated the analysis. DM patients are under regular medical surveillance, similar to that of patients with MGUS. Furthermore, PN is a well-known feature of DM, and DM patients often undergo PN screening during follow-up, presenting a more appropriate comparison group.

In a secondary analysis, we assessed the association of PN and MGUS progression and death. We included all participants with MGUS and considered PN as the exposure. We then followed them until death or the diagnosis of MM, Waldenström's macroglobulinemia (WM), and AL in four separate analyses while censoring at the other endpoints or loss to follow-up. In order to prevent immortal time bias in those participants who developed PN after MGUS diagnosis, we included PN as a time-dependent covariate in a Cox proportional hazard regression model. The models were adjusted for age, sex, and year of MGUS diagnosis, as well as for DM when assessing risk of death.

A total of 15,351 participants with MGUS and 58,619 matched controls were included in the study. The prevalence of PN was higher in participants with MGUS than controls (6.5% *vs.* 2.8%) (Table 1). The reported prevalence of PN varies widely but more recent observational studies estimate the prevalence at 15-20%.[7] Therefore, these findings, based on clinical diagnoses of PN, indicate under-recognition of PN during the clinical care of individuals with MGUS.

Individuals with MGUS had 2.7-fold risk of PN compared to matched controls (HR=2.7; 95% confidence interval [95%CI]: 2.4-3.1; $P<0.001$). After stratification for DM, we found MGUS and DM to be associated with higher risk of PN as compared to controls without MGUS and DM (MGUS alone: HR=3.0; 95%CI: 2.6-3.4; $P<0.001$ and DM alone: HR=3.6; 95%CI: 3.2-4.2; $P<0.001$).

Table 1. Baseline characteristics of study participants in the original cohort and after additional stratification for diabetes mellitus (DM) as well as results of a Cox proportional hazard regression model of risk of peripheral neuropathy (PN) for each group.

	Original cohort		After DM stratification			
	MGUS	Control	MGUS alone	DM alone	MGUS+DM	Control
N	15,351	58,619	12,818	7,953	2,533	50,666
Median age (years)	73	72	72	74	74	72
Age range (years)	18-104	18-101	18-104	26-97	26-95	18-101
% male	51%	51%	50%	58%	58.1	50%
N by year of inclusion						
1986-1995	22%	22%	22%	23%	22%	21%
1996-2005	33%	33%	33%	35%	31%	33%
2006-2013	45%	45%	45%	42%	47%	46%
PN	996 (6.5%)	1,644 (2.8%)	681 (5.3%)	620 (7.8%)	315 (12.4%)	1024 (2.0%)
before MGUS/matching (% of PN)	549 (55%)	770 (47%)	376 (55%)	310 (50%)	173 (55%)	460 (45%)
Median follow-up (years)	4.0	6.1	4.1	6.1	3.7	6.1
Risk of PN in HR (95% CI)[1]	2.7 (2.4-3.1)***	Reference	–	–	–	–
	–	–	3.0 (2.6-3.4)***	3.6 (3.2-4.2)***	7.5 (6.3-9.1)***	Reference
	–	–	0.8 (0.7-1.0)*	Reference	2.1 (1.7-2.5)***	0.3 (0.2-0.3)***

[1]Adjusted for sex, age, and year of inclusion. *$P<0.05$, ***$P<0.001$, MGUS: monoclonal gammopathy of undetermined significance; HR: hazard ratio, 95% CI: 95% confidence interval; N: number.

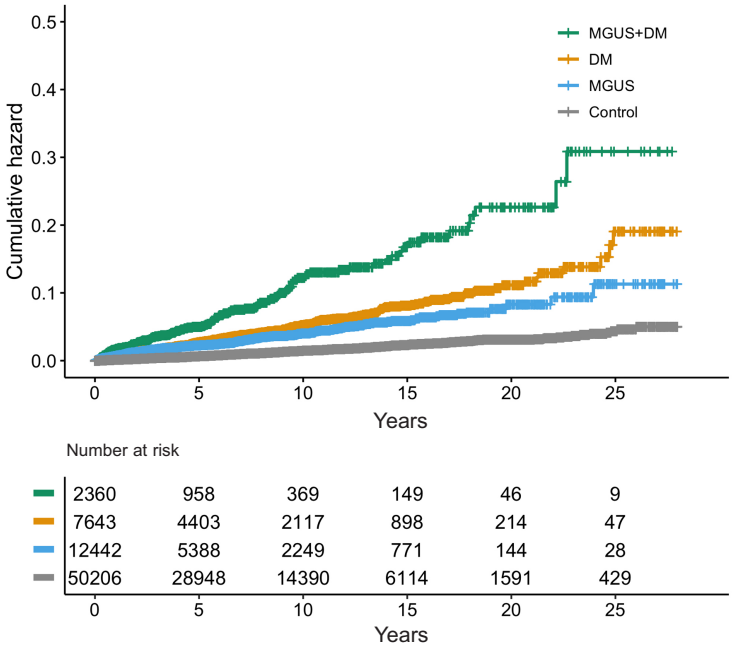


Figure 1. Kaplan-Meier graph illustrating the cumulative hazard of peripheral neuropathy (PN) throughout the study period by assigned study group. MGUS: monoclonal gammopathy of undetermined significance; DM: diabetes mellitus.

MGUS was associated with a 0.8-fold risk of PN as compared to DM (HR=0.8; 95%CI: 0.7-0.9, *P*=0.02). Participants with MGUS and DM had a 2.1-fold risk of PN as compared to those with DM alone (MGUS and DM: HR= 2.1; 95%CI: 1.7-2.5; *P*<0.001) (Table 1 and Figure 1). Although these findings could suggest a synergistic effect of MGUS and DM, it is more likely that excess PN caused by MGUS is being detected during DM or MGUS follow-up in individuals with DM and MGUS as compared to those with DM alone. These findings indicate that PN is truly associated with MGUS, contradicting previous findings that questioned this.[4]

In the secondary analysis, 1,368 participants progressed to MM, 449 progressed to WM, and 173 progressed to AL (Table 2). PN was associated with lower risk of MM (HR=0.7; 95%CI: 0.5-0.9; *P*=0.02) but was not associated with WM progression (HR=1.3; 95%CI: 0.9-1.9; *P*=0.2). PN has been shown to be more common in IgM MGUS3 that rarely progresses to MM, but rather to WM,[8] potentially leading to selection bias. Unfortunately, isotype data are not available for this cohort making it difficult to interpret these results. However, these findings could indicate that PN is unlikely to be associated with increased risk of MM or WM, suggesting that the development of PN in MGUS might be unrelated to progression of the underlying plasma cell disorder.

Interestingly, we found PN to be associated with a 2.9-fold risk of MGUS progression to AL (HR=2.9; 95%CI: 1.8-4.6; *P*<0.001). Furthermore, we found that nine out of the 11 individuals (82%) with PN at diagnosis who later progressed to AL did so within a year of MGUS diagnosis. Diagnosis of AL can be difficult, leading to under-recognition and a delay in diagnosis of AL.[9] Furthermore, virtually all cases of AL are preceded by MGUS,[10] so it is likely that these participants had AL, not MGUS, at inclusion. These findings stress the importance of a thorough evaluation for AL in individuals with MGUS and PN, especially at MGUS diagnosis.

We found PN to be associated with a 1.3-fold risk of death in MGUS (HR=1.3; 95%CI: 1.2-1.5; *P*<0.001). When associated with other disorders, PN can lead to falls and fractures[11-13] which might contribute to this increased risk of death. However, PN is also associated with various other diseases that might lead to increased risk of death, such as other cancers and alcohol misuse.[6] Therefore, it is unclear whether this represents a causal relationship. Further studies are needed to validate these findings.

Our study has several strengths. We included a nationwide population of 15,351 MGUS cases and 58,619 matched controls diagnosed over a 28-year period. Data were acquired with high accuracy and completeness from well-established registries. As far as we know, this is the largest study of MGUS-associated PN so far. Secondly, by including clinical data from routine care, the study provides an insight into the real-world care of individuals with MGUS. Finally, by also stratifying participants for DM, we mitigated detection bias that would otherwise have affected the results of this type of study.

The study also has important limitations. Firstly, PN diagnoses were acquired from diagnostic coding without data on the underlying symptoms or diagnostic tests, relying on detection, and accurate diagnosis of PN by physicians. By stratifying for DM, we mitigated some of the effects of any unequal detection and reporting of PN in the cohort. Secondly, study participants were not screened for MGUS, but were diagnosed during the work-up of other medical problems, leading to biased selection of participants with other medical problems into the MGUS group. Furthermore, MGUS might have been diagnosed as a result of PN. However, this applies to all real-world MGUS populations, and individuals with PN before MGUS diagnosis were excluded from analyses

Table 2. Baseline characteristics, rates and incidence of progression, and risk of progression to MM, WM, and AL as well as death for study participants with MGUS with and without PN as assessed by Cox proportional hazard regression with PN as a time dependent covariate.

	MGUS without PN	MGUS and PN
N	14,355	996
Male	51%	63%
Median age	73	69
Median follow-up (years)	4.0	3.0
Progressed to MM (incidence[1])	1,328 (1.6)	40 (1.0)
HR (95%CI)[2]	–	0.7 (0.5-0.9)*
Progressed to WM (incidence[1])	422 (0.5)	27 (0.7)
HR (95%CI)[2]	–	1.2 (0.8-1.8)
Progressed to AL (incidence[1])	153 (0.2)	21 (0.5)
HR (95%CI)[2]	–	2.3 (1.5-3.7)***
Death (mortality rate[1])	5,522 (6.8)	315 (8.0)
HR (95%CI)[3]	–	1.3 (1.2-1.5)***

[1]Incidence per 100 person-years. [2]Adjusted for sex, age, and year of inclusion. [3]Adjusted for sex, age, year of inclusion, and diabetes mellitus (DM). ***P<0.001, MGUS: monoclonal gammopathy of undetermined significance; PN: peripheral neuropathy; MM: multiple myeloma; WM: Waldenström's macroglobulinemia; AL: amyloid light-chain amyloidosis; HR: hazard ratio; 95%CI: 95% confidence interval.

assessing risk of PN. Thirdly, and unfortunately, immunoglobulin isotype data are not available for this cohort, and some isotypes, especially IgM, might be associated with higher risk of PN and skew the average risk for the whole cohort so that it might not be representative for each isotype. Furthermore, this limits the interpretation of analyses of progression to MM and WM. Prospective studies including screening for MGUS and PN are needed to validate these findings. We are currently conducting a population-based screening study for MGUS (*clinincaltrials.gov identifier: NCT03327597*). A substudy is ongoing assessing the prevalence, symptoms, clinical impact, and associated disease factors of MGUS-associated PN.

In conclusion, in this large population-based study, including 15,351 MGUS individuals and 58,619 matched controls, we found that a significant proportion of individuals with MGUS have clinically evident PN (6.5%) and that PN is truly associated with MGUS. In addition, our findings suggest under-recognition of PN in the real-world care of individuals with MGUS. Interestingly, we found PN to be associated with a 2.9-fold risk of AL and that PN is not associated with increased risk of MM or WM. PN was associated with increased risk of death, but multiple confounders make it impossible to establish a causal relationship. When associated with other disorders, PN leads to falls, fractures,[11-13] and lower quality of life.[14] It is, therefore, reasonable to assume that PN causes considerable morbidity in MGUS that may go unrecognized. Our findings should help increase awareness of MGUS as a cause of PN among all clinicians and promote closer monitoring of individuals with MGUS for symptoms of PN.

Sæmundur Rögnvaldsson,[1] Villhjálmur Steingrímsson,[1] Ingemar Turesson,[2] Magnus Björkholm,[3] Ola Landgren[4] and Sigurður Yngvi Kristinsson[1,3]

[1]Faculty of Medicine, University of Iceland, Reykjavík, Iceland; [2]Skåne University Hospital, Malmö/Lund, Sweden; [3]Department of Medicine, Karolinska University Hospital Solna and Institutet, Stockholm, Sweden and [4]Myeloma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA

Correspondence:
SAEMUNDUR ROGNVALDSSON - saer2@hi.is

doi:10.3324/haematol.2019.239632

Funding: this research was supported by grants from the University of Iceland Research Fund, Icelandic Centre for Research (RANNIS), Landspitali University Hospital Research Fund, and Karolinska Institutet Foundations. Funding support for this publication was provided by the Memorial Sloan Kettering Core Grant (P30 CA008748) and the Perelman Family Foundation in collaboration with the Multiple Myeloma Research Foundation (MMRF) for OL. SR is a PhD candidate at the University of Iceland and this work is submitted in partial fulfilment of the requirement for a PhD.

References

1. Kyle RA, Therneau TM, Rajkumar SV, et al. Prevalence of monoclonal gammopathy of undetermined significance. N Engl J Med. 2006;354(13):1362-1369.
2. Dispenzieri A, Katzmann JA, Kyle RA, et al. Prevalence and risk of progression of light-chain monoclonal gammopathy of undetermined significance: a retrospective population-based cohort study. Lancet. 2010;375(9727):1721-1728.
3. Gosselin S, Kyle RA, Dyck PJ. Neuropathy associated with monoclonal gammopathies of undetermined significance. Ann Neurol. 1991;30(1):54-61.
4. Bida JP, Kyle RA, Therneau TM, et al. Disease associations with monoclonal gammopathy of undetermined significance: a population-based study of 17,398 patients. Mayo Clin Proc. 2009;84(8):685-693.
5. Kristinsson SY, Bjo M, Goldin LR, McMaster ML, Turesson I, Landgren O. Risk of lymphoproliferative disorders among first-degree relatives of lymphoplasmacytic lymphoma / Waldenström macroglobulinemia patients: a population-based study in Sweden. Blood. 2008;112(8):3052-3056.
6. Hanewinckel R, van Oijen M, Ikram MA, van Doorn PA. The epidemiology and risk factors of chronic polyneuropathy. Eur J Epidemiol. 2016;31(1):5-20.
7. Steiner N, Schwärzler A, Göbel G, Löscher W, Gunsilius E. Are neurological complications of monoclonal gammopathy of undetermined significance underestimated? Oncotarget. 2017;8(3):5081-5091.
8. Kyle RA, Therneau TM, Rajkumar SV, et al. Long-term follow-up of IgM monoclonal gammopathy of undetermined significance. Blood. 2003;102(10):3759-3764.
9. Lousada I, Comenzo RL, Landau H, Guthrie S, Merlini G. Light chain amyloidosis: patient experience survey from the Amyloidosis Research Consortium. Adv Ther. 2015;32(10):920-928.
10. Weiss BM, Hebreo J, Cordaro D, Roschewski MJ, Abbott KC, Olson SW. Monoclonal gammopathy of undetermined significance (MGUS) precedes the diagnosis of AL amyloidosis by up to 14 years. Blood. 2011;118(21):1827.
11. Strotmeyer ES, Cauley JA, Schwartz AV, et al. Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults. Arch Intern Med. 2005;165(14):1612.
12. Timar B, Timar R, Gaita L, Oancea C, Levai C, Lungeanu D. The impact of diabetic neuropathy on balance and on the risk of falls in patients with type 2 diabetes mellitus: a cross-sectional study. PLoS One. 2016;11(4):e0154654.
13. Marshall TF, Zipp GP, Battaglia F, Moss R, Bryan S. Chemotherapy-induced-peripheral neuropathy, gait and fall risk in older adults following cancer treatment. J Cancer Res Pract. 2017;4(4):134-138.
14. Benbow SJ, Wallymahmed ME, MacFarlane IA. Diabetic peripheral neuropathy and quality of life. QJM. 1998;91(11):733-737.

Paper II

Received: 2 February 2021 | Revised: 8 April 2021 | Accepted: 8 April 2021

DOI: 10.1111/ejh.13633

ORIGINAL ARTICLE

European Journal of Haematology | WILEY

Untangling fracture risk in monoclonal gammopathy of undetermined significance: A population-based cohort study

Sæmundur Rögnvaldsson[1] | Thor Aspelund[1] | Sigrún Thorsteinsdóttir[1,2] | Ingemar Turesson[3] | Magnus Björkholm[4] | Ola Landgren[5] | Sigurður Yngvi Kristinsson[1]

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

[2]Department of Haematology, Rigshospitalet, Copenhagen, Denmark

[3]Skåne University Hospital, Malmö/Lund, Sweden

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

[5]Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, USA

Correspondence
Sæmundur Rögnvaldsson, Sturlugata 8, 101 Reykjavík, Iceland.
Email: saer2@hi.is

Funding information
University of Iceland Research Fund; Icelandic Centre for Research; Swedish Cancer Society; Landspitali University Hospital Research Fund; Karolinska Institutet Foundations; Memorial Sloan Kettering Core Grant, Grant/Award Number: P30 CA008748; Perelman Family Foundation in Collaboration with the Multiple Myeloma Research Foundation

Abstract

Objective: Monoclonal gammopathy of undetermined significance (MGUS) is the asymptomatic precursor of multiple myeloma (MM). Lytic bone lesions and fractures are hallmarks of MM and although there are no lytic lesions in MGUS, it has also been associated with fractures. The causes of fractures in MGUS are currently unclear but potential causes include inherent MGUS bone disease, undiagnosed MM, and peripheral neuropathy (PN). We therefore conducted a large population-based study including 8395 individuals with MGUS and 30 851 matched controls from Sweden.

Methods: Data on fractures, PN, and confounders were acquired from high-quality registers in Sweden.

Results: Monoclonal gammopathy of undetermined significance and PN were independently associated with fractures (hazard ratio [HR]: 1.29; 95% confidence interval [95% CI]: 1.21-1.37; $P < .001$ and HR: 1.34; 95% CI: 1.16-1.55; $P < .001$). Imminent MGUS progression increased the risk of fractures (odds ratio: 1.66; 95% CI: 1.27-2.16; $P < .001$). Fractures were not associated with long-term risk of MGUS progression (HR: 1.08; 95% CI: 0.77-1.53; $P = .64$).

Discussion: Based on these findings, we speculate that MGUS leads to fractures through at least 3 independent mechanisms: undetected MGUS progression to MM, MGUS inherent bone disease, and PN through falls. These findings highlight the need for further study of MGUS inherent bone disease and can inform further research into fracture prevention in MGUS.

KEYWORDS

bone disease, fractures, monoclonal gammopathy of undetermined significance, multiple myeloma, neuropathy

Plain Language Summary

What is the new aspect of your work?

People with monoclonal gammopathy of undetermined significance (MGUS), a precursor disease of multiple myeloma, have an increased risk of fractures. This study identifies potential underlying causes of these fractures.

What is the central finding of your work?

Based on our findings, we speculate that fractures in MGUS have at least three independent causes: the development of multiple myeloma from MGUS, microscopic bone changes in MGUS that are not related to multiple myeloma, and damage to the peripheral nerves (peripheral neuropathy) leading to falls which has been associated with MGUS previously.

What is (or could be) the specific clinical relevance of your work?

The study identifies potential targets for interventions that might decrease the risk of fractures in MGUS. For example, physical therapy might decrease the fracture risk with peripheral neuropathy and early detection of MGUS progression to multiple myeloma might prevent multiple myeloma fractures.

Significance Statement

Individuals with monoclonal gammopathy of undetermined significance (MGUS), a precursor to multiple myeloma, a bone marrow cancer, have an increased risk of fractures. The causes of these fractures are unclear. This paper identifies three independent causes of fractures in MGUS of which two may be clinically actionable. (a) Undetected MGUS progression to multiple myeloma which may be prevented by closer follow-up, (b) MGUS related peripheral neuropathy leading to falls which may be prevented with balance training, and (c) MGUS inherent bone disease which required further study. The study findings can therefore inform research into methods of fracture prevention in MGUS.

1 | INTRODUCTION

Monoclonal gammopathy of undetermined significance (MGUS) is the precursor condition of multiple myeloma (MM) and related disorders.[1,2] MGUS is defined by the presence of serum monoclonal immunoglobulins or an abnormal free immunoglobulin light chain ratio in the absence of symptoms or biomarkers of MM or more advanced disorders, including MM-related bone lesions and fractures.[3] MGUS is relatively common in the general population, with a prevalence of 4.2% in white populations over the age of 50.[4,5] The main clinical implication of MGUS is a 1%-1.5% risk of progression to MM and related disorders per year.[6,7] Until progression, MGUS is often referred to as being asymptomatic. However, MGUS has been shown to be associated with important clinical outcomes such as peripheral neuropathy (PN),[8] arterial and venous thrombosis,[9] fractures,[10-12] infections,[13] and increased risk of death.[14]

Individuals with MGUS have, in several independent studies, been shown to have an increased risk of fractures.[10-12] However, the exact underlying explanation for this increased fracture risk is unclear and previous studies have had contradicting findings. The most common predisposing factor for fractures in the general population is osteoporosis[15] and although early reports suggested that osteoporosis was more prevalent in MGUS,[16-18] a recent meta-analysis and a high-quality population-based study of bone mineral density in a screened MGUS population contradicted these findings.[11,19] Some authors have found MGUS to be associated with an abnormal bone microarchitecture unrelated to osteoporosis and characterized by increased bone volume, reduced cortical bone thickness, increased endocortical area, higher cortical porosity, and reduced bone strength.[11,20,21] The clinical meaning of these microscopic architectural changes, however, remain unclear. Finally, since MM carries a significant diagnostic delay, fractures are common in the months preceding MM diagnosis that is caused by active MM bone disease.[22] This may lead to an observed risk of fractures in MGUS that is actually caused by undetected progression to MM.

A subset of individuals with MGUS have been reported to develop PN and we recently showed in a large population-based study with over 73 000 participants, that PN is truly associated with MGUS.[23] PN is characterized by pain and/or sensory and motor deficits brought on by impairment of peripheral nerve function.[24] The prevalence of PN among individuals with MGUS varies greatly in the literature and has been reported to be from 3% to 70%.[8,23,25-31] The role of PN in the risk for falls and fractures is well established in relation to several other disorders, like diabetes mellitus.[32-35] We speculated that PN might contribute to the fracture risk observed in MGUS. However, we are not aware of any studies addressing the role of PN in MGUS fracture risk. We were therefore motivated to conduct a large population-based study including 8649 individuals with MGUS and 31 272 matched controls to further untangle these potentially multifactorial causes of fractures in MGUS.

2 | METHODS

2.1 | Study cohort

Monoclonal gammopathy of undetermined significance cases diagnosed during clinical care in Sweden were enrolled from an MGUS

registry created from the records of a network of hematology and oncology centers in Sweden and the Swedish Patient Registry that has been described previously.[36,37] In order to limit the effect of different diagnostic coding systems, enrollment started in 1999, 2 years after the implementation of the International Classification of Diseases 10 (ICD-10) in Sweden. Enrollment stopped at the end of 2012. Participants who progressed from MGUS to MM or other lymphoproliferative disease within a year of MGUS diagnosis were excluded to avoid undetected malignancy. At the date of inclusion, 4 controls from the general population that were alive and free of known lymphoproliferative disease at that date, were matched to each MGUS case by year of birth, gender, and county of residence. In some cases, four controls were not found, reducing the number to those available. Cases with no available controls were excluded. Data were collected for participants until death or the end of 2013, whichever occurred first.

2.2 | Swedish registries

Data from nationwide, central Swedish government registries were cross-linked to participants using the unique national registration number assigned to all residents of Sweden. *The Swedish Patient Registry* contains data on ICD coded diagnoses in the universal Swedish healthcare system as well as dates of admission and discharge.[38] Since 2001, the registry also records outpatient encounters, including ICD diagnoses and dates of outpatient visit, including primary care visits. *The Swedish Cause of Death Registry* comprises the dates and underlying causes of all deaths in Sweden.[39] *The Swedish Cancer Registry* contains data on all cancers diagnosed in Sweden with registration of cancer cases being compulsory for Swedish physicians since 1958. For MM, the coverage rate has been reported to be 95%.[40]

2.3 | Peripheral neuropathy and fracture diagnoses

Diagnoses of PN and fractures were acquired from the *Swedish Patient Registry* using ICD-10 codes as specified by the Swedish National Board of Health and Welfare (*Socialstyrelsen*) with the date of admission or outpatient visit defined as the date of diagnosis. PN was included regardless of registered underlying cause since the precise cause can, in practice, be difficult to ascertain. Acute inflammatory neuropathies, such as Guillain-Barré neuropathy were excluded. ICD-10 diagnostic codes used for PN were E10.4, E11.4, E12.4, E13.4, E14.4, G13.0, G60.3, G61.8, G61.9, G62, G63, R20.0, R20.1, R20.2, and R20.8.

Fractures were considered the outcome in the study in three categories: *any fractures* (ICD-10 codes: S02, S12, S22, S32, S42, S52, S62, S72, S82, S92), *peripheral fractures* (ICD-10 codes: S02, S52, S62, S82, S92), and *axial fractures* (ICD-10 codes: S12, S22, S32, S42, S72). Since the fractures may be coded repeatedly in relation to readmission or rehabilitation, fractures within 6 months from a fracture of the same body part were considered the same fracture.

2.4 | Statistical analysis

Participants were followed until their first fracture after inclusion in 3 separate analyses for any fractures, peripheral fractures only, and axial fractures only. Participants were censored at death as recorded in the *Swedish Cause of Death Registry*, end of follow-up at the end of 2013, or at the time of a fracture not considered the outcome in that particular analysis. Additionally, we censored participants who progressed to MM or related disorders 1 year before progression as recorded in the *Swedish Cancer Registry*. To avoid immortal time bias, PN was included as a time-dependent covariate in all models as some participants developed PN after inclusion.

To adjust for potential confounders, we included age, sex, year of inclusion, previous fractures in the preceding 2 years, and having any alcohol use related ICD-10 diagnosis into a propensity score for MGUS and PN using multinomial logistic regression essentially dividing them into 4 groups: individuals with MGUS, individuals with MGUS and PN (MGUS + PN), individuals without MGUS (Control), and individuals without MGUS who have PN (Control + PN). Using the generated propensity score, inverse probability weighted Cox regression was performed with MGUS as a covariate and PN as a time-dependent covariate. All analyses were additionally performed after stratification for sex with new propensity scores calculated for each stratified analysis. The interaction for PN and MGUS was evaluated for all analyses. All models were checked for proportional hazards using Schoenfeld's global tests.

In order to evaluate the role of MGUS progression in fracture risk, we performed 2 additional analyses. In the first additional analysis, we evaluated the fracture risk in the year prior to MGUS progression in a nested case-control study. We identified participants with MGUS who progressed to MM or related disorders and matched them to 3 individuals with MGUS that were alive and free of progression at the same time in follow-up. Matching was done by sex, age, and year of MGUS diagnosis (±1 year). Logistic regression was performed to estimate odds ratios (OR) of having any, peripheral, or axial fractures in the year prior to progression compared to a similar year without progression in the controls. In the second additional analysis, we evaluated the risk of progression 1 year after fracture. We identified all participants with MGUS who had a fracture and were alive, free of progression, and in follow-up 1 year after a fracture. We then matched them with 3 other individuals with MGUS by sex and age who were alive, free of progression or fractures, and in follow-up at the same time in follow-up. This way age, sex, and time with MGUS, which may affect the risk of progression, was equally distributed between the groups in the analysis. We followed this cohort for progression to MM or related disorders. Cox regression was then used to estimate the hazard ratio (HR) of progression for those who had a fracture compared with those that did not. Cases where 3 matched controls could not be found were excluded in both analyses.

All analyses were performed in using R[41] using the *survival*,[42] *survminer*,[43] and *ipw*[44] packages.

TABLE 1 The baseline characteristics of the study participants and number and types of fractures over the study period

	MGUS	MGUS + PN	Control	Control + PN
n person years	39 559	2263	174 844	3168
n individuals	8052	597	30 423	849
PN after inclusion (%)	—	254 (43%)	—	419 (49%)
Age (median)	71	68	70	71
Age (range)	18-97	28-89	18-97	23-96
Male	52%	63%	53%	62%
Year of diagnosis				
1999-2003	29%	20%	28%	24%
2004-2007	29%	29%	29%	29%
2008-2012	43%	51%	43%	48%
Median follow-up[a]	4.1 years	3.7 years	5.1 years	3.6 years
Median potential follow-up[b]	4.8 years	4.1 years	5.8 years	4.1 years
Previous alcohol use (%)	242 (3%)	31 (5%)	720 (2%)	47 (6%)
Previous fracture (%)[c]	428 (5%)	33 (6%)	1138 (4%)	44 (5%)
All participants				
All fractures (rate[c])	1402 (3.5)	89 (3.9)	4535 (2.6)	120 (3.8)
Peripheral fractures (rate[c])	545 (1.4)	38 (1.7)	1978 (1.1)	46 (1.5)
Axial fractures (rate[c])	887 (2.2)	54 (2.4)	2647 (1.5)	76 (2.4)
Males				
All fractures (rate[c])	560 (2.9)	48 (3.4)	1718 (1.9)	60 (3.0)
Peripheral fractures (rate[c])	188 (1.0)	17 (1.2)	667 (0.7)	20 (1.0)
Axial fractures (rate[c])	383 (2.0)	32 (2.3)	1078 (1.2)	40 (2.0)
Females				
All fractures (rate[c])	842 (4.2)	41 (4.8)	2817 (3.3)	60 (5.1)
Peripheral fractures (rate[c])	357 (1.8)	21 (2.5)	1311 (1.5)	26 (2.2)
Axial fractures (rate[c])	504 (2.5)	22 (2.6)	1569 (1.8)	36 (3.0)

Note: Study participants who develop PN after inclusion are included in the non-PN groups until the point of PN diagnosis and are counted there as well.

Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; PN, peripheral neuropathy.

[a]As evaluated by the Kaplan-Meier estimator for any outcome.

[b]As evaluated by the Kaplan-Meier estimator for censoring events.

[c]Rate per 100 person years.

2.5 | Sensitivity analyses

In order to adjust for comorbidities, a known risk factor for fractures,[45] we performed 2 sensitivity analyses which included markers of comorbidity and general frailty into the propensity score. Firstly, we included previous diagnoses of the disorders included in the Charlson comorbidity index as binomial variables.[46] Secondly, we included hospital admissions lasting 4 days or longer in the previous 2 years as a general marker of frailty and need of medical care.

3 | RESULTS

A total of 8395 individuals with MGUS and 30 851 matched controls were included in the study with 597 and 849 having PN before or after the time of inclusion in the MGUS and control groups, respectively. There were slightly more males (53%) than females. Participant acquisition increased over the study period (Table 1). PN was associated with recorded alcohol use ($P < .001$) and previous fractures ($P = .02$) (Table 1).

Individuals with MGUS had a 1.29-fold risk of any fracture independently of PN (HR: 1.29; 95% confidence interval [95% CI]: 1.21-1.37; $P < .001$). This increased risk of any fracture was also observed in both males and females separately with males having a higher relative risk increase (males: HR: 1.39; 95% CI 1.27-1.53; $P < .001$ and females: HR: 1.22; 95% CI: 1.13-1.31; $P < .001$). The absolute risk increase was similar for males and females (males: 1.9 vs 2.9 fractures per 100 person years. females: 3.3 vs 4.2 fractures per 100 person years). MGUS was associated with both peripheral and axial fractures but was more strongly associated with axial fractures (peripheral: HR: 1.18; 95% CI: 1.08-1.30 $P < .001$; axial: HR: 1.37 95% CI: 1.27-1.48; $P < .001$). The results were similar for males and females (Table 2).

Peripheral neuropathy was associated with a 1.34-fold increased risk of fractures independently of MGUS (HR: 1.34; 95% CI: 1.16-1.55; $P < .001$). Both males and females with PN had an increased risk of fractures (males: HR: 1.39; 95% CI: 1.14-1.69; $P = .001$; females: HR: 1.35 95% CI: 1.10-1.65; $P = .004$). There was no significant interaction between MGUS and PN affecting the risk of fractures (HR: 0.90; 95% CI: 0.68-1.2; $P = .49$) indicating no effect modification. PN was associated with axial and peripheral fractures with similar risk increases (peripheral: HR: 1.36; 95% CI: 1.09-1.71 $P = .007$; axial: HR: 1.34; 95% CI: 1.12-1.61; $P = .001$). The results were similar for males and females (Table 2).

Sensitivity analyses adjusting for comorbidities showed essentially the same results (data not shown).

A total of 2068 individuals who progressed from MGUS to MM or related disorders and 6204 matched controls were included in the first additional analysis. Of those, 87 (4.2%) progressing MGUS individuals and 160 (2.6%) matched controls had a fracture in the year observed. The risk of fractures was 1.66-fold in individuals who were about to progress as compared to matched controls with MGUS (OR: 1.66; 95% CI: 1.27-2.16; $P < 0001$). Their risk of peripheral fractures was not significantly increased (OR: 1.15; 95% CI: 0.68-1.87; $P = .60$) but they had a 1.94-fold risk of axial fractures (OR: 1.94; 95% CI: 1.44-2.62; $P < .001$).

Into the second additional analysis, we included 1041 individuals with fractures in MGUS and 3123 matched MGUS controls. Among these participants, 171 (4.1%) progressed to MM or related disorders. Fractures (more than 1 year before MGUS progression) were not associated with progression (HR: 0.95; 95% CI: 0.66-1.36; $P = .77$) (Figure 1).

4 | DISCUSSION

In this large population-based study including more than 8000 MGUS individuals and their almost 31 000 matched controls, we found that MGUS and PN are 2 independent risk factors for fractures. These findings indicate that PN contributes to the previously observed fracture risk associated with MGUS, increasing the risk of fractures by 34%. The risk increase associated with PN was similar for peripheral and axial fractures. Because individuals with PN in other studies have poorer balance and increased risk of falls,[32-34] we speculate that this association of PN and falls in MGUS is caused by an increased risk of falls.

Monoclonal gammopathy of undetermined significance was associated with a 29% increased risk of fractures, even after adjusting for PN. Importantly, in this analysis we excluded fractures in the year before MGUS progression to MM or related disorders, thereby controlling for increased fractures due to undetected MM bone disease. This period, which we found to be associated with a 66% increased risk of fractures has been included in previous studies which have described slightly higher risk estimates for fractures in MGUS than found in our study.[10,12] Furthermore, we found that these MGUS fractures were not associated with progression of MGUS. These findings indicate that MGUS is associated with an inherent fracture

TABLE 2 Calculated HRs and 95% CIs of the different fracture types for MGUS and PN before and after stratification by sex

	Any fracture		Peripheral fracture		Axial fracture	
	HR (95% CI)	*P*	HR (95% CI)	*P*	HR (95% CI)	*P*
All participants						
MGUS	1.29 [1.21-1.37]	<.001	1.18 [1.08-1.30]	<.001	1.37 [1.27-1.48]	<.001
PN	1.34 [1.16-1.55]	<.001	1.36 [1.09-1.71]	.007	1.34 [1.12-1.61]	.001
Males						
MGUS	1.39 [1.27-1.53]	<.001	1.23 [1.05-1.44]	.009	1.51 [1.34-1.69]	<.001
PN	1.39 [1.14-1.69]	.001	1.38 [0.99-1.93]	.057	1.37 [1.07-1.75]	.012
Females						
MGUS	1.22 [1.13-1.31]	<.001	1.14 [1.02-1.28]	.022	1.28 [1.16-1.41]	<.001
PN	1.35 [1.10-1.65]	.004	1.40 [1.04-1.88]	.027	1.36 [1.04-1.77]	.025

Note: All analyses were adjusted for age, year of inclusion, registered alcohol use, and any fracture in the 2 years preceding inclusion using inverse probability weighted Cox regression. Analyses including all participants were also adjusted for sex.

Abbreviations: 95% CI, 95% confidence interval; HR, hazard ratio; MGUS: monoclonal gammopathy of undetermined significance; PN, peripheral neuropathy.

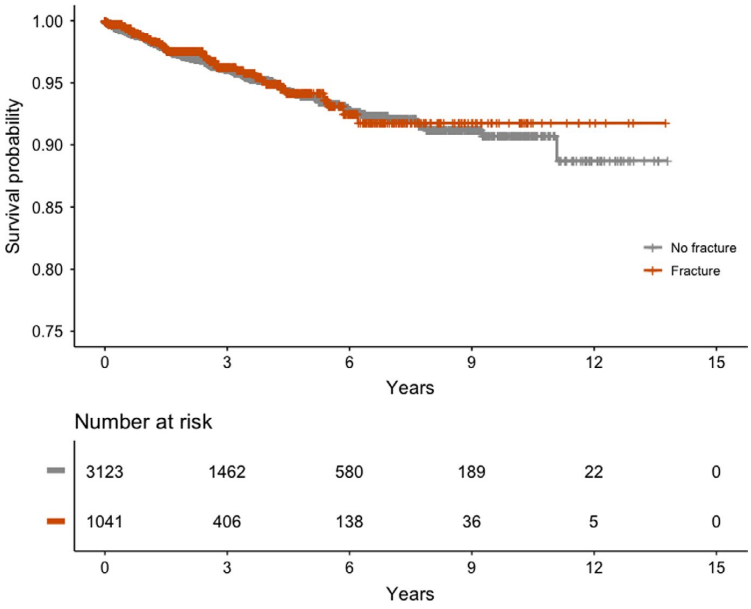


FIGURE 1 A Kaplan-Meyer graph illustrating long-term risk of monoclonal gammopathy of undetermined significance (MGUS) progression in those with MGUS who had a fracture compared to those who did not. The time in this analysis starts at 1 year after the fracture or at the same time in MGUS follow-up for the corresponding controls. Note that the y axis is restricted to 1-0.75 [Colour figure can be viewed at wileyonlinelibrary.com]

risk that is unrelated to MM bone disease or PN. This observed risk in MGUS may be due to the previously described changes in bone microarchitecture found in some individuals with MGUS.[11,20,21] Interestingly, MGUS was associated with both peripheral and axial fractures, albeit more strongly with axial fractures (18% vs 37%) while MGUS progression was only associated with axial fractures. This further indicates that MGUS inherent fracture risk is different to the fracture risk associated with MM bone disease which typically leads to axial fractures.[22]

Monoclonal gammopathy of undetermined significance and PN was associated with an increased risk of fractures in both males and females. This is in contrast with some previous studies that have only found an increased fracture risk in males with MGUS.[11] Indeed, males with MGUS had a higher relative risk increase of fractures than females. However, due to the higher baseline risk of fractures in postmenopausal women,[15] the increase in absolute fracture risk was similar. Interestingly, PN was associated with a similar relative increase in fractures risk in males and females translating to a greater increase in absolute fracture risk for females. These findings indicate that PN is associated with more fractures in women than in men.

Our study has some strengths. We included a whole nation population with MGUS and matched controls over a 13-year period. Secondly, by censoring participants 1 year before progression of MGUS to MM and related disorders and closing study inclusion a year before the end of available data, we were less likely to include MM associated fractures. Our study therefore gives a clearer picture of MGUS specific fracture risk that may have been over-estimated in previous studies. Thirdly, the same diagnostic coding system was used over the whole study period and the preceding 2 years limiting any period effects of different classification for fractures and importantly for PN. Finally, by including alcohol use and previous fractures in the models as well as comorbidities in 2 different sensitivity analyses, we were able to adjust for important confounders.

This study also has important limitations. Firstly, diagnoses of PN and fractures are acquired from physician registered diagnostic codes without any underlying data, therefore relying on the detection, diagnostic skill, and correct registration of these disorders by Swedish physicians. Secondly, isotype data for MGUS were not available. Thirdly, similar to most other MGUS cohorts, all cases of MGUS were diagnosed incidentally during work-up for other illnesses leading to selection of individuals with other medical problems that predispose to both PN and fractures leading to confounding. In an effort to minimize this bias, we adjusted for comorbidities in 2 different sensitivity analyses that showed essentially the same results. However, it is likely that some residual confounding remains. Despite these important limitations, we believe that due to the very specific nature of studying the role of PN in MGUS fracture risk it would be unfeasible to perform a similar study in any dataset that we are currently aware of.

In conclusion, in this large population-based study including almost 8500 individuals with MGUS and their more than 30 000 matched controls, we found that the causes of previously observed increased fracture risk in MGUS are likely multifactorial. We found fractures in MGUS to be associated with both PN and progression, and that MGUS remained associated with an increased fracture risk after controlling for these factors. We therefore speculate that fractures in MGUS have at least 3 independent causes; undetected MM bone disease, MGUS-specific fractures due to MGUS bone disease, and PN by leading to increased falls. Fractures are a major cause of morbidity and mortality, especially in the elderly where the prevalence of MGUS is high.[47] Based on these findings, it might be possible to mitigate MGUS fracture risk by several measures. Firstly, by

close follow-up of individuals with MGUS and early detection and intervention of MGUS progression, it may be possible to prevent MM bone disease and associated fractures. Secondly, although there are few evidence-based therapies for MGUS-associated PN,[48,49] physiotherapeutic interventions may mitigate the associated fracture risk of PN.[50] However, such interventions would need further study in interventional trials. Our findings also highlight the need for further study of MGUS inherent bone disease, its causes, detection, and potential therapeutic interventions.

ACKNOWLEDGEMENTS

This research was supported by grants from the University of Iceland Research Fund, Icelandic Centre for Research (RANNIS), the Swedish Cancer Society, Landspitali University Hospital Research Fund, and Karolinska Institutet Foundations. For OL: funding support for this publication was provided by the Memorial Sloan Kettering Core Grant (P30 CA008748) and the Perelman Family Foundation in Collaboration with the Multiple Myeloma Research Foundation (MMRF). SR is a PhD candidate at the University of Iceland and this work is submitted in partial fulfilment of the requirement for a PhD.

CONFLICT OF INTEREST

OL has received research funding from: National Institutes of Health (NIH), US Food and Drug Administration (FDA), Multiple Myeloma Research Foundation (MMRF), International Myeloma Foundation (IMF), Leukemia and Lymphoma Society (LLS), Perelman Family Foundation, Rising Tides Foundation, Amgen, Celgene, Janssen, Takeda, Glenmark, Seattle Genetics, Karyopharm; Honoraria/ad boards: Adaptive, Amgen, Binding Site, BMS, Celgene, Cellectis, Glenmark, Janssen, Juno, Pfizer; and serves on Independent Data Monitoring Committees (IDMCs) for clinical trials lead by Takeda, Merck, Janssen. SYK has received research funding from: Celgene, Amgen. Other authors have no relevant conflicts-of-interest to disclose.

AUTHOR CONTRIBUTION

SYK and SR designed the study and obtained data. SR performed the analyses with statistical input from TA. ST provided the additional design input. SR, SYK, ST, TA, MB, IT, and OL involved in the analyses and the interpretation of the results, read and gave comments, and approved the final manuscript written by SR and SYK. All authors accessed the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis.

DATA AVAILABILITY STATEMENT

The data included in study is built on multiple population-based registries including the medical records of Swedish citizens. Although the data has been made unidentifiable before analysis, ethical board approval does not permit data sharing.

ORCID

Sæmundur Rögnvaldsson https://orcid.org/0000-0003-2162-8414
Sigrún Thorsteinsdóttir https://orcid.org/0000-0001-5017-3530
Ingemar Turesson https://orcid.org/0000-0002-4115-8010
Sigurður Yngvi Kristinsson https://orcid.org/0000-0002-4964-7476

REFERENCES

1. Landgren O, Kyle RA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. *Blood*. 2009;113:5412-5417.
2. Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM. A monoclonal gammopathy precedes multiple myeloma in most patients. *Blood*. 2009;113:5418-5422.
3. Kyle RA, Durie BGM, Rajkumar SV, et al. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. *Leukemia*. 2010;24:1121-1127.
4. Kyle RA, Therneau TM, Vincent Rajkumar S, et al. Prevalence of monoclonal gammopathy of undetermined significance. *N Engl J Med*. 2006;354:1362-1369.
5. Dispenzieri A, Katzmann JA, Kyle RA, et al. Prevalence and risk of progression of light-chain monoclonal gammopathy of undetermined significance: a retrospective population-based cohort study. *Lancet*. 2010;375:1721-1728.
6. Kyle RA, Therneau TM, Rajkumar SV, et al. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. *N Engl J Med*. 2002;346:564-569.
7. Kyle RA, Therneau TM, Rajkumar SV, et al. Long-term follow-up of IgM monoclonal gammopathy of undetermined significance. *Blood*. 2003;102:3759-3764.
8. Gosselin S, Kyle RA, Dyck PJ. Neuropathy associated with monoclonal gammopathies of undetermined significance. *Ann Neurol*. 1991;30:54-61.
9. Kristinsson SY, Pfeiffer RM, Björkholm M, et al. Arterial and venous thrombosis in monoclonal gammopathy of undetermined significance and multiple myeloma: a population-based study. *Blood*. 2010;115:4991-4998.
10. Kristinsson SY, Tang M, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance and risk of skeletal fractures: a population-based study. *Blood*. 2010;116:2651-2655.
11. Thorsteinsdottir S, Lund SH, Lindqvist EK, et al. Bone disease in monoclonal gammopathy of undetermined significance: results from a screened population-based study. *Blood Adv*. 2017;1:2790-2798.
12. Melton LJ, Rajkumar SV, Khosla S, Achenbach SJ, Oberg AL, Kyle RA. Fracture risk in monoclonal gammopathy of undetermined significance. *J Bone Miner Res*. 2004;19:25-30.
13. Kristinsson SY, Tang M, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance and risk of infections: a population-based study. *Haematologica*. 2012;97:854-858.
14. Kristinsson SY, Björkholm M, Andersson TML, et al. Patterns of survival and causes of death following a diagnosis of monoclonal gammopathy of undetermined significance: a population-based study. *Haematologica*. 2009;94:1714-1720.
15. Cummings SR, Nevitt MC, Browner WS, et al. Risk factors for hip fracture in white women. *N Engl J Med*. 1995;332:767-773.
16. Abrahamsen B, Andersen I, Christensen SS, Skov Madsen J, Brixen K. Utility of testing for monoclonal bands in serum of patients with suspected osteoporosis: retrospective, cross sectional study. *BMJ*. 2005;330:818.
17. Edwards BJ, Langman CB, Bunta AD, Vicuna M, Favus M. Secondary contributors to bone loss in osteoporosis related hip fractures. *Osteoporos Int*. 2008;19:991-999.

18. Bouvard B, Royer M, Chappard D, Audran M, Hoppé E, Legrand E. Monoclonal gammopathy of undetermined significance, multiple myeloma, and osteoporosis. *Jt Bone Spine*. 2010;77:120-124. https://doi.org/10.1016/j.jbspin.2009.12.002
19. Veronese N, Luchini C, Solmi M, Sergi G, Manzato E, Stubbs B. Monoclonal gammopathy of undetermined significance and bone health outcomes: a systematic review and exploratory meta-analysis. *J Bone Miner Metab*. 2018;36:128-132.
20. Ng AC, Khosla S, Charatcharoenwitthaya N, et al. Bone microstructural changes revealed by high-resolution peripheral quantitative computed tomography imaging and elevated DKK1 and MIP-1a levels in patients with MGUS. *Blood*. 2011;118:6529-6535.
21. Farr JN, Zhang W, Kumar SK, et al. Altered cortical microarchitecture in patients with monoclonal gammopathy of undetermined significance. *Blood*. 2014;123:647-649.
22. Melton LJ, Kyle RA, Achenbach SJ, Oberg AL, Rajkumar SV. Fracture risk with multiple myeloma: a population-based study. *J Bone Miner Res*. 2005;20:487-493.
23. Rögnvaldsson S, Steingrímsson V, Turesson I, Björkholm M, Landgren O, Kristinsson SY. Peripheral neuropathy and monoclonal gammopathy of undetermined significance: a population-based study including 15,351 cases and 58,619 matched controls. *Haematologica*. 2020;105:2679-2681. https://doi.org/10.3324/haematol.2019.239632
24. Dyck PJ, Thomas PK. *Peripheral Neuropathy*. 4th ed. Philadelphia, PA: Elsevier Inc; 2005.
25. Isobe T, Osserman EF. Pathologic conditions associated with plasma cell dyscrasias: a study of 806 cases. *Ann N Y Acad Sci*. 1971;190:507-518.
26. Nobile-Orazio E, Barbieri S, Baldini L, et al. Peripheral neuropathy in monoclonal gammopathy of undetermined significance: prevalence and immunopathogenetic studies. *Acta Neurol Scand*. 1992;85:383-390.
27. Ösby E, Noring L, Hast R, Kjellin KG, Knutsson E, Sidén Å. Benign monoclonal gammopathy and peripheral neuropathy. *Br J Haematol*. 1982;51:531-539.
28. Steiner N, Schwärzler A, Göbel G, Löscher W, Gunsilius E. Are neurological complications of monoclonal gammopathy of undetermined significance underestimated? *Oncotarget*. 2017;8:5081-5091.
29. Vrethem M, Cruz M, Wen-Xin H, Malm C, Holmgren H, Ernerudh J. Clinical, neurophysiological and immunological evidence of polyneuropathy in patients with monoclonal gammopathies. *J Neurol Sci*. 1993;114:193-199.
30. Bida JP, Kyle RA, Therneau TM, et al. Disease associations with monoclonal gammopathy of undetermined significance: a population-based study of 17,398 patients. *Mayo Clin Proc*. 2009;84:685-693.
31. Johansen P, Leegaard OF. Peripheral neuropathy and paraproteinemia: an immunohistochemical and serologic study. *Clin Neuropathol*. 1985;4:99-104.
32. Brown SJ, Handsaker JC, Bowling FL, Boulton AJM, Reeves ND. Diabetic peripheral neuropathy compromises balance during daily activities. *Diabetes Care*. 2015;38:1116-1122.
33. Richardson JK, Hurvitz EA. Peripheral neuropathy: a true risk factor for falls. *J Gerontol A Biol Sci Med Sci*. 1995;50A:M211-M215.
34. Timar B, Timar R, Gaita L, Oancea C, Levai C, Lungeanu D. The impact of diabetic neuropathy on balance and on the risk of falls in patients with type 2 diabetes mellitus: a cross-sectional study. *PLoS One*. 2016;11:e0154654.
35. Strotmeyer ES, Cauley JA, Schwartz AV, et al. Nontraumatic fracture risk with diabetes mellitus and impaired fasting glucose in older white and black adults. *Arch Intern Med*. 2005;165:1612.
36. Kristinsson SY, Bjo M, Goldin LR, Mcmaster ML, Turesson I, Landgren O. Risk of lymphoproliferative disorders among first-degree relatives of lymphoplasmacytic lymphoma/Waldenström macroglobulinemia patients: a population-based study in Sweden. *Blood*. 2008;112:3052-3056.
37. Landgren O, Kristinsson SY, Goldin LR, et al. Risk of plasma cell and lymphoproliferative disorders among 14621 first-degree relatives of 4458 patients with monoclonal gammopathy of undetermined significance in Sweden. *Blood*. 2009;114:791-795.
38. Anonymous. *Kvalitet och innehåll i patientregistret—Utskrivningar från slutenvården 1964–2007 och besök i specialiserad öppenvård (exklusive primärvårdsbesök) 1997–2007*. Stockholm, Sweden: Socialstyrelsen; 2009.
39. Brooke HL, Talbäck M, Hörnblad J, et al. The Swedish cause of death register. *Eur J Epidemiol*. 2017;32:765-773.
40. Turesson I, Linet MS, Björkholm M, et al. Ascertainment and diagnostic accuracy for hematopoietic lymphoproliferative malignancies in Sweden 1964–2003. *Int J Cancer*. 2007;121:2260-2266.
41. R Development Core Team R. *R: A Language and Environment for Statistical Computing*. 2011. https://doi.org/10.1007/978-3-540-74686-7
42. Therneau T. A package for survival analysis in S. R package version. *Survival*. 2012;454:455-456.
43. Kassambara A, Kosinski M, Biecek P, Fabian S. survminer: Drawing Survival Curves using 'ggplot2' (R package) version 0.4.4. 2019. https://cran.r-project.org/package=survminer. Accessed September 23, 2020.
44. van der Wal WM, Geskus RB. Ipw: an R package for inverse probability weighting. *J Stat Softw*. 2011. https://doi.org/10.18637/jss.v043.i13
45. Kanis JA, Borgstrom F, De Laet C, et al. Assessment of fracture risk. *Osteoporos Int*. 2005;16:581-589.
46. Charlson M, Szatrowski TP, Peterson J, Gold J. Validation of a combined comorbidity index. *J Clin Epidemiol*. 1994;47:1245-1251.
47. Nazrun AS, Tzar MN, Mokhtar SA, Mohamed IN. A systematic review of the outcomes of osteoporotic fracture patients after hospital discharge: morbidity, subsequent fractures, and mortality. *Ther Clin Risk Manag*. 2014;10:937-948.
48. Chaudhry HM, Mauermann ML, Rajkumar SV. Monoclonal gammopathy–associated peripheral neuropathy: diagnosis and management. *Mayo Clin Proc*. 2017;92:838-850.
49. Stork ACJ, Lunn MPT, Nobile-Orazio E, Notermans NC. Treatment for IgG and IgA paraproteinaemic neuropathy. *Cochrane Database Syst. Rev*. 2015;3:CD005376.
50. Streckmann F, Zopf EM, Lehmann HC, et al. Exercise intervention studies in patients with peripheral neuropathy: a systematic review. *Sports Med*. 2014;44(9):1289-1304. https://doi.org/10.1007/s40279-014-0207-5

How to cite this article: Rögnvaldsson S, Aspelund T, Thorsteinsdóttir S, et al. Untangling fracture risk in monoclonal gammopathy of undetermined significance: A population-based cohort study. *Eur J Haematol*. 2021;107:137–144. https://doi.org/10.1111/ejh.13633

Paper III

Rögnvaldsson et al. *Blood Cancer Journal* (2021)11:94
https://doi.org/10.1038/s41408-021-00480-w

Blood Cancer Journal

ARTICLE Open Access

Iceland screens, treats, or prevents multiple myeloma (iStopMM): a population-based screening study for monoclonal gammopathy of undetermined significance and randomized controlled trial of follow-up strategies

Sæmundur Rögnvaldsson[1], Thorvardur Jon Love[1], Sigrun Thorsteinsdottir[1,2], Elín Ruth Reed[1], Jón Þórir Óskarsson[1], Íris Pétursdóttir[1], Guðrún Ásta Sigurðardóttir[1], Brynjar Viðarsson[3], Páll Torfi Önundarson[1,3], Bjarni A. Agnarsson[1,3], Margrét Sigurðardóttir[3], Ingunn Þorsteinsdóttir[3], Ísleifur Ólafsson[3], Ásdís Rósa Þórðardóttir[1], Elías Eyþórsson[3], Ásbjörn Jónsson[3], Andri S. Björnsson[4], Gunnar Þór Gunnarsson[1,5], Runólfur Pálsson[1,3], Ólafur Skúli Indriðason[1,3], Gauti Kjartan Gíslason[1], Andri Ólafsson[1], Guðlaug Katrín Hákonardóttir[1], Manje Brinkhuis[1], Sara Lovísa Halldórsdóttir[1], Tinna Laufey Ásgeirsdóttir[6], Hlíf Steingrímsdóttir[3], Ragnar Danielsen[3], Inga Dröfn Wessman[4], Petros Kampanis[7], Malin Hulcrantz[8], Brian G. M. Durie[9], Stephen Harding[7], Ola Landgren[10] and Sigurður Yngvi Kristinsson[1,3]

Abstract

Monoclonal gammopathy of undetermined significance (MGUS) precedes multiple myeloma (MM). Population-based screening for MGUS could identify candidates for early treatment in MM. Here we describe the Iceland Screens, Treats, or Prevents Multiple Myeloma study (iStopMM), the first population-based screening study for MGUS including a randomized trial of follow-up strategies. Icelandic residents born before 1976 were offered participation. Blood samples are collected alongside blood sampling in the Icelandic healthcare system. Participants with MGUS are randomized to three study arms. Arm 1 is not contacted, arm 2 follows current guidelines, and arm 3 follows a more intensive strategy. Participants who progress are offered early treatment. Samples are collected longitudinally from arms 2 and 3 for the study biobank. All participants repeatedly answer questionnaires on various exposures and outcomes including quality of life and psychiatric health. National registries on health are cross-linked to all participants. Of the 148,704 individuals in the target population, 80 759 (54.3%) provided informed consent for participation. With a very high participation rate, the data from the iStopMM study will answer important questions on MGUS, including potentials harms and benefits of screening. The study can lead to a paradigm shift in MM therapy towards screening and early therapy.

Introduction

Monoclonal gammopathy of undetermined significance (MGUS) is characterized by the presence of monoclonal immunoglobulins (M proteins) or an abnormal ratio of free immunoglobulin light chains (FLC) in the blood[1]. MGUS can be classified by the type of M proteins present. Non-IgM MGUS is the most common type and is defined by the presence of IgG, IgA, and rarely IgD or IgE M proteins[2]. IgM MGUS is defined by the presence of IgM M proteins[3]. Light-chain (LC) MGUS is defined by an abnormal

Correspondence: Sigurður Yngvi Kristinsson (sigyngvi@hi.is)
[1]Faculty of Medicine, Univeristy of Iceland, Reykjavík, Iceland
[2]Dept of Hematology, Rigshospitalet, Copenhagen, Denmark
Full list of author information is available at the end of the article

FLC-ratio, indicating an excess of monoclonal FLCs in the absence of M proteins[4]. Non-IgM MGUS and LC-MGUS are caused by monoclonal bone marrow plasma cells (BMPCs) and are the precursor of multiple myeloma (MM), a malignancy of BMPCs[5,6]. IgM MGUS is caused by monoclonal lymphoplasmacytic lymphocytes and is a precursor to other lymphoproliferative disorders (LP), most notably Waldenström's macroglobulinemia (WM), and rarely MM[3]. In addition, MGUS of all types, especially LC-MGUS, can precede amyloid light chain amyloidosis (AL)[7]. Prior studies suggest a 1% annual risk of progressing from MGUS and LC-MGUS to frank malignancy[1,3,4,8].

Before progressing to MM or WM, MGUS is believed to pass through a smoldering MM or WM phase (SMM and SWM), which is associated with a higher disease burden than MGUS and LC-MGUS but without MM or WM related organ damage[1]. Smoldering disease carries a higher risk of progression to active disease than MGUS. Retrospective data from the Mayo Clinic suggest that the risk of progression from SMM to MM is 10% per year for the first five years[9], and that the risk of progression of SWM to WM is 60% within 10 years[10].

Currently, consensus guidelines recommend indefinite follow-up in MGUS, SMM, and SWM. However, there is no data available from prospective studies or randomized trials regarding optimal clinical management[1,11–13]. Three recent observational studies from Sweden and the US have consistently demonstrated that individuals with known MGUS prior to the diagnosis of MM have 13–15% better overall survival in MM[14–16]. These observations indicate that clinical follow-up of precursor disease leads to earlier detection and diagnosis of MM, resulting in fewer patients presenting with symptomatic end-organ damage at the time of MM diagnosis, which may have contributed to the observed better overall survival.

In the clinical setting, the optimal timing of therapy in MM has been a subject of debate. Traditionally, therapy has been reserved for those with MM-related end-organ damage, however, in 2014 the definition of MM was expanded to also include myeloma-defining biomarkers in asymptomatic individuals[8]. With the advent of newer, more effective, and less toxic drugs, survival has improved dramatically in MM[17–19]. Three separate randomized controlled trials starting therapy at the stage of SMM have shown improved progression-free survival, and one study showed superior overall survival[20–22]. Importantly, these studies have shown more favorable toxicity profiles than earlier trials[23]. In light of these findings some authors now recommend early treatment in high-risk SMM[24,25]. However, only 2.7–6.0% of MM patients have previously identified precursor disease, which limits the implementation of early treatment in most MM patients[14,16]. This raises the question of whether population-based screening and follow-up of MGUS could improve the outcomes in MM by identifying candidates for early treatment. However, there is no evidence supporting the implementation of asymptomatic screening for MGUS, and screening is not currently recommended. To address this question, we have launched a population-based screening study with a subsequent randomized controlled trial (RCT) evaluating the risks and benefits of screening and follow-up of MGUS patients.

Here, we describe the design and recruitment of the Iceland Screens, Treats, or Prevents Multiple Myeloma study (iStopMM), a population-based screening study of MGUS and the disorders it precedes and RCT of follow-up strategies.

Methods

Approval

The study protocol, all information material, biobank, and questionnaires were approved by the Icelandic National Bioethics Committee (Number 16–022, date: 2016-04-26) with approval from the Icelandic Data Protection Agency. Access to national healthcare registries has been approved by the Icelandic Directorate of Health and the Icelandic Cancer Society. The study was preregistered on ClinicalTrials.gov (ClinicaTrials.gov identifier: NCT03327597).

Recruitment and screening

The study's inclusion criteria were being born in 1975 or earlier and residing in Iceland on the 9th of September 2016, as registered in the Icelandic National Registry. Eligible individuals were invited to participate in the iStopMM study ($n = 148{,}711$). A letter containing a detailed information brochure and consent form was mailed to them and an extensive campaign on social and conventional media was launched introducing the study to the Icelandic public. This campaign was followed by phone calls to those who had not yet signed up for the study. Participants could provide informed consent through three different mechanisms: (1) returning a signed informed consent form by mail, (2) registering electronically using a participation code included in the invitation letter, or (3) through a secure internet gateway provided by the Icelandic government (island.is), which is accessible to all residents through a secure electronic authentication process. The only exclusion criterion was previously known LP, other than MGUS.

After enrollment, serum samples for screening are collected alongside the collection of blood during clinical care in the universal Icelandic healthcare system, including blood banks (Fig. 1). The study team in collaboration with Landspítali—The National University Hospital of Iceland (LUH), developed an electronic system linking participant data to the central laboratory network of all major and smaller urban healthcare institutions, which covers at least 92% of all Icelandic residents. The system

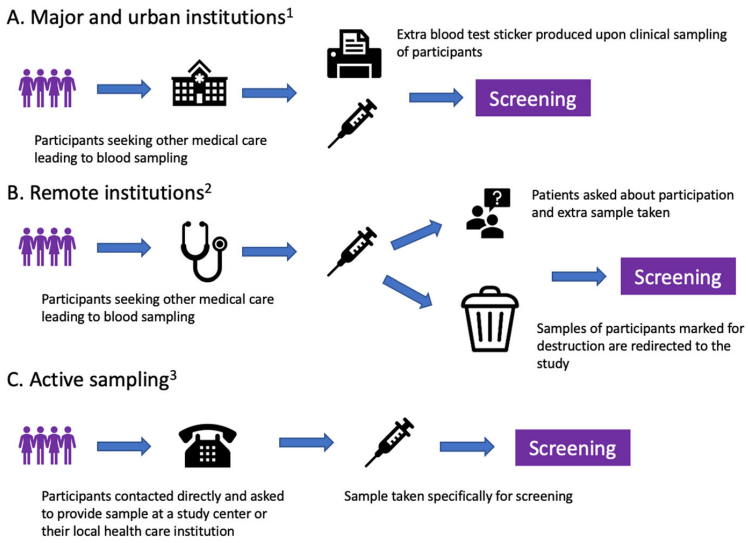


Fig. 1 Methods of blood sample acquisition. A and **B** describe passive sampling starting during the fall of 2016, and **C** describes active sampling beginning during the fall of 2019. 1: Reykjavik Capital Area, Akureyri, Ísafjörður, Reykjanes Peninsula, Akranes, Healthcare Institution of Northern Iceland, Healthcare Institution of South Iceland, blood banks 2: Neskaupsstaður, Healthcare institution of West Iceland, Healthcare Institution of East Iceland. 3: Available for all Icelandic residents.

notifies healthcare workers to take an extra blood sample for the study at the point of clinical blood sampling. For smaller rural institutions and private clinics, a manual system was developed whereby laboratory technicians crosslink left-over samples marked for destruction to registered participants and in some cases ask their patients if they are participants in the study and draw an additional sample for the study. To capture samples from participants who do not require clinical blood sampling, an active sampling drive was initiated after three years of passive sample collection.

All samples are sent to the clinical laboratory at LUH in Reykjavik, Iceland where serum is aliquoted into identical sample tubes and assigned an anonymous study identification number. The laboratory uses TC automation and aliquoter (Thermo Scientific®, MA, USA) for sample handling. Samples are then sent to The Binding Site laboratory in Birmingham, UK where all samples are screened for M protein by capillary zone electrophoresis (CZE; Helena Laboratories, Texas, USA) and for FLC, immunoglobulins (IgG, IgA, and IgM), and total protein by Freelite® and Hevylite® assays performed on an Optilite® turbidimeter (The Binding Site Group Ltd, Birmingham, UK). Immunofixation electrophoresis (IFE; Helena Laboratories, TX, USA) is performed on samples with clear or suspected M protein bands by CZE and/or abnormal FLC results. The CZE and IFE gels are assessed independently by at least two experienced observers.

Randomization and study arms

Participants with an M protein or pathological FLC results are considered eligible for the RCT and are randomized into three study arms in a dynamic, non-predetermined manner (Fig. 2). To avoid skewed distribution of high-risk MGUS and LC MGUS, randomization is carried out by blocks of having an M protein >1.5 g/dL and having LC-MGUS. Participants in arm 1 are not informed of their MGUS status and continue to receive conventional healthcare as if they had never been screened. Arm 2 follows current guidelines for follow-up, stratified by low and non-low risk MGUS[1]. Arm 3 follows a more intensive strategy that is not risk-stratified (see below).

Participants with an M protein ≥3.0 g/dL or an FLC ratio ≥100 are not eligible for randomization but are all called in for evaluation since they have, by definition, more advanced disease than MGUS[1,8,10]. Participants with previously diagnosed MGUS cannot be randomized to arm 1, as they are aware of their MGUS status, and are thus randomized to arms 2 or 3 and will not be included in comparisons with arm 1.

Initial assessment and follow-up

Initial assessment and follow-up of participants in arms 2 and 3 and participants diagnosed with more advanced disease (SMM, SWM, MM, AL, or other LP) at screening is performed in the iStopMM study clinic in Reykjavík, Iceland. Temporary clinics are also regularly established in

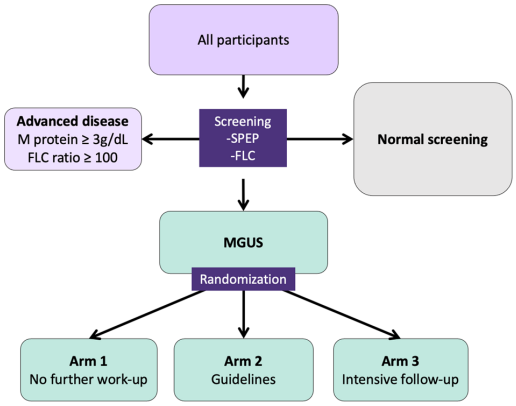


Fig. 2 A flowchart outlining the study design for screening and randomization of individuals with MGUS. MGUS Monoclonal gammopathy of undetermined significance, SPEP Serum protein electrophoresis, FLC Free light chain assay, SMM Smoldering multiple myeloma, MM Multiple myeloma.

Akureyri, Ísafjörður, Húsavík, and Egilsstaðir for complete geographical coverage. All participants who are called into the clinic are seen by specialized study nurses and those with more advanced disease are also seen by a physician. The participants undergo a clinical interview and thorough clinical examination and are given detailed oral and written information about their diagnosis and prognosis.

Participants in arm 2 with non-IgM MGUS or LC-MGUS are stratified by having low-risk MGUS or not. These participants are then followed according to guidelines including plain skeletal surveys and bone marrow sampling for those with non-low risk MGUS or when clinically indicated[1]. All participants in arm 3 follow an intensive follow-up schedule regardless of risk, including bone marrow sampling and whole-body low-dose computerized tomography (WB-LDCT). Participants in arm 2 and 3 with IgM MGUS undergo a computerized tomography (CT) of the abdomen. Diagnostics and follow-up intervals for arms 2 and 3 are shown in Table 1. Participants with smoldering or active disease at baseline or later are followed according to guidelines. This includes intensive follow-up every 4 months or sooner if clinically indicated with annual bone marrow samples and WB-LDCT, as well as magnetic resonance imaging (MRI) if no bone lesions are seen on WB-LDCT. Participants who develop intermediate to high-risk SMM, MM, or other related disorders that require treatment are offered participation in a treatment trial (ClinicalTrials.gov identifier: NCT03815279) or referred to the hematology unit at LUH or Akureyri Hospital for evaluation, treatment, and follow-up.

To detect AL, urine samples are tested for proteinuria in participants visiting the study clinic. In addition, participants in arm 3 and those with more advanced disease are tested for cardiac markers (Table 1). Those with significant proteinuria and decreased kidney function of unclear etiology are referred to a nephrologist for further evaluation. Those with abnormal cardiac markers not explained by known comorbidities are referred to a cardiologist for clinical evaluation and echocardiography. Bone marrow biopsies are stained with Congo red for the presence of amyloid fibrils in all these cases and another testing for AL is performed as clinically indicated.

After each visit, participant's test results and clinical findings are thoroughly reviewed by the primary investigator and the clinic staff with respect to their disease status and progression at regular clinical decision meetings. Additional testing including repeat bone marrow sampling, imaging, blood sampling, or clinical evaluation is ordered as clinically indicated at or between protocol visits. Diagnoses of SMM, MM, SWM, WM, AL, and other LP are made according to current diagnostic criteria[1,8,26,27].

Imaging

Plain radiographs, WB-LDCT, and CT of the abdomen are performed in LUH and Akureyri Hospital. MRI is performed in LUH and Akureyri Hospital. All radiological images are reviewed independently by two physicians, one in specialty training and a senior radiologist at LUH. The radiological assessments are blinded and any discordance in findings is discussed and solved by the two physicians.

Bone marrow samples

Bone marrow sampling is performed by study nurses that have been trained, both locally and in an accredited facility in the United Kingdom (The Royal Marsden Hospital, London, UK). Samples are collected as bone marrow smears and as trephine biopsies. Bone marrow smears are stained with Giemsa stain and jointly evaluated by two senior hematologists at LUH reporting the percentage of BMPCs or lymphoplasmacytic lymphocytes, lymphoid infiltrates, and sample quality. Trephine biopsies are stained with hematoxylin and eosin, as well as for CD138 before being evaluated by two senior hematopathologists at LUH. The sample with the higher percentage of BMPCs/lymphocytic infiltration at each sampling time is used to guide follow-up.

Questionnaires

Immediately following informed consent, participants were asked to complete questionnaires on psychiatric symptoms (e.g., anxiety and depressive symptoms) and life satisfaction to establish a baseline prior to screening[28–30]. Throughout the study period, all participants, regardless of screening status, are asked to complete the same questionnaires electronically at predefined intervals, as

Table 1 Clinical assessment, imaging, and laboratory studies included for participants in the different study arms of the iStopMM study as per protocol.

Test	Arm 2–low risk and LC-MGUS	Arm 2–non-low risk	Arm 3–All	SMM and SWM	MM and WM
Physical exam[a]	First visit	First visit	Each visit	Each visit	At diagnosis
Blood sampling					
SPEP FLC assay	Each visit	Each visit	Each visit	Each visit	At diagnosis
CBC	First visit	Each visit	Each visit	Each visit	At diagnosis
Total calcium Albumin Creatinine	First visit	First visit	Each visit	Each visit	At diagnosis
CRP LDH ß2M	–	–	Each visit	Each visit	At diagnosis
TnT pro-BNP	–	–	Annually	Annually	At diagnosis
Bone marrow					
Smear Biopsy	As clinically indicated	0 months Except if LC	0 and 60 months	Annually	At diagnosis
Urine					
Protein dipstick	First visit	First visit	–	–	–
UPEP	If positive dipstick or if previously abnormal	If positive dipstick or if previously abnormal	–	–	–
Albumin/ creatinine ratio	–	–	Annually	Annually	At diagnosis
ECG	–	–	Annually	Annually	At diagnosis
Imaging					
WB-LDCT	–	–	0 and 60 months in LC- and non-IgM	Annually in LC- and non-IgM	At diagnosis of MM
Plain X-ray of bones	As clinically indicated	First visit in LC- and non-IgM	–	–	–
CT abdomen	–	First visit to IgM	0 and 60 months in IgM	Annually in IgM	At diagnosis of WM
MRI of bones	–	–	–	As clinically indicated	–
Follow-up	Every 2–3 years	Annual	Annual	Every 4–6 months	Single-visit

Note that additional sampling and imaging were permitted as clinically indicated and decided at regularly scheduled clinical decision meetings.
SMM smoldering multiple myeloma, *SWM* smoldering Waldenströms macroglobulinemia, *MM* multiple myeloma, *WM* Waldenströms macroglobulinemia, *SPEP* serum protein electrophoresis, *FLC* free light chains, *CBC* complete blood count, *CRP* C-reactive protein, *LDH* Lactate dehydrogenase, *ß2M* ß-2-microglobulin, *TnT* Troponin T, *pro-BPN* pro-Brain natriuretic peptide, *UPEP* Urine protein electrophoresis, *ECG* electrocardiogram, *WB-LDCT* whole-body low-dose computerized tomography, *CT* Computerized tomography, *MRI* magnetic resonance imaging, *LC* Light chain.

well as additional questionnaires on psychiatric health, pain, neuropathic symptoms, and more (Table 2).

Those who visit the study clinic (arms 2 and 3, and individuals with more advanced disease) answer more extensive questionnaires at each clinic visit and annually. Those who are randomized to arm 1 or are screened negative continue to receive the same annual questionnaires. One-time questionnaires, e.g., baseline characteristics, employment history, resilience, social support, and adverse childhood experiences are sent to all participants by email (Table 2).

Currently, 72 918 (90%) of all participants have provided their email addresses. All non-valid email addresses are reviewed by study staff and participants who visit the study clinic are asked to provide a valid email. Participants are reminded to answer the questionnaires in three separate emails.

Table 2 Questionnaires sent to participants by email or answered at the study clinic.

Questionnaire	Subject	Validated?	All	Arm 1 and normal screening		Arm 2 and 3 and advanced disease[a]	
			At registration	One time	Annually	One time	Each visit
Background							
Anthropomorphic data	Weight, height etc.	NA		✓		✓	
Social history[b]	Socioeconomic status	NA		✓		✓	
Medical history[c]	Medical history			✓		✓	
Habits[d]	Environment	NA		✓		✓	
Industrial exposure	Environment	NA		✓		✓	
Quality of life							
PHQ9	Depression	Yes	✓		✓		✓
GAD-7	Anxiety	Yes	✓		✓		✓
SWLS	Quality of life	Yes	✓		✓		✓
Other questions of happiness and wellbeing	Quality of life	No	✓		✓		✓
SF-36	Health-related quality of life	Yes			✓		✓
PSS-10	Stress and anxiety	Yes			✓		✓
PCL-5 (MGUS specific)	PTSD from MGUS diagnosis	Yes					✓
PCL-5 (nonspecific)	PTSD other	Yes			✓		
Symptoms							
BPI	Pain	Yes			✓		✓
NSS	Neuropathy	Yes			✓		✓
DN4	Neuropathy	Yes			✓		✓
Symptoms of PMR	PMR	No			✓		✓
Social background							
MSPSS	Social support	Yes		✓		✓	
CD-RISC-10ICE	Resilience	Yes		✓		✓	
ACE	Childhood traumatic events	Yes		✓		✓	
LEC	Lifetime traumatic events	Yes		✓		✓	

Note that all participants were asked to answer four questionnaires when providing informed consent electronically or if they provided an email address in their written consent form.
Questionnaires were not sent to participants who did not provide an email address and were not called into the study.
PHQ9 patient health questionnaire, *GAD-7* General anxiety disorder, *SWLS* satisfaction with life scale, *SF-36* 36-item short-form survey, *PSS-10* perceived stress scale, *PCL-5* post-traumatic stress disorder checklist for DSM-5, *BPI* brief pain inventory, *NSS* neuropathy symptom scale, *DN4* Douleur neuropathique. *PMR* polymyalgia rheumatica, *MSPSS* Multidimensional scale of social support, *CD-RISC-10ICE* Connor-Davidson resilience scale. *ACE* adverse childhood events. *LEC* Lifetime events checklist.
✓Showing the timing of the questionnaire in that row is the time/frequency assigned to that column.
[a]Including MM, WM, SMM, and SWM.
[b]Employment, marital status, education, income, and residence.
[c]Including obstetric history for women.
[d]Including smoking and alcohol intake.

Registry crosslinking

Several national healthcare-related registries exist in Iceland that can be accurately crosslinked using a government-issued national identification number. Data from these registries are linked to all participants in the iStopMM study at least twice each year. The following registries are linked to the study datasets: (1) The Icelandic Cancer Registry includes information on all cancers diagnosed in Iceland. It has been mandatory for all physicians and pathologists to register diagnoses of cancer

Table 3 Biosamples included in the study biobank and when they are obtained from participants.

Sample	Arm 2–Low risk	Arm 2–Non-low risk	Arm 3–All	SMM and SWM–4-month follow-up	SMM and SWM–6-month follow-up	MM and WM
Bone marrow						
Sorted and unsorted cells[a]	None	0 and 60 months	0 and 60 months	Annually	Annually	At diagnosis
Plasma	None	0 and 60 months	0 and 60 months	Annually	Annually	At diagnosis
Blood						
Cell-free plasma (EDTA tube)	0 months	Annually	Annually	Every 4 months	Every 6 months	At diagnosis
Plasma (Li-Hep tubes)	0 months	Annually	Annually	Every 4 months	Every 6 months	At diagnosis
Serum (SST tubes)	0 months	Annually	Annually	Every 4 months	Every 6 months	At diagnosis
Blood RNA (PaxGene® tube)	0 months	0 months	0 months	0 months	0 months	At diagnosis
Lymphocytes (CPT tube)	0 months	0 and 60 months	0 and 60 months	0 and 60 months	0 and 60 months	At diagnosis
Urine	0 months	0 months	0 months	Annually	Annually	At diagnosis

SMM smoldering multiple myeloma, *SWM* smoldering Waldenströms macroglobulinemia, *MM* multiple myeloma, *WM* Waldenströms macroglobulinemia.
[a]Buffy coat from the bone marrow samples. Unsorted in IgM MGUS but stored as CD138+ and CD138− fractions using magnetic-activated cell sorting (MACS) in Non-IgM MGUS and LC-MGUS.

since 1955 and it is virtually complete with high diagnostic accuracy and timeliness[31]; (2) The Icelandic Causes of Death Registry includes all deaths in Iceland including the date and the presumed causes of death. Registration has been mandatory since 1971; (3) The Icelandic Prescription Medicines Registry includes all prescriptions, including whether the prescriptions were filled or not. in Iceland since 2002; (4) The Icelandic Hospital Discharge Registry includes all inpatient admissions in Iceland from 1999 with the dates of admission and discharge, as well as international classification of diseases (ICD) codes for the diagnoses made by treating physicians. The registry also includes outpatient visits at hospitals, including emergency rooms since 2010; (5) The Icelandic Registry of Primary Health Care Contacts includes all primary care visits and registered ICD-coded diagnoses for all primary care encounters in Iceland since 2004; (6) The Icelandic Central Laboratory Database comprises laboratory test results from all major clinical laboratories in Iceland stored in a central database since 1999, including all blood tests for participants prior to participation and during follow-up in the study; (7) All medical records at LUH, the only tertiary care medical center in Iceland and the general acute care hospital for the vast majority of Icelandic residents. This includes clinical notes, anthropometric data, written radiology and pathology reports, microbiology and virology test results, and all other documented clinical data.

Biobanking

Blood samples drawn at each clinic visit are biobanked including cell-free plasma, serum, and plasma. Bone marrow samples are collected for biobanking in parallel to bone marrow sampling. Urine and blood in Blood-RNA tubes (PAXgene^{TM}) tubes and in mononuclear cell preparation tubes (BD Vacutainer® CPT^{TM}) are collected at sparser timepoints (Table 3). Samples are processed onsite and aliquoted at the study laboratory in Reykjavík, Iceland, and bone marrow samples separated into plasma and buffy coats. The bone marrow buffy coats from non-IgM MGUS and LC-MGUS are further separated into a plasma cell-enriched CD138+ fraction and a CD 138− fraction by Magnetic-activated cell sorting (MACS) using CD138 MicroBeads and an autoMACS pro cell separator (Miltenyi Biotec, Bergisch Gladbach, Germany). All cell fractions are cryopreserved and stored in liquid nitrogen. Other biobanking samples are frozen and stored in a secure state-of-the-art robotic biobanking facility in Reykjavík, Iceland, and cataloged using unique study identification numbers.

Study monitoring

A study monitor was appointed to review the study protocol and regularly assessed the conduction of the study

for compliance with relevant good clinical practice (GCP) principles. An independent data monitoring committee was established including two clinicians and a statistician that are not associated with the study. Interim analyses assessing safety and efficacy data are performed biannually. Additional interim analyses are scheduled when 500 subjects with MGUS have been followed for 6 months and when 100 participants with MGUS have died. When participants who have been randomized have been followed for five years, or if interim analysis shows a difference in the overall survival between arm 1 compared to arms 2 and 3, arm 1 will be discontinued. At that time the participants in arm 1 are unblinded to their MGUS status and offered a choice between randomization to arms 2 or 3, or clinical follow-up in the Icelandic healthcare system.

Study endpoints

The primary endpoint of the study is the overall survival of individuals with MGUS receiving follow-up (arms 2 and 3) compared to those not receiving any follow-up within the study (arm 1) after 5 years of follow-up. Secondary endpoints are cause-specific survival due to MM or other LPs, psychiatric health and well-being, and cost-effectiveness of screening. In addition, study data will be crosslinked to registries and samples in the biobank providing a large dataset for future studies.

Assuming that 3360 individuals with MGUS are identified and the hazard ratio (HR) for the primary outcome is 0.81 as previously described[32] the study has 77.2% power to reject the null hypothesis of HR = 1 at 5 years of follow-up and 89.3% power at 7 years of follow-up at an alpha level of 0.05.

Results

A pilot recruitment phase was started in Akranes (population 7411) in Western Iceland on September 15th, 2016, to ensure that informational materials and processes of recruitment functioned as planned. After minor adjustments, the whole-nation recruitment phase commenced on November 15th, 2016, and continued until February 20th, 2018.

A total of 148,704 individuals born in 1975 and earlier resided in Iceland when enrollment started, constituting the target population of the study. During the 15 months of recruitment, a total of 80,759 (54.3%) individuals provided informed consent for participation in the study (Fig. 3). Written informed consent was provided by 26% of participants while 74% provided informed consent electronically.

Of registered participants, 46% were male and 54% female constituting participation rates of 51% and 58%, respectively. Participation was highest (64%) among those between the ages of 60–79 but was lower (46%) in those between the ages of 40–49 and lowest (18%) among those over the age of 90 years old. The majority of participants (59%) were residents of the Reykjavik Capital Area with 18% and 23% of participants residing in other urban centers (more than 5000 inhabitants) and in rural areas, respectively. The participation rates were higher among those not residing in the Reykjavík Capital Area (60% versus 51% in the Reykjavík Capital Area; Table 4).

A total of 548 (0.7%) of participants had previously known LP before enrollment and were therefore excluded and 246 (0.3%) had previously known MGUS before enrollment. At the close of study enrollment on February 20th, 2018, a total of 190,382 hospital admissions since

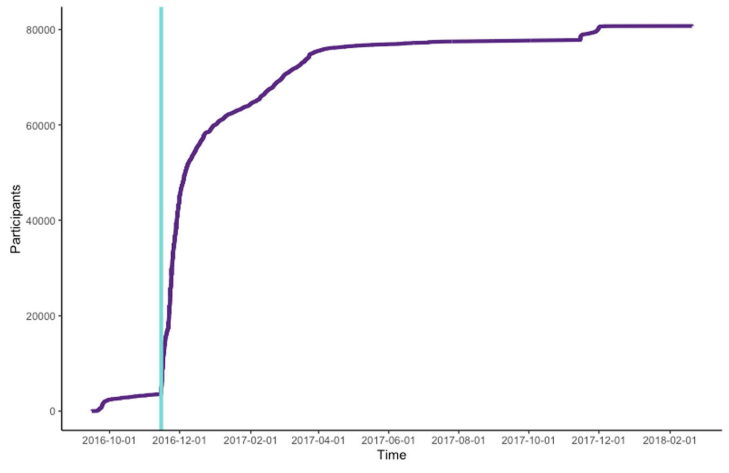


Fig. 3 Participant enrollment over the recruitment period. The light green line represents the end of the pilot period and the initiation of nationwide recruitment.

Table 4 The age, sex, and geographical distribution of participants and the target population, as well as available national registry data at the close of study recruitment.

	Registered participants	Target population
n	80,759	148,704
% females	54%	51%
median age[a]	59	57
Age range[a]	40–104	40–107
Participation rate		
All	54%	–
Males	51%	–
Females	58%	–
Age group (male/female)[a]		
40–49 (%)	21.2%/23.7%	27.4%/26.0%
50–59 (%)	27.7%/29.9%	29.4%/28.7%
60–69 (%)	28.4%/26.1%	23.4%/22.4%
70–79 (%)	16.6%/14.4%	12.9%/13.3%
80–89 (%)	5.7%/5.3%	6.0%/7.8%
>90 (%)	0.4%/0.5%	0.9%/1.8%
Place of residence		
Reykjavik Capital Area	58.7%	62.9%
Other urban centers[b]	17.5%	15.6%
Rural	23.3%	21.1%
Missing	0.6%	0.4%
Known MGUS[c]	246 (0.3%)	–
Previous LP[d]	548 (0.7%)	–
Data from registries[e]		
n hospital admissions	190,382	–
n primary care visits	8,187,805	–
n cancers diagnoses	10,328	–
n prescriptions	15,839,376	–

[a]Age at the time of study initiation on September 9th, 2016.
[b]Urban centers with >5000 inhabitants outside the Capital area.
[c]As registered before study enrollment in the Icelandic Cancer Registry since 1955, Icelandic Central Laboratory Database since 1999. and a registry of MGUS cases at Icelandic Private Clinics.
[d]As recorded before study enrollment in the Icelandic Cancer Registry since 1955.
[e]As recorded in national registries at the close of study enrollment on February 20th, 2018.

1999, 8,187,805 primary health care visits since 2004, 10,328 cancer diagnoses since 1955, and 15,839,376 medication prescriptions in the national registries.

Discussion

The iStopMM study is the first nationwide population-based, prospective screening study, and RCT among individuals with MGUS and the disorders it precedes. A total of 80,755 participants, 54.3% of the whole Icelandic population, born 1975 and earlier have enrolled in the iStopMM study. The high participation rate can be attributed to the extensive promotional effort undertaken in social and conventional media across Iceland where participation in scientific studies has historically been high[33–35]. In addition, using innovative solutions such as electronic informed consent and sampling parallel to clinical blood draws for screening, participants could easily sign-up and did not need to schedule a blood draw specifically for the study.

MGUS was first described as "benign gammopathy" by Dr. Jan Waldenström in 1960[36] and later defined as MGUS by Dr. Robert Kyle in 1978[37]. Since then, screening studies in Olmstead county[2] and the National Health and Nutrition Examination Survey in the US[38,39], in Ghana[40], and the PLCO-NCI Cancer Screening Trial[41] have fundamentally changed our understanding of MGUS and the disorders it precedes. These studies have provided important evidence directing the course of clinical and basic science in the field and guided the management of individuals with MGUS. The iStopMM study builds upon these studies with nationwide screening and detailed clinical assessment and follow-up of individuals with MGUS within an RCT. Through this design, the iStopMM study aims to evaluate the potential harms and benefits of population-based screening while also providing evidence for the optimal diagnostic approach and follow-up of individuals with MGUS.

Guidelines currently recommend screening for cancers of the breast, cervix, colon, lungs, and prostate[42]. Cancer screening is controversial due to the high number of individuals needed to be screened to improve clinical outcomes and the high level of false-positive results that may lead to overtreatment, a lower sense of wellbeing, and even psychiatric illness[43]. In fact, a diagnosis of active cancer, including MM, has been associated with psychiatric disorders[44] and suicide[45,46]. However, the role of screening in these outcomes is not known and such effects have not been shown to result from the diagnosis of pre-cancerous conditions like MGUS[47,48]. All participants of the iStopMM study are closely monitored for their psychiatric well-being using multiple psychometrically sound questionnaires. This will provide high-quality evidence on the potential psychological harms of MGUS screening that may have wider implications for cancer screening in general. Widely accepted criteria for when population-based disease screening is appropriate was developed by Wilson and Jungner in 1968[49] and recently expanded further[50]. As detailed in Table 5, most of these criteria are already filled by MM. However, there are still important questions that need to be answered, most notably whether the benefits of screening outweigh

Table 5 Application of the Wilson and Jungner criteria and the additional recently proposed emerging criteria to multiple myeloma.

Criteria	Applies to MM?	Comment
Original criteria[49]		
The condition sought should be an important health problem	Yes	MM is the second most common hematological malignancy with 31,810 new cases and 12,770 attributed deaths in 2018 in the United States alone[53]
There should be an accepted treatment with recognized disease	Yes	Treatment for MM is widely available and international organizations recommending specific care for MM[54]
Facilities for diagnosis and treatment should be available	Yes	This at least applies to developed countries
There should be a recognizable or early symptomatic stage	Yes	MGUS and SMM are clearly established entities[1] and precede all cases of MM[5,6]
There should be a suitable test or examination	Yes	SPEP, IFE, and FLC assays are sensitive and specific tests for MM and its precursors and can easily be repeated to confirm the diagnosis[55]
The test should be acceptable to the population	Yes	Screening is done by a blood test which is widely acceptable
The natural history of the condition, including development from latent to declared disease, should be adequately understood	Yes	Although there is still much to learn about the underlying pathogenesis of MM, a wealth of literature on the subject exists[56]. Furthermore, the natural history of MM and its development from precursor disorders is adequately understood with studies including decades of follow-up available[57]
There should be an agreed policy on whom to treat as patients	Yes	Although this is currently a moving target, there are clear guidelines on whom to treat, i.e., those with end-organ damage or myeloma defining events. In light of recent evidence, however, treatment might become available at even earlier stages[20,21,58]. If and when such early treatment is appropriate, there are institutions in place that will include such treatment in their guidelines
The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditures on medical care as a whole	Unknown	There are currently no screening studies available for MM and its precursor conditions and a cost-benefit analysis is not available. This will be addressed as part of the iStopMM study
Case finding should be a continuing process and not a "once and for all" project	Yes	Since blood sampling for screening can be carried out at any time MM screening can be a continuing process
Emerging screening criteria[50]		
The screening program should respond to a recognized need	Yes	Although survival in MM has dramatically improved in recent years[17–19] the disease remains a major burden on affected individuals and healthcare systems[59]
The objectives of screening should be defined at the outset	Yes	The objectives of screening for MM are clear: providing earlier treatment for MM
There should be a defined target population	Unknown	Currently, a well-defined target population for screening does not exist. This is addressed with regards to age, sex, and various other measures in the iStopMM study. However, due to the dominant white ethnicity of the Icelandic population, race cannot be addressed in the iStopMM study. Another study, the PROMISE study, focuses on the impact of screening in individuals of African descent. (ClinicalTrials.gov Identifier: NCT03689595)

Table 5 continued

Criteria	Applies to MM?	Comment
There should be scientific evidence of screening program effectiveness	Unknown	The objective of the iStopMM study is to provide this evidence
The program should integrate education, testing, clinical services, and program management	Yes	There are excellent patent resources available in MM and its precursor disorders. Any screening program would be able to fulfill this criterion
There should be quality assurance, with mechanisms to minimize potential risks of screening	Yes	This organizational issue can be solved in MM screening since there are clear response criteria[60] and accepted relevant endpoints like survival available for MM
The program should ensure informed choice, confidentiality, and respect for autonomy	Yes	This is a practical issue that does not require scientific proof of concept, although such proof is provided in the iStopMM trial
The program should promote equity and access to screening for the entire population	Yes	Since the cost of MM screening is relatively low and requires no specialized equipment at the point of patient care, equity in testing is therefore feasible. Follow-up for precursor disorders and treatment for MM can however be expensive and could lead to inequity in non-universal healthcare systems
Program evaluation should be planned from the outset	Yes	The practical issue of evaluation is possible for MM as proven by the methodology described above
The overall benefits of screening should outweigh the harm	Unknown	This is the principal study objective of the iStopMM study

the associated harms and costs. The results of the iStopMM study will provide answers to these outstanding questions on whether population-based screening is warranted in MM.

Current clinical consensus guidelines for MGUS are not based on RCT data but rather on observational studies and expert opinions[1,11–13]. By conducting an RCT of different follow-up strategies, the iStopMM study aims to provide high-quality evidence for the optimal follow-up in MGUS. This includes the role of clinical assessment, questionnaires on symptoms, imaging, blood, bone marrow, and urine sampling. In addition, for research purposes, these clinical parameters are crosslinked to past and future testing in the Universal Icelandic healthcare, as well as health-related endpoints such as all cancers and death. Furthermore, novel testing modalities like next-generation flow cytometry of plasma cells in the blood and bone marrow[51] and their microenvironment, mass spectrometry[52], and single-cell, and germline genetics will be utilized to investigate their role in clinical management and to gain insight into the pathogenesis of MGUS and the biological processes involved in its progression to more advanced disorders. This is even further supplemented by the study´s extensive biobank, which includes blood, bone marrow, and urine samples collected repeatedly over the study period that can be retrieved at a later date for all participants or for participants of particular interest. With this extensive dataset and biobank, the iStopMM results will generate one of the most complete datasets on MGUS to date, providing unique opportunities for future studies.

The iStopMM study has some limitations. Firstly, the study is performed in Iceland which has a highly genetically homogenous white population and generalization of the study findings in non-white populations is somewhat limited. Secondly, by offering early treatment the natural history of MGUS progression to MM is affected. The main ethical issue of the study is that participants in arm 1 are not made aware of their MGUS status. These participants will not gain the potential benefits of screening but will also not be exposed to the potential harms of screening including psychological harms. These participants will continue receiving care in the universal Icelandic healthcare system and may be diagnosed there. Importantly, participants with markers of advanced disease at screening are not randomized to arm 1. Arm 1 will also be followed closely in regular interim analyses and will be unblinded if shown to have inferior survival.

In conclusion, using a novel and innovative recruitment methodology, including electronic informed consent and sampling parallel to clinical blood draws, as well as social and conventional media campaigns, over 80,000 individuals, more than half of the eligible Icelandic population, have enrolled in the iStopMM study. By population-based screening, follow-up of individuals with MGUS within an RCT, and early treatment in MM, the iStopMM study will generate large datasets and sample collections that will impact our basic understanding of MGUS and the

disorders it precedes. Furthermore, it holds promise to fundamentally change the paradigm of MM treatment from late treatment in MM patients with end-organ damage to screening and early intervention, improving the overall survival and quality of life for patients worldwide.

Acknowledgements
The iStopMM study is funded by the Black Swan Research Initiative by the International Myeloma Foundation and the Icelandic Centre for Research (grant agreement No 173857). Furthermore, this project has received funding from the European Research Council (ERC) under the European Union's Horizon 2020 research and innovation programme (grant agreement No 716677). Screening tests are performed by The Binding Site Ltd. Birmingham, UK. Additional funding is provided by the University of Iceland, Landspítali University Hospital, and the Icelandic Cancer Society. Crosslinking of study data to national registries is performed by the Icelandic Directorate of Health and the Icelandic Cancer Society. The study is made possible by the hundreds of nurses, laboratory technicians, and physicians around Iceland who collect blood samples from participants for screening or during follow-up and provide clinical care that is not part of the study. Icelandic and International myeloma patient organizations including Perluvinir, the Icelandic myeloma patient organization, and the International Myeloma Foundation have provided the project with important perspectives and networks. The information technology department and clinical laboratory staff at LUH made the complex sampling processes required for the study possible. The staff at Loftfar, Aton, Miðlun and Hvíta Húsið played an integral part in the development of recruiting strategies and media campaigns. Decode genetics have generously provided the study team with important insights and access to their state-of-the-art facilities. Presidents Vigdís Finnbogadóttir and Guðni Th Jóhannesson are thanked for their public support of the study. The iStopMM team including Sigurlína H Steinarsdóttir, Hera Hallbera Björnsdóttir, Tinna Hallsdóttir, Ingibjörg Björnsdóttir, and Eva Brynjarsdóttir are particularly acknowledged for their hard and dedicated work. Special thanks go to the residents of Akranes, Iceland who participated in the pilot and proof-of-concept of the study's recruitment. Most importantly, acknowledgments go to the thousands of Icelanders who have generously provided their informed consent for the study, answered questionnaires, provided blood samples, and undergone diagnostic testing and follow-up.

Author details
[1]Faculty of Medicine, Univeristy of Iceland, Reykjavík, Iceland. [2]Dept of Hematology, Rigshospitalet, Copenhagen, Denmark. [3]Landspítali University Hospital, Reykjavík, Iceland. [4]Faculty of Psychology, University of Iceland, Reykjavik, Iceland. [5]Akureyri Hospital, Akureyri, Iceland. [6]Faculty of Economics, University of Iceland, Reykjavik, Iceland. [7]The Binding Site, Birmingham, West Midlands, UK. [8]Memorial Sloan Kettering Cancer Center, New York, NY, USA. [9]Cedar-Sinai Samual Oschin Cancer Center, Los Angeles, CA, USA. [10]Sylvester Comprehensive Cancer Center, University of Miami, Miami, FL, USA

Author contributions
The manuscript was written by S.R. and S.Y.K. with additional input from the other coauthors. The study concept was developed by S.Y.K., O.L., and S.H. All the coauthors contributed to the scientific and practical design of the iStopMM study.

Conflict of interest
P.K. is an employee of The Binding Site. BGMD has done consultancy for Amgen, Janssen, Celgene, Takeda. S.H. is the director of The Binding Site. O.L. has received research funding from: National Institutes of Health (NIH), National Cancer Institute (NCI), U.S. Food and Drug Administration (FDA), Multiple Myeloma Research Foundation (MMRF), International Myeloma Foundation (IMF), Leukemia and Lymphoma Society (LLS), Perelman Family Foundation, Rising Tide Foundation, Amgen, Celgene, Janssen, Takeda, Glenmark, Seattle Genetics, Karyopharm; Honoraria/ad boards: Adaptive, Amgen, Binding Site, BMS, Celgene, Cellectis, Glenmark, Janssen, Juno, Pfizer; and serves on Independent Data Monitoring Committees (IDMCs) for clinical trials lead by Takeda, Merck, Janssen, Theradex. S.Y.K. has received research funding from International Myeloma Foundation, European Research Council, Icelandic Center for Research (Rannís), Amgen, Celgene. The remaining authors declare no competing interests.

Publisher's note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41408-021-00480-w.

Received: 9 December 2020 Revised: 31 March 2021 Accepted: 13 April 2021
Published online: 17 May 2021

References

1. Kyle, R. A. et al. Monoclonal gammopathy of undetermined significance (MGUS) and smoldering (asymptomatic) multiple myeloma: IMWG consensus perspectives risk factors for progression and guidelines for monitoring and management. *Leukemia* **24**, 1121–1127 (2010).
2. Kyle, R. A. M. D. et al. Prevalence of monoclonal gammopathy of undetermined significance. *N. Engl. J. Med.* **354**, 1362–1369 (2006).
3. Kyle, R. A. et al. Long-term follow-up of IgM monoclonal gammopathy of undetermined significance. *Blood* **102**, 3759–3764 (2003).
4. Dispenzieri, A. et al. Prevalence and risk of progression of light-chain monoclonal gammopathy of undetermined significance: a retrospective population-based cohort study. *Lancet* **375**, 1721–1728 (2010).
5. Landgren, O. et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. *Blood* **113**, 5412–5417 (2009).
6. Weiss, B. M., Abadie, J., Verma, P., Howard, R. S. & Kuehl, W. M. A monoclonal gammopathy precedes multiple myeloma in most patients. *Blood* **113**, 5418–5422 (2009).
7. Weiss, B. M. et al. Monoclonal gammopathy of undetermined significance (MGUS) precedes the diagnosis of AL amyloidosis by up to 14 years. *Blood* **118**, 1827 (2011).
8. Rajkumar, S. V. et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol.* **15**, e538–e548 (2014).
9. Kyle, R. A. et al. Clinical course and prognosis of smoldering (asymptomatic) multiple myeloma. *N. Engl. J. Med.* **356**, 2582–2590 (2007).
10. Kyle, R. A. et al. Progression in smoldering Waldenström macroglobulinemia: long-term results. *Blood* **119**, 4462–4466 (2012).
11. van de Donk, N. W. C. J. et al. The clinical relevance and management of monoclonal gammopathy of undetermined significance and related disorders: Recommendations from the European Myeloma Network. *Haematologica* **99**, 984–996 (2014).
12. Bird, J. et al. UK myeloma forum (UKMF) and nordic myeloma study group (NMSG): Guidelines for the investigation of newly detected M-proteins and the management of monoclonal gammopathy of undetermined significance (MGUS). *Br. J. Haematol.* **147**, 22–42 (2009).
13. Berenson, J. R. et al. Monoclonal gammopathy of undetermined significance: a consensus statement: Guideline. *Br. J. Haematol.* **150**, 28–38 (2010).
14. Sigurdardottir, E. E. et al. The role of diagnosis and clinical follow-up of monoclonal gammopathy of undetermined significance on survival in multiple myeloma. *JAMA Oncol.* **1**, 168–174 (2015).
15. Go, R. S., Gundrum, J. D. & Neuner, J. M. Determining the clinical significance of monoclonal gammopathy of undetermined significance: a SEER-medicare population analysis. *Clin. Lymphoma Myeloma Leuk.* **15**, 117–186 (2015).
16. Goyal, G. et al. Impact of prior diagnosis of monoclonal gammopathy on outcomes in newly diagnosed multiple myeloma. *Leukemia* **33**, 1273–1277 (2019).
17. Thorsteinsdottir, S. et al. Dramatically improved survival in multiple myeloma patients in the recent decade: results from a Swedish population-based study. *Haematologica* **103**, e412–e415 (2018).
18. Kumar, S. K. et al. Improved survival in multiple myeloma and the impact of novel therapies. *Blood* **111**, 2516–2520 (2008).
19. Kristinsson, S. Y., Landgren, O., Dickman, P. W., Derolf, Å. R. & Björkholm, M. Patterns of survival in multiple myeloma: a population-based study of patients diagnosed in Sweden from 1973 to 2003. *J. Clin. Oncol.* **25**, 1993–1999 (2007).

20. Mateos, M.-V. et al. Lenalidomide plus dexamethasone for high-risk smoldering multiple myeloma. *N. Engl. J. Med.* **369**, 438–447 (2013).
21. Lonial, S. et al. Randomized trial of lenalidomide versus observation in smoldering multiple myeloma. *J. Clin. Oncol.* **38**, 1126–1137 (2019).
22. Landgren, C. O. et al. Daratumumab monotherapy for patients with intermediate-risk or high-risk smoldering multiple myeloma: a randomized, open-label, multicenter, phase 2 study (CENTAURUS). *Leukemia* **34**, 1840–1852 (2020).
23. Zhao, A. L. et al. Early or deferred treatment of smoldering multiple myeloma: a meta-analysis on randomized controlled studies. *Cancer Manag. Res.* **11**, 5599–5611 (2019).
24. Landgren, O. Shall we treat smoldering multiple myeloma in the near future? *Hematology* **1**, 194–204 (2017).
25. Kapoor, P. & Rajkumar, S. V. Smoldering multiple myeloma: to treat or not to treat. *Cancer J.* **25**, 65–71 (2019).
26. Castillo, J. J. et al. Recommendations for the diagnosis and initial evaluation of patients with Waldenström Macroglobulinaemia: a Task Force from the 8th International Workshop on Waldenström Macroglobulinaemia. *Br. J. Haematol.* **175**, 77–86 (2016).
27. WHO. *International Statistical Classification of Diseases and Related Health Problems*, 10th revision (ICD-10). (2016).
28. Spitzer, R. L., Kroenke, K., Williams, J. B. W. & Löwe, B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch. Intern. Med.* **166**, 1092–1097 (2006).
29. Martin, A., Rief, W., Klaiberg, A. & Braehler, E. Validity of the brief patient health questionnaire mood scale (PHQ-9) in the general population. *Gen. Hosp. Psychiatry* **28**, 71–77 (2006).
30. Diener, E., Emmons, R. A., Larsem, R. J. & Griffin, S. The satisfaction with life scale. *J. Pers. Assess.* **49**, 71–75 (1985).
31. Sigurdardottir, L. G. et al. Data quality at the Icelandic Cancer Registry: comparability, validity, timeliness and completeness. *Acta Oncol.* **51**, 880–889 (2012).
32. Kristinsson, S. Y. et al. Patterns of survival and causes of death following a diagnosis of monoclonal gammopathy of undetermined significance: a population-based study. *Haematologica* **94**, 1714–1720 (2009).
33. Harris, T. B. et al. Age, gene/environment susceptibility-reykjavik study: multidisciplinary applied phenomics. *Am. J. Epidemiol.* **165**, 1076–1087 (2007).
34. Gudbjartsson, D. F. et al. Large-scale whole-genome sequencing of the Icelandic population. *Nat. Genet.* **47**, 435–444 (2015).
35. Jónsson, H. et al. Whole genome characterization of sequence diversity of 15,220 Icelanders. *Sci. Data* **21**, 170115 (2017).
36. Waldenström, J. Studies on conditions associated with disturbed gamma globulin formation (gammopathies). *Harvey Lect.* **56**, 211–231 (1960).
37. Kyle, R. A. Monoclonal gammopathy of undetermined significance. Natural history in 241 cases. *Am. J. Med.* **64**, 814–826 (1978).
38. Landgren, O. et al. Prevalence of myeloma precursor state monoclonal gammopathy of undetermined significance in 12372 individuals 10-49 years old: a population-based study from the National Health and Nutrition Examination Survey. *Blood Cancer J* **7**, e618 (2017).
39. Landgren, O. et al. Racial disparities in the prevalence of monoclonal gammopathies: a population-based study of 12 482 persons from the national health and nutritional examination survey. *Leukemia* **28**, 1537–1542 (2014).
40. Landgren, O. et al. Prevalence of monoclonal gammopathy of undetermined significance among men in Ghana. *Mayo Clin. Proc.* **82**, 1468–1473 (2007).
41. Landgren, O. et al. Association of immune marker changes with progression of monoclonal gammopathy of undetermined significance to multiple myeloma. *JAMA Oncol.* **5**, 1293–1301 (2019).
42. Smith, R. A. et al. Cancer screening in the United States, 2019: a review of current American Cancer Society guidelines and current issues in cancer screening. *CA Cancer J. Clin.* **69**, 184–210 (2019).
43. Adami, H. O., Kalager, M., Valdimarsdottir, U., Bretthauer, M. & Ioannidis, J. P. A. Time to abandon early detection cancer screening. *Eur. J. Clin. Invest.* **49**, e13062 (2019).
44. Lu, D. et al. Clinical diagnosis of mental disorders immediately before and after cancer diagnosis: a nationwide matched cohort study in Sweden. *JAMA Oncol.* **2**, 1188–1196 (2016).
45. Fang, F. et al. Suicide and cardiovascular death after a cancer diagnosis. *N. Engl. J. Med.* **366**, 1310–1318 (2012).
46. Hultcrantz, M. et al. Incidence and risk factors for suicide and attempted suicide following a diagnosis of hematological malignancy. *Cancer Med.* **4**, 147–154 (2015).
47. Korfage, I. J. et al. How distressing is referral to colposcopy in cervical cancer screening? *Gynecol. Oncol.* **132**, 142–148 (2014).
48. Taghizadeh, N. et al. Health-related quality of life and anxiety in the PAN-CAN lung cancer screening cohort. *BMJ Open* **9**, 1–10 (2019).
49. Wilson, J. M. G. & Jungner, G. World Health Organization. *Principles and Practice of Screening for Disease*. (World Health Organization, 1968).
50. Andermann, A., Blancquaert, I., Beauchamp, S. & Déry, V. Revisiting Wilson and Jungner in the genomic age: a review of screening criteria over the past 40 years. *Bull. World Health Organ.* https://doi.org/10.2471/BLT.07.050112 (2008).
51. Flores-Montero, J. et al. Immunophenotype of normal vs. myeloma plasma cells: Toward antibody panel specifications for MRD detection in multiple myeloma. *Cytom. Part B* **90**, 61–78 (2016).
52. Mills, J. R., Barnidge, D. R. & Murray, D. L. Detecting monoclonal immunoglobulins in human serum using mass spectrometry. *Methods* **81**, 56–65 (2015).
53. Siegel, R. L., Miller, K. D. & Jemal, A. Cancer statistics, 2018. *CA Cancer J. Clin.* **68**, 7–30 (2018).
54. Ludwig, H. et al. International Myeloma Working Group recommendations for global myeloma care. *Leukemia* **28**, 981–992 (2014).
55. Bradwell, A. R. et al. Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. *Clin. Chem.* **47**, 673–680 (2001).
56. Anderson, K. C. & Carrasco, R. D. Pathogenesis of myeloma. *Annu. Rev. Pathol. Mech. Dis.* **6**, 249–274 (2011).
57. Kyle, R. A. et al. Long-term follow-up of monoclonal gammopathy of undetermined significance. *N. Engl. J. Med.* **378**, 241–249 (2018).
58. Korde, N. et al. Treatment with carfilzomib-lenalidomide-dexamethasone with lenalidomide extension in patients with smoldering or newly diagnosed multiple myeloma. *JAMA Oncol.* https://doi.org/10.1001/jamaoncol.2015.2010 (2015).
59. Cowan, A. J. et al. Global burden of multiple myeloma: a systematic analysis for the global burden of disease study 2016. *JAMA Oncol.* **4**, 1221–1227 (2018).
60. Kumar, S. et al. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. *Lancet Oncol.* **17**, e328–e346 (2016).

Paper IV

Blood Cancer Journal www.nature.com/bcj

ARTICLE OPEN

Check for updates

Monoclonal gammopathy of undetermined significance and COVID-19: a population-based cohort study

Saemundur Rognvaldsson[1,2,9], Elias Eythorsson[2,9], Sigrun Thorsteinsdottir[1,3], Brynjar Vidarsson[2], Pall Torfi Onundarson[1,2], Bjarni A. Agnarsson[1,2], Margret Sigurdardottir[2], Ingunn Thorsteinsdóttir[2], Isleifur Olafsson[1,2], Hrafnhildur L. Runolfsdottir[2], Dadi Helgason[2], Arna R. Emilsdottir[2], Arnar S. Agustsson[1,2], Aron H. Bjornsson[1,2], Gudrun Kristjansdottir[2], Asdis Rosa Thordardottir[1], Olafur Skuli Indridason[1,2], Asbjorn Jonsson[4], Gauti Kjartan Gislason[1], Andri Olafsson[1], Hlif Steingrimsdottir[2], Petros Kampanis[5], Malin Hultcrantz[6], Brian G. M. Durie[7], Stephen Harding[5], Ola Landgren[8], Runolfur Palsson[1,2], Thorvarður Jon Love[1,2] and Sigurdur Yngvi Kristinsson[1,2]✉

Multiple myeloma (MM) patients have increased risk of severe coronavirus disease 2019 (COVID-19) when infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Monoclonal gammopathy of undetermined significance (MGUS), the precursor of MM has been associated with immune dysfunction which may lead to severe COVID-19. No systematic data have been published on COVID-19 in individuals with MGUS. We conducted a large population-based cohort study evaluating the risk of SARS-CoV-2 infection and severe COVID-19 among individuals with MGUS. We included 75,422 Icelanders born before 1976, who had been screened for MGUS in the Iceland Screens Treats or Prevents Multiple Myeloma study (iStopMM). Data on SARS-CoV-2 testing and COVID-19 severity were acquired from the Icelandic COVID-19 Study Group. Using a test-negative study design, we included 32,047 iStopMM participants who had been tested for SARS-CoV-2, of whom 1754 had MGUS. Among these participants, 1100 participants, tested positive, 65 of whom had MGUS. Severe COVID-19 developed in 230 participants, including 16 with MGUS. MGUS was not associated with SARS-CoV-2 infection (Odds ratio (OR): 1.05; 95% confidence interval (CI): 0.81–1.36; $p = 0.72$) or severe COVID-19 (OR: 0.99; 95%CI: 0.52–1.91; $p = 0.99$). These findings indicate that MGUS does not affect the susceptibility to SARS-CoV-2 or the severity of COVID-19.

Blood Cancer Journal (2021)11:191 ; https://doi.org/10.1038/s41408-021-00580-7

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first detected in Wuhan, China in 2019 [1], and has since developed into a global pandemic. The clinical presentation of the associated coronavirus disease 19 (COVID-19) varies from mild disease to multi-organ failure and death [2]. Risk factors for severe COVID-19 have been identified, including age, male sex, and several comorbidities including cancer [3].

Patients with multiple myeloma (MM), a malignancy of bone marrow plasma cells, are at a particularly high risk of developing severe illness when infected by SARS-CoV-2 [4–7]. Potential pathobiological mechanisms have been suggested, including immunosuppressive therapy, inherent suppression and dysregulation of humoral and cellular immunity, and MM-associated kidney disease. Currently, high disease burden, and severe hypogammaglobulinemia have been associated with increased risk for severe COVID-19 in MM patients, whilst treatement-related factors have not [4–7].

The precursor condition of MM, monoclonal gammopathy of undetermined significance (MGUS) [8, 9] is characterized by the presence of monoclonal immunoglobulins (M proteins) or free light chains (FLC) in the serum without MM-defining clinical or biological markers [10]. MGUS is common, affecting 4.2% of the general population over 50 years of age [11, 12]. MGUS has been associated with a similar but milder inherent immune dysfunction as MM, including significant defects in both humoral and cellular immunity [13] and relatively high rates of hypogammaglobulinemia (25%) [11, 14]. Furthermore, MGUS has been associated with a two-fold risk of bacteremia and almost three-fold risk of viral infections [15]. MGUS has also been associated with thrombosis [16] and kidney disease [17], both of which are features of and risk factors for severe COVID-19 [3]. Therefore, it has been speculated that individuals with MGUS might have an increased risk of SARS-CoV-2 infection and severe COVID-19 [18]. In a recently reported small ($n = 7$) case series of individuals with MGUS who were infected with SARS-CoV-2, five required hospitalization and one died [19]. However, no systematic data on MGUS and COVID-19 have been published to date.

MGUS is usually asymptomatic and is most often diagnosed incidentally during evaluation of unrelated medical problems. This

[1]Faculty of Medicine, University of Iceland, Reykjavik, Iceland. [2]Landspítali–The National University Hospital of Iceland, Reykjavik, Iceland. [3]Rigshospitalet, Copenhagen, Denmark. [4]Akureyri Hospital, Akureyri, Iceland. [5]The Binding Site ltd, Birmingham, UK. [6]Memorial Sloan-Kettering Cancer Center, New York City, NY, USA. [7]Cedar-Sinai Samual Oschin Cancer Center, Los Angeles, CA, USA. [8]Sylvester Cancer Center, University of Miami, Miami, FL, USA. [9]These authors contributed equally: Saemundur Rognvaldsson, Elias Eythorsson. ✉email: sigyngvi@hi.is

Received: 3 September 2021 Revised: 1 November 2021 Accepted: 11 November 2021
Published online: 01 December 2021

leads to a biased selection of individuals with other comorbidities that may generate false associations between MGUS and various diseases. In fact, a previous study of a screened MGUS cohort in the US could not confirm many of the disease associations found in clinical cohorts [20], highlighting the need to use screened MGUS cohorts to assess the association of MGUS with other diseases, including COVID-19.

Here, we report the first study of COVID-19 in individuals with MGUS using data from the ongoing Iceland Screens Treats or Prevents Multiple Myeloma study (iStopMM), which has already screened 75,422 Icelanders for MGUS. The objective of the study was to evaluate the risk of SARS-CoV-2 infection and severe COVID-19 in individuals with MGUS. Based on the previous literature we hypothesized that MGUS might increase the risk of SARS-CoV-2 infection and severe COVID-19.

METHODS

Ethical approval

The iStopMM study, data collection for Icelandic patients with SARS-CoV-2 infection, and crosslinking of healthcare data has been approved by the Icelandic National Bioethics Committee (VSN 16-022, date: 2016-04-26; VSN 20-078, date: 2021-05-26) with additional approval from the Icelandic Data Protection Agency. The iStopMM study has been registered on ClinicalTrials.gov (ClinicaTrials.gov identifier: NCT03327597).

COVID-19 in Iceland

The first case of SARS-CoV-2 infection in Iceland was diagnosed on February 28, 2020. The Icelandic authorities implemented an aggressive testing strategy early in the pandemic that included targeted testing based on clinical suspicion, open invitation population screening, and random screening for SARS-CoV-2 among asymptomatic persons [21]. As the pandemic continued, random screening was discontinued, while self-ordered testing with same-day results became available to all and double screening of individuals in quarantine and persons arriving at the border was initiated. All SARS-CoV-2 testing was done by real-time quantitative polymerase chain reaction (qPCR) of simultaneously acquired oropharyngeal and nasopharyngeal swabs. Through this approach, Iceland has consistently ranked among the nations with the highest level of testing in the world. In total, 6,126 individuals were found to be SARS-CoV-2-positive by qPCR in Iceland between February 28 and December 31, 2020 [22].

All SARS-CoV-2-positive individuals were centrally registered and immediately contacted by the COVID-19 Outpatient Clinic at Landspitali–The National University Hospital at the time of diagnosis. If the diagnostic sample was obtained during asymptomatic screening, a repeat qPCR test of a nasopharyngeal sample and a blood test for SARS-CoV-2-antibodies were performed within 24 h. All persons who were considered to have an active SARS-CoV-2 infection were isolated and enrolled into telehealth monitoring at the COVID-19 Outpatient Clinic. Monitoring consisted of serial telephone interviews conducted by either a nurse or physician using a standardized data entry form. Patients reporting concerning symptoms were evaluated at the clinic and admitted to the hospital if needed. Patients were monitored for at least 14 days after their first positive qPCR and until they had been asymptomatic for at least seven days. This comprehensive systematic approach of combined community and clinical care has previously been described in detail [23].

Study cohort

The study cohort was comprised of participants in the iStopMM study who had been screened for MGUS before December 31, 2020 and were alive and had not been diagnosed with MM and related disorders, including smoldering MM requiring treatment, before February 28, 2020. iStopMM is a population-based screening study for MGUS and randomized trial of follow-up strategies in Iceland. All Icelanders born in 1975 ($n = 148{,}704$) and earlier were invited to participate and 80,759 (54%) accepted and provided informed consent between September 2016 and February 2018. Serum samples were collected from 75,422 study participants (93%) between September 9, 2016 and December 31 2020 and screened for MGUS by capillary zone electrophoresis (CZE), immunofixation electrophoresis (IFE), and serum FLC assay. The iStopMM study design has previously been described in detail [24].

Study design and statistical analysis

The primary exposure was MGUS as determined by M protein detectable on CZE and confirmed by IFE or an abnormal FLC ratio (kappa/lambda ratio <0.26 and lambda >26.3 g/L or a kappa/lambda ratio >1.65 and kappa >19.4 g/L). Those with MGUS were further subdivided into heavy chain-MGUS (HC-MGUS) and light-chain MGUS (LC-MGUS) subgroups. All analyses were carried out separately for MGUS, HC-MGUS, and LC-MGUS. When included as a covariate, age was modeled with a four-knot restricted cubic spline.

In the first analysis, we evaluated whether there was an association between MGUS and testing positive for SARS-CoV-2 using a test-negative study design. Participants from the study cohort who had been tested at least once for SARS-CoV-2 between February 28 and December 31, 2020, were included. Those who had at least one positive qPCR test for SARS-CoV-2 were considered to be infected. The association of MGUS and SARS-CoV-2 infection was evaluated using logistic regression adjusting for sex and age.

In the second analysis, we evaluated the association between MGUS and severe COVID-19. Participants from the previous analysis who tested positive for SARS-CoV-2 were included. Those who were hospitalized for other medical problems or were living in a nursing home at the time of testing were excluded. Participants were followed until discharge from telehealth monitoring or until they had developed severe COVID-19. Severe COVID-19 was defined as the composite outcome of requiring an emergency outpatient visit, requiring hospital admission, or death (emergency outpatient visit or worse). Additionally, we conducted an analysis where severe COVID-19 was defined as hospital admission or death (hospital admission or worse). The association of MGUS with severe COVID-19 was assessed using logistic regression, adjusting for sex and age.

Two sensitivity analyses were performed. In the first sensitivity analysis, we evaluated the association of MGUS and SARS-CoV-2 testing in the whole study cohort using logistic regression, adjusting for sex and age. In the second sensitivity analysis, we repeated the first analysis and included the entire study cohort regardless of whether the participants had been tested for SARS-CoV-2 or not.

All analyses were carried out in R, version 3.6.3, using the *rms* package [25]. The code used for this study and its output have been published online at https://osf.io/kfdg9/.

RESULTS

Of the 75,422 participants who had been screened for MGUS, 1,854 had died and 693 had been diagnosed with MM and related disorders before the study period. A total of 32,047 participants, of whom 1,754 (5.5%) had MGUS, had been tested for SARS-CoV-2 (Fig. 1). Those who had MGUS were older (mean age 66.3 vs 59.1 years, $p < 0.001$) and more likely to be male (50% vs 41%, $p < 0.001$) than those who did not have MGUS. Of those tested, 1,100 (3.4%) were positive for SARS-CoV-2, including 65 who had MGUS. After adjusting for sex and age, MGUS was not found to be associated with SARS-CoV-2 infection (odds ratio (OR): 1.05; 95% confidence interval (CI): 0.81–1.36; $p = 0.72$). The findings were similar for HC- and LC-MGUS (Table 1 and Fig. 2). Sensitivity analysis that included the whole study chort showed essentially the same results (Supplemental Table). There was no significant association between MGUS and the rate of SARS-CoV-2 testing (Supplemental Table).

Among 1100 persons who tested positive for SARS-CoV-2, 40 were hospitalized for other medical problems or were residing in a nursing home at the time of SARS-CoV-2 testing (Fig. 1). Of the remaining 1060 cases, 56 had MGUS. During follow-up, 16 (29%) individuals with MGUS and 214 (21%) without the disorder developed severe COVID-19. We did not find MGUS to be associated with severe COVID-19 when defined as emergency outpatient visit or worse (OR: 0.99; 95%CI: 0.52–1.91; $p = 0.99$) or as hospital admission or worse, (OR: 1.13; 95%CI: 0.52–2.46; $p = 0.76$). The findings were similar for HC- and LC-MGUS (Table 2 and Fig. 3).

DISCUSSION

This large nationwide cohort study is the first population-based study evaluating COVID-19 susceptibility and severity among

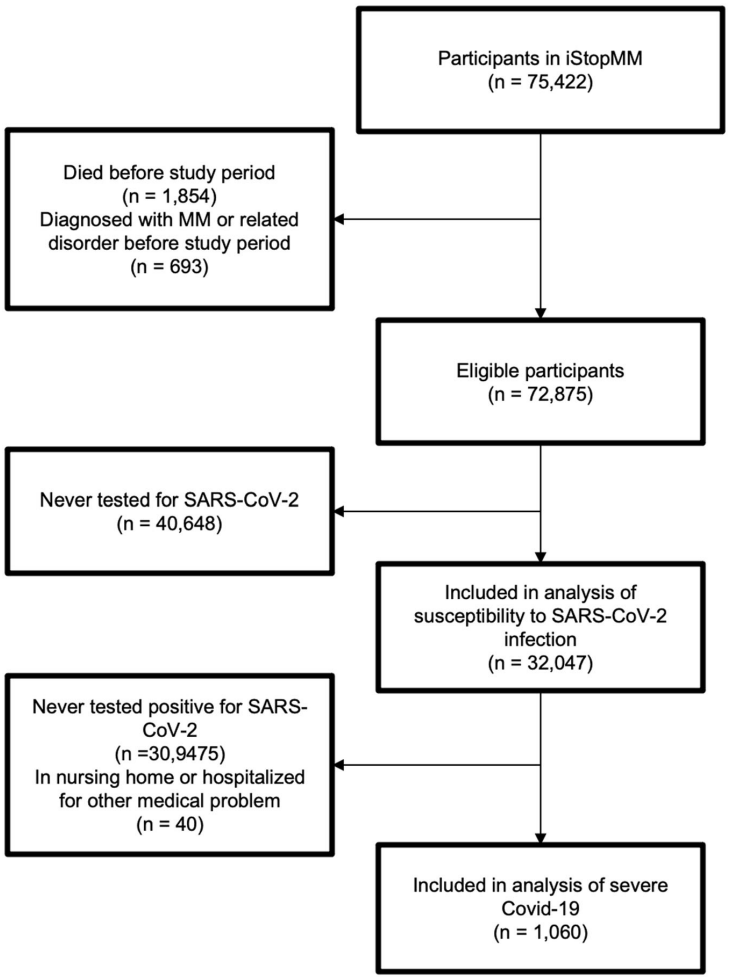


Fig. 1 Participant selection. Flowchart demonstrating the inclusion and exclusion of participants.

Table 1. Baseline characteristics of study participants tested for SARS-CoV-2 and the association between MGUS and SARS-CoV-2 infection.

	No MGUS	MGUS	HC-MGUS	LC-MGUS
n	30,293	1754	1140	614
Mean age (SD)	59 (10)	66 (11)	65.9 (11)	66.9 (11)
Men	12,283 (41%)	880 (50%)	571 (50%)	309 (50%)
Person-years	24,882	1431	–	–
SARS-CoV-2-positive	1035 (3.4%)	65 (3.7%)	41 (3.6%)	24 (3.9%)
OR of SARS-CoV-2 positivity (95% CI)[a]	Ref	1.05 (0.80-1.36)	1.02 (0.74-1.40)	1.11 (0.73-1.69)

MGUS monoclonal gammopathy of undetermined significance, *HC-MGUS* heavy chain MGUS, *LC-MGUS* light chain MGUS, *SD* standard deviation, COVID-19 coronavirus disease 2019, *OR* odds ratio, *CI* confidence interval, *Ref* reference.
[a] Adjusted for age and sex.

persons with MGUS. Although previous authors have speculated that MGUS may impact the risk of SARS-CoV-2 infection and especially the risk of severe COVID-19 [18], we did not find MGUS to be associated with contracting SARS-CoV-2 or developing severe COVID-19 once infected.

MGUS was not associated with an increased risk of SARS-CoV-2 infection. This contradicts previous studies that have found individuals with MGUS to have an increased risk of infections, including viral infections [15]. This might indicate that this increased risk of viral infections does not apply to COVID-19.

However, these studies were based on individuals with incidentally diagnosed MGUS, which may have biased the sample to include individuals with more comorbid conditions. By contrast, our study is based on a cohort of persons who were screened for MGUS and underwent a high rate of SARS-CoV-2 testing (42% being tested at least once) and no difference in the frequency of testing between those with and without MGUS. This minimizes the possibility of detection bias due to MGUS in this study.

Individuals with MGUS did not have an increased risk of severe COVID-19 compared to those without MGUS. This is in contrast to MM, which has been associated with severe COVID-19 [4–7]. Severe COVID-19 is believed to be caused by immune dysregulation and hyperactivation [26]. Disease factors rather than treatment factors in MM have been associated with severe COVID-19 and it has been speculated that immune dysregulation inherent in MM increases the risk of severe COVID-19 [4–7]. It is therefore unexpected that we did not find an increased risk of severe COVID-19 in individuals with MGUS, who have been shown to have similar, although milder, dysregulation of humoral and cellular immune function [13]. These findings indicate that more severe immune dysregulation, as seen in MM, contributes to the risk of severe COVID-19 or that the effects of treatment factors have been underestimated in previous studies.

This study has several strengths. Firstly, the entire study population was screened for MGUS, thereby eliminating the selection bias present in most other MGUS cohorts where disorder is primarily diagnosed in those who have other medical problems and are therefore likely to have a greater burden of comorbid conditions. Furthermore, the non-MGUS group had been tested for MGUS removing potential false negatives from that study group. Secondly, the comprehensive and aggressive SARS-CoV-2 testing strategy employed in Iceland has yielded a high case capture rate compared to other nations. Thirdly, data on diagnosis and follow-up of COVID-19 cases, which has been collected by the same clinical team, are virtually complete, and centrally registered for the whole nation. Finally, the study included a large number of persons who represent a significant proportion of the nation's

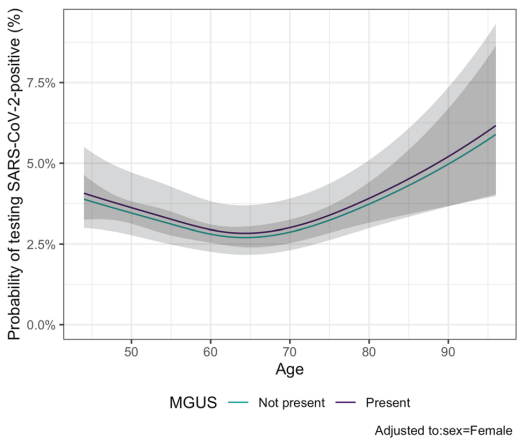


Fig. 2 Probability of testing SARS-CoV-2 positive. Probability of individuals with or without MGUS of testing positive for SARS-COV-2 according to age, adjusted to female sex.

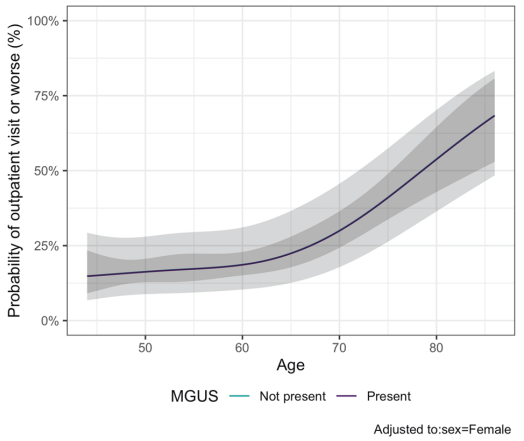


Fig. 3 Probability of severe COVID-19. Probability of individuals with or without MGUS having severe COVID-19 according to age, adjusted to female sex. Severe COVID-19 is defined as the composite outcome of emergency outpatient visits, hospital admission, or death.

Table 2. Baseline characteristics of SARS-CoV-2-positive participants and the association between MGUS and severity of COVID-19.

	No MGUS	MGUS	HC-MGUS	LC-MGUS
n	1004	56	35	21
Mean age (SD)	59 (10)	65 (11)	65 (12)	65 (10)
Men	448 (45%)	30 (54%)	22 (63%)	8 (38%)
Person-days	16,589	962	–	–
Emergency outpatient visit	176 (18%)	12 (20%)	7 (20%)	5 (24%)
Hospital admission	105 (11%)	11 (20%)	8 (23%)	3 (14%)
Intensive care unit admission	20 (2%)	3 (5%)	2 (6%)	1 (5%)
Death	5 (1%)	0 (0%)	0 (0%)	0 (0%)
Emergency outpatient visit or worse	214 (21%)	16 (29%)	10 (29%)	6 (29%)
OR (95% CI)[a]	Ref	0.99 (0.52–1.91)	0.90 (0.39–2.08)	1.10 (0.39–3.10)
Hospital admission or worse	106 (11%)	11 (20%)	8 (23%)	3 (14%)
OR (95% CI)[a]	Ref	1.13 (0.52–2.46)	1.25 (0.49–3.19)	0.83 (0.21–3.29)

MGUS monoclonal gammopathy of undetermined significance, *HC-MGUS* heavy chain MGUS, *LC-MGUS* light chain MGUS, *SD* standard deviation, *COVID-19* Coronavirus disease 2019, *OR* odds ratio, *CI* confidence interval, *Ref* reference.
[a] Adjusted for age and sex.

population making the findings likely to be generalizable to similar populations.

This study also has limitations. Firstly, we included individuals based on blood testing alone which does not completely exclude more advanced diseases than MGUS. However, those who had MM or related disease, including smoldering MM in treatment, were excluded. Secondly, despite being the largest study to date with more than 75,422 participants, the number of events was relatively low, and therefore, the study might be underpowered to detect a modest increase in risk. Thirdly, due to the relative scarcity of participants with MGUS and SARS-CoV-2 infection and the low mortality from COVID-19 in Iceland, hard endpoints were too rare for this analysis requiring the use of composite outcomes. Finally, the Icelandic population is highly genetically homogenous and mostly white, limiting generalization in non-white populations.

In conclusion, in this large population-based cohort study including 75,422 individuals screened for MGUS, we did not find MGUS to be a risk factor for contracting SARS-CoV-2 or developing severe COVID-19. These findings are important since MGUS is the most common precursor condition of hematological malignancy, affecting millions of individuals worldwide [11, 12]. The findings provide guidance for how physicians should counsel their patients with MGUS about their risks during the COVID-19 pandemic.

REFERENCES

1. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020;395:497–506. https://doi.org/10.1016/S0140-6736(20)30183-5.
2. Guan W-J, Ni Z-Y, Hu Y, Liang W-H, Ou C-Q, He J-X, et al. Clinical characteristics of coronavirus disease 2019 in China. N. Engl J Med. 2020;382:1708–20. https://doi.org/10.1056/nejmoa2002032.
3. Chen R, Liang W, Jiang M, Guan W, Zhan C, Wang T, et al. Risk factors of fatal outcome in hospitalized subjects with coronavirus disease 2019 from a nationwide analysis in China. Chest. 2020;158:97–105. https://doi.org/10.1016/j.chest.2020.04.010.
4. Hultcrantz M, Richter J, Rosenbaum CA, Patel D, Smith EL, Korde N, et al. COVID-19 infections and clinical outcomes in patients with multiple myeloma in New York City: A Cohort Study from Five Academic Centers. Blood Cancer Discov. 2020;1:234–43. https://doi.org/10.1158/2643-3230.bcd-20-0102.
5. Wang B, Van Oekelen O, Mouhieddine TH, Del Valle DM, Richter J, Cho, HJ et al. A tertiary center experience of multiple myeloma patients with COVID-19: lessons learned and the path forward. J Hematol Oncol. 2020:13. https://doi.org/10.1186/s13045-020-00934-x.
6. Chari A, Samur MK, Martinez-Lopez J, Cook G, Biran N, Yong K, et al. Clinical features associated with COVID-19 outcome in multiple myeloma: first results from the International Myeloma Society data set. Blood. 2020;136:3033–40. https://doi.org/10.1182/blood.2020008150.
7. Martínez-López J, Mateos M-V, Encinas C, Sureda A, Hernández-Rivas JÁ, Lopez De La Guía, A et al. Multiple myeloma and SARS-CoV-2 infection: clinical characteristics and prognostic factors of inpatient mortality. Blood Cancer J. 2020:10. https://doi.org/10.1038/s41408-020-00372-5.
8. Landgren O, Kyle RA, Pfeiffer RM, Katzmann JA, Caporaso NE, Hayes RB, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. Blood. 2009;113:5412–5417. https://doi.org/10.1182/blood-2008-12-194241.
9. Weiss BM, Abadie J, Verma P, Howard RS, Kuehl WM. A monoclonal gammopathy precedes multiple myeloma in most patients. Blood. 2009;113:5418–22. https://doi.org/10.1182/blood-2008-12-195008.
10. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos M-V, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15:e538–e548. https://doi.org/10.1016/s1470-2045(14)70442-5.
11. Kyle RA, Therneau TM, Rajkumar SV, Larson DR, Plevak MF, Offord JR, et al. Prevalence of monoclonal gammopathy of undetermined significance. N. Engl J Med. 2006;354:1362–1369. https://doi.org/10.1056/nejmoa054494.
12. Dispenzieri A, Katzmann JA, Kyle RA, Larson DR, Melton LJ 3rd, Colby CL, et al. Prevalence and risk of progression of light-chain monoclonal gammopathy of undetermined significance: a retrospective population-based cohort study. Lancet. 2010;375:1721–1728. https://doi.org/10.1016/s0140-6736(10)60482-5.
13. Tete SM, Bijl M, Sahota SS & Bos NA. Immune defects in the risk of infection and response to vaccination in monoclonal gammopathy of undetermined significance and multiple myeloma. Frontiers Immunol. 2014:5. https://doi.org/10.3389/fimmu.2014.00257.
14. Gregersen H, Madsen KM, Sørensen HT, Schønheyder HC, Ibsen JS, Dahlerup JF. The risk of bacteremia in patients with monoclonal gammopathy of undetermined significance. Eur J Haematol. 2009;61:140–144. https://doi.org/10.1111/j.1600-0609.1998.tb01075.x.
15. Kristinsson SY, Tang M, Pfeiffer RM, Bjorkholm M, Goldin LR, Blimark C, et al. Monoclonal gammopathy of undetermined significance and risk of infections: a population-based study. Haematologica. 2012;97:854–858. https://doi.org/10.3324/haematol.2011.054015.
16. Kristinsson SY, Pfeiffer RM, Björkholm M, Goldin LR, Schulman S, Blimark C, et al. Arterial and venous thrombosis in monoclonal gammopathy of undetermined significance and multiple myeloma: a population-based study. Blood. 2010;115:4991–4998. https://doi.org/10.1182/blood-2009-11-252072.
17. Leung N, Bridoux F, Hutchison CA, Nasr SH, Cockwell P, Fermand J-P, et al. Monoclonal gammopathy of renal significance: when MGUS is no longer undetermined or insignificant. Blood. 2012;120:4292–4295. https://doi.org/10.1182/blood-2012-07-445304.
18. Jain A, Ramasamy K. Potential 'significance' of monoclonal gammopathy of 'undetermined significance' during COVID-19 pandemic. Blood Cells Mol Dis. 2020;85:102481–102481. https://doi.org/10.1016/j.bcmd.2020.102481.
19. Gonzalez-Lugo JD, Bachier-Rodriguez L, Goldfinger M, Shastri A, Sica RA, Gritsman K et al. A case series of monoclonal gammopathy of undetermined significance and COVID-19. Br J Haematol. 2020:190. https://doi.org/10.1111/bjh.16906.
20. Bida JP, Kyle RA, Therneau TM, Melton LJ, Plevak MF, Larson DR, et al. Disease associations with monoclonal gammopathy of undetermined significance: a population-based study of 17,398 patients. Mayo Clin Proc. 2009;84:685–93. https://doi.org/10.4065/84.8.685.
21. Gudbjartsson DF, Helgason A, Jonsson H, Magnusson OT, Melsted P, Norddahl GL, et al. Spread of SARS-CoV-2 in the Icelandic Population. N. Engl J Med. 2020;382:2302–15. https://doi.org/10.1056/nejmoa2006100.
22. Icelandic Directorate of Health. *COVID-19 in Iceland - Statistics*, https://www.covid.is/data.
23. Helgason D, Eythorsson E, Olafsdottir LB, Agustsson T, Ingvarsdottir S, Sverrisdottir S et al. Beating the odds with systematic individualized care: nationwide prospective follow-up of all patients with COVID-19 in Iceland. J Internal Med. 2020. https://doi.org/10.1111/joim.13135.
24. Rögnvaldsson S, Löve TJ, Thorsteinsdottir S, Reed ER, Óskarsson JÞ, Pétursdóttir Í, et al. Iceland screens, treats, or prevents multiple myeloma (iStopMM): a population-based screening study for monoclonal gammopathy of undetermined significance and randomized controlled trial of follow-up strategies. Blood Cancer J. 2021;11:94. https://doi.org/10.1038/s41408-021-00480-w.
25. Harrell FE Jr Regression modeling strategies: with applications to linear models, logistic and ordinal regression, and survival analysis. Springer; 2015.
26. Yao C, Bora SA, Parimon T, Zaman T, Friedman OA, Palatinus JA et al. Cell-type-specific immune dysregulation in severely Ill COVID-19 patients. Cell Rep. 2021:34, https://doi.org/10.1016/j.celrep.2020.108590.

ACKNOWLEDGEMENTS

The iStopMM study is funded by the Black Swan Research Initiative by the International Myeloma Foundation, the European Research Council, and the Icelandic Center for Research. Screening tests were performed by The Binding Site Ltd, Birmingham, UK. Additional funding for the iStopMM study is provided by the University of Iceland, Landspítali—The National University Hospital of Iceland, and the Icelandic Cancer Society. The iStopMM study is made possible the nurses, data scientists, and other staff of the study as well as by the hundreds of nurses and laboratory technicians who had collected blood samples for the study. Most importantly, our gratitude goes to the thousands of Icelanders who have generously provided their informed consent for the study, answered questionnaires, provided blood samples, and undergone diagnostic testing and follow-up.

AUTHOR CONTRIBUTIONS

S.R. and S.Y.K. wrote the manuscript with input from E.E. and R.P. The study was designed by S.R. and E.E. under the supervision of S.Y.K. and R.P. E.E. acquired and crosslinked data and performed statistical analysis. S.R., E.E., S.T., B.V., P.T.O., B.A.A., M. S., I.T., I.O., A.J., P.K., A.R.T., G.K., G.K.G., A.O., H.S., M.H., B.G.M.D., O.L., T.J.L., R.P., O.S.I., S.H. and S.Y.K. contributed significantly to the design and implementation of the iStopMM study. E.E., H.L.R., D.H., A.R.E., A.S.A., A.H.B., O.S.I. and R.P. contributed significantly to the collection of data on COVID-19 that was curated by E.E. All authors reviewed and edited the manuscript and approved the final manuscript. S.R. and E.E. had an equal contribution to the study and are both considered first authors of the paper.

COMPETING INTERESTS

P.K. is an employee of The Binding Site. M.H. has received research funding from Amgen and Daiichi Sankyo, has provided consultancy for Intellisphere LLC and Curio Science LLC, and served on an advisory committee for GlaxoSmithKline. B.G.M.D. has provided consultancy for Amgen, Janssen, Celgene, Takeda. S.H. is the director of The Binding Site. O.L. has received research funding from National Institutes of Health (NIH), National Cancer Institute (NCI), U.S. Food and Drug Administration (FDA), Multiple Myeloma Research Foundation (MMRF), International Myeloma Foundation (IMF), Leukemia and Lymphoma Society (LLS), Perelman Family Foundation, Rising Tide Foundation, Amgen, Celgene, Janssen, Takeda, Glenmark, Seattle Genetics, Karyopharm; and received honorariacompensation for advisory boards from Adaptive, Amgen, Binding Site, BMS, Celgene, Cellectis, Glenmark, Janssen, Juno, Pfizer; and serves on Independent Data Monitoring Committees (IDMCs) for clinical trials lead by Takeda, Merck, Janssen, Theradex. S.Y.K. has received research funding from International Myeloma Foundation, European Research Council, Icelandic Center for Research (Rannís), Amgen, Celgene. Other coauthors have nothing to disclose.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41408-021-00580-7.

Correspondence and requests for materials should be addressed to Sigurdur Yngvi Kristinsson.

Appendix

	MGUS (N=8052)	MGUS+PN (N=597)	Control (N=30423)	Control+PN (N=849)
AIDS	11 (0.1%)	0 (0%)	6 (0%)	0 (0%)
Cerebrovascular disease	820 (10.2%)	74 (12.4%)	2543 (8.4%)	126 (14.8%)
Heart failure	728 (9.0%)	48 (8.0%)	1502 (4.9%)	70 (8.2%)
Chronic liver disease	79 (1.0%)	6 (1.0%)	64 (0.2%)	4 (0.5%)
Chronic lung disease	800 (9.9%)	66 (11.1%)	1918 (6.3%)	87 (10.2%)
Connective tissue disease	498 (6.2%)	30 (5.0%)	646 (2.1%)	28 (3.3%)
Dementia	150 (1.9%)	7 (1.2%)	485 (1.6%)	13 (1.5%)
Diabetes mellitus	202 (2.5%)	53 (8.9%)	567 (1.9%)	104 (12.2%)
Diabetes mellitus with end-organ disease	289 (3.6%)	80 (13.4%)	726 (2.4%)	163 (19.2%)
Hemiplegia or paraplegia	33 (0.4%)	8 (1.3%)	99 (0.3%)	7 (0.8%)
Myocardial infarction	649 (8.1%)	54 (9.0%)	2198 (7.2%)	103 (12.1%)
Moderate to severe liver disease	24 (0.3%)	2 (0.3%)	14 (0.0%)	1 (0.1%)
Moderate to severe kidney disease	421 (5.2%)	43 (7.2%)	290 (1.0%)	18 (2.1%)
Peptic ulcer disease	314 (3.9%)	25 (4.2%)	716 (2.4%)	30 (3.5%)
Peripheral vascular disease	223 (2.8%)	23 (3.9%)	523 (1.7%)	24 (2.8%)
Neoplasm	1219 (15.1%)	85 (14.2%)	3586 (11.8%)	123 (14.5%)
Malignant neoplasm	15 (0.2%)	0 (0%)	34 (0.1%)	1 (0.1%)
Previous admission ≥4 days in preceding 2 years	2191 (27.2%)	194 (32.5%)	4128 (13.6%)	216 (25.4%)

MGUS: Monoclonal gammopathy of undetermined significance (MGUS); PN: Peripheral neuropathy; AIDS: Acquired immunodeficiency syndrome.

Supplemental table 1: The rate of the comorbidity classes as defined in the Charlson comorbidity index and the rate of previous admissions lasting more than four days in the two years preceding inclusion.

	Any fracture		***Peripheral fracture***		***Axial fracture***	
	HR (95% CI)	*p*	*HR (95% CI)*	*p*	*HR (95% CI)*	*p*
Adjusted for Charlson comorbidity index comorbidities						
All						
MGUS	1.25 (1.18-1.33)	<0.001	1.16 (1.06-1.28)	0.002	1.32 (1.22-1.42)	<0.001
PN	1.33 (1.14-1.55)	<0.001	1.34 (1.04-1.71)	0.02	1.32 (1.08-1.61)	0.007
Males						
MGUS	1.33 (1.21-1.47)	<0.001	1.21 (1.03-1.42)	0.02	1.42 (1.26-1.6)	<0.001
PN	1.37 (1.1-1.71)	0.005	1.31 (0.9-1.92)	0.16	1.37 (1.04-1.8)	0.023
Females						
MGUS	1.2 (1.11-1.29)	<0.001	1.14 (1.01-1.28)	0.03	1.24 (1.12-1.37)	<0.001
PN	1.32 (1.05-1.66)	0.02	1.36 (0.97-1.92)	0.08	1.29 (0.96-1.75)	0.1
Adjusted for admission lasting ≥ 4 days within 2 years						
All						
MGUS	1.26 (1.18-1.34)	<0.001	1.18 (1.08-1.3)	<0.001	1.32 (1.22-1.42)	<0.001
PN	1.34 (1.15-1.55)	<0.001	1.38 (1.09-1.75)	0.01	1.33 (1.1-1.62)	0.004
Males						
MGUS	1.36 (1.24-1.5)	<0.001	1.23 (1.05-1.45)	0.01	1.45 (1.29-1.63)	<0.001
PN	1.37 (1.11-1.69)	0.003	1.4 (0.99-2)	0.06	1.32 (1.02-1.72)	0.04
Females						
MGUS	1.19 (1.1-1.28)	<0.001	1.15 (1.02-1.29)	0.02	1.22 (1.11-1.36)	<0.001
PN	1.34 (1.09-1.66)	0.006	1.41 (1.03-1.92)	0.03	1.35 (1.02-1.79)	0.04

MGUS: Monoclonal gammopathy of undetermined significance; PN: Peripheral neuropathy; HR: Hazard ratio; 95% CI: 95% Confidence interval.

Supplemental table 2: A table with the estimated hazard ratios (HR) for fractures in those with monoclonal gammopathy of undetermined significance (MGUS) and peripheral neuropathy (PN) after adjusting for comorbidities in two separate sensitivity analyses.