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MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE: NATURAL COURSE AND COMORBIDITIES

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Monoclonal Gammopathy of Undetermined Significance: Natural Course and Comorbidities
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To Reinhold and Åsa.

ABSTRACT

Monoclonal gammopathy of undetermined significance (MGUS) is a plasma cell disorder characterized by an overproduction of monoclonal immunoglobulins. MGUS is asymptomatic but clinically relevant since annually 0.5-1.5% of individuals with MGUS will develop multiple myeloma (MM) or another malignant lymphoproliferative disease. Individuals with MGUS are followed for signs of progression, however, so far this management strategy has never been evaluated. Results from previous studies have shown that individuals with MGUS have inferior survival and increased risk of thrombosis compared to individuals without MGUS, yet all studies to date have been performed on clinically established cohorts of MGUS patients, introducing a high risk of selection bias. Recently, a new entity called light-chain MGUS (LC-MGUS) has been identified. Very little is known about the epidemiology and clinical course of LC-MGUS.

In order to establish a clinically informative, correct, and easily applicable definition of LC-MGUS, and describe the prevalence of MGUS and LC-MGUS in the population, we performed a large population-based screening study. We screened more than 11,000 individuals from the Icelandic AGES-Reykjavik Study cohort and the American PLCO Study cohort. The prevalence of MGUS was 4.8-5.2%. Based on findings from the two cohorts and on statistical analysis of normal distributions, we propose a revised definition of LC-MGUS; (1) an abnormal free light-chain ratio (<0.26 or >1.65), (2) an elevated involved light chain concentration (40 mg/L or higher), (3) no M-protein on serum protein electrophoresis or immunofixation, and (4) no evidence of end-organ damage that can be attributed to a lymphoproliferative disorder. The prevalence of LC-MGUS in our study using this definition was 0.9-1.0%. The prevalence of LC-MGUS increased with age (p<0.001), was higher in men (p<0.001), and more common among blacks (2.9%) than whites (0.7%) or Asian/Pacific Islanders (0.2%). The revised definition of LC-MGUS captures the condition in fewer but clinically relevant individuals.

We conducted three population-based studies with the purpose of studying the natural course and survival of individuals with MGUS and LC-MGUS. We used the Icelandic AGES-Reykjavik Study cohort of 5,764 individuals, including 300 individuals with MGUS and 52 individuals with LC-MGUS, as well as a Swedish cohort of 18,768 MGUS patients. Through the Swedish Cancer Register we identified all patients with MM diagnosed from 1976 to 2013, as well as randomly sampled population-based controls. Individuals with MGUS had a 1.2-fold (95% confidence interval (CI) 1.04-1.4) and individuals with LC-MGUS had a 1.6-fold (1.2-2.3) increased risk of death compared to individuals without MGUS, during a median follow-up time of almost ten years. The risk remained increased after progression to lymphoproliferative disease was taken into account. We found a personal history of autoimmune disease to increase the risk of death significantly in both individuals with MM (hazard ratio (HR) = 1.2, 1.2-1.3) and individuals with MGUS (HR = 1.4, 1.3-1.4). These findings could be due to an underlying genetic susceptibility for both plasma cell disorders and other conditions, such as autoimmune disease, or to the overproduction of light chains causing organ damage. We found that MM patients with prior knowledge of MGUS had a better overall survival (median survival 2.8 years) than MM patients without prior knowledge of MGUS (median survival 2.1 years). Among MM patients with a prior knowledge of MGUS, a low M-protein concentration at MGUS diagnosis was predictive of worse survival in MM (HR = 1.9, 1.1-3.0), possibly due to patients with low M-protein concentration being followed less frequently. Our findings support the recommendations of regular clinical follow-up of individuals with MGUS, regardless of M-protein concentration.

In further analysis of the AGES-Reykjavik Study cohort, we assessed the causes of death and risk of thrombosis among individuals with MGUS and LC-MGUS and found an increased risk of death from cancer (HR = 1.8, 1.6-2.3) and from heart disease (HR = 1.4, 1.1-1.8), adjusted for age and sex. We found that a history of thrombosis was more common in individuals with LC-MGUS (25%) than individuals with MGUS (10%) or without MGUS (12%), and that individuals with LC-MGUS had an increased risk of a history of arterial thrombosis especially (crude odds ratio (OR) = 2.5, 95% CI 1.3-4.9), compared to individuals without MGUS. During a median follow-up time of almost nine years, we detected an almost two-fold risk of arterial thrombosis in individuals with LC-MGUS compared to individuals without MGUS (crude HR = 1.9, 1.1-3.2). No increased risk of venous thrombosis was detected in individuals with MGUS or LC-MGUS. Our results suggest that previously detected increased risks of thrombosis in MGUS have been due to confounding factors. Our findings on LC-MGUS point towards an elevated risk of arterial, but not venous, thrombosis.

In future investigations, we suggest attention is focused on characterizing the clinical, genetic, and biochemical profiles of LC-MGUS, with the purpose of understanding the connection to cancer, to heart disease, and to thrombosis.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Kristinsson SY, **Lindqvist EK**, Lund SH, Costello R, Burton D, Korde N, Hoffman JN, Purdue MP, Mailankody S, Steingrimsdottir H, Murata K, Björkholm M, Eiriksdottir G, Launer LJ, Harris TB, Hultcrantz M, Gudnason V, Landgren O. *Light-chain monoclonal gammopathy of undetermined significance: a new definition.* Manuscript 2016.

II. **Lindqvist EK**, Lund SH, Costello R, Burton D, Korde N, Mailankody S, Björkholm M, Gudnason V, Eiriksdottir G, Launer LJ, Harris TB, Hultcrantz M, Landgren O, Kristinsson SY. *The increased risk of dying in individuals with monoclonal gammopathy of undetermined significance is caused by malignant progression and heart disease.* Manuscript 2016.

III. **Lindqvist EK**, Landgren O, Lund SH, Turesson I, Hultcrantz M, Goldin L, Björkholm M, Kristinsson SY. *History of autoimmune disease is associated with impaired survival in multiple myeloma and monoclonal gammopathy of undetermined significance: a population-based study.* Ann Hematol. 2016 Nov 2. [Epub ahead of print]

IV. Sigurdardottir EE, Turesson I, Lund SH, **Lindqvist EK**, Mailankody S, Korde N, Björkholm M, Landgren O, Kristinsson SY. *The role of diagnosis and clinical follow-up of monoclonal gammopathy of undetermined significance (MGUS) on survival in multiple myeloma.* JAMA Oncol. 2015;1(2):168-174.

V. **Lindqvist EK**, Lund SH, Costello R, Burton D, Korde N, Mailankody S, Björkholm M, Gudnason V, Eiriksdottir G, Launer LJ, Harris TB, Hultcrantz M, Landgren O, Kristinsson SY. *Monoclonal gammopathy of undetermined significance and risk of arterial and venous thrombosis: results from a population-based study.* Manuscript 2016.

CONTENTS

LIST OF ABBREVIATIONS

AGES	Age, Gene/Environment Susceptibility study
CI	Confidence interval
FLC	Free light chain
HR	Hazard ratio
IFE	Immunofixation electrophoresis
Ig	Immunoglobulin
LC-MGUS	Light-chain monoclonal gammopathy of undetermined significance
MGUS	Monoclonal gammopathy of undetermined significance
MM	Multiple myeloma
M-protein	Monoclonal protein
OR	Odds ratio
PLCO	Prostate, Lung, Colon and Ovarian Cancer study
SPEP	Serum protein electrophoresis

1 INTRODUCTION

1.1 LYMPHOPROLIFERATIVE DISORDERS

All cells of the blood are derived from common hematopoietic progenitor cells that reside mainly in the bone marrow and give rise to lymphoid, myeloid, erythroid, monocyte, and megakaryocyte lineages.[1-3] The cell designated for the lymphoid pathway will eventually develop into a B lymphocyte, a T lymphocyte, an NK-cell or a dendritic cell. Lymphoproliferative disorders such as chronic lymphocytic leukemia and lymphoma are cancers of the blood and the lymph nodes, derived from cells in different stages of the lymphoid pathway, and a majority of these malignancies stem from B lymphocytes.[4,5]

B lymphocytes from the bone marrow differentiate into plasma cells that produce immunoglobulins, commonly known as antibodies, and are the basis of the humoral immune system.[1,2,6,7] Immunoglobulins consist of two heavy polypeptide chains of either gamma, alpha, mu, delta, or epsilon type, and two light polypeptide chains of either kappa or lambda type (Figure 1).[7,8]
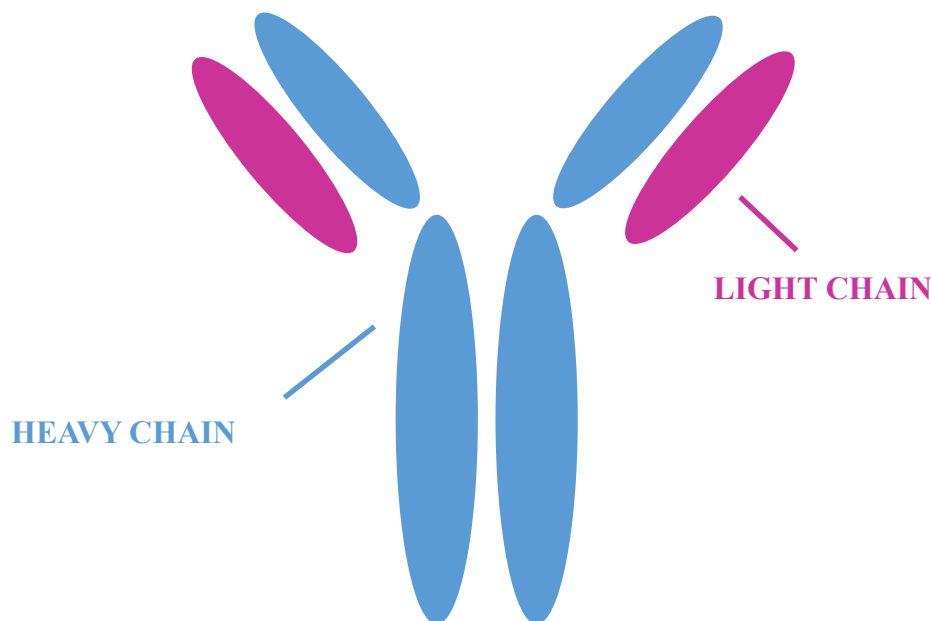
Figure 1. Basic structure of an immunoglobulin (antibody) with heavy chains (blue) and light chains (purple).

Under normal circumstances, a large diversity of plasma cells are present in the blood, secreting a variety of immunoglobulins – polyclonal secretion.[9] In plasma cell disorders, a clonal proliferation of plasma cells leads to overproduction of a single clone of immunoglobulins – so called monoclonal immunoglobulins.[4,10] The monoclonal overload in the blood can be detected as a monoclonal band, known as an M-protein, on serum protein electrophoresis (SPEP).[4] The clinical manifestations of different plasma cell disorders are due to the expansion of neoplastic cells in the bone marrow, in the blood, or in other lymphatic or non-lymphatic organs, and to the secretion of immunoglobulins. In Bence Jones proteinuria, monoclonal light chains are secreted in the urine and can be detected by urine protein electrophoresis or urine immunofixation.[11,12]

1.2 MULTIPLE MYELOMA

1.2.1 Definition and epidemiology

Multiple myeloma (MM) is the most common lymphoproliferative disorder after non-Hodgkin lymphoma, and accounts for about 10% of all hematological malignancies.[13,14] Worldwide incidence rates vary from 0.7 to 3.3 per 100,000 person-years.[13,15,16] MM is characterized by a monoclonal proliferation of plasma cells in the bone marrow (Figure 2), M-protein in the blood or urine, and end-organ damage such as anemia.[4,5,10] Common clinical manifestations among patients include fatigue and bone pain, due to underlying anemia and osteolytic lesions.[17,18] MM is a disease of the elderly, with a median age at diagnosis of approximately 70 years.[19,20] It is more common in African-Americans than in Caucasians, and men are more frequently affected than women.[15,21,22]
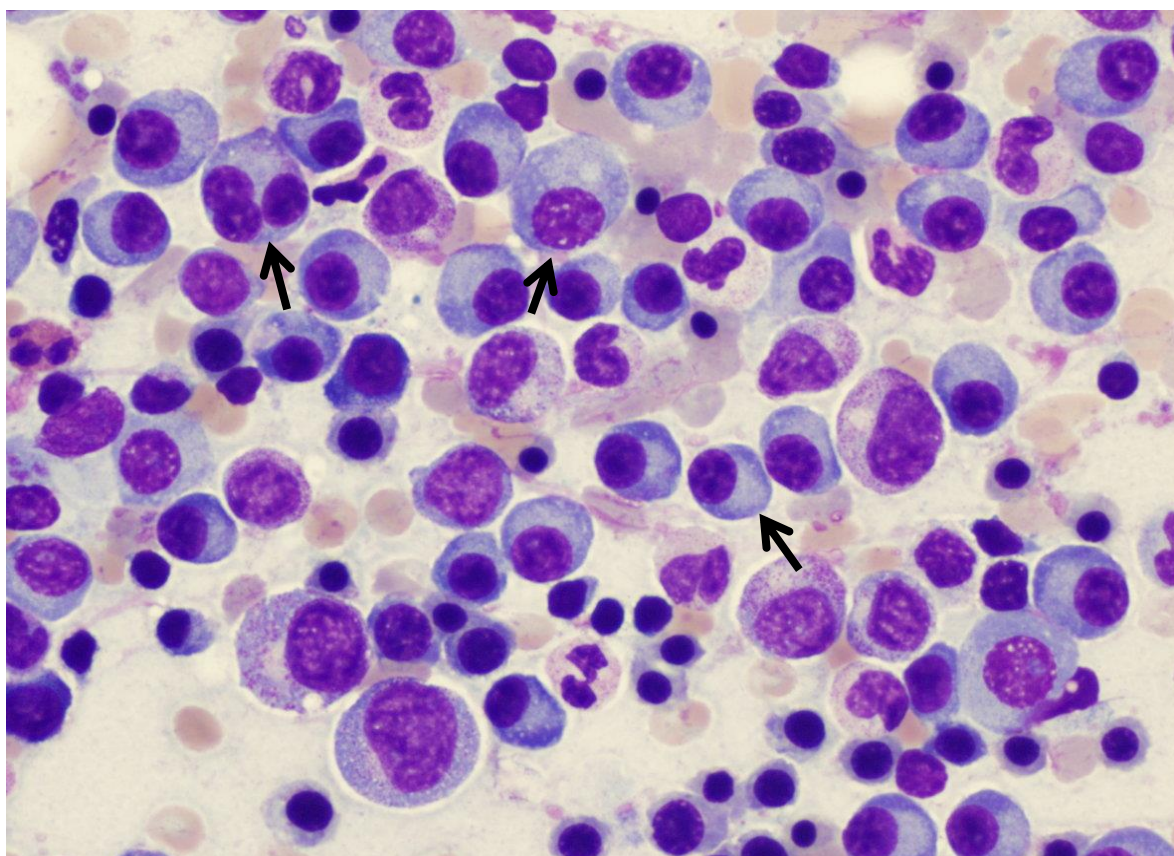
Figure 2. A picture of the bone marrow from a patient with multiple myeloma. The bone marrow is infiltrated with plasma cells/plasma blasts of different maturation (arrows).

The International Myeloma Working group diagnostic criteria for MM have recently been updated and now include at least 10% of clonal plasma cells in the bone marrow, presence of a myeloma defining event, or specific biomarkers (Table 1). An early stage of MM is smoldering MM, when the patient has no myeloma defining events, but a serum M-protein

of ≥30 g/L or urinary M-protein ≥500 mg per 24 hours, and/or clonal bone marrow plasma cells between 10 and 60%.[17]

Table 1. Diagnostic criteria for multiple myeloma[17]

1) Clonal bone marrow plasma cells ≥10% *and*	Or biopsy-proven bony or extramedullary plasmacytoma
2) Myeloma defining events: evidence of end organ damage such as:	Hypercalcemia
	Renal insufficiency
and	Anemia
	Bone lesions
3) Biomarkers of malignancy:	Clonal bone marrow plasma cells ≥60%
	Involved:uninvolved serum free light chain ratio ≥100
	>1 focal lesions on MRI studies[a]

[a]MRI: magnetic resonance imaging

1.2.2 Etiology and pathogenesis

The etiology of MM is largely unknown. However, there is evidence for a role of genetic factors, such as studies showing familial aggregation of MM,[23-28] racial disparities in incidence,[21,22] and germline single-nucleotide polymorphisms that have been associated with an increased risk of developing MM.[29] Both autoimmune disease and infectious disease have been linked to an increased risk of developing MM, suggesting chronic or powerful immune system stimulation could play a role.[24,30-32] Furthermore, dietary factors have been investigated, and the consumption of fish and seafood has been shown to decrease the risk of MM, suggesting that environmental factors also play a part in the etiology.[33]

The development of MM is an intricate process involving genetic changes in the plasma cell as well as in the bone marrow microenvironment.[34] Early hits in myelomagenesis include either chromosome 14 (*IGH* locus) translocations or hyperdiploidy, which are observed at a very early stage.[35] Cytogenetic aberrations are evaluated in routine clinical practice for risk stratification, see below.[36] Furthermore, recent sequencing studies have revealed a complex genetic landscape in MM including somatic mutations in *KRAS, NRS, FAM46C, BRAF, TP53, TRAF3, DIS3, CYLD,* and more.[37-39] Recently, a large genome-wide association study confirmed nine previously known risk loci and discovered eight new loci.[40] Epigenetic abnormalities, including deregulation of methylation pathways, have also been shown to contribute to the initiation or the progression of MM.[41]

MM is consistently preceded by a precursor condition called monoclonal gammopathy of undetermined significance (MGUS), see below.[42,43]

1.2.3 Treatment and prognosis

The cornerstone of MM treatment was for a long time melphalan-prednisone; a combination of an alkylating agent and a corticosteroid leading to a median overall survival of between two and four years.[44-46] In the last fifteen years, the treatment arsenal has been expanded with novel agents, such as proteasome inhibitors, immunomodulatory agents such as thalidomide, lenalidomide, and pomalidomide, interferons, monoclonal antibodies, histone deacetylase inhibitors, and kinesin spindle protein inhibitors, as well as with stem cell transplantation.[47-52] As a result, survival in MM has improved significantly, and median overall survival is now more than six years.[53-56] In 2015, three new agents for multiple myeloma were approved by the US Food and Drug Administration; ixazomib, daratumumab, and elotuzumab, and treatment strategies are moving away from chemotherapy and towards novel agents only.[57]

A number of prognostic factors have been identified, such as type and concentration of the M-protein, C-reactive protein, albumin, β2-microglobulin, serum free light chains (FLC), and cytogenetic abnormalities.[58-62] The Revised International Staging System is a prognostic model for newly diagnosed MM patients, and takes into account albumin, β2-microglobulin, lactate dehydrogenase, and chromosomal abnormalities as detected by interphase fluorescent in situ hybridization, such as deletion(17p), translocation(4;14), and translocation(14;16).[63] Furthermore, it is well established that a higher comorbidity score, as measured by Freiburg Comorbidity Index, the Charlson Comorbidity Index, or the Hematopoietic Cell Transplant Comorbidity Index, predicts an inferior survival in patients with MM and is an independent risk factor for death even when the Revised International Staging System is taken into account.[64-69]

1.2.4 Thrombosis

The association between cancer and venous thrombosis is well established, leading to a higher mortality.[70-74] It has been suggested that neoplastic cells, possibly because of tumor cell hypoxia, activate the coagulation system by stimulating the production of various procoagulants and angiogenic factors such as tissue factor. Tumor cells as well as chemotherapeutic agents can injure endothelial cells which also activates the coagulation system.[71,75,76] Consequently, primary thromboprophylaxis with low molecular weight heparin is recommended to some, but not all, cancer patients.[72,73]

After the introduction of novel treatment agents for MM, an increased risk of venous thrombosis was observed, primarily in patients treated with immunomodulatory drugs.[77-80] Consequently, thromboprophylaxis with aspirin, low molecular weight heparin, or warfarin is recommended for MM patients who receive treatment regiments based on immunomodulatory drugs together with steroids.[80] It was initially believed that the increased risk of venous thrombosis in MM was solely a complication of the treatment. However, findings of an increased risk of venous thrombosis in the precursor state MGUS, and in MM patients before the introduction of novel agents, suggest that the plasma cell disorder in itself is also associated with an increased risk of thrombosis.[81,82] Furthermore, MM is associated with an elevated risk of arterial thrombosis, although this risk appears to be smaller than the

risk of venous thrombosis and has only been examined in two previous studies.[82,83] The increased risk of thrombosis in MM patients persists up to ten years after MM diagnosis, and has been demonstrated to affect survival negatively.[82,83]

Risk factors for venous thrombosis in MM include advanced age, obesity, personal or family history of venous thrombosis, presence of central venous catheter, trauma, surgery, immobility, use of tamoxifen or hormone replacement, thrombophilia, and comorbidities such as cardiac disease, renal impairment, or autoimmune disease.[80] More active disease and higher levels of M-protein have been linked to a stronger procoagulant state.[84,85] A variety of factors seem to be involved in venous thrombosis in MM, among them increased blood viscosity and inflammatory cytokines.[86] It appears that, similar to patients with other malignancies, patients with MM display changes in their coagulation status such as longer prothrombin times, and higher levels of D-dimer, factor VIII, tissue factor, fibrinogen, and von Willenbrand factor.[85,87]

1.3 MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE

1.3.1 Definition

Monoclonal gammpathy of undetermined significance (MGUS) is a condition where there is an M-protein on SPEP without evidence of MM, amyloidosis, Waldenström macroglobulinemia, or other lymphoproliferative disorder (Figure 3).[5,17]
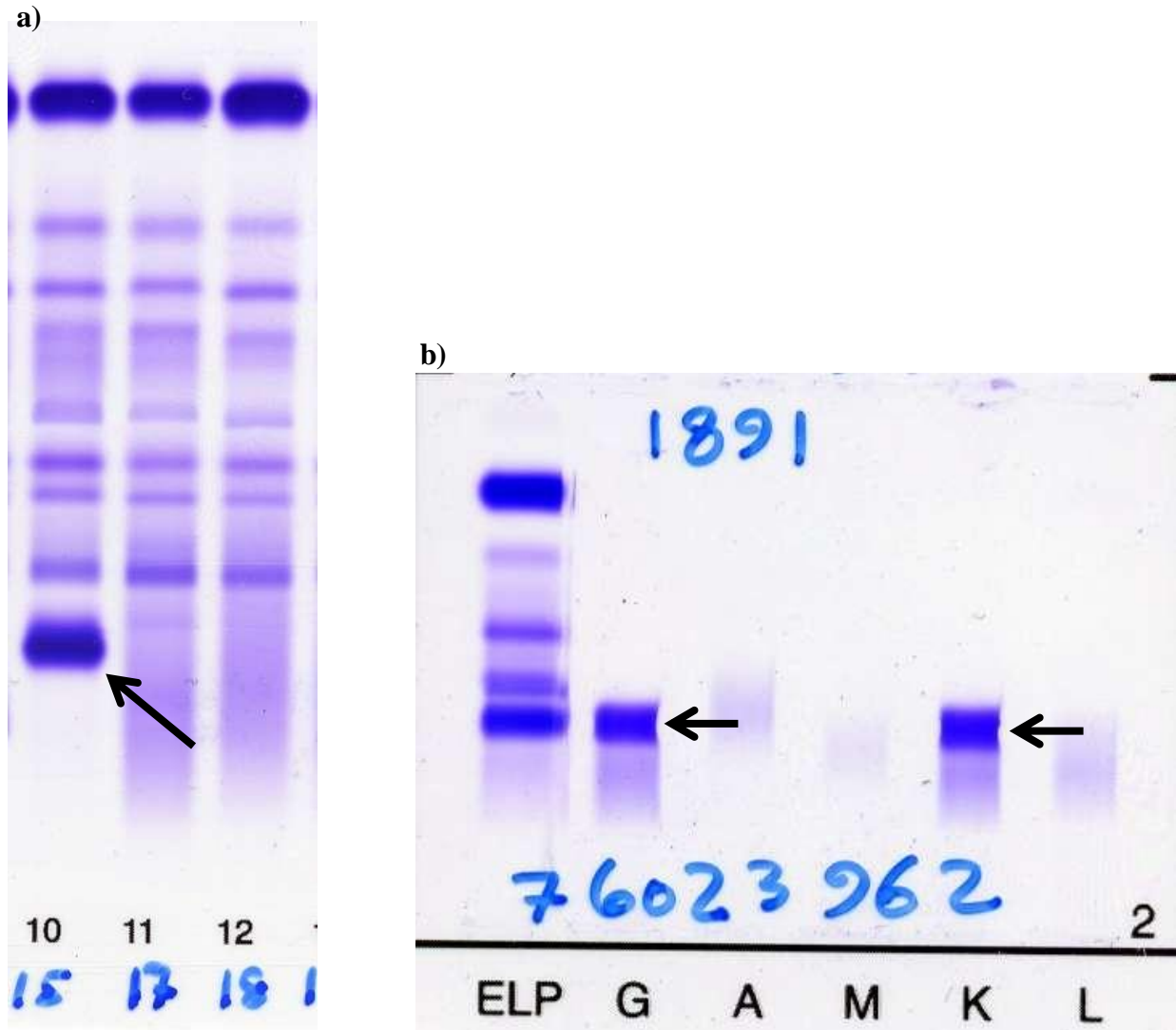
Figure 3. Serum electrophoresis panel (a) and immunofixation (b). The electrophoresis panel (a) contains one electrophoresis showing an M-protein (arrow), one with polyclonal hypergammaglobulinemia (middle), and one normal electrophoresis (right). The immunofixation (b) shows IgG kappa M-protein (arrows).

For the diagnosis of MGUS, the following is required: a serum M-protein concentration of less than 30 g/L, less than 10% of clonal plasma cells in the bone marrow, and absence of end-organ damage that can be attributed to a lymphoproliferative disorder (Table 2).[17]

Table 2. Diagnostic criteria for MGUS[17]

Non-IgM MGUS:	
1) Serum M-protein <30 g/L	
2) Clonal bone marrow plasma cells <10%	
3) Absence of end-organ damage attributable to a lymphoproliferative disorder, such as:	Hypercalcemia
	Renal insufficiency
	Anemia
	Bone lesions
	Amyloidosis
IgM MGUS:	
1) Serum M-protein <30 g/L	
2) Bone marrow lymphoplasmacytic infiltration <10%	
3) Absence of end-organ damage attributable to a lymphoproliferative disorder, such as:	Anemia
	Constitutional symptoms
	Hyperviscosity
	Lymphadenopathy
	Hepatosplenomegaly

MGUS is in its nature asymptomatic, and so is the previously mentioned early stage of MM called smoldering MM. The difference between the two is in the concentration of M-protein in serum and the proportion of clonal plasma cells in the bone marrow, as detected through blood samples and bone marrow aspirations, respectively.[5,88] The M-protein secreted by the clonal plasma cells in MGUS determines the MGUS isotype, and can be of gamma (G), alpha (A), mu (M), delta (D), or epsilon (E) type, depending on the heavy chain of the immunoglobulin, in combination with kappa or lambda, depending on the light chain. In the diagnostic criteria, MGUS is divided into non-IgM MGUS and IgM-MGUS.[17] Recently, a new entity of MGUS called light-chain MGUS has been described (see below).

1.3.2 Epidemiology

Similar to MM, the prevalence of MGUS is highly associated with age. MGUS is present in 2.4-3.5% of white Caucasian populations over the age of 50 years.[22,89-93] According to findings from one study, the prevalence is as high as 5.3% in people older than 70 years.[89] MGUS appears to be more common in men than in women.[89,90] Due to its asymptomatic nature, cases of MGUS are usually found *en passant* during workup for some other condition, and the large majority of cases in the population are likely to go undetected. The only means to accurately estimate of the true prevalence of MGUS in the population is through screening. A few screening studies have been performed to date, on populations of different ethnicity, age and gender.[89,90,93 92] Kyle et al. screened 21,463 residents 50 years or older in Olmsted County, Minnesota, and found an MGUS prevalence of 3.2%.[89] Landgren et al. screened 917 men in Ghana and found a prevalence of 5.8%, and later screened 12,482 participants in the

NHANES and NHANES III studies and found a prevalence of 2.4%.[90,92] Finally, Eisele et al. determined the prevalence of MGUS to be 3.5% in a screened German population of 4,702 individuals.[93] Based on these findings, it seems reasonable to conclude that MGUS is a quite common, undetected condition among the elderly.

1.3.3 Etiology and pathogenesis

The etiology of MGUS is largely unknown. As in MM, there are ethnic disparities in the incidence patterns, where MGUS is more common in African-Americans than in Caucasians, and more common in Caucasians than in Asians.[22,90,92] Given the findings of increased risk of MGUS in first-degree relatives of patients with MM or MGUS, a role for genetic factors seems plausible.[25,94] Exposure to pesticides, such as Agent Orange, have also been linked to increased risk of developing MGUS, demonstrating an impact of environmental factors.[95,96] It has been shown that both a personal history of autoimmune disease, inflammatory condition, or infections, as well as a family history of autoimmune disease increase the risk of developing MGUS, suggesting a shared susceptibility for these conditions.[21,30] How autoimmune disease affects survival in MGUS is unknown.

1.3.4 Prognosis and survival

MM is consistently preceded by MGUS, however, not all individuals with MGUS develop MM or any other lymphoproliferative disorder.[42,43] The fact that MGUS can develop into a malignant disorder is what makes it clinically relevant, despite its asymptomatic state. At progression, non-IgM MGUS in the majority of cases evolves into MM, primary AL amyloidosis, or other lymphoproliferative disorders, and IgM MGUS progresses mainly to Waldenström macroglobulinemia.[97-100] The risk of progression to MM has been investigated in a few prospective studies, based on clinically established cohorts of individuals with MGUS. The Mayo cohort included 241 MGUS patients, followed up to 39 years. The overall risk of progression to any lymphoproliferative disorder was 1.5% per year, with a cumulative risk of 27%.[98] In another cohort of 1,384 MGUS patients from Minnesota, the annual risk of progression to any lymphoproliferative disorder was 1.0%, with a cumulative probability of progression of 30% at 25 years.[99] Results from a Swedish study following 728 MGUS patients showed an annual risk of progression to MM of 0.5%, with a 30-year cumulative risk of 10.6%.[97] In conclusion, the annual risk progression appears to be 0.5-1.0% per year.

Treatment is currently not recommended for MGUS. Expert opinions recommend indefinite follow-up of individuals with MGUS, with the hope of catching malignant progression early. The recommendations for follow-up are initially six months after diagnosis and then annually or every two to three years depending on risk stratification.[101,102] No investigators have shown results supporting these recommendations, such as better survival in MM after follow-up of MGUS.

According to the International Myeloma Working Group risk stratification of MGUS, low-risk MGUS is characterized by an M-protein <15 g/L, isotype IgG, and a normal FLC ratio (0.26-1.65 mg/L).[101] Additional risk stratification systems include the one presented by

Pérez-Persona et al., which defines low-risk MGUS as <95% bone marrow aberrant plasma cells, and no DNA aneuploidy, as assessed through flow cytometry, and the most recent scoring system developed by Turesson et al., in which low-risk MGUS is described by a normal FLC ratio, an M-protein <15 g/L, and presence of immunoparesis (reduction of noninvolved immunoglobulin isotype levels.)[97,103] Additionally, several other biological characteristics of the clone, along with detectable Bence Jones proteinuria and a high erythrocyte sedimentation rate (ESR), increase the risk of progression from MGUS to a lymphoproliferative disorder.[48,104]

As mentioned, the majority of individuals with MGUS will never develop a malignant disorder, and in that sense, MGUS has been considered a benign condition. However, results from studies on clinically established cohorts suggest that individuals with MGUS have a higher mortality.[98,100,105,106] A Dutch study found individuals with MGUS to have an inferior survival compared to population-based controls, even in the absence of progression to lymphoproliferative disorder, and also found serum albumin levels predictive of survival.[100] Gregersen et al. found that malignant transformation only explained around 20% of the excess mortality observed in their Danish cohort of MGUS patients, noting an increased mortality from several other causes of death including heart disease and lung disease, throughout the follow-up period.[105] In a large Swedish study, the 4,259 MGUS patients in the cohort had an increased risk of death not only from hematologic disorders, but also from bacterial infections, heart disease, liver disease, and renal disease, compared to matched controls.[55] However, these findings are all based on clinically established cohort of MGUS patients, and, as previously noted, cases of MGUS are usually detected during workup for some other disease. Consequently, clinically based MGUS cohorts are likely to have more comorbidities than the general population. The findings of decreased survival in these individuals are not necessarily due to MGUS in itself, but are just as likely to be explained by the underlying comorbidities that lead to the finding of MGUS initially. Thus, it is currently unknown whether MGUS ascertained through screening is associated with excess mortality.

1.3.5 Thrombosis

Several investigators have found an increased risk of venous thrombosis in individuals with MGUS compared to the general population.[81,82,107,108] In addition, Kristinsson et al. reported an increased risk of arterial thrombosis in 5,395 individuals with MGUS compared to matched controls without MGUS[82]. Conversely, Za et al. found that the rate of arterial and venous thrombotic events in a retrospective cohort of 1,491 MGUS patients did not appear higher than that reported in the general population, however, no control group was used in that study.[109]

In regard to MGUS isotype, individuals with IgG MGUS have in one study been shown to be less prone to develop thrombosis than other MGUS isotypes,[108] and in another study individuals with IgG or IgA MGUS had a greater risk than individuals with IgM MGUS.[82]

All previous studies on thrombosis in MGUS have been performed on clinically established cohorts, and are, as previously mentioned, at risk of bias due to comorbidities.

1.3.6 Light-chain monoclonal gammopathy of undetermined significance

Recently, a new entity called light-chain MGUS (LC-MGUS) has been described.[17,110] In LC-MGUS, there is only secretion of either kappa or lambda FLC. There is no monoclonal immunoglobulin heavy chain expression, no end-organ damage attributable to lymphoproliferative disorder, clonal bone marrow plasma cells of <10%, and urinary M-protein of <500 mg/24 hours.[17] Individuals with LC-MGUS are at an increased risk of progression to LC-MM or amyloid light-chain amyloidosis.[17,43,110]

The current definition of LC-MGUS is an abnormal FLC ratio (< 0.26 or > 1.65), and an increased level of the involved light chain of more than 19.4 mg/L in kappa FLC and more than 26.3 mg/L in lambda FLC.[17] Since FLCs are cleared from the serum by the kidneys, a decrease in renal function leads to an increase in serum FLC levels, which is why a modified, extended range of what is considered normal FLC ratio has been suggested for individuals with renal failure (0.37-3.1).[110-112] The upper and lower limits of kappa and lambda were established using the normal distribution of free light chains in 282 individuals.[113]

Currently, the information available regarding LC-MGUS is based on only two studies.[93,110] Dispenzieri et al used FLC assay, IFE, and SPEP to screen 18,357 individuals of 50 years and older in Olmsted County and found 146 cases of LC-MGUS, corresponding to a prevalence of 0.8% (Table 3).[110] The prevalence of LC-MGUS was, as in MGUS, higher in men than in women. They found the prevalence of LC-MGUS to be increasing with age up to around 80 years of age in men, after which it levelled off, whereas in women the prevalence of LC-MGUS increased consistently throughout the higher age-groups. The highest incidence was thus found among men aged 70-79 years (1.7%) and women aged 80-89 years (1.5%). The modified, renal reference range was applied but not used for the definition. Only three cases in the cohort progressed during follow-up, and all to LC-MM.

Eisele et al screened 4,702 serum samples from German men and women of the Heinz Nixdorf Recall Study, aged 45-75 years, and found 34 cases of LC-MGUS, a prevalence of 0.7% (Table 3).[93] The prevalence of LC-MGUS was higher in men than in women. During a median follow-up time of five years, none of the LC-MGUS cases progressed. The risk of progression to lymphoproliferative disease thus appears to be smaller in LC-MGUS than in MGUS.

The knowledge of LC-MGUS is thus very limited, with information on prevalence based on two studies only and little information about risk of progression.

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Table 3. Prevalence of LC-MGUS

	Dispenzieri et al, 2010[110]	Eisele et al, 2012[93]
Number of participants	18,357	4,702
Number of LC-MGUS	146	34
Prevalence (total)	0.8%	0.7%
Age 40-49 years	-	0.3%
Age 50-59 years	0.5%	0.2%
Age 60-69 years	0.8%	1.0%
Age 70-79 years	1.1%	1.7%
Age 80-89 years	1.3%	-
Median age	68 years (range 50-96)	67 years (range 47-74)
Kappa LC-MGUS	108 (74%)	28 (82%)
Lambda LC-MGUS	38 (26%)	6 (18%)
Median concentration of involved FLC	176 mg/L	-
Number who progressed	3	0
Risk of progression	0.3 per 100 person-years	-

2 AIM

Overall aim

The overall aim was to increase our understanding of MGUS and LC-MGUS, with the purpose of improving the management of patients with these conditions.

Hypotheses

1) The diagnostic criteria for LC-MGUS can be improved to increase accuracy and better capture cases of clinical importance.

2) MGUS and LC-MGUS is a common condition in an elderly population and its prevalence can be quantified through screening using SPEP, IFE, and FLC analysis

3) Individuals with MGUS and LC-MGUS have inferior survival compared to individuals without MGUS

4) Autoimmune disease has a negative effect on survival in MGUS and in MM

5) Clinical follow-up of individuals with MGUS leads to better survival in MM

6) The causes of death in individuals with MGUS and LC-MGUS differ from individuals without MGUS.

7) Individuals with MGUS and LC-MGUS are at increased risk of arterial and venous thrombosis

3 PREVALENCE OF MGUS AND LIGHT-CHAIN MGUS (I)

Our aims of the study (Paper I) presented below were to quantify the prevalence of MGUS and LC-MGUS in a screened population, and to simplify and improve the diagnostic criteria for LC-MGUS.

3.1 METHODOLOGICAL CONSIDERATIONS

In order to quantify the prevalence of MGUS and LC-MGUS in the population, and to determine an adequate and useful definition of LC-MGUS, we used two population-based cohorts (see below) and screened for MGUS and LC-MGUS using SPEP, IFE (Helena Laboratories, Beaumont, Texas, USA), and FLC analysis (FREELITE, The Binding Site Ltd, Birmingham, UK). The FLC assay measures free kappa and free lambda light-chain concentrations as well as the kappa-to-lambda ratio (FLC ratio).[113] Individuals whose samples contained one or more M-protein bands on SPEP and/or IFE were considered to have MGUS, and those samples were subjected to IFE to determine the MGUS isotype, and M-protein concentration was measured. Since different lymphoproliferative disorders also can have an M-protein on SPEP, individuals with a lymphoproliferative diagnosis at study baseline were identified and excluded. In each cohort, the prevalence of MGUS was calculated in crude number and as in percent of the entire cohort, along with numbers and prevalence of different isotypes.

Renal function for all individuals in the AGES-Reykjavik Study cohort was calculated as glomerular filtration rate (GFR) in milliliters per minute per $1.73m^2$, using the Modification of Diet in Renal Disease formula, which takes into account creatinine level, age, and sex.[114] Analyses for the prevalence of LC-MGUS were performed based on the previous definition of LC-MGUS, with and without the renal reference range, and exploratory analyses using the normal distribution of kappa and lambda values.[110] The results from the different analyses were used to create new cut-off values for FLC concentrations, which were then applied to the cohorts. The prevalence of LC-MGUS, and the number of kappa and lambda LC-MGUS, was determined in each step. In the American cohort, the prevalence of LC-MGUS was estimated in whites, blacks, and Asian/Pacific Islanders separately.

Methodologically, the use of SPEP and IFE to detect MGUS is uncontroversial. However, the criteria for MGUS, according to the International Myeloma Working Group, require low prevalence of clonal plasma cells in the bone marrow and absence of end-organ damage attributable to plasma cell disease. [17] Alas, the serum analyses were performed on stored samples, and we did not have the opportunity to examine all screened patients with regard to end-organ damage or bone marrow testing. This is a limitation of the screening method used and it is not inconceivable that some of the individuals with positive SPEP and/or IFE were in fact in the early stages of an undetected lymphoproliferative disorder. FLC analysis is the suggested and the only approach to detect LC-MGUS.

3.1.1 AGES-Reykjavik Study cohort

The cohort of the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik Study) is based on a prospective study of 30,795 Icelandic men and women.[115] It originated as the Reykjavik Study in 1967, and was initiated by the Icelandic Heart Association. Men and women born 1907-1934 and living in Reykjavik were invited to participate in an elaborately designed examination schedule divided into six stages, spanning over almost 30 years (1967-1996). The purpose of the Reykjavik Study was to prospectively study cardiovascular disease, and to identify risk factors for cardiovascular disease. Some of the findings from the Reykjavik Study include establishing family history of myocardial infarction, erythrocyte sedimentation rate, C-reactive protein as independent risk factors for myocardial infarction, and showing a family history of lung cancer to be an independent risk factor for lung cancer.[116-119]

In 2002, individuals were randomly selected from survivors of the Reykjavik Study, and 5,764 individuals (a response rate of 75%) were re-examined for the AGES-Reykjavik Study.[115] The purpose of the AGES-Reykjavik Study was to study environmental and genetic contributions to diseases of the elderly, with special focus on four biologic systems: vascular, neurocognitive, musculoskeletal, and body composition/metabolism. The study design allows for the combination of midlife data from the Reykjavik Study and old-age data from AGES-Reykjavik, for life course study. Participants in the AGES-Reykjavik Study examination in 2002-2006 completed a questionnaire, underwent clinical examination, laboratory testing, and radiological examinations.

The AGES-Reykjavik Study cohort is longitudinal and ongoing, and information on incidence of disease, date of death, and cause of death is collected annually through hospital, nursing home, and mortality records. End of follow-up for the analyses of outcome in the present study was March 31, 2014. Study baseline was date of first visit in AGES-Reykjavik Study. To capture individuals who progressed to a lymphoproliferative disorder, we used information from the Icelandic Cancer Registry and hospital records. For individuals who progressed, individual medical records were assessed and diagnosis of lymphoproliferative disorder was validated.

3.1.2 PLCO cohort

The Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial is a large, randomized trial designed to determine if screening for these four cancers can decrease the mortality in these diseases.[120,121] The trial recruited from 1992 to 2001 around 155,000 volunteers, aged 55 to 74 years, who were randomized to attend either a cancer screening regimen, or to routine medical care. For the present study, a random sample of 5,916 individuals were selected from participants in the screening arm of the trial who had available serum and were not diagnosed with a lymphoproliferative disorder prior to study entry or during follow-up (end of follow-up January 1st, 2012). The sampling was performed within strata by sex, age at baseline, and race. No further exclusion criterion was applied. Study baseline was date of blood sample collection.

3.2 RESULTS AND DISCUSSION

From the AGES-Reykjavik cohort of 5,725 included participants, MGUS was identified, through one or several M-protein bands on SPEP, in 300 individuals, corresponding to a prevalence of 5.2% (Table 4).

Among the 5,916 individuals of the PLCO cohort, 283 cases of MGUS were identified, corresponding to a prevalence of 4.8%. The prevalence of MGUS was highest in the 70-79 years age group, and higher in men than in women (p<0.001), in both cohorts. The median age in the AGES-Reykjavik Study cohort was higher than in the PLCO cohort, with more individuals in the 70-79 years age group, which is likely the explanation behind the slightly higher prevalence (5.2%) in the AGES-Reykjavik Study cohort compared to the PLCO cohort (4.8%). The prevalence of MGUS is known to increase with age.[22,89,93,110] The prevalence of MGUS in our study cohorts is similar to that found in previous studies, which confirms the prevalence of MGUS to be 4-5% in a population over 60 years of age.[22,89-93]

In the AGES-Reykjavik Study cohort, 453 individuals (7.9%) had a pathological FLC ratio but normal SPEP, and 275 of these (4.8%) also had an increased concentration of the light chain involved (>19.4 mg/L kappa, or >26.3 mg/L lambda). This resulted in 264 kappa and 11 lambda cases of LC-MGUS, a kappa prevalence of 96%, according to the previous definition.[110] When applying the modified, renal reference range (0.37-3.1) to individuals with an impaired renal function (GFR <60 mL/min), this resulted in 135 individuals with LC-MGUS with a kappa prevalence of 92%. Only applying the renal reference range to individuals with severe renal failure (GFR <30 mL/min) increased the number of LC-MGUS to 249, with persisting high kappa prevalence of 96%. Similarly, applying the previous definition of LC-MGUS to the PLCO cohort, 286 cases of LC-MGUS were identified with a kappa prevalence of 95%.

Table 4. Results from analysis of the AGES (5,725 participants) and PLCO (5,916 participants) cohorts

	AGES			PLCO		
	MGUS[a]	LC-MGUS[b]	No MGUS	MGUS	LC-MGUS	No MGUS
Total n[c] of cases (%)	300 (5.2)	52 (0.9)	5,373 (93.9)	283 (4.8)	57 (0.96)	5,576 (94.3)
Men, n (%)	159 (53.0)	34 (65.4)	2,226 (41.4)	201 (71.0)	41 (71.9)	3,452 (61.9)
Women, n (%)	141 (47.0)	18 (34.6)	3,147 (58.6)	82 (29.0)	16 (28.1)	2,124 (38.1)
Median age, years (range)	78 (67-93)	82 (69-96)	76 (66-98)	72 (60-80)	71 (60-79)	69 (59-80)
Age group, n (%)						
Less than 70 years	21 (7.0)	1 (1.9)	529 (9.9)	107 (37.8)	22 (38.6)	2,876 (51.6)
70-79 years	148 (49.3)	16 (30.8)	3,039 (56.6)	173 (61.1)	35 (61.4)	2,685 (48.2)
80-89 years	120 (40.0)	33 (63.5)	1,679 (31.2)	3 (1.1)	0 (0)	15 (0.3)
90 years and older	11 (3.7)	2 (3.8)	126 (2.3)	0 (0)	0 (0)	0 (0)
MGUS isotype, n (%)						
IgG	159 (53.0)	N.A.[d]	N.A.	179 (63.3)	N.A.	N.A.
IgA	27 (9.0)	N.A.	N.A.	32 (11.3)	N.A.	N.A.
IgM	81 (27.0)	N.A.	N.A.	55 (19.4)	N.A.	N.A.
IgD	1 (0.3)	N.A.	N.A.	0 (0)	N.A.	N.A.
Biclonal	32 (10.7)	N.A.	N.A.	17 (6.0)	N.A.	N.A.
M-protein concentration, n (%)[e]						
>15.0 g/L	17 (5.7)	N.A.	N.A.	9 (3.2)	N.A.	N.A.
<15.0 g/L	147 (49.0)	N.A.	N.A.	207 (73.1)	N.A.	N.A.
missing	136 (45.3)	N.A.	N.A.	67 (23.7)	N.A.	N.A.
FLC[f] ratio						
0.26-1.65	168 (56.0)	0 (0.0)	4972 (92.5)	176 (62.2)	0 (0)	5,347 (95.9)
<0.26 / >1.65	132 (44.0)	52 (100.0)	401 (7.5)	107 (37.8)	57 (100)	229 (4.1)
Race						
White	N.A.	N.A.	N.A.	185 (65.4)	27 (47.4)	3,787 (67.9)
Black	N.A.	N.A.	N.A.	70 (24.7)	28 (49.1)	857 (15.4)
Asian/Pacific Islander	N.A.	N.A.	N.A.	28 (9.9)	2 (3.5)	932 (16.7)

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]n: number, [d]N.A.: not applicable, [e]available for 164 subjects in AGES, for 216 subjects in PLCO, [f]FLC: free light chain.

Considering the surprisingly high prevalence of LC-MGUS and the kappa-biased results, we performed additional analyses using the normal distribution and the log-transformed values of kappa and lambda. We used the entire AGES-Reykjavik Study and PLCO cohorts, excluding the 300 and 283 individuals with MGUS, respectively, and determined the 2.5th and the 97.5th percentiles of kappa concentration, lambda concentration, and FLC-ratio (Figure 4). These were found to be very similar in the two cohorts: 7.7-40.6 mg/L and 7.3-37.3 mg/L for kappa, 7.2-36.3 mg/L and 6.8-32.3 mg/L for lambda, and 0.6-2.0 and 0.6-1.8 for the FLC-ratio, in the AGES-Reykjavik Study and the PLCO cohorts, respectively.
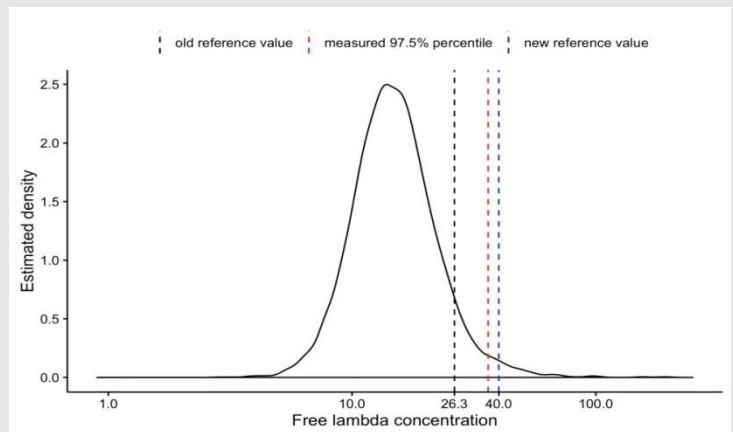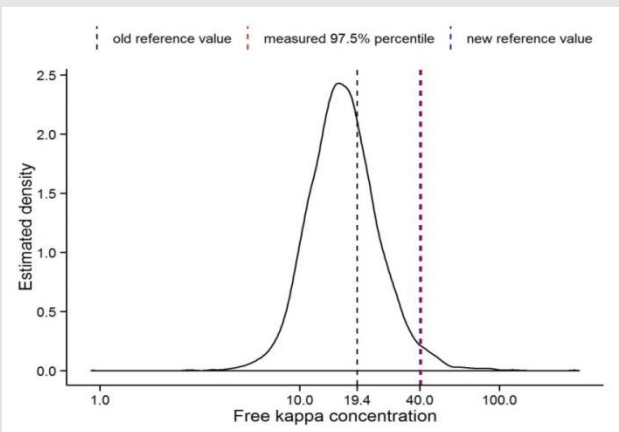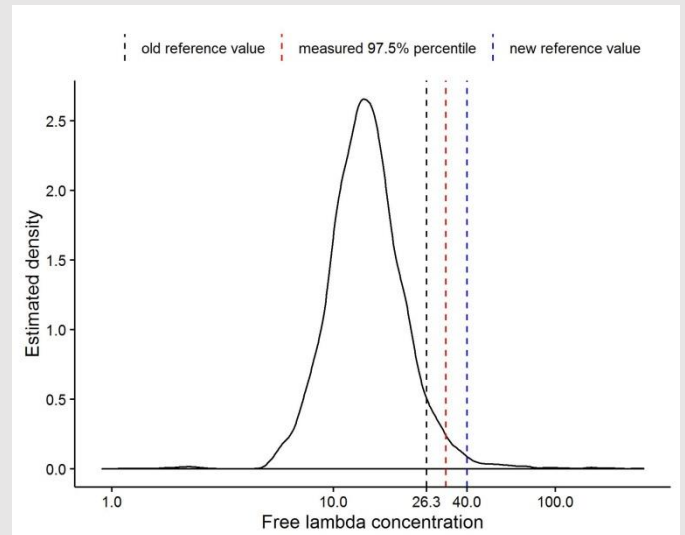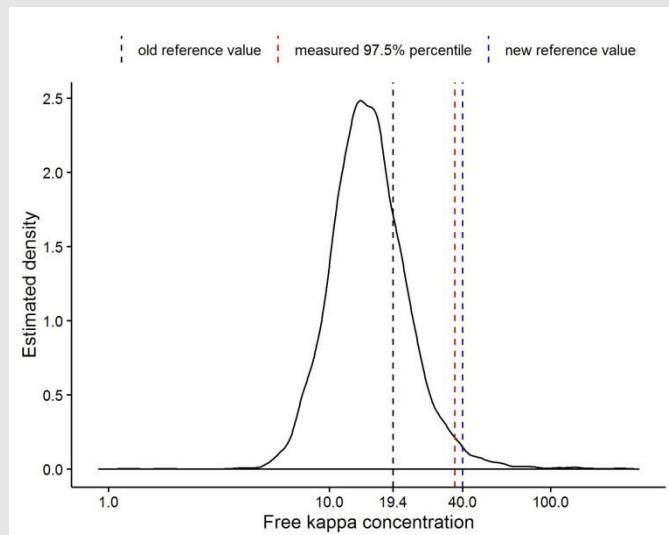

Figure 4. The distribution of serum free kappa and lambda concentrations among individuals without M-protein on serum protein electrophoresis (the 97.5th percentile, prior cut-offs for LC-MGUS, and 40 mg/L are marked) in a) the AGES-Reykjavik Study and b) the PLCO Study. The x axis is on a log transformed scale with base 10.

With the purpose of evaluating the requirement for a renal reference range, which has previously been suggested, we examined the 2.5th and the 97.5th percentiles of FLC concentration and FLC-ratio among individuals with different degrees of impaired renal function in the AGES-Reykjavik cohort. In the 2,131 individuals with moderately impaired renal function (GFR ≥30 or ≤60 mL/min), the percentiles were 0.9-43.6 mg/L for kappa, 8.0-38.1 mg/L for lambda, and 0.7-2.0 for FLC-ratio. In the 105 individuals with severely impaired renal function (GFR <30 mL/min), the percentiles were 14.8-112.6 mg/L for kappa, 13.0-160.3 mg/L for lambda, and 0.5-2.2 for FLC-ratio. Concluding that the 2.5th and the 97.5th percentiles of FLC concentration and FLC-ratio in moderately impaired renal function were very similar to the values of individuals with normal renal function, and the 2.5th and 97.5th percentiles of FLC-ratio was essentially unchanged even in severe renal function, we decided that the suggested renal reference range (0.37-3.1) is of little use in the definition of LC-MGUS. Similarly, Dispenzieri et al. also concluded that the normal reference range allowed for the greatest consistency.[110]

Finally, we evaluated the effect of using the 97.5th percentile as a cut-off for normal values of the involved light-chain, instead of the previously suggested levels (19.4 mg/L for kappa and 26.3 mg/L for lambda). For simplicity, since the 97.5th percentile was close to 40 mg/L in both cohorts, this was chosen as the upper limit for both kappa and lambda. We then used a definition of LC-MGUS as a pathological FLC-ratio (unchanged from the previously suggested normal range of 0.26-1.65), regardless of renal function, in combination with an increased concentration of more than 40 mg/L of the light-chain involved. This resulted in 52 LC-MGUS cases in the AGES-Reykjavik Study cohort, a prevalence of LC-MGUS of 0.9%, and 57 cases in the PLCO cohort, a prevalence of 1.0% (Table 4). The kappa prevalence was 79% and 74% in the two cohorts, respectively.

The LC-MGUS prevalence in our study of 0.9-1.0% is similar to the prevalence found in the only two studies that have previously been performed.[93,110] The prevalence of LC-MGUS in the PLCO cohort was highest among blacks (2.9%), and lowest among Asian/Pacific Islanders (0.2%), compared to white Caucasians (0.7%), a similar race distribution to that previously described for MGUS.[22,90,92]

MGUS and LC-MGUS are of clinical importance due to the risk of malignant transformation. An accurate definition of LC-MGUS is thus of great importance, and needs to be sensitive enough to identify clinically relevant patients, who are at risk of progression and could benefit from clinical follow-up, yet specific enough not to include too many individuals who will never develop a malignant disease and have nothing to gain from follow-up. To assess the sensitivity of our revised definition, we examined the incidence of progression to lymphoproliferative disorder in the AGES-Reykjavik Study cohort. During follow-up time, three individuals who fulfilled both the previous and our revised criteria progressed: one to LC-MM, one to amyloid light-chain amyloidosis, and one to diffuse large B-cell lymphoma. One individual developed diffuse large B-cell lymphoma three years after baseline, and had an elevated FLC-ratio at baseline, but had an impaired renal function (GFR = 50 mL/min) and did thus not fulfill either the previous or the revised criteria for LC-MGUS. In summary, compared to the prior definition of LC-MGUS, the new definition that we are suggesting did not miss any additional cases of LC-MGUS.

We believe our study lays a strong foundation for a revised definition of LC-MGUS, based on normal distributions just like the original suggestion, but on a larger and more heterogeneous population (Table 5).[113] Our new definition of LC-MGUS differs from the

22

previously suggested foremost in precision, but also in simplicity; it is easier to apply since the cut-off is the same for both kappa and lambda (40 mg/L), and does not depend on renal function.[93,110,113] Applying our revised criteria on our two cohorts, considerably fewer individuals are diagnosed with LC-MGUS than would have been the case with the previous definition, and although it still successfully identifies the individuals who will later develop a clinically relevant lymphoproliferative disorder.

Table 5. New revised criteria for LC-MGUS[a]

Abnormal free light-chain ratio (< 0.26 or > 1.65)[b,c]
Elevated involved light chain (40 mg/L or higher)
No immunoglobulin heavy chain M-spike by SPEP/IFE[d]
No evidence of end-organ damage that can be attributed to lymphoproliferative disorder

[a]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [b]values based on the serum Freelite assay (The Binding Site Group, Birmingham, UK), [c]regardless of renal function, [d]SPEP: serum protein electrophoresis and IFE: immunofixation.

MGUS and LC-MGUS are challenging conditions to study, due to their asymptomatic nature. A screened cohort study such as the present one is the only way to gain an accurate estimate of the true prevalence in the population. The major strengths of our study is its size, based on more than 11,000 participants, the heterogeneity of the cohorts; with different age groups, different ethnicities, and both genders represented, and the screening efforts underlying the findings of MGUS and LC-MGUS. Limitations include, as previously mentioned, the inability to perform bone marrow samples and clinical assessment on all individuals, lack of information on renal function and on progression in the PLCO cohort, and a limited number of patients who progressed in the AGES-Reykjavik Study cohort during follow-up which makes it difficult to assess the risk of progression. Furthermore, participants in the AGES-Reykjavik Study were selected survivors from the original Reykjavik Study cohort, and are likely to be healthier than the general population.

In conclusion, in this large international investigation, based on two independent, screened cohorts, we have determined that among the elderly, the prevalence of MGUS and LC-MGUS is 4.8-5.2% and 0.9-1.0%, respectively. We suggest a revised definition of LC-MGUS that captures the clinically relevant individuals and can be used regardless of renal function. The present study adds significantly to the field considering that until date, knowledge of FLC levels in general and of LC-MGUS in particular has been based on few studies, containing small numbers of individuals with LC-MGUS, all studies restricted to Caucasians.[93,110,113] The major clinical implication of the revised definition is that considerably fewer individuals will be diagnosed with LC-MGUS, which will decrease unnecessary health-care costs as well as reduce the burden of anxiety among affected patients. Ideally, our proposed definition would be replicated in a larger cohort study with even longer follow-up, but in the meantime we recommend the diagnostic criteria for LC-MGUS to be revised according to our suggestions.

4 NATURAL COURSE AND SURVIVAL (II, III, IV)

In our investigations on natural course and survival, our aims were to compare the survival of individuals with MGUS and LC-MGUS to individuals without MGUS, to determine whether autoimmune disease has a negative effect on survival in MGUS and in MM, and to establish whether clinical follow-up of individuals with MGUS leads to better survival in MM.

4.1 METHODOLOGICAL CONSIDERATIONS

In order to study the natural course and survival of MGUS and LC-MGUS we conducted three population-based studies, using an Icelandic cohort of individuals with MGUS and LC-MGUS, and a Swedish cohort of MGUS patients.

The Icelandic cohort of the AGES-Reykjavik Study was described previously. To examine survival in MGUS and in LC-MGUS, we used the entire cohort, including the 300 individuals with MGUS and 52 individuals with LC-MGUS that we had detected through screening, using the revised definition of LC-MGUS. The participants in AGES-Reykjavik Study are followed prospectively, with information on incidence of disease and date of death collected annually through medical records and registers. End of follow-up for the analysis of survival was March 2014. We excluded individuals who had a lymphoproliferative disorder as cause of death without previous diagnosis of lymphoproliferative disorder, and individuals with shorter follow-up time than ten days.

For the analysis of survival in MM and in MGUS after autoimmune disease, and survival in MM depending on previously known MGUS, we used Swedish cohorts of MM patients and MGUS patients. In Sweden, all physicians are obliged to report each case of cancer to the nationwide Swedish Cancer Register. We identified 8,367 MM patients diagnosed from 2000 to 2013 in the Swedish Cancer Register.[122] We also used 18,768 patients with MGUS, diagnosed from 1988 to 2013, identified from an MGUS cohort established through a national network of hematologists and oncologists, and the Swedish Inpatient and Outpatient Registers.[123] For each patient, four population-based control subjects were randomly sampled, and information on autoimmune disease in patient and controls were collected from the Swedish Inpatient Register.[30,124]

For the analysis of survival after progression to MM, depending on known MGUS status before diagnosis of MM, we used again the Swedish Cancer Register to identify 14,404 MM patients diagnosed from 1976 to 2005. We cross-linked them against the MGUS cohort mentioned previously, to determine who among the MM patients had a previously known, and thus with great likelihood clinically followed, MGUS. Survival was compared, from time of MM diagnosis, between patients with and without prior knowledge of MGUS. Information on date and cause of death was gathered from the Swedish Cause of Death Register. The completeness and diagnostic accuracy are high for the Swedish registers used.[125,126]

In all of the analyses, difference in survival was estimated using Kaplan Meier model, the log rank test, and Cox regression model.[127-129] Adjustments were made for potential confounders such as age and gender, as well as M-protein isotype and M-protein concentration, when this was available. For analyses using Cox regression model, the proportional hazards assumption was tested using plotting of the Schoenfeld residuals, a formal statistical test, and through introducing time-varying covariates into the model. The

correlation between continuous variables and categorical variables was assessed using Pearson product moment correlation coefficient and chi-squared tests, respectively. Statistical results were considered significant at $p < 0.05$.

We were able to perform these large cohort studies owing to the high quality Swedish registers and to access to the Icelandic AGES-Reykjavik Study cohort. Despite the fact that the analysis was performed retrospectively, the design is prospective in the sense that individuals in all mentioned cohorts were followed and information on outcomes was collected in the forward directionality from time of diagnosis/blood sample collection. The cohort study design is an excellent methodology to study rare exposures such as MGUS or LC-MGUS, and has the further advantage of excluding recall bias, since ascertainment of exposure status was performed through registers and, in the case of the AGES-Reykjavik Study cohort, through screening. Additionally, the prospective cohort study design allows for speculations of causality among detected associations in a way that cross-sectional studies do not. One methodological limitation is that the Swedish MGUS cohort was not established through screening, but through a national network and through registers.

4.2   RESULTS AND DISCUSSION

When studying the survival of individuals in the AGES-Reykjavik Study cohort (Paper II), we found that the five-year survival rates for individuals with MGUS, LC-MGUS, and no MGUS were 76% (95% CI 0.71-0.81), 52% (0.4-0.6), and 84% (0.82-0.84), respectively (Figure 5).

When adjusted for age and sex, individuals with MGUS and LC-MGUS had a significantly higher risk of death (HR = 1.2, 1.04-1.4, and HR = 1.6, 1.2-2.3, respectively) compared to individuals without MGUS during a median follow-up time of 9.7 years. The increased risk of death persisted after exclusion of individuals who progressed to lymphoproliferative disease, although the increased risk was then statistically significant only for LC-MGUS.
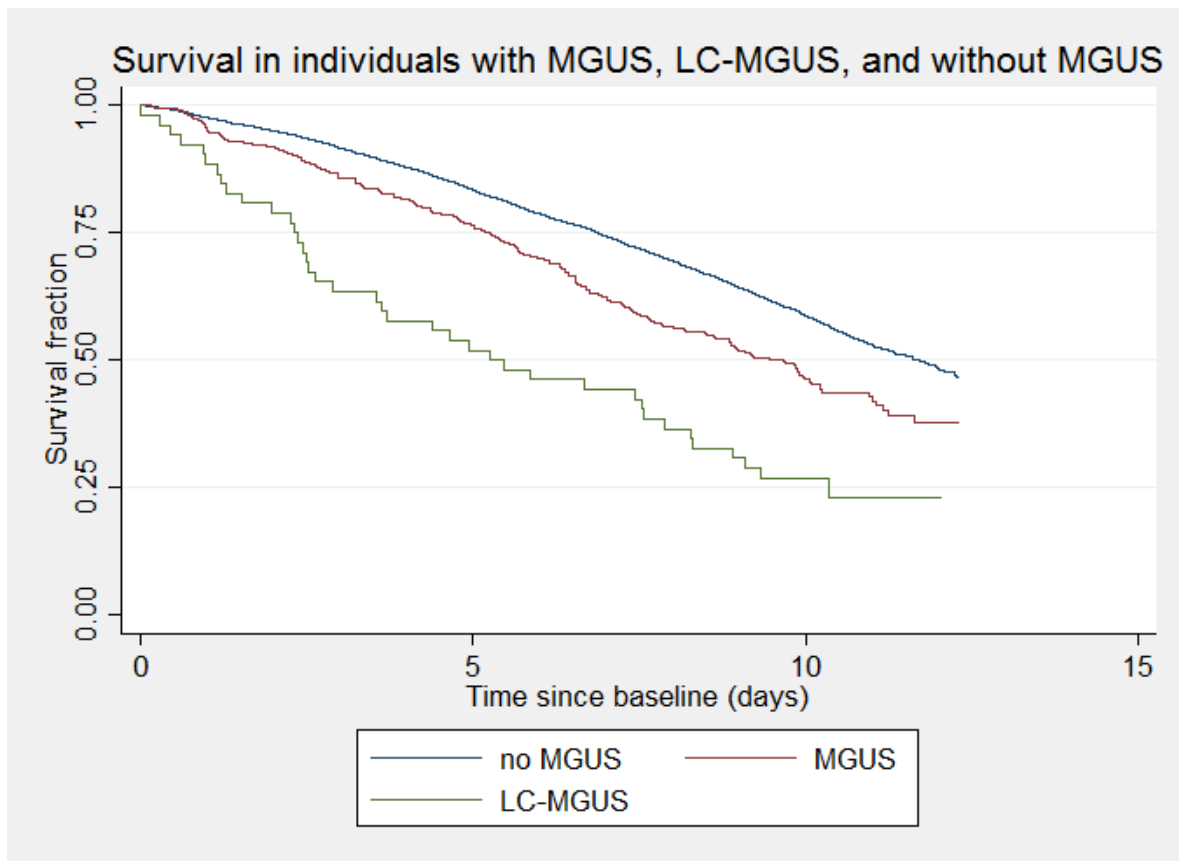
Figure 5. Survival in individuals with MGUS and LC-MGUS compared to individuals without MGUS.

We investigated the effects of different MGUS isotypes, M-protein concentration, and FLC ratio, and found that individuals with MGUS isotype A had a significantly increased risk of death compared to individuals with other isotypes (HR = 1.8, 1.1-2.9). Moreover, a high M-protein concentration or an abnormal FLC ratio did not statistically increase the risk of death when adjusted for age and sex.

The finding that individuals with LC-MGUS and MGUS have an increased risk of death compared to individuals without MGUS is interesting, since the individuals under study were diagnosed with MGUS and LC-MGUS through screening, and not clinically detected during workup for some other condition. This is, to our knowledge, the first investigation where results show inferior survival in MGUS in a screened cohort. Our results are in line with findings from previous studies from non-screened cohorts, but are unlikely to be explained by the comorbidities inevitably present in clinical cohorts of MGUS patients.[98,100,105,106] Individuals with LC-MGUS were older at baseline (82 years) than individuals with MGUS (78 years) or without MGUS (76 years), but since age was adjusted for in the analyses, it is unlikely to explain the inferior survival of individuals with LC-MGUS.

With regard to the increased risk of death in individuals with LC-MGUS, it is not necessarily the monoclonal FLCs that cause the mortality, but it could also be due to the overload of FLCs regardless of clonality. It has been shown that in the general population, non-clonal FLCs predict decreased overall survival, independently of renal function, sex, and

age.[130] An increase in polyclonal FLCs is seen in autoimmune diseases such as rheumatoid arthritis and Sjögren's syndrome[131]

One reason for the increased risk of death in individuals with MGUS and LC-MGUS could of course be progression to a lymphoproliferative disorder, which either goes clinically unnoticed, or for some reason is not noted in medical records or registers. When individuals who developed a lymphoproliferative disorder during follow-up were excluded from the analyses, the risk of death remained increased for LC-MGUS (HR = 1.5, 1.1-2.1) and MGUS (HR = 1.1, 0.9-1.3), although the risk estimate was no longer statistically significant for the latter. Since the individuals in the AGES-Reykjavik Study cohort were not planned to be re-examined in the study after baseline, with the goal of detecting early signs of malignancy, and individual medical records were not routinely evaluated, cases of undetected progression to lymphoproliferative disorder in our cohort cannot be entirely excluded. However, several factors speak against this, including the rareness of lymphoproliferative disorders, the completeness of the registries used, and the Icelandic health care system which is well-functioning and provides affordable health care to the entire population.

We believe that the inferior survival noticed among individuals with MGUS and LC-MGUS in our cohort is indeed a true reflection of an increased risk of death in these disorders, and is not caused by malignant progression. The increased risk could be due to an underlying genetic susceptibility for both plasma cell disorders and other conditions, or to the overproduction of light chains causing previously undetected organ damage.

In the Swedish cohorts, a personal history of autoimmune disease was found in 16% of MM patients and in 21% of MGUS patients, compared to 13% in MM controls and 12% in MGUS controls, respectively (Paper III). In individuals with both MM (HR = 1.2, 95% CI 1.2-1.3) and MGUS (HR = 1.4, 1.3-1.4), a decreased survival was associated with a personal history of autoimmune disease (Figure 6). In particular, a history of ulcerative colitis had a stronger negative impact on survival in MM than in controls. The effect of autoimmunity on survival was not different between individuals with different MGUS isotypes or M-protein concentration, or between men and women.
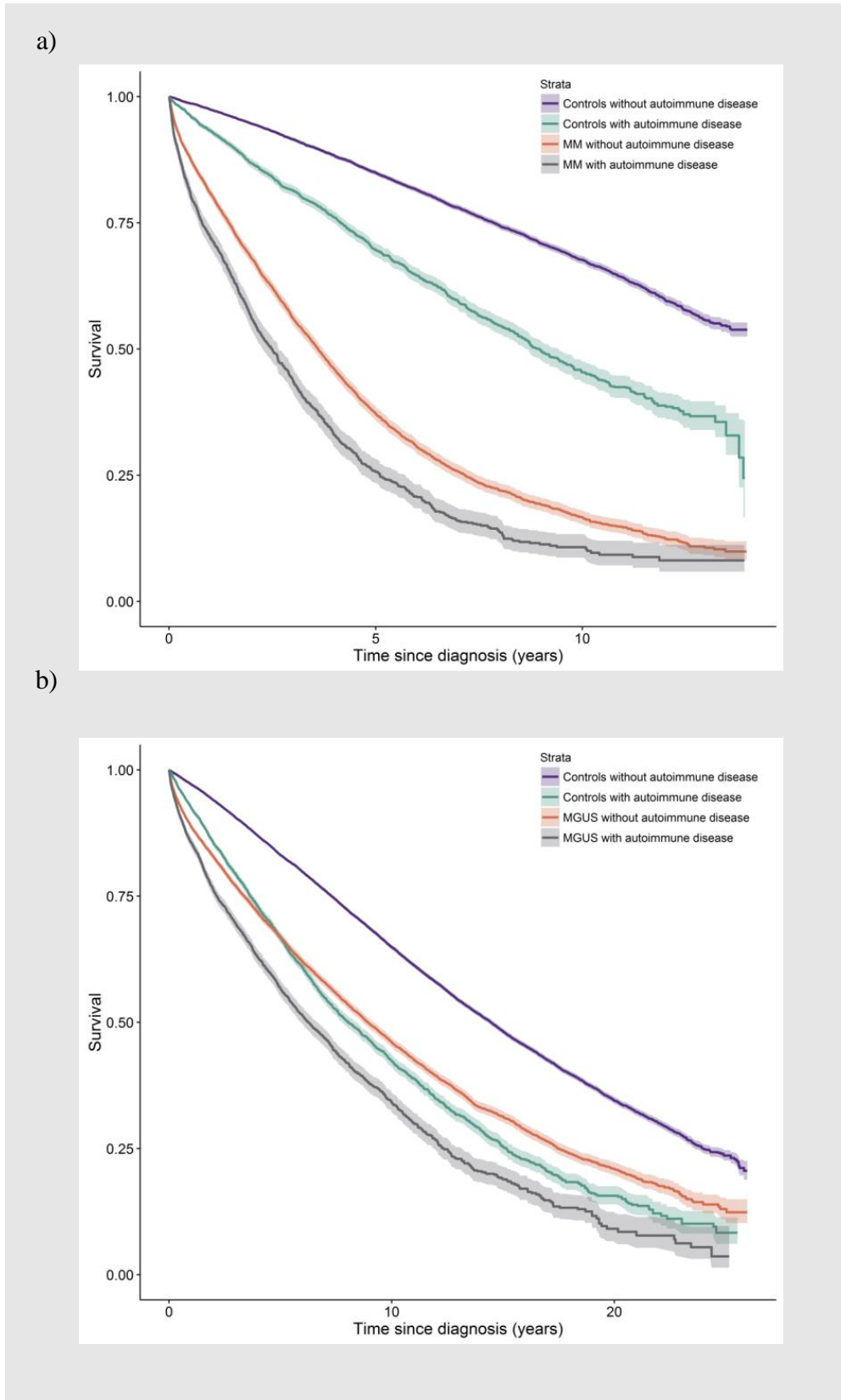
a)

b)

Figure 6. Survival in MM (a) and in MGUS (b) with and without a personal history of autoimmune disease, compared to controls.

The observation that a personal history of autoimmune disease is associated with an increased risk of death in both individuals with MM and MGUS could also be due to shared genetic susceptibility for plasma cell disorders and autoimmune disease, or to cumulative comorbidity in the individual. We know that autoimmunity is associated with an increased risk of developing MGUS and MM, and possibly, autoimmunity triggers a more severe form of MM – but this could not account for the increased risk of death detected in individuals with MGUS who do not progress.[21,30]

We studied survival among 14,798 MM patients, whereof 394 had previously been diagnosed with MGUS (Paper IV). We found that patients with MM with prior knowledge of MGUS had significantly better overall survival (median survival 2.8 years) than MM patients without prior knowledge of MGUS (median survival 2.1 years), even though the former had more comorbidities (p<0.001) (Figure 7). The results were similar for cause-specific survival, where the risk of dying from MM was lower (HR = 0.75, 0.6-0.9) for MM patients with a prior knowledge of MGUS compared to those without prior knowledge of MGUS.
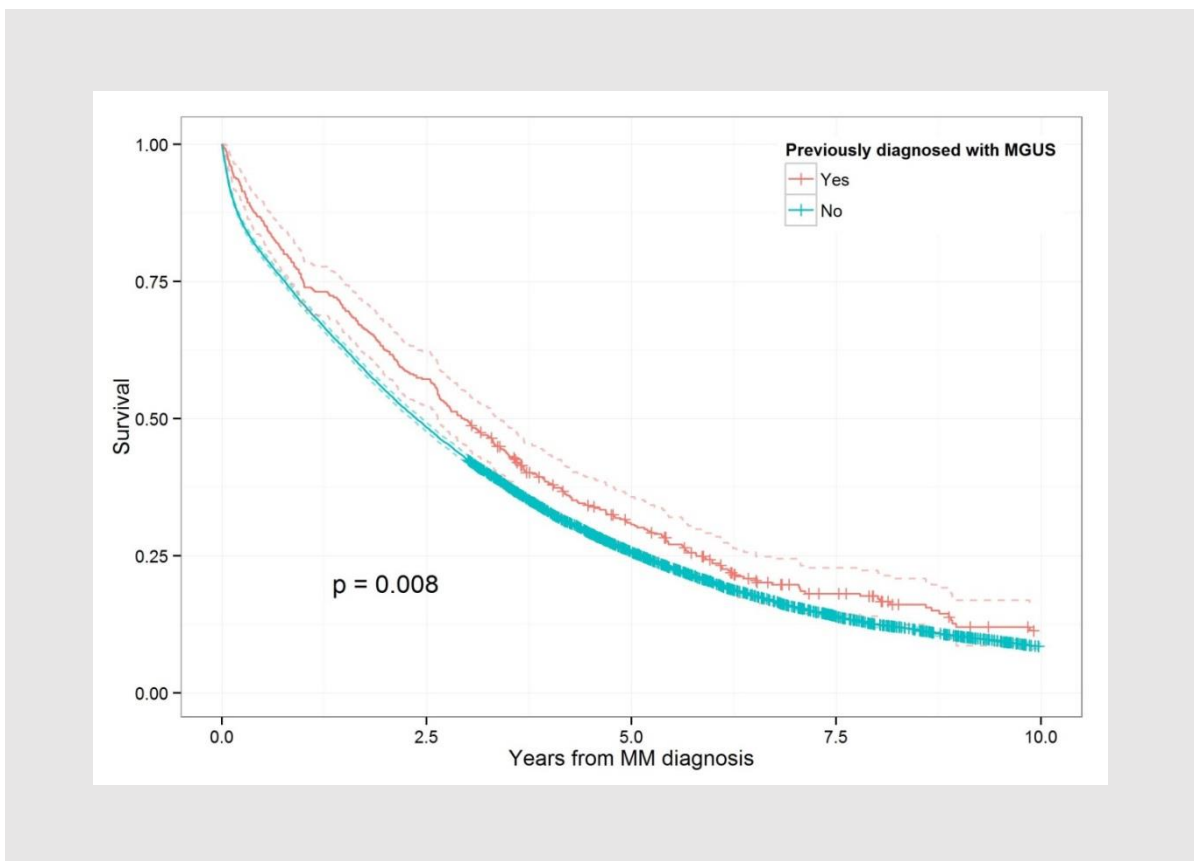
Figure 7. Survival in MM patients with and without prior knowledge of MGUS.

Furthermore, we found that among MM patients with prior knowledge of MGUS, a low M-protein concentration at MGUS diagnosis was predictive of inferior survival in MM (HR = 1.86, 1.1-3.0), and the median time from MGUS to MM diagnosis was shorter for those with a high M-protein concentration. There was no difference in survival between different MGUS isotypes.

In the cohort under study, the risk of death was 14% lower in MM patients with a prior diagnosis of MGUS, which confirms results from a recent publication demonstrating a 13% better overall survival.[132] This makes a strong case for the benefit of clinical follow-up of individuals with MGUS. Curiously, a low M-protein concentration predicted inferior survival in MM, which is contradictory to the current belief that a high M-protein concentration increases the risk of progression from MGUS to MM.[101] One reason for this could be current guidelines recommending less frequent monitoring of low-risk MGUS patients, which could lead to a delayed diagnosis of progression to a lymphoproliferative disorder.[101] If this were the case, it would speak against current guidelines and argue for regular and prevalent follow-up of individuals with MGUS regardless of risk profile.

One reason behind the detected difference in survival could be that the MM patients with a prior knowledge of MGUS do not in fact *survive* longer, but rather are diagnosed earlier, and therefore *followed* (from detection of disease until end of follow-up) longer, a so called lead-time bias. If this were the case, MM patients with a prior knowledge of MGUS would be diagnosed at an earlier age. To investigate this, we compared median age at diagnosis in the two groups, which was similar, and furthermore we compared days between diagnosis of MGUS and diagnosis of MM, and age at diagnosis of MM, and the correlation was low (0.03 by Pearson's product moment correlation coefficient).

We found that the MM patients with prior knowledge of MGUS had higher prevalence of comorbidities than did MM patients without prior knowledge of MGUS, which confirms the previously stated assumption that clinically detected MGUS is often the result of workup for an unrelated disease, and underlines the need for studies based on screened, population-based cohorts. It also suggests that in individuals with MGUS detected through screening, the benefit of clinical follow-up would be even greater.

In summary, we found that individuals with LC-MGUS and MGUS have a higher risk of death than individuals without MGUS, that a history of autoimmune disease predicts a decreased survival in both MM and MGUS, and that prior knowledge of MGUS is associated with an improved survival in MM.

The major strength of our studies on the natural course of MGUS and LC-MGUS, and on the survival in MGUS and in MM, is the cohort study design, and in particular the screened cohort design of the studies on the participants of the AGES-Reykjavik Study. Furthermore, the cohorts under study were all large and population-based, and exposures such as prior knowledge of MGUS, a history of autoimmune disease, and other comorbidities, were established through registers, thus eliminating recall bias on behalf of the participants. Limitations include the inability to validate individual medical records as well as absence of information on potentially important confounders in the studies on MM patients, such as treatment and risk stratification score.

Our findings are of clinical importance, since they suggest that greater attention should be paid to comorbidity in MGUS and MM, and that follow-up of MGUS patients is important in order for early detection and treatment of malignant disease. It would be interesting to further characterize, in detail, the comorbidities and biomarkers in a large cohort of individuals with MGUS and LC-MGUS, to determine whether concurrent conditions, autoimmune disease especially, or risk factors can explain the inferior survival demonstrated in these studies.

5 CAUSES OF DEATH AND COMORBIDITIES (II, V)

We aimed to study the causes of death of individuals with MGUS and LC-MGUS, and compare them to individuals without MGUS, and to examine the risk of arterial and venous thrombosis in individuals with MGUS and LC-MGUS.

5.1 METHODOLOGICAL CONSIDERATIONS

We conducted a cohort study using the participants of the AGES-Reykjavik Study, which has been described previously (Table 6).

Table 6. Characteristics of the study participants			
	MGUS[a]	**LC-MGUS[b]**	**No MGUS**
Total n[c] of cases (%)	297 (5.2)	52 (0.9)	5367 (93.9)
Men, n (%)	158 (53.2)	34 (65.4)	2222 (41.4)
Women, n (%)	139 (46.8)	18 (34.6)	3145 (58.6)
Median age, years (range)	78 (67-93)	82 (69-96)	76 (66-98)
Age group, n (%)			
Less than 70 years	21 (7.1)	1 (1.9)	528 (9.8)
70-79 years	146 (49.2)	16 (30.8)	3037 (56.6)
80-89 years	119 (40.0)	33 (63.5)	1677 (31.2)
90 years and older	11 (3.7)	2 (3.8)	125 (2.3)
MGUS isotype, n (%)			
IgG	158 (53.2)	N.A.	N.A.[d]
IgA	27 (9.1)	N.A.	N.A.
IgM	79 (26.6)	N.A.	N.A.
IgD	1 (0.3)	N.A.	N.A.
Biclonal	32 (10.8)	N.A.	N.A.
M-protein concentration, n			
>15.0 g/L	17 (5.7)	N.A.	N.A.
<15.0 g/L	145 (48.8)	N.A.	N.A.
Information missing	135 (45.5)	N.A.	N.A.
FLC[e] ratio			
0.26-1.65	167 (56.2)	0 (0.0)	N.A.
<0.26 / >1.65	130 (43.8)	52 (100.0)	N.A.

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]n: number, [d]NA: not applicable, [e]FLC: free light chain.

To study causes of death, we used a previously described categorization, and estimated mortality rate ratios for each cause of death using Cox proportional hazards model.[55,127] The cause-specific mortality estimates show the actual risk in the cohort of death from the causes

investigated. However, in a cohort of elderly individuals such as the one at hand, the participants are subject to several potential events that could impede the occurrence of other events. One example is, when looking at risk of death from malignant disease, one individual who dies from heart disease at five years of follow-up might have died from malignant disease at eight years of follow-up, if they had not been subjected to this so called competing event. If we are interested to know the real-world risk of malignant disease, for example, then we must take into account that some participants will, due to other causes of death, not be under risk to experience this event.

One approach to cause-specific hazards is to model the cause-specific hazard of each event or category of events separately, using a standard Cox regression model, and treat other (competing) events as censored observations. Another approach is Fine and Gray's extension of that method, which models the cumulative incidence function.[133] The cumulative incidence function is the probability sub-distribution function of failure from a specific cause.[134] Each of these methods can provide useful insights about the variables in the model. We were mostly interested in the pure effects of MGUS and LC-MGUS on the different causes of death, which are most easily visualized through the cause-specific hazard model. However, in real world scenarios, patients in particular will be interested to know what their actual risk is, considering all other causes that might play a role. We therefore chose to use both models in our analyses on cause of death.

To examine the risk of thrombosis, we had access to incidence of venous and arterial thrombosis in all subjects, both as first occurrence in the health care records used, and as cause of death. Information on incidence of disease had been collected for all participants from nine years before study baseline, and all through follow-up with a median follow-up time of 8.8 years (Figure 7). However, due to data limitations, we only had access to the first incidence of thrombosis for each individual. This was indeed our outcome of interest, although, as a consequence, all individuals who had a history of thrombosis before study baseline were censored from experiencing an event during follow-up, unless that event was cause of death (we had access to cause of death for all individuals).
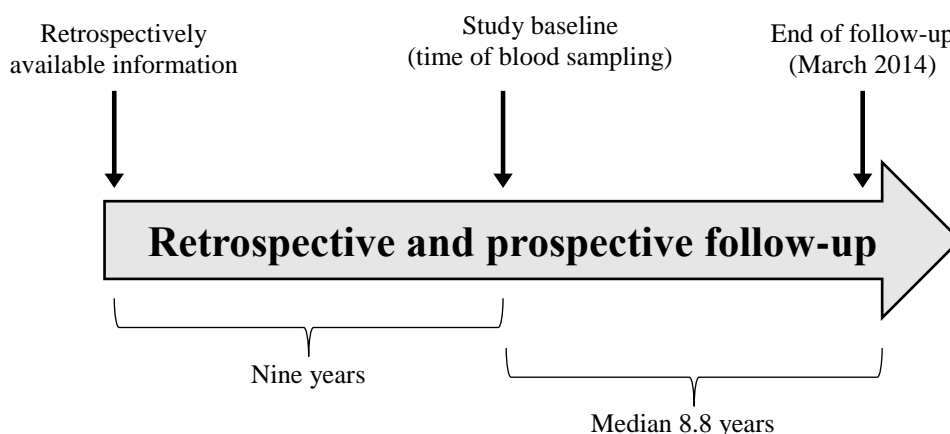

Figure 7. Study design of the AGES-Reykjavik Study cohort.

We aimed to work around this data limitation in two ways. Firstly, we did a cross-sectional analysis of study baseline information of the participants. Through logistic regression, we evaluated the association of a history of thrombosis, as assessed by a diagnosis of thrombosis from health care records as well as self-reported in the questionnaire, and MGUS status, and adjusted for age and sex as well as risk factors for venous and arterial thrombosis, respectively. Secondly, we used a Cox regression model to estimate the risk of incidence of thrombosis during follow-up. When assessing the risk of venous thrombosis (defined as pulmonary embolism, deep vein thrombosis, or other venous thrombosis), individuals with a history of venous thrombosis at baseline were excluded, and adjustments were made for comorbidity with obesity and cancer.[71,72,135] For the risk of arterial thrombosis (defined as acute coronary syndrome, ischemic stroke, or arterial embolism), individuals with a history of arterial thrombosis at baseline were excluded, and adjustments were made for known risk factors diabetes mellitus type II, hypertension, smoking, family history of arterial thrombosis, and serum cholesterol level.[136-141]

An alternative method would have been to move study baseline nine years back, and under the assumption that all individuals with MGUS or LC-MGUS detected through screening in 2002-2004 also had this condition nine years prior, to perform a retrospective prospective analysis using all individuals in the cohort. However, we chose to avoid this methodology, considering that even though MGUS is known to be present for a long time, it is impossible for us to know when it appeared in these individuals. Furthermore, such a study design would introduce an immortal time bias that would limit the generalizability of the results.

5.2 RESULTS AND DISCUSSION

In the analysis of causes of death (Paper II), we found that individuals with MGUS and LC-MGUS had an increased risk of death from the categories any cancer (HR = 1.7, 1.3-2.3, and HR = 2.3, 1.2-4.3, respectively) and any heart disease (HR = 1.4, 1.1-1.8, and HR = 1.8, 1.1-3.0), compared to individuals without MGUS. We noted with interest that the estimates were higher for LC-MGUS than for MGUS, even though the CIs were overlapping. Furthermore, individuals with LC-MGUS also had an increased risk of death from myeloid malignancy, amyloidosis, biliary/pancreatic disease, and psychiatric illness, compared to individuals without MGUS; although very few deaths occurred in these groups (Table 7).

The elevated risk of death from cancer was unsurprising to us, seeing as patients who progress from MGUS to lymphoproliferative disorders are likely to die from their malignant disease. Since we were interested in the survival patterns and causes of death among individuals with MGUS and LC-MGUS who did not undergo malignant transformation, the 56 subjects in the cohort who progressed to a lymphoproliferative disorder during follow-up were excluded.

Interestingly, the risk of death from any cancer (HR = 2.1, 1.1-4.1) and from the category any heart disease (HR = 1.8, 1.01-3.0) was still increased for LC-MGUS. The risks for MGUS were no longer statistically significant (any cancer HR = 1.3, 0.9-1.8, any heart disease HR = 1.3, 0.99-1.7). The category any heart disease comprised two subgroups; ischemic heart disease and other heart disease. Trying to characterize these risks further, we

looked at specific conditions in each category; a limited analysis due to very few deaths from each condition. We did however see that the risk of conditions in the subgroup other heart disease accounted for the increased risk in both LC-MGUS (other heart disease: HR = 2.5, 1.1-5.8) and MGUS (HR = 1.8, 1.1-2.7). The subgroup other heart disease included conditions such as rheumatic heart disease, cardiac valve disease, hypertension, cardiac failure, and different arrhythmias of the heart. Our analyses on competing risks did not substantially change the estimates.

Table 7. Risk of selected causes of death in MGUS and LC-MGUS, compared to without MGUS.

Cause of death	MGUS[a]			LC-MGUS[b]			No MGUS
	N.	HR[c]*	95% CI[d]	N.	HR*	95% CI	No.
Any cancer	51	1.7	1.3-2.3	10	2.3	1.2-4.3	552
Any hematologic malignancy	16	11.2	6.0-20.8	2	10.7	2.5-45.7	28
Multiple myeloma	11	∞	N.A.[e]	1	∞	N.A.	0
Waldenström macroglobulinemia	2	∞	N.A.	0	-	-	0
Other LD[f]	2	4.1	0.9-18.8	0	-	-	10
Myeloid malignancy	0	-	-	1	11.9	1.5-96.3	11
Any solid tumor	35	1.2	0.9-1.7	8	1.9	0.9-3.8	524
Amyloidosis	0	-	-	1	104.4	6.0-1826.7	1
Heart disease	61	1.4	1.1-1.8	15	1.8	1.1-3.0	745
Ischemic heart disease	37	1.1	0.8-1.5	9	1.3	0.7-2.5	510
Other heart disease	24	1.8	1.2-2.8	6	2.5	1.1-5.8	235
Psychiatric illnesses	3	0.6	0.2-1.8	3	3.3	1.1-10.7	97

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]HR: hazard ratio, [d]CI: confidence interval, [e]N.A.: not applicable, [f]LD: lymphoproliferative disorder. *Estimates are adjusted for age and sex.

The finding of an increased risk of death from heart disease is in line with findings in one previous study on causes of death in MGUS.[105] Our finding that the increased risk of death from heart disease is particularly prominent in individuals with LC-MGUS is especially interesting, and point at an increased risk of cardiovascular disease in individuals with LC-MGUS. This could be due to an undetected genetic, or environmental, susceptibility for both LC-MGUS and diseases of the heart or vascular system, or a common pathogenesis, or that one condition predisposes for the other. The association we found is no ground for conclusions on causality, but one might speculate that the FLC in individuals with LC-MGUS are somehow involved in the pathogenesis of cardiovascular disease. If this is the case, it is not necessarily the monoclonality in itself that is the underlying cause, but it could also be the overload of FLC that causes disease. It has been shown that elevated levels of polyclonal FLC are associated with increased mortality and, furthermore, predicts cardiovascular events in patients with diabetes.[142,143] A common etiology, or a strong connection between inflammation and atherosclerosis, could be one underlying

explanation.[144] Naturally, another explanation for the observed association could be that the individuals with LC-MGUS in our cohort suffer from undetected amyloidosis, which is causing both monoclonal FLC and heart disease.

At study baseline, a history of thrombosis was present in 30 (10.1%) individuals with MGUS, in 13 (25.0%) individuals with LC-MGUS, and in 642 (12.0%) of individuals without MGUS (Paper V). Arterial thrombosis was more common than venous thrombosis in all groups. The risk of having had an arterial thrombosis at baseline was significantly increased for individuals with LC-MGUS (OR = 2.5, 1.3-4.9), and remained increased in a model adjusted for age and sex (OR = 2.0, 1.03-3.8), but not when additional risk factors were added to the model (OR = 1.9, 0.93-3.8), which could be interpreted as a power issue (Table 8).

Similarly, during follow-up, the risk of experiencing an arterial thrombosis was almost doubled (HR = 1.9, 1.1-3.2) for individuals with LC-MGUS, compared to individuals without MGUS. When adjusting for age, sex, and risk factors for arterial thrombosis, the risk estimate was not statistically significant (HR = 1.3, 0.7-1.3).

No increased risk of venous thrombosis was found in individuals with MGUS or with LC-MGUS, compared to individuals without MGUS, in the retrospective or the prospective analysis.

Table 8. A history of thrombosis at baseline in individuals with LC-MGUS, compared to individuals without MGUS.

	LC-MGUS[a]				No MGUS[b]
	N.[c]	OR[d] (95% CI[e])			N.
		Crude	Adjusted for age and sex	Multivariate analysis[†]	
Any thrombosis	13 (25.0%)	2.5 (1.3-4.6)	1.9 (1.00-3.6)	1.9 (0.94-3.7)	642 (12.0%)
Arterial thrombosis	12 (23.1%)	2.5 (1.3-4.9)	2.0 (1.03-3.8)	1.9 (0.93-3.8)	565 (10.5%)
Venous thrombosis	2 (3.9%)	2.5 (0.6-10.5)	2.0 (0.5-8.5)	2.0 (0.5-8.5)	84 (1.6%)

[a]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [b]MGUS: monoclonal gammopathy of undetermined significance, [c]N.: number of individuals, [d]OR: odds ratio, [e]CI: confidence interval. [†]Results adjusted for age, sex, smoking, hypertension, cholesterol, diabetes mellitus type II, and family history of arterial thrombosis, and age, sex, body mass index, previous or current cancer, and family history of venous thrombosis, respectively.

To summarize, our findings regarding risks of arterial and venous thrombosis are conflicting; our inability to detect an increased risk of thrombosis in individuals with MGUS is contradictory to findings reported in previous studies, where MGUS is associated with increased risks of thrombosis.[81,82] Our analysis could not detect an increased risk of a history of either arterial or venous thrombosis in individuals with MGUS at baseline, and no increased risk during follow-up was observed. We believe that the previously published investigations have been biased due to clinically detected MGUS, and it is the comorbidity in these MGUS patients that has been the true driver behind the risk of thrombosis previously observed.

The findings of an increased risk of arterial thrombosis, both at baseline and prospectively, in individuals with LC-MGUS, should be interpreted with caution. The

observed increased risks were not statistically significant in a multivariate analysis. This could be due to no real excess risk of thrombosis in LC-MGUS, and that the increased risk we detected at baseline was confounded by age or other variables insufficiently for. Another plausible explanation is that our analysis suffers from a power issue, and a larger, prospective study of more individuals and longer follow-up time is necessary before any strong conclusions can be drawn. However, the findings of an elevated risk of arterial thrombosis among individuals with LC-MGUS are especially interesting in light of the previous findings on heart disease being a prominent cause of death among individuals with LC-MGUS. The results from our analyses on thrombosis lend strength to the previously mentioned theory of an increased risk of cardiovascular disease in individuals with LC-MGUS, due to common susceptibility, shared pathogenesis, or that one condition inclines the individual towards the other.

The major limitation of our investigations detailed above was, as initially explained, the limitation of data on thrombotic events. We had access only to information on first thrombotic event, and on causes of death. Consequently, all subjects with a first incidence of any thrombosis before study baseline (and detection of MGUS or LC-MGUS), could have one or several more events of thrombosis during follow-up without our knowledge, up until date of death, when causes of death was available for all deceased individuals. This, naturally, introduced a selection bias to our study design, and we tried to compensate for this by restricting the prospective analysis only to those participants with no listed or reported prior venous or arterial thrombosis, respectively. Of course, the possibility remains of them having had an unnoted thrombosis before nine years prior to baseline, but this should then be the case for participants in all study groups (MGUS, LC-MGUS, and without MGUS) alike – unless, of course, individuals in one group such as LC-MGUS are much more prone to thrombosis, experience their events earlier than the other groups in the cohort, and are then either protected from further thrombosis due to treatment, or more prone to develop several more incidences of thrombosis. This is information we do not have access to.

A further limitation of this investigation is, as previously stated, the inability to validate individual medical records, but importantly, there are some strengths as well. The cohort design, although impaired by the data limitations discussed, is still an advantageous study design for the study of the impact of exposures on outcomes of interest. We had the possibility to adjust for several risk factors in our analyses. Furthermore, important risk factors and covariates in the model were primarily assessed through register data, and not through self-reporting, thus minimizing recall bias.

In conclusion, we found that individuals with MGUS or LC-MGUS who do not progress to a lymphoproliferative disorder still have an increased risk of death from cancer and from heart disease. The increased risk of death from heart disease is higher in LC-MGUS than in MGUS, and stems from elevated risk of death from the subgroup non-ischemic heart disease. Furthermore, we found that the risk of a history of thrombosis was higher in LC-MGUS, but not in MGUS, compared to individuals without MGUS. We found that the increased risk was due to arterial thrombosis and not venous thrombosis.

Our findings during these investigations are of clinical importance in the sense that they speak against the previously held belief that individuals with MGUS are at an increased risk of thrombosis. However, the findings that individuals with MGUS and LC-MGUS alike are at increased risk of death from both cancer and heart disease are troubling and lends further strength to the recommendations of clinical follow-up of these patients. Additionally, this is

to our knowledge the first time survival, cause of death and risk of thrombosis is studied in individuals with LC-MGUS, and the results that point towards increased risk of thrombosis in general, and arterial thrombosis in particular, should be of interest to clinical hematologists as well as the scientific community. Our findings indicate that MGUS and LC-MGUS are two clinically distinct conditions, with differing risk profiles, and that between MGUS and LC-MGUS, it is the latter condition that is the most hazardous – which further underlines the absolute need for an adequate and easily applicable definition of LC-MGUS. However, LC-MGUS is less common than MGUS, affecting only 1% of the elderly population. Bearing in mind that the risk estimates in our study are modest, the true absolute difference in survival between individuals with LC-MGUS and individuals without MGUS might not be of clinical relevance. Nevertheless, with better prediction models and a more thorough understanding of how the underlying causes of increased risk of heart disease and thrombosis interact with LC-MGUS, in the future we might be able to single out individuals with LC-MGUS who could benefit from cardiovascular risk prevention strategies.

6 FUTURE DIRECTIONS

In the future, we hope to see MGUS and LC-MGUS even further characterized, for example through a large screening study with long follow-up time. If future investigations can isolate risk factors for mortality and morbidity in these conditions, prediction models could perhaps be developed, and possibly even treatment of high risk MGUS and/or LC-MGUS could be considered.

Furthermore, we recommend scientific attention to be focused on LC-MGUS, to characterize the clinical, genetic, and biochemical profiles of this condition, with the purpose of understanding the connection to cancer, to heart disease, and to thrombosis. With a better understanding of the pathogenesis, morbidity and mortality in individuals with LC-MGUS might be decreased, or even prevented.

7 SUMMARY AND CONCLUSIONS

MGUS and LC-MGUS can be found through screening in approximately 5% and 1% in a cohort of elderly individuals (age range 59-98 years), respectively. Here we suggest a revised definition of LC-MGUS that is easily applicable, captures fewer individuals yet all with a clinically relevant condition, and can be used regardless of renal function. The proposed definition will decrease unnecessary health-care costs as well as reduce the burden of anxiety among affected patients and their families.

Individuals with MGUS and LC-MGUS appear to have inferior survival compared to individuals without MGUS, even when they do not progress to a lymphoproliferative disorder. In addition, individuals with LC-MGUS seem to have inferior survival than those with MGUS.

A preceding autoimmune disease has a negative effect on survival in MGUS and also in MM, and the effect persists regardless of M-protein isotype or M-protein concentration.

MM patients with a prior established MGUS have a better survival than those without. Among the former, a low M-protein concentration at MGUS diagnosis predicts inferior MM survival. Our findings lend support to the recommendations of a close clinical follow-up of all individuals with MGUS.

MGUS and LC-MGUS seem to be two clinically distinct conditions, with differing risk profiles. Individuals with MGUS and LC-MGUS have an increased risk of death from cancer and from heart disease. The elevated risk is greater in LC-MGUS than in MGUS, and appears to stem from non-ischemic heart disease.

Overall, individuals with MGUS do not seem to have an increased risk of venous or arterial thrombosis. However, individuals with LC-MGUS appear to have an elevated risk of arterial thrombosis. This could be indicative of an underlying genetic susceptibility to cardiovascular disease and LC-MGUS, or to the FLC overload in LC-MGUS contributing to cardiovascular morbidity.

A future more detailed characterization of the genetic, biochemical, and clinical profile of LC-MGUS, will hopefully better explain the association with cancer, heart disease, thrombosis, and inferior survival observed in this condition.

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Light-chain Monoclonal Gammopathy of Undetermined Significance: A New Definition

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Running title: Light-chain MGUS: a new definition

ABSTRACT

Introduction
Limited information is available regarding light-chain monoclonal gammopathy of undetermined significance (LC-MGUS). Our objective was to establish the most clinically useful and correct definition of LC-MGUS and describe its distribution between age-groups, sexes, and different ethnical groups.

Methods and Material
In total, 5,725 individuals from the AGES-Reykjavik Study and 5,916 individuals from the Prostate, Lung, Colorectal and Ovarian (PLCO) Study were screened by subjecting blood samples to serum protein electrophoresis, immunofixation, and free light-chain assays. Statistical modeling was performed taking into account distributions of normal values of kappa and lambda, renal function, and free light-chain ratio.

Results
Applying the previous definition of LC-MGUS resulted in a 4.8% prevalence of LC-MGUS, whereof 96% kappa LC-MGUS. Based on findings from the two cohorts, an improved definition of LC-MGUS was developed: (1) an abnormal free light-chain ratio (<0.26 or >1.65), (2) an elevated involved light chain (40 mg/L or higher), (3) no M-protein on serum protein electrophoresis or immunofixation, and (4) no evidence of end-organ damage that can be attributed to the plasma cell proliferative disorder. Using this definition, 52 and 57 LC-MGUS cases were identified in the two cohorts, respectively. The prevalence was 0.9-1.0%. The prevalence of LC-MGUS increased with age (p<0.001), was higher in men (p<0.001), and differed between whites, blacks, and Asian/Pacific Islanders (0.7%, 2.9%, and 0.2%, respectively). LC-MGUS cases progressed to light-chain multiple myeloma, AL-amyloidosis, and diffuse large B cell lymphoma.

Conclusion
The revised definition will lead to fewer individuals being diagnosed with LC-MGUS, which will eliminate the burden of anxiety among false positive cases, and decrease unnecessary health-care costs from clinical work-up and lifelong monitoring.

INTRODUCTION

Monoclonal gammopathy of undetermined significance (MGUS) is defined by a monoclonal-(M)-protein on serum protein electrophoresis (SPEP) or immunofixation electrophoresis (IFE) without underlying multiple myeloma (MM) or other lymphoproliferative malignancy.[1] MM and amyloidosis are consistently preceded by MGUS.[2-4] Current expert opinions recommend indefinite follow-up of individuals with MGUS.[5,6] Indeed, in support of these opinion documents, two recent population-based studies independently showed that early detection of MM (through follow-up of MGUS) is associated with a 13-14% better survival, compared to patients presenting with MM without a previously known MGUS diagnosis.[7,8]

Approximately 20% of MM patients do not have a detectable M-protein in blood or in urine, but expression of light chains in blood and in urine (so called light-chain MM).[9] Similar to MM with an M-protein being consistently preceded by MGUS[2], light-chain MM is consistently preceded by light-chain MGUS (LC-MGUS), detectable through analysis of free light chains in the blood.[2,3]

Currently, limited information is available regarding LC-MGUS. [9,10] Based on the results from a retrospective cohort study, the proposed definition of LC-MGUS was a pathological ratio of the concentration between free light chains kappa and lambda (FLC-ratio; normal reference: 0.26-1.65) in combination with an increased concentration of the light-chain involved (free kappa >19.4 mg/L or free lambda >26.3 mg/L)[9], or outside the normal reference 0.37-3.1 in individuals with renal failure, without underlying malignant plasma cell disorder or M-protein on SPEP.[11,12] The upper and lower limits of kappa and lambda were established using the normal distribution of free light chains in 282 individuals.[13]

We conducted FLC analysis by screening two large independent population-based cohorts including more than 11,000 individuals with up to 9 years of follow-up, and included detailed information on renal function. Given the lack of data on LC-MGUS across racial groups, we further assessed the prevalence of LC-MGUS by race. Based on extensive analyses in this work, we provide an updated definition of LC-MGUS, taking into account production of the involved light-chain and at the same time minimizing the risk of missing individuals with monoclonal disease who will later progress to lymphoproliferative disease.

METHODS

Exploratory cohort - AGES Reykjavik Study cohort

We performed a large, population-based cohort study using the longitudinal cohort of the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik Study), which has been described previously.[14] Briefly, the Reykjavik Study is a longitudinal cohort study of men and women (30,795) born 1907-1934 in Iceland who were followed from 1967 onward in the Reykjavik Study by the Icelandic Heart Association. In 2002, 5,764 persons randomly chosen from survivors of the Reykjavik Study cohort were re-examined for the AGES-Reykjavik Study, with a response rate of 75%. Participants in the AGES-Reykjavik Study examination in 2002-2006 completed a questionnaire, underwent clinical examination,

laboratory testing, and radiological examinations. Study baseline was date of first visit in AGES-Reykjavik Study. The participants in the cohort are followed prospectively, and information on incidence of disease and date of death is collected annually through hospital, nursing home, and mortality records. End of follow-up for the analyses on survival and lymphoproliferative outcome was March 31, 2014. The study was approved by the National Bioethics Committee of Iceland (VSN-00-063-V35), the Icelandic Data Protection Authority, the institutional review board for the NIH National Institute on Aging in the USA, and the National Ethics Committee of Stockholm, Sweden. Signed informed consent was obtained from all participants.

Testing for monoclonal proteins

Blood samples from all participants, collected in 2002-2006, were screened for M-protein using SPEP and IFE (Helena Laboratories, Beaumont, Texas, USA) as well as free light chain analysis (FLC analysis) (FREELITE®, The Binding Site Ltd, Birmingham, UK) in 2012-2013. The FLC assay measures free kappa and lambda light-chain concentrations and the kappa to lambda ratio.[13] Individuals with a lymphoproliferative diagnosis at baseline were identified and excluded. We used the standard definition of MGUS, which denotes presence of one or more M-protein bands on SPEP and/or IFE.

Explorative analyses for optimal LC-MGUS definition - AGES-Reykjavik Study cohort

Analyses based on previous definition of LC-MGUS were performed, in addition to analyses taking renal function into account and using the 97.5th percentile as the proxy for the upper normal limit for kappa and lambda and created new cut-off using these definitions. The prevalence of LC-MGUS was determined in each step.

First, we performed an analysis based on the previously suggested definition of LC-MGUS, where samples were defined as LC-MGUS if they had a pathological FLC ratio (outside of reference range 0.26-1.65) in combination with an absence of M-protein on SPEP or IFE, and an increase in the involved light-chain (kappa >19.4 mg/L or lambda > 26.3 mg/L), regardless of kidney function.

Secondly, we applied the same method to samples from individuals with normal kidney function, but applied the renal reference range (0.37-3.1) to individuals with moderate (glomerular filtration rate, GFR, <60 mL/min) or severe kidney failure (GFR <30 mL/min).

Thirdly, we drew kernel estimates of the density function, with individuals with MGUS excluded, to investigate the empirical statistical distribution of normal values. We identified the 97.5th percentile as the proxy for the upper normal limit for kappa and lambda and for the FLC ratio, in the entire cohort as well as in individuals with kidney failure only, respectively.

In a fourth step, the results from these analyses were used to create new cut-off values for the increased light-chain concentrations, based on the 97.5th percentile, and these new values were applied to the whole cohort as detailed above.

In each step, the prevalence of LC-MGUS was determined, as well as the number of cases of kappa light-chain and lambda LC-MGUS. Difference in prevalence between men and women was assessed with a chi-squared test.

Ambiguous reactions were detected on SPEP for eight samples, of which two had visible IgG bands on IFE and were grouped as MGUS. The other six were grouped as LC-MGUS if they fulfilled the criteria for LC-MGUS or as no MGUS if they did not. A suspected M-protein band was observed on SPEP for four subjects who, on serum IFE, had free kappa or lambda only and no heavy chain expression. These were grouped as LC-MGUS if they fulfilled the criteria for LC-MGUS, or no MGUS if they did not.

Progression of LC-MGUS

To capture individuals who progressed to lymphoproliferative disease, we used information from the Icelandic Cancer Registry and hospital records. Medical records for individuals who progressed were assessed and clinical information recorded, including kappa/lambda clonality in the tumor sample when available. All lymphoproliferative diagnoses were validated and medical history and clinical status noted.

Validation of LC-MGUS definition - PLCO cohort

Individuals from the Prostate, Lung, Colorectal and Ovarian (PLCO) cancer screening trial were used for replication. The PLCO study population has been described previously.[15,16] In summary, from 1992 to 2001 more than 150,000 participants aged 55 to 74 were randomized to either attend a cancer screening regimen for the included cancer forms, or to routine medical care. For the current analyses, individuals were selected among white, black, and Asian/Pacific Islander participants in the screening arm of the trial who had available serum and were not diagnosed with a lymphoproliferative malignancy prior to study entry or during follow-up (through January 2012). Black and Asian/Pacific Islander participants were over-sampled for this selection. Among those who met the study inclusion criteria, a random sample of participants were selected within strata by sex and age at baseline for each racial group. A total of 5,916 individuals were selected, including 3,999 whites, 955 blacks, and 962 Asian/Pacific Islanders. As described above for the explorative analyses in the AGES-Reykjavik Study, we performed analyses based on the previously published definition of LC-MGUS.[9,10] To investigate whether the cut-off values found in the AGES-Reykjavik Study were similar in the PLCO Study, we performed analyses to investigate the distribution of normal values, where 97.5 percentile of the population was estimated for the FLC ratio, the kappa concentration, and the lambda concentration in the subset of PLCO controls defined above. We then applied the new LC-MGUS definition to the cohort. The prevalence of LC-MGUS was determined with each definition, as well as the number of cases of kappa LC-MGUS and lambda LC-MGUS. The prevalence of LC-MGUS was estimated in whites, blacks, and Asian/Pacific Islanders separately.

RESULTS

From the AGES-Reykjavik study cohort of 5,764 participants, 16 participants were excluded due to missing blood samples, one was excluded due to missing consent form, and 22 patients with lymphoproliferative disorders (MM, Waldenström macroglobulinemia, chronic lymphocytic leukemia, amyloidosis, or lymphoma) at baseline were excluded. Blood samples from the remaining 5,725 participants were analyzed for MGUS and 300 (5.2%) had one, two, or three M-protein bands on SPEP (Table 1). Median follow-up time was 8.8 years.

Table 1. Characteristics of the 5,725 included participants from the AGES-Reykjavik Study and 5,916 included participants from the PLCO Study

	AGES			PLCO		
	MGUS[a]	LC-MGUS[b]	No MGUS	MGUS	LC-MGUS	No MGUS
Total n[c] of cases (%)	300 (5.2)	52 (0.9)	5,373 (93.9)	283 (4.8)	57 (0.96)	5,576 (94.3)
Men, n (%)	159 (53.0)	34 (65.4)	2,226 (41.4)	201 (71.0)	41 (71.9)	3,452 (61.9)
Women, n (%)	141 (47.0)	18 (34.6)	3,147 (58.6)	82 (28.0)	16 (28.1)	2,124 (38.1)
Median age, years (range)	78 (67-93)	82 (69-96)	76 (66-98)	72 (60-80)	71 (60-79)	69 (59-80)
Age group, n (%)						
** Less than 70 years**	21 (7.0)	1 (1.9)	529 (9.9)	107 (37.8)	22 (38.6)	2,876 (51.6)
** 70-79 years**	148 (49.3)	16 (30.8)	3,039 (56.6)	173 (61.1)	35 (61.4)	2,685 (48.2)
** 80-89 years**	120 (40.0)	33 (63.5)	1,679 (31.2)	3 (1.1)	0 (0)	15 (0.3)
** 90 years and older**	11 (3.7)	2 (3.8)	126 (2.3)	0 (0)	0 (0)	0 (0)
MGUS isotype, n (%)						
** IgG**	159 (53.0)	N.A.[d]	N.A.	179 (63.3)	N.A.	N.A.
** IgA**	27 (9.0)	N.A.	N.A.	32 (11.3)	N.A.	N.A.
** IgM**	81 (27.0)	N.A.	N.A.	55 (19.4)	N.A.	N.A.
** IgD**	1 (0.3)	N.A.	N.A.	0 (0)	N.A.	N.A.
** Biclonal**	32 (10.7)	N.A.	N.A.	17 (6.0)	N.A.	N.A.
M-protein concentration, n (%)[e]						
** >15.0 g/L**	17 (10.4)	N.A.	N.A.	9 (4.2)	N.A.	N.A.
** <15.0 g/L**	147 (89.6)	N.A.	N.A.	207 (95.8)	N.A.	N.A.
FLC[f] ratio						
** 0.26-1.65**	168 (56.0)	0 (0.0)	4972 (92.5)	176 (62.2)	0 (0)	5,347 (95.9)
** <0.26 / >1.65**	132 (44.0)	52 (100.0)	401 (7.5)	107 (37.8)	57 (100)	229 (4.1)
Race						
** White**	N.A.	N.A.	N.A.	185 (65.4)	27 (47.4)	3,787 (67.9)
** Black**	N.A.	N.A.	N.A.	70 (24.7)	28 (49.1)	857 (15.4)
** Asian/Pacific Islander**	N.A.	N.A.	N.A.	28 (9.9)	2 (3.5)	932 (16.7)

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]n: number, [d]N.A.: not applicable, [e]available for 164 subjects in AGES, for 216 subjects in PLCO, [f]FLC: free light chain analysis.

LC-MGUS in AGES-Reykjavik Study cohort

Among 5,725 individuals, a total of 453 participants (7.9%) had a pathological FLC-ratio (normal reference: 0.26-1.65) but normal SPEP. Out of these, 275 participants (4.8% of the whole AGES-Reykjavik Study) had a pathological FLC-ratio in combination with an increased concentration of the light chain involved (kappa >19.4 mg/L or lambda >26.3 mg/L), resulting in a total of 264 kappa and 11 lambda cases, thus a kappa prevalence of 96% (Figure 1a).

Secondly, we applied the same method, but using the renal reference range of the FLC-ratio (0.37-3.1) in combination with an increased concentration of involved light-chain (>19.4 mg/L or >26.3 mg/L, respectively) to all individuals with a moderately to severely impaired (GFR<60 mL/min) renal function. This resulted in 135 individuals with LC-MGUS, 124 kappa and 11 lambda, with a kappa prevalence of 92%. We then applied the renal reference range only to individuals with severe renal failure (GFR<30 mL/min), which resulted in 249 kappa LC-MGUS and 10 lambda LC-MGUS, with a kappa prevalence of 96%.

Given the kappa-biased results and the corresponding high prevalence of LC-MGUS in the AGES-Reykjavik Study cohort, we performed additional cut-off defining analyses. In the entire cohort, excluding the 300 individuals with MGUS, the 2.5th and the 97.5th percentile of the FLC-ratio was 0.6-2.0, of the kappa concentration 7.7-40.6 mg/L, and the lambda concentration 7.2-36.3 mg/L (Figure 1a). In individuals with moderately impaired renal function (GFR $\geqslant$30-<60 mL/min), 2,131 individuals, the 2.5th to 97.5th percentiles of the FLC-ratio, kappa and lambda concentrations were 0.7-2.0, 9.0-43.6, and 8.0-38.1, respectively. Out of these, 32 individuals had a skewed FLC-ratio in combination with a concentration of the light-chain involved of more than 40.0 mg/L. In individuals with severely impaired renal function (GFR<30 mL/min), 105 individuals, the 2.5th to 97.5th percentile were 0.5-2.2, 14.8-112.6, and 13.0-160.3, respectively. Out of these, 11 individuals had a skewed FLC-ratio in combination with a concentration of the light chain involved >40.0 mg/L.

Finally, as the distribution of log-transformed kappa and lambda values resembled the normal distribution, we evaluated the effect of using the 97.5th percentile as a cut-off for normal values for the involved light-chain. Using a definition of LC-MGUS as a pathological FLC-ratio (normal reference: 0.26-1.65), regardless of renal function, in combination with an increased concentration of more than 40.0 mg/L of the light-chain involved, resulted in 52 LC-MGUS cases, of which 41 were kappa LC-MGUS and 11 lambda LC-MGUS, with a kappa prevalence of 79%. According to the new definition, the prevalence of MGUS (5.2%) and LC-MGUS (0.9%) was 6.1% using the new revised LC-MGUS criteria (Table 1).

Among individuals younger than 70 years, the prevalence of MGUS and LC-MGUS was 3.8% and 0.2%, respectively. Among individuals 70-79 years, the prevalence of MGUS and LC-MGUS was 4.6% and 0.5%, respectively. Among individuals 80-89 years, the prevalence of MGUS and LC-MGUS was 6.6% and 1.8%, respectively. Among individuals 90 years or older, the prevalence of MGUS and LC-MGUS was 7.9% and 1.4%, respectively (Figure 2a). The prevalence of LC-MGUS was higher in men (0.6%) than women (0.3%) (p<0.001).

Figure 1. The distribution of serum free kappa and lambda concentrations among individuals without M-protein on serum protein electrophoresis (the 97.5[th] percentile, prior cut-offs for LC-MGUS, and 40 mg/L are marked) in a) the AGES-Reykjavik Study and b) the PLCO Study. The x axis is on a log transformed scale with base 10.
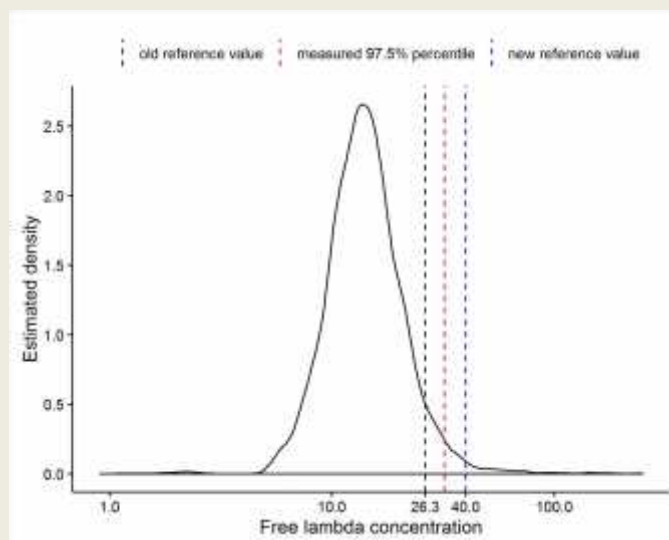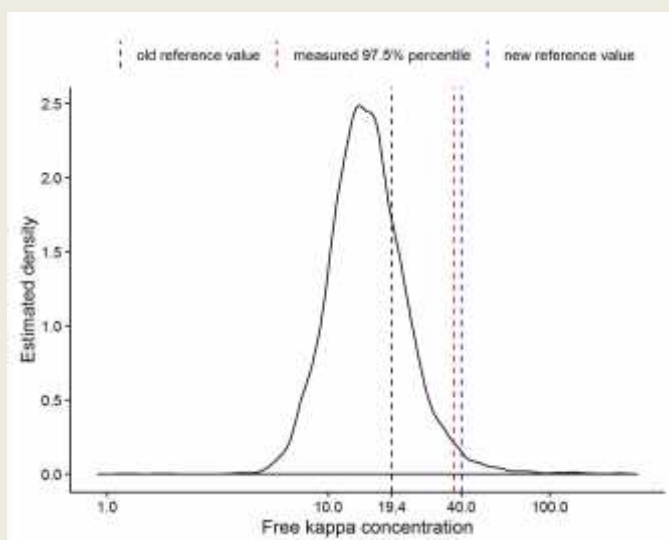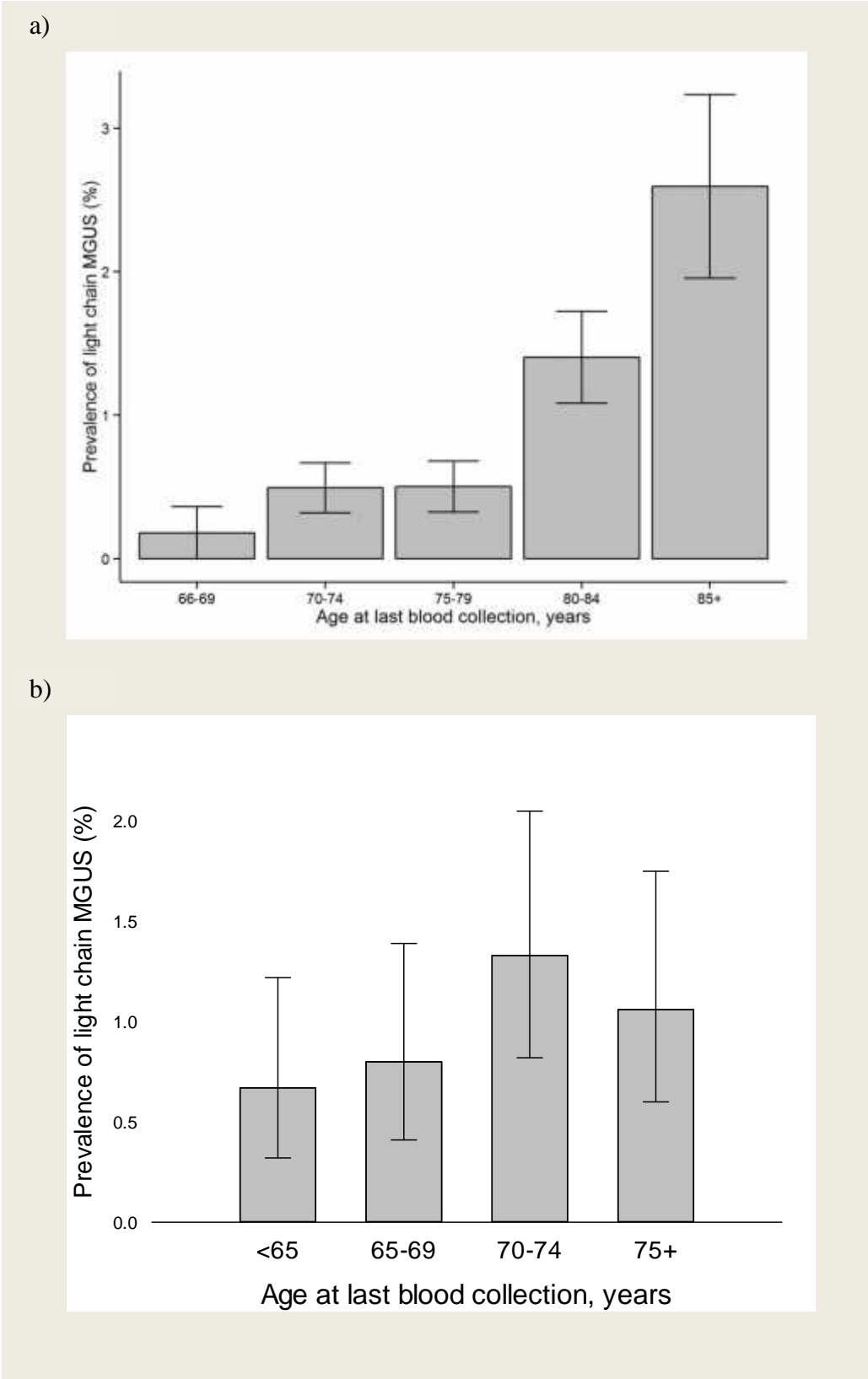
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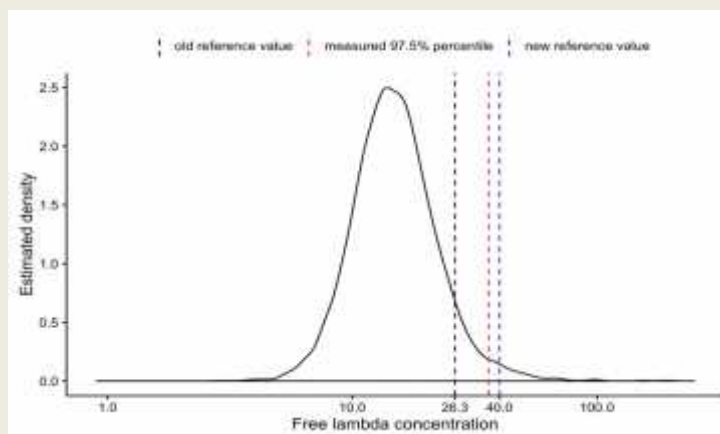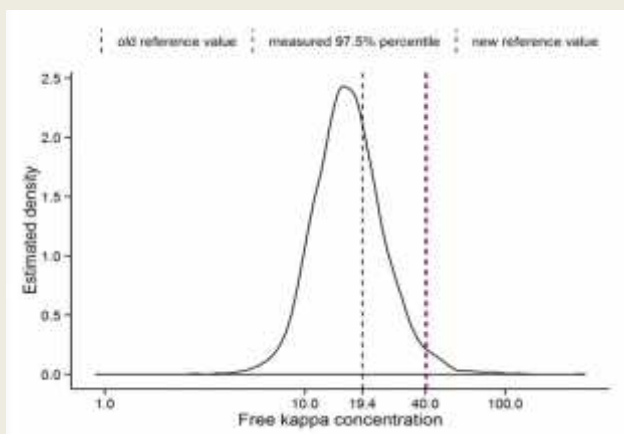
b)

Figure 2. The prevalence of LC-MGUS in a) the AGES-Reykjavik Study and b) the PLCO Study, stratified by age and last blood collection (PLCO).

Progression of LC-MGUS in AGES-Reykjavik Study cohort

Three individuals (patients 1, 2 and 3), fulfilled the previous criteria for LC-MGUS [9] and subsequently progressed to a lymphoproliferative disorder; one with light-chain MM, one with AL-amyloidosis, and one with diffuse large B-cell lymphoma (DLBCL) (Table 2). These individuals are all captured as LC-MGUS by our new revised definition (Table 3).

Table 2. Individuals who progressed from LC-MGUS[a] to lymphoproliferative disease in the AGES-Reykjavik Study

ID	Free kappa conc.[b]	Free lambda conc.*	FLC[c] ratio	Age	Sex	Status	LP[d] dx[e]	Days to dx	GFR[f]	Prev.[g] definition	New definition
1	31.07	151.07	0.21	77	M	dead	Amyloidosis	542	66.7	Yes	Yes
2	604.05	16.10	37.51	73	M	dead	MM[h]	709	31.8	Yes	Yes
3	43.12	23.51	1.83	72	M	dead	DLBCL	1,079	67.6	Yes	Yes
4	24.87	13.08	1.90	75	F	alive	DLBCL[i]	1,138	50.3	No	No

[a]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [b]in mg/L, [c]FLC: free light chain, [d]LP: lymphoproliferative disease, [e]dx: diagnosis, [f]GFR: glomerular filtration rate in milliliters per minute per 1.73 m^2, calculated through the Modification of Diet in Renal Disease formula, which takes into account creatinine level, age, and sex[30], [g]Prev.: previous, [h]MM: multiple myeloma, [i]DLBCL: diffuse large B-cell lymphoma.

A 72-year-old woman (patient 4) developed DLBCL 1,138 days (3.1 years) after the blood sample was drawn and had at that time neither MGUS nor LC-MGUS. She had an elevated FLC-ratio at the time of blood sampling and a kappa concentration of 24.9 mg/L and lambda of 13.1 mg/L. Her GFR was 50 mL/min, and therefore she did not fulfill either the prior[9] or the new criteria for LC-MGUS.

Table 3. New revised criteria for LC-MGUS[a]

1) Abnormal free light-chain ratio (normal reference: 0.26-1.65)[b,c]
2) Elevated involved light chain (40 mg/L or higher)
3) No immunoglobulin heavy chain M-spike by SPEP/IFE[d]
4) No evidence of end-organ damage that can be attributed to lymphoproliferative disorder

[a]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [b]values based on the serum Freelite assay (The Binding Site Group, Birmingham, UK), [c]regardless of renal function, [d]SPEP: serum protein electrophoresis and IFE: immunofixation.

Validation in PLCO cohort

Among 5,916 control subjects from the PLCO cohort, a total of 283 (4.8%) had MGUS (Table 1, data presented for time of blood sample collection). Of the remaining 5,633, a total of 286 (5.1%) had an abnormal FLC-ratio along with an increased involved light-chain. Out of these, 271 were kappa and 15 were lambda, with a kappa prevalence of 95%. As shown in Figure 1b, in individuals without MGUS, the 97.5th percentile of kappa and lambda was 37.3 mg/L and 32.3 mg/L, respectively. The 2.5th percentile of kappa and lambda was 7.3 mg/L and 6.8 mg/L, respectively. The 2.5th and the 97.5th percentile of the FLC-ratio was 0.6 and 1.8.

Using the cut-off of 40.0 mg/L for the involved light-chain together with an abnormal FLC ratio (normal reference 0.26-1.65) resulted in 57 individuals with LC-MGUS, of which 42 were kappa and 15 lambda, with a kappa prevalence of 74% (Table 1, and Figure 1b).

The prevalence of LC-MGUS among whites was 0.7% (27 out of 3,999), among blacks 2.9% (28 out of 955), and among Asian/Pacific Islanders 0.2% (2 out of 962). The prevalence of LC-MGUS increased with age and was 0.7% (10 out of 1,502), 0.8% (12 out of 1,503), 1.3% (20 out of 1,502), and 1.1% (15 out of 1,409), in the age groups <65 years, 65-69, 70-74, and >75 years of age, respectively (Figure 2b).

DISCUSSION

The importance of MGUS as a precursor condition to MM and related lymphoproliferative diseases has been well established, but the significance of LC-MGUS is less defined.[2,3] In this large international investigation based on two independent screening studies, including more than 11,000 individuals from Europe and the USA, we developed a revised and improved definition of LC-MGUS based on four criteria: (1) an abnormal FLC-ratio (normal reference: 0.26-1.65), (2) an elevated involved light chain (40 mg/L or higher), (3) no M-protein by SPEP/IFE, and (4) no underlying lymphoproliferative disease (Table 3). This revised and improved definition is valid independently of the individual's kidney function.

MGUS is a difficult condition to study, due to its asymptomatic nature, and LC-MGUS even more so since the there is no gold standard definition in the same way as presence of an M-protein on SPEP is for MGUS. Indeed, until date, knowledge of free light chain in general and LC-MGUS in particular has been based on small studies restricted to Caucasians.[9,10,13] We were motivated to perform a large screening study to establish the most clinically useful and correct definition of LC-MGUS, and describe its distribution between age-groups, sexes, and different ethnical groups, and we consider it reasonable to change the previously suggested definition in light of new data from this population-based study.

The revised and improved definition of LC-MGUS was reached through a large screening effort, extensive statistical modeling including data on kidney function, and robust study design allowing replication of the results in two independent cohorts. The consequences are that we are still able to catch the important cases of LC-MGUS, but alongside fewer "false positives". The direct clinical implications are that the revised definition will lead to considerably fewer individuals being diagnosed with LC-MGUS. This, in turn, will reduce the

burden of anxiety among (false positive) cases that do not meet the revised and improved definition but only have an abnormal FLC-ratio. On a population-wide health care level, this will decrease unnecessary costs driven by clinical work-up and lifelong monitoring. Using the previous definition, for every 100,000 individuals subjected to FLC analysis, 5,096 individuals would be diagnosed with LC-MGUS and monitored clinically for signs of progression, whereof 4,100 followed unnecessarily. The new definition, however, will accurately identify around 958 individuals per 100,000 FLC analyses performed, who need clinical monitoring and information about risk of progression. Thus, our new, improved definition saves more than 4,000 individuals per 100,000 tested from pointless examinations and possible anxiety.

When applying the previous definition of LC-MGUS, the prevalence of LC-MGUS was substantially higher (4.8%) than previously reported (0.6%), with an excess of kappa-restricted cases suggesting a well-described kappa bias.[9,13] In contrast to previous studies, we were able to use information on renal function in our modeling, however, applying the renal reference range to individuals with renal failure did not remove the kappa bias. The finding that the 97.5[th] percentile of the distribution of kappa and lambda levels was higher than previously thought indicated 40 mg/L to be a more meaningful cut-off, which is well in line with findings from traditional MGUS where it has been estimated that at least 200 grams of tumor cells are needed to be picked up as a band on SPEP.[17] The previously suggested upper limits for free kappa (19.4 mg/L) and lambda (26.3 mg/L) light chains were also established using reference intervals and the normal distribution in a small population, however, these were based on only 282 individuals of various ages.[13] Our results were successfully replicated in a large independent US study, the PLCO cohort, and the prevalence of LC-MGUS was established to be 0.9% in both cohorts. To ensure the clinical validity of the revised LC-MGUS definition, analyses in relation to outcomes were performed, and demonstrated that clinically important cases of LC-MGUS were captured accurately.

This is the first study to estimate racial disparity in the prevalence of LC-MGUS. We found that the prevalence of LC-MGUS was almost five times higher in blacks compared to whites. There is a marked racial disparity in the incidence of MM and MGUS, with African-American blacks and men from Ghana having a twofold or greater risk of MM and MGUS compared with whites.[18-23] Further studies are required to assess the risk of progression from LC-MGUS among blacks and evaluate possible differences in prognosis and progression in relation to ethnic groups. In addition, this disparity may affect counseling efforts. Similar to patterns established for MGUS[24], we found that the prevalence of LC-MGUS increases by older age and is more common in men.

As opposed to the two previously published studies, we had information on GFR in almost all individuals in the AGES-Reykjavik Study, and could therefore evaluate the impact of kidney function on LC-MGUS. We found that by using a higher cut-off (40 mg/L), the definition was not further improved by applying the separate renal reference range. We thus propose that as far as LC-MGUS is concerned, our definition can be used regardless of renal function. For the purpose of patients with severe renal failure, additional work is needed to validate our data.

The results from our study establish LC-MGUS as a precursor to light-chain MM and AL-amyloidosis. The importance of LC-MGUS is based on the fact that it is a precursor

condition. Therefore, it is of major importance that a strict definition does not miss individuals with monoclonal disease and a high risk of progression. Importantly, our revised and improved definition captures these individuals. In addition, the patients presented in the original paper by Dispenzieri et al. who progressed all had the involved light chain concentration of 40 mg/L or above, and would thus also be captured in our revised definition.[9] However, in the study by Dispenzieri et al, one patient who later developed MM was not captured using the then-applied definition. Importantly, by reviewing the literature, we found that among 151 cases of light-chain MM reported, which included information on FLC measurements, only 3 (2%) had a concentration of the involved light chain of less than 40 mg/L.[25-29] This highlights the fact that by increasing the specificity of LC-MGUS, individuals that have already developed light-chain MM are of very low risk to be missed. Taken together, none of the individuals with LC-MGUS that were captured as progressing to MM in the Mayo study[9], the German study[10], or in our large cohort, would be missed according to the new revised version definition.

Our study has several strengths, including the population-based cohort design and the screening approach, as well as complete follow-up. The individuals in our studies were all examined according to a standardized protocol. Also, all the FLC analyses were performed in the same laboratory, and the individuals in the study were followed prospectively without researcher knowledge of FLC or SPEP results, thus minimizing surveillance bias. Another major strength of our study is the almost identical results in two independent cohorts of patients from two continents. Limitations include lack of information on renal function in the PLCO cohort, relatively few patients that progressed, and few patients with severe renal failure (n=105). The small number of patients who developed lymphoproliferative disease, and the small number of patients in subgroups by sex and race, are limiting, and an even larger population-based study with longer follow-up time would be better suited to answer questions regarding progression and survival. However, due to the high costs and extensive efforts required for such a study, we are likely to have to wait for a long time for such a study to be conducted and results obtained. The participants from the AGES-Reykjavik study were selected from survivors from the original cohort, with a 75% response rate, and are likely to be healthier than the general population. However, none of the participants were excluded because of comorbidities, and are thus likely to be similar to the patients encountered in real-world clinical practice.

In summary, we provide a revised, improved and stricter definition of LC-MGUS involving (1) an abnormal FLC-ratio (normal reference: 0.26-1.65), (2) an elevated involved light chain (40 mg/L or higher), (3) no M-protein by SPEP/IFE, and (4) no underlying lymphoproliferative disease. The prevalence of LC-MGUS is lower in white populations than in black populations, and we have established it as an important disease entity and a precursor of light-chain MM and amyloidosis. As previously recommended for individuals with MGUS, we suggest individuals with LC-MGUS should be followed indefinitely for progression. Finally, future studies should be aimed at exploring in more detail the outcome of these individuals and determining a risk score for progression, for which a prospective MGUS screening study is needed.

AUTHORSHIP CONTRIBUTIONS

SYK, EKL, and OL designed the study. RC and DB performed the laboratory analyses. SHL, EKL and JNH performed the statistical analyses. SYK, EKL and OL wrote the report. All authors were involved in the planning of the study, the interpretation of results, and the preparation of the final manuscript.

DISCLOSURE OF CONFLICTS OF INTERESTS

The other authors declare no competing interests.

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The Increased Risk of Dying in Individuals with Monoclonal Gammopathy of Undetermined Significance is Caused by Malignant Progression and Heart Disease

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ABSTRACT

Introduction

Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic precursor condition to multiple myeloma and other lymphoproliferative disorders. Previous studies on clinically established cohorts have suggested inferior survival in individuals with MGUS.

Methods

We studied the population-based, longitudinal cohort of the AGES-Reykjavik Study, consisting of 5,764 elderly Icelandic men and women, among which 300 individuals with MGUS and 52 individuals with light chain MGUS (LC-MGUS) had previously been identified through screening. Survival and causes of death in individuals with MGUS and LC-MGUS was compared to individuals without MGUS (n = 5,367) using a Kaplan Meier model, Cox proportional hazards model, and the Fine and Gray model for competing risks analysis.

Results

After a median follow-up time of 9.7 years, individuals with MGUS had a higher risk of death (hazard ratio (HR) = 1.2, 95% confidence interval (CI) 1.04-1.4) than individuals without MGUS, as did individuals with LC-MGUS (HR = 1.6, 1.2-2.3). After excluding those who developed a lymphoproliferative disorder during follow-up, the risk of death in MGUS and LC-MGUS remained increased. The 5-year survival rates for those without MGUS, MGUS, and LC-MGUS were 83.5% (95% CI 0.82-0.84), 76.4% (0.71-0.81), and 51.9% (0.38-0.64), respectively. Adjusted for age and sex, individuals with MGUS and LC-MGUS had an increased risk of death from cancer (HR = 1.8, 1.6-2.3) and from heart disease (HR = 1.4, 1.1-1.8).

Conclusions

In this large, population-based cohort study, we demonstrate for the first time in a screened cohort that individuals with MGUS and LC-MGUS have an increased risk of death compared to individuals without MGUS. The increased risk of death was primarily explained by malignant progression and by heart disease. Our findings suggest that both MGUS and LC-MGUS increase the risk of death, and that the two conditions are clinically distinct where LC-MGUS is the more hazardous condition. Attention should be directed towards finding the underlying reasons for the increased risk of death in general and specifically from heart disease in these individuals.

INTRODUCTION

Multiple myeloma (MM) is a hematological malignancy where abnormal plasma cells proliferate and accumulate in the bone marrow.[1] All cases of MM are preceded by the asymptomatic condition monoclonal gammopathy of undetermined significance (MGUS).[2,3] MGUS is thus a premalignant condition defined by the presence of a monoclonal immunoglobulin (M-protein) in serum of less than 30 g/L, with less than 10% monoclonal plasma cells in the bone marrow, and no evidence of MM or other lymphoproliferative disease.[1] The diagnosis requires the absence of hypercalcemia, renal insufficiency, anemia, or skeletal lytic lesions (the CRAB criteria), which can be attributed to underlying plasma cell disorder.[1] MGUS is clinically relevant since the risk of progression to lymphoproliferative disorders is on average 1% per year.[4,5]

Since MGUS by its nature is asymptomatic and likely to remain undiscovered, information on etiology, prevalence and survival is limited. Known risk factors for MGUS include high age, African-American ethnicity, exposure to pesticides, a family history of MGUS or MM or of autoimmune disease, and a personal history of autoimmune disease, infection or inflammatory disorder.[6-12] The prevalence of MGUS is estimated, based on a few screening studies as well as clinically established cohorts, to be approximately 2.4-3.5% in people aged 50 years or older.[7,13-15]

Recently, light chain MGUS (LC-MGUS) has been identified as a precursor condition leading to light-chain MM.[13,14] LC-MGUS is defined by an abnormal free light chain (FLC) ratio, with no expression of heavy immunoglobulin chains, together with increased concentration of the involved light chain.[13] We recently suggested a new definition of LC-MGUS, with stricter criteria than the previous definition, which tended to overestimate the prevalence of kappa restricted LC-MGUS.(Paper I) The prevalence of LC-MGUS has been estimated to be 0.7 – 0.8%.[13,14] No study on survival in LC-MGUS has been published to date.

Results from prior studies on clinically established cohorts have suggested that individuals with MGUS have an increased risk of death.[16-20] However, these findings could be explained by the underlying comorbidities that are likely to exist in clinically diagnosed MGUS patients, leading to the detection of MGUS in the work-up for other diseases. No study investigating the survival or causes of death in individuals with MGUS identified by screening has previously been performed.

Since individuals with MGUS carry an increased risk of progression to lymphoproliferative disease, it is of clinical relevance to know the true effect of MGUS on survival, which previously has only been investigated in clinically-based cohort studies. In this study we aimed at determining the effect of MGUS and LC-MGUS on survival in a screened, elderly population, as well as determine causes of death in MGUS and LC-MGUS.

METHODS

Study Population

This is a cohort study based on the screened longitudinal cohort of the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik Study), which has been described previously.[21] Briefly, the Reykjavik Study is a longitudinal cohort study of men and women (30,795) born 1907-1934 in Iceland who were followed from 1967 onward in the Reykjavik Study by the Icelandic Heart Association. In 2002, 5,764 persons randomly chosen from survivors of the Reykjavik Study cohort were re-examined for the AGES-Reykjavik Study. Participants in the AGES-Reykjavik Study examination in 2002-2006 completed a questionnaire, undertook a clinical examination, and underwent laboratory testing and radiological examinations. The participants are followed prospectively, and information on incidence of disease, date of death, and cause of death is collected annually through hospital, nursing home, and mortality records. End of follow-up for this study was March 2014.

Blood samples from the participants in the AGES-Reykjavik Study were screened between 2012 and 2014 for M-protein using serum protein electrophoresis (SPEP) and free light chain (FLC) analysis. MGUS was defined as a presence of M-protein band on SPEP, and those samples were also subjected to immunofixation (IFE). The definition of LC-MGUS was based on results from FLC analysis, and was defined as a pathological FLC ratio (reference range 0.26-1.65) in combination with an increased concentration of more than 40.0 mg/L of the involved light chain.

Exclusion criteria were diagnosis of a lymphoproliferative disease at baseline, missing consent form, and missing blood samples for SPEP, FLC, or IFE analysis. Having a lymphoproliferative disorder as cause of death without a previous diagnosis of a lymphoproliferative disorder was also an exclusion criterion, since it would be impossible to determine when the disease appeared and if it was really the true cause of death. A minimum follow-up time of ten days was required for inclusion in this study.

The study was approved by the National Bioethics Committee of Iceland (VSN-00-063-V35), the Icelandic Data Protection Authority, the institutional review board for the NIH National Institute on Aging in the USA, and the Regional Ethical Review Board in Stockholm, Sweden. Written informed consent was obtained from all participants.

Statistical Analysis

Survival of individuals with MGUS and individuals with LC-MGUS was compared to individuals without MGUS by calculating hazard ratios (HRs) and 95% confidence intervals (CIs), based on Cox proportional hazards model adjusting for age and gender, and by using Kaplan Meier analysis. Another model was fitted which included only those with information on serum albumin level, as low serum albumin levels have previously been shown to predict poorer survival in MGUS.[17] A low serum albumin level was defined as <35 g/L, which is the lower threshold for the normal range. The underlying time scale was time from study entry. Survival ratios were computed as measures of 1-, 3-, and 5-year survival. Difference between

survival curves was tested using the log rank test. The proportional hazards assumption was tested using plotting of the Schoenfeld residuals, through a formal statistical test, and through introducing time-varying covariates into the model. We tested for interactions between the variables in the model by introducing interaction terms in the model.

Causes of death were analyzed according to a previously described categorization (Appendix 1) and mortality rate ratios were estimated for each cause of death using Cox's proportional hazards model.[20] A separate model was estimated for each category of death.

In a secondary analysis, the Fine and Gray regression model was used to estimate cause-specific mortality rates for different causes of death in individuals with MGUS compared to the individuals without. In this model the different causes of death were treated as competing events and the probability of death from each cause was estimated as a function of time. This is known as the cumulative incidence function and is obtained through transformation of the cause-specific mortality rates estimated through the regression model.[22]

All calculations were performed using STATA version 13 (StataCorp 2013 Stata Statistical Software: Collage Station, TX, USA).

RESULTS

Overall, 5,716 individuals were included in the survival analyses, whereof 297 individuals with MGUS, 52 individuals with LC-MGUS, and 5,367 individuals without MGUS. From the original AGES-Reykjavik Study cohort of 5,764 individuals, 16 were excluded due to missing blood samples, one was excluded due to missing consent form, 22 were excluded due to a lymphoproliferative disorder at baseline, one was excluded due to follow-up time less than 10 days, and eight were excluded due to a lymphoproliferative disorder as cause of death without previous diagnosis of lymphoproliferative disorder. The demographic and clinical characteristics of all participants are shown in Table 1. At study baseline, the median age of individuals with LC-MGUS (82 years) was higher than that of individuals with MGUS (78 years) or without MGUS (76 years). A majority of individuals with LC-MGUS were male (65.4% compared to 53.2% of MGUS and 41.4% of individuals without MGUS, respectively).

Among the individuals with MGUSs, IgG (158 individuals, 53.2%) was the most common isotype, followed by IgM (79 individuals, 26.6%) (Table 1). Information on MGUS isotype was available for all study participants. Information on M-protein concentration was available for 162 (54.2%) of the MGUS individuals, and the mean concentration was 7.7 g/L (range 0.0-28.8 g/L).

Table 1. Characteristics of the study participants

	MGUS[a]	LC-MGUS[b]	No MGUS
Total n[c] of cases (%)	297 (5.2)	52 (0.9)	5367 (93.9)
Males, n (%)	158 (53.2)	34 (65.4)	2222 (41.4)
Females, n (%)	139 (46.8)	18 (34.6)	3145 (58.6)
Median age, years (range)	78 (67-93)	82 (69-96)	76 (66-98)
Age group, n (%)			
Less than 70 years	21 (7.1)	1 (1.9)	528 (9.8)
70-79 years	146 (49.2)	16 (30.8)	3037 (56.6)
80-89 years	119 (40.0)	33 (63.5)	1677 (31.2)
90 years and older	11 (3.7)	2 (3.8)	125 (2.3)
MGUS isotype, n (%)			
IgG	158 (53.2)	N.A.	N.A.[d]
IgA	27 (9.1)	N.A.	N.A.
IgM	79 (26.6)	N.A.	N.A.
IgD	1 (0.3)	N.A.	N.A.
Biclonal	32 (10.8)	N.A.	N.A.
M-protein concentration, n (%)			
>15.0 g/L	17 (10.5*)	N.A.	N.A.
<15.0 g/L	145 (89.5*)	N.A.	N.A.
FLC[e] ratio			
0.26-1.65	167 (56.2)	0 (0.0)	N.A.
<0.26 / >1.65	130 (43.8)	52 (100.0)	N.A.

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]n: number, [d]NA: not applicable, [e]FLC: free light chain analysis. *In percent of individuals (162) with available information.

Patterns of survival

After a median follow-up time of 9.7 years, 168 deaths (56.6%) had been observed in the MGUS group, 39 (75.0%) in the LC-MGUS group, and 2,433 (45.3%) in the no MGUS group.

The 1-year crude survival rates for individuals without MGUS, with MGUS, and with LC-MGUS were 97.7%, 95.6%, and 88.5%, respectively. The 3-year crude survival rates were 91.7%, 85.9%, and 63.5%, and the 5-year crude survival rates were 83.5%, 76.4%, and 51.9%, respectively (Figure 1 and Table 2). The difference in survival between the groups was statistically significant at five years by p<0.001.

Figure 1. Kaplan-Meier estimates of the survival in MGUS[a], LC-MGUS[b], and no MGUS.

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance.

Table 2. Survival probabilities at 1, 3, and 5 years of follow-up for MGUS, LC-MGUS, and no MGUS.

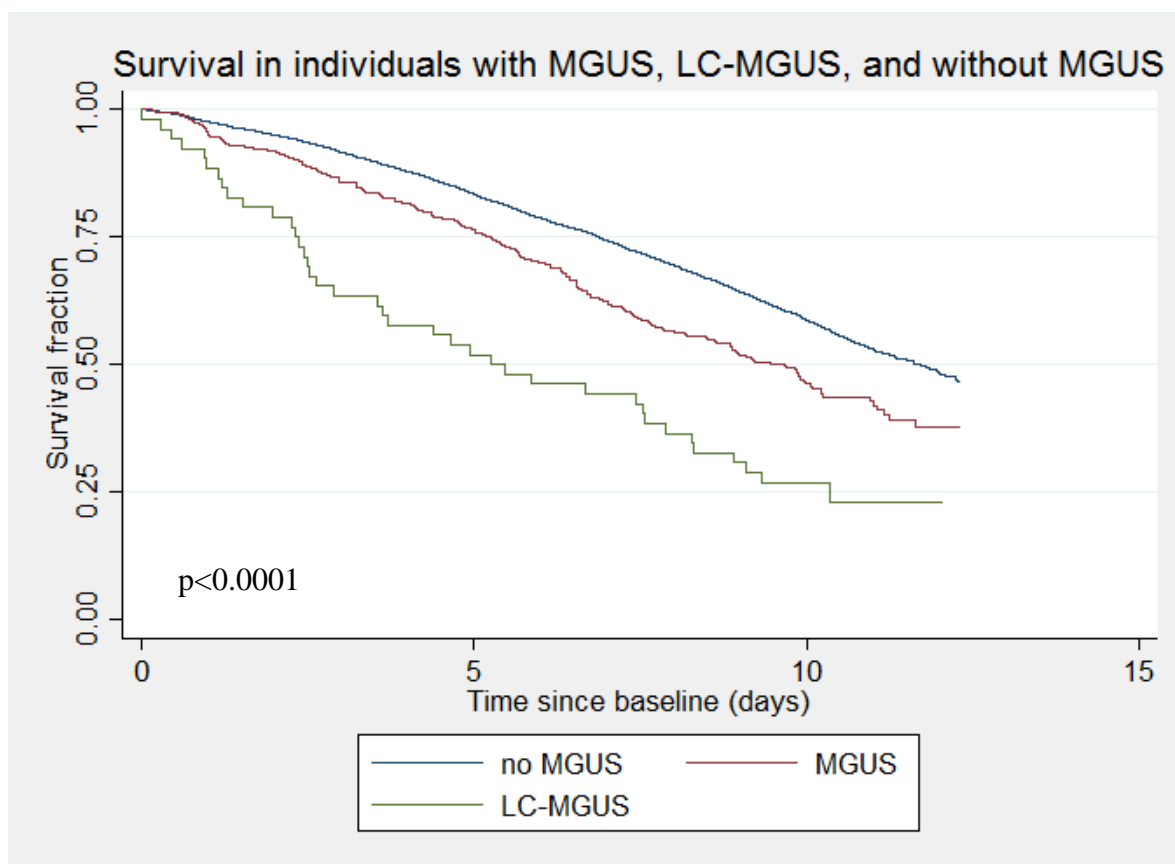|	No MGUS[a] Survival (95% CI)	MGUS Survival (95% CI)	LC-MGUS[b] Survival (95% CI)
1-year survival	97.7% (0.97-0.98)	95.6% (0.93-0.97)	88.5% (0.76-0.95)
3-years survival	91.7% (0.91-0.92)	85.9% (0.81-0.89)	63.5% (0.49-0.75)
5-years survival	83.5% (0.82-0.84)	76.4% (0.71-0.81)	51.9% (0.38-0.64)

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance.

Individuals with MGUS had a significantly higher risk of death (HR = 1.2, 95% CI 1.04-1.4), as did individuals with LC-MGUS (HR = 1.6, 1.2-2.3), compared to individuals without MGUS, adjusted for age and gender. When grouped together, the risk of death in MGUS and LC-MGUS was HR = 1.3 (1.1-1.5), compared to individuals with no MGUS (Table 3).

Table 3. Risk of death in MGUS and LC-MGUS, compared to no MGUS.

	MGUS[a] HR[c]* (95% CI[d])	LC-MGUS[b] HR* (95% CI)	MGUS and LC-MGUS combined HR* (95% CI)
Risk of death	1.2 (1.04-1.4)	1.6 (1.2-2.3)	1.3 (1.1-1.5)
Risk of death, excl.**	1.1 (0.93-1.3)	1.5 (1.09-2.1)	1.2 (1.004-1.4)

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]HR: hazard ratio, [d]CI: confidence interval. *Estimates are adjusted for age and sex.** excl.: those who progressed to lymphoproliferative disorder excluded

Serum albumin was available for 3,216 individuals (56.3%). A low serum albumin level, as a categorical variable, was associated with a significantly increased risk of death (crude HR = 3.5, 2.4-5.2). Looking only at individuals with LC-MGUS or MGUS, individuals with a low serum albumin level did not have a statistically increased risk of death compared to individuals without a low serum albumin level (HR = 2.4, 0.8-7.7 for MGUS, HR = 4.1, 0.8-19.6 for LC-MGUS). In individuals without MGUS, a low serum albumin level was associated with an increased risk of death (HR = 2.8, 1.8-4.4).

When looking at individuals with MGUS only, the risk of death was significantly increased in individuals with MGUS isotype A (HR = 1.8, 1.1-2.9) compared to individuals with other isotypes. No inferior survival was shown in individuals with a low (below 15 g/L) M-protein concentration compared to individuals with a high (above 15 g/L) M-protein concentration (HR = 0.9, 0.5-1.8). When comparing survival in MGUS with versus without an abnormal FLC ratio, the crude risk was increased (HR = 1.4, 1.1-1.9), but did not reach statistical significance (HR = 1.2, 0.92-1.7) when adjusted for age and sex.

When excluding 56 individuals who progressed to lymphoproliferative disorders during follow-up, the increased risk overall of death in MGUS was not statistically significant (HR = 1.1, 0.9-1.3), whereas the estimate for LC-MGUS was similar (HR = 1.5, 1.1-2.1) (Table 3). The results of the analyses on serum albumin, MGUS isotype, concentration, and FLC ratio were not significantly affected with the 56 individuals were removed.

All analyses were rerun with the underlying time scale changed to attained age, with no effect on the results (data not shown).

Causes of Death

Subjects with any MGUS (both MGUS and LC-MGUS) had a significantly increased risk of death from any cancer (HR = 1.9, 1.4-2.3), any hematologic malignancy (HR = 11.1, 6.1-

20.3), any heart disease (ischemic heart disease and other heart disease grouped together) (HR = 1.4, 1.1-1.8), and other heart disease (HR = 1.9, 1.3-2.8) (Table 4).

Table 4. Causes of death in MGUS, light chain MGUS, and any MGUS

Cause of death _Subgroup_	No MGUS[a]	MGUS			LC-MGUS[b]			Any MGUS		
	No.	No.	HR[c]*	95% CI[d]	No.	HR*	95% CI	No.	HR*	95% CI
Any cancer	552	51	1.7	1.3-2.3	10	2.3	1.2-4.3	61	1.8	1.4-2.3
Any hematologic malignancy	28	16	11.2	6.0-20.8	2	10.7	2.5-45.7	18	11.1	6.1-20.3
Multiple myeloma	0	11	∞	N.A.[e]	1	∞	N.A.	12	∞	N.A.
Waldenström's macroglobulinemia	0	2	∞	N.A.	0	-	-	2	∞	N.A.
Other lymphoproliferative malignancy	10	2	4.1	0.9-18.8	0	-	-	2	3.7	0.8-16.9
Myeloid malignancy	11	0	-	-	1	11.9	1.5-96.3	1	1.4	0.2-11.3
Any solid tumor	524	35	1.2	0.9-1.7	8	1.9	0.9-3.8	43	1.3	0.96-1.8
Amyloidosis	1	0	-	-	1	104.4	6.0-1826.7	1	13.7	0.8-227.4
Infections	110	6	0.99	0.4-2.3	2	1.6	0.4-6.5	8	1.1	0.5-2.3
Tuberculosis	4	0	-	-	0	-	-	0	-	-
Bacterial infection	97	3	0.6	0.2-1.8	2	1.7	0.4-7.9	5	0.8	0.3-1.9
Pneumonia	83	3	0.6	0.2-2.0	2	2.0	0.5-8.1	5	0.9	0.3-2.2
Heart disease	745	61	1.4	1.1-1.8	15	1.8	1.1-3.0	76	1.4	1.1-1.8
Ischemic heart disease	510	37	1.1	0.8-1.5	9	1.3	0.7-2.5	46	1.1	0.8-1.5
Other heart disease	235	24	1.8	1.2-2.8	6	2.5	1.1-5.8	30	1.9	1.3-2.8
Vascular disease	305	24	1.4	0.9-2.1	1	0.3	0.1-2.3	25	1.2	0.8-1.9
Venous thromboembolism (pulmonary embolism, DVT[f])	11	1	1.7	0.2-13.5	0	-	-	1	1.5	0.2-11.9
Cerebrovascular disease	262	20	1.4	0.9-2.1	1	0.4	0.1-2.7	21	1.2	0.8-1.9
Peripheral vascular disease	31	3	1.6	0.5-5.3	0	-	-	3	1.4	0.4-4.5
Gastrointestinal disease	76	0	-	-	1	1.4	0.2-10.3	1	0.2	0.1-1.5
Bowel disease	60	0	-	-	0	-	-	0	-	-
Liver disease	6	0	-	-	0	-	-	0	-	-
Biliary/pancreatic disease	10	0	-	-	1	12.9	1.4-99.6	1	1.8	0.2-14.1
Pulmonary disease	113	3	0.5	0.2-1.5	3	2.9	0.9-9.2	6	0.8	0.4-1.8
Musculoskeletal disease	8	1	2.5	0.3-20.5	0	-	-	1	2.2	0.3-18.1
Endocrine disorders	37	1	0.5	0.07-3.6	0	-	-	1	0.4	0.1-3.1
Diabetes	30	0	-	-	0	-	-	0	-	-
Other hematologic disorders	3	1	6.2	0.6-60.8	0	-	-	1	5.2	0.5-51.9
Psychiatric illnesses	97	3	0.6	0.2-1.8	3	3.3	1.1-10.7	6	1.0	0.4-2.2
Neurological disease	248	11	0.8	0.4-1.5	0	-	-	11	0.7	0.4-1.3
Renal disease	52	3	0.99	0.3-3.2	2	4.9	0.95-16.5	5	1.4	0.6-3.5
All others	78	3	0.7	0.2-2.1	2	2.5	0.6-10.3	5	0.9	0.4-2.3

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]HR: hazard ratio, [d]CI: confidence interval, [e]N.A.: not applicable, [f]DVT: deep vein thrombosis. *Estimates are adjusted for age and sex.

Individuals with LC-MGUS, but not those with MGUS, had an increased risk of death from myeloid malignancy, amyloidosis, biliary/pancreatic disease, and psychiatric illness, compared to individuals without MGUS. Very few deaths occurred in these groups.

When the 56 individuals who progressed to lymphoproliferative disorders were excluded, the risk of death from any cancer was still significantly increased for LC-MGUS (HR = 2.1, 1.1-4.1), but not for MGUS (HR = 1.3, 0.9-1.8). The risk of death from any heart disease remained increased for MGUS (HR = 1.3, 0.99-1.7) and for LC-MGUS (HR = 1.8, 1.01-3.0), as did the risk of death from other heart disease for both MGUS (HR = 1.8, 1.1-2.7) and LC-MGUS (HR = 2.5, 1.1-5.8). The risk of death from any hematologic malignancy was no longer significant (HR = 1.9, 0.4-8.1).

In a competing risks analysis, excluding the 56 individuals who progressed to lymphoproliferative disorders, the risk estimates were essentially the same (data not shown).

DISCUSSION

In this large, population-based study of more than 5,700 participants, with almost ten years of follow-up, we demonstrate for the first time in a screened cohort that individuals with MGUS and LC-MGUS have a 30% increased risk of death compared to individuals without MGUS. Furthermore, the results suggest that the inferior survival cannot be attributed solely to progression to lymphoproliferative diseases. We also found a poorer survival in LC-MGUS than in MGUS, with a 5-year survival of only 52%. Specifically, we show that among causes of death, the risk of death from heart disease is increased in both MGUS and LC-MGUS.

We found that individuals with MGUS had a 21% increased risk of death compared to individuals with no MGUS, a risk that was mainly, but not entirely, accounted for by progression to lymphoproliferative disease. We and others have previously shown a decreased life-expectancy in clinically established MGUS cohorts,[16-19] but this study is, to our knowledge, the first to detect an inferior survival in MGUS patients in a population-based, screened cohort. This is highly relevant, since in a clinical cohort an inferior survival could be explained by underlying disease that led to the detection of MGUS, considering that MGUS is an asymptomatic condition. In this cohort, however, the MGUS in these individuals was detected through screening the entire cohort.

We found that individuals with LC-MGUS had 1.6-fold increased risk of death, a higher risk estimate than that for individuals with MGUS, and only slightly lowered by accounting for progression to lymphoproliferative disorder. The predicted 5-year survival in LC-MGUS was only 52%, compared to 84% among the individuals without MGUS. The finding that the risk is higher in LC-MGUS than in MGUS, despite statistical adjustment for the higher age in LC-MGUS, is very interesting. It strengthens the hypothesis that MGUS and LC-MGUS are clinically distinct, and suggests that LC-MGUS is the less benign condition of the two, regardless of progression to lymphoproliferative disorders. We have speculated that this could be due to different underlying pathogenesis in MGUS and in LC-MGUS, where LC-MGUS tends to occur in individuals with more comorbidities, or that the FLC overload in LC-MGUS are involved in the etiology of other conditions that ultimately increase the risk of death. This needs to be investigated further.

We found that a low serum albumin level was associated with an increased risk of death in the entire cohort, however, when examining the effect of serum albumin by MGUS status, we discovered that a low serum albumin level was only associated with a significantly increased risk of death in individuals without MGUS. In individuals with MGUS and with LC-MGUS, we could not find a statistically increased risk of death in those with a low level of serum albumin compared to those without. Whether serum albumin affects the progression rate from MGUS or LC-MGUS to lymphoproliferative disorders remains to be investigated.

Among causes of death, hematological malignancy was predictably dominating among MGUS subjects, both conventional MGUS and LC-MGUS; however, malignant transformation alone did not explain the increased risk of death of LC-MGUS in particular. These results are in line with the findings of Schaar et al, where an increased risk of death among individuals with MGUS was observed, which was not completely explained by progression to lymphoproliferative disease.[17] However, we also found that individuals with MGUS and LC-MGUS combined had an increased risk of death from heart disease, which remained statistically increased after exclusion of those who progressed to lymphoproliferative disease. The increased risk of death from heart disease is thus not explained by progression to malignant disease, or the treatment thereof, and warrants further study. The results are in line with our previous findings of increased risk of both venous and arterial thrombosis in MGUS, as well as the finding by Gregersen et al that individuals with MGUS are at higher risk of death from heart disease.[18,23,24] The explanation for the increased risk of death in heart disease in individuals with MGUS might lie in a common genetic or environmental susceptibility behind both MGUS and heart disease, or that one condition predisposes for the other. It could also be that the individuals with LC-MGUS and MGUS in our cohort suffer from undetected amyloidosis, which would increase their risk of heart disease. Another explanation could be the polyclonality itself. Elevated levels of non-monoclonal FLC are associated with increased mortality and have also been shown to predict cardiovascular events in patients with diabetes.[25,26]

We found an increased risk of death from myeloid malignancy, amyloidosis, biliary/pancreatic disease, and psychiatric illnesses in individuals with LC-MGUS, however, these estimates are based on as few as one to three deaths per group, and should be interpreted with caution.

Our study has several strengths. It is based on a large, population-based cohort of elderly people, who are well characterized with regard to extensive health-related factors and medical conditions, and with almost a decade of follow-up time. The screening approach, where MGUS status was determined through performing SPEP and FLC analysis on all participants, is an important strength adding to the validity of our study. Similarly, all analyses were performed by the same laboratory, the loss to follow-up in the cohort is minimal, and the causes of death were attained from high-quality registers with strict procedures. In our analysis we were able to adjust for important risk factors such as age, sex, and knowledge of progression to lymphoproliferative disease. We also performed a competing risks analysis of causes of death, where the estimated did not differ greatly from the results from the Cox regression model. Considering the low number of subjects and known causes of death in each group, the results from the competing risks analysis is unlikely to give useful estimates for crude mortality in this cohort.

However, some limitations need to be kept in mind when interpreting the results. Even though this is a screened study, bias might be present in the form of selective survival and participation. The median age in our cohort is rather high (77 years), possibly representing a selection of the population that is unusually healthy. Also, the group under study is an exclusively Northern European, Caucasian study group, and considering knowledge on MGUS variance across ethnic groups our findings may not be representative of all individuals with MGUS.[6,7] We did not have the possibility to perform IFE on all samples that were normal on serum protein electrophoresis but fulfilled the criteria for LC-MGUS. Thus, the categories conventional MGUS and LC-MGUS might overlap in that some of the samples in LC-MGUS might have had a visible M-protein band on IFE and would then have been classified as conventional MGUS. For this reason among others, we have chosen to present results from analysis not only for MGUS and LC-MGUS separately, but also for all MGUS. We did not perform bone marrow examinations on any of the study participants, and were thus unable to truly distinguish between MGUS and what could have been smoldering multiple myeloma. Furthermore, in the cause of death analyses, some groups have very few deaths, and the results should be interpreted with caution.

In conclusion, this study demonstrates a 30% increased risk of death in MGUS subjects with 9.7 years of follow-up, and is to our knowledge the first study to demonstrate inferior survival in MGUS in a screened population-based cohort. Furthermore, our results imply that the inferior survival in individuals with MGUS could in part be due to an increased risk of heart disease, and that individuals with LC-MGUS are at an increased risk compared to individuals with MGUS. Taken together, our findings suggest that conventional MGUS and LC-MGUS are two different entities with different survival patterns. Attention should be directed towards finding the underlying reasons for increased risk of death from heart disease in MGUS and LC-MGUS

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AUTHORSHIP CONTRIBUTIONS

EK Lindqvist, SY Kristinsson, O Landgren and SH Lund designed the study. G Eiriksdottir, V Gudnason, LJ Tauner and TB Harris obtained the data. R Costello and D Burton performed the serum analyses. M Björkholm, M Hultcrantz, N Korde and S Mailankody were involved in the designing of the study and in the management of the data. EK Lindqvist performed the statistical analyses. EK Lindqvist and SY Kristinsson wrote the report. All the authors were

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

DISCLOSURE OF CONFLICTS OF INTERESTS

The other authors declare no competing interests.

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Appendix 1: Categorization of Causes of Death

Groups and subgroups	ICD codes
Any cancer	C00-C97, D37-D48
Any hematologic malignancy	C81-C96, D45-D47
Multiple myeloma	C90
Waldenström's macroglobulinemia	C88
Other lymphoproliferative malignancy	C81-C85, C91
Myeloid malignancy	C92-C94
Any solid tumor	C00-C26, C30-C41, C43-C58, C60-C80, C97, D37-D48 (except D45-D47)
Amyloidosis	E85
Infection	A00-B99, I33, I38, J00-J22, G00-G09, L00-L08, M00-M03, M86
Tuberculosis	A15-A19, B90
Bacterial infection	A00-A05, A20-A56, A65-A79, B95-B96, G01, G04-G07, J13-J17.0, J18, I33, L00-L03, M00-M02, M86
Pneumonia	J13-J18
Heart disease	I00-I25, I27-I52 (except I33 and I38)
Ischemic heart disease	I20-25
Other heart disease	I00-I19, I27-I52 (except I33 and I38)
Vascular disease	I26, I60-I99
Venous thromboembolism (pulmonary embolism and DVT)	I26, I80-I82, I87
Cerebrovascular	I60-I69
Peripheral vascular disease	I70-I79, I83-I86, I88-I99
Gastrointestinal disease	K00-K93
Bowel disease	K00-K67, K90-K93
Liver disease	K70-K77
Biliary/pancreatic disease	K80-K87
Pulmonary disease	J30-J99
Musculoskeletal disease	M05-M85, M87-M99
Endocrine/metabolic disorders	E00-E90 (except E85)
Diabetes	E10-E14
Other hematologic disorders	D50-D89
Psychiatric illnesses	F00-F99
Neurological disease	G10-G99
Renal disease	N00-N50
All others	all other codes

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ORIGINAL ARTICLE

History of autoimmune disease is associated with impaired survival in multiple myeloma and monoclonal gammopathy of undetermined significance: a population-based study

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Abstract Multiple myeloma (MM) is a plasma cell disorder preceded by monoclonal gammopathy of undetermined significance (MGUS). Incidence of MM and MGUS is higher among patients with autoimmune disease. The aim of this study was to determine whether a history of autoimmunity has an impact on survival in MM and MGUS. Using high-quality national Swedish registries, we identified 8367 patients with MM, 18,768 patients with MGUS, and 110,251 matched control subjects, and obtained information on previous autoimmune disease in patients and controls. Cox regression was used to calculate hazard ratios (HRs) for overall survival with 95 % confidence intervals (CIs). In patients with MM and a prior autoimmune disease, the risk of death was significantly increased, HR = 1.2 (95 % CI 1.2–1.3) compared to MM patients with no history of autoimmunity. In MGUS patients, a prior autoimmune disease was associated with a significantly 1.4-fold elevated risk of death (95 % CI 1.3–1.4). When analyzing different types of autoimmune diseases, a history of ulcerative colitis had a stronger impact on survival in MM than in controls. Our findings that a history of autoimmune disease has a negative impact on survival in MM and MGUS could be due to shared underlying common genetic factors, or that patients with a history of autoimmunity develop more severe cases of MM and MGUS, or cumulative comorbidity in the individual. Our results suggest that more attention should be paid to comorbidity as a prognostic factor in MGUS and MM, and underlines the need for studies aimed at tailoring therapy according to comorbidity.

Keywords Autoimmunity · Multiple myeloma · MGUS · Survival · Population-based

Introduction

Multiple myeloma (MM) is a chronic plasma cell disorder, characterized by a monoclonal proliferation of plasma cells in the bone marrow coupled with an overproduction of monoclonal (M-) protein [1]. Clinical manifestations of MM include osteolytic lesions, anemia, renal failure, and hypercalcemia [2]. Monoclonal gammopathy of undetermined significance (MGUS) is an asymptomatic, pre-malignant condition with an average risk of progression to MM or other lymphoproliferative disorders of 1 % per year [3].

The etiology of MM and MGUS is largely unknown. Familial risk factors have been identified, such as an increased risk of MM and MGUS in first-degree relatives of patients with these disorders [4–7]. This, together with ethnic disparities in the incidence patterns [8, 9], suggests a role for genetic factors in the etiology. Furthermore, high age and male gender have been found to be risk factors for MGUS, as have environmental risk factors such as exposure to pesticides and herbicides [10–12].

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Autoimmune diseases include a variety of conditions, which jointly affect 5 to 10 % of the population [13]. Autoimmune diseases are characterized by increased activation of T or B cells toward own antigens (autoreactivity), causing local or systemic symptoms [14]. A history of autoimmunity increases the risk of certain malignancies [15–19], possibly due to chronic stimulation of the immune system; however, the complete underlying mechanisms are unknown. We and several investigators have shown that a personal history of autoimmune disease is associated with a significantly increased risk of MGUS and to some extent MM [20–22]. Our research group recently presented results showing a family history of autoimmune disease to be associated with a significantly increased risk of MGUS [20], suggesting a common genetic susceptibility between autoimmunity and plasma cell disorders.

Autoimmune disease is a predictor of poor survival in the general population, especially among women [23, 24]. A history of a few specific autoimmune diseases has been found to increase mortality in patients with certain digestive tract cancers [17], but does not seem to influence prognosis in other cancer types, e.g., lung cancer [18]. In a large study on patients with Hodgkin lymphoma, a prior diagnosis of autoimmune disease was associated with poorer survival [25]. In a smaller study on Swedish MM patients, the effect of 33 different autoimmune diseases on survival was analyzed, and only a history of rheumatic fever was associated with a decreased survival, although results were limited due to small numbers [22].

To increase knowledge in this field, we conducted a population-based study to determine whether a personal history of autoimmune disease has an impact on survival in MM and MGUS.

Methods

Registries, patients, and control subjects

Patients in Sweden with MM are treated by physicians at hospital-based hematology or oncology centers. All physicians in Sweden are obliged to report each case of incident cancer to the nationwide Swedish Cancer Register [26]. The completeness and diagnostic accuracy of the register is high (>93 %) for MM [27]. We identified all patients with a diagnosis of MM diagnosed from January 1, 2000, through December 31, 2013, in the nationwide Swedish Cancer Register. We established an MGUS cohort that has been described previously [20] consisting of MGUS patients retrieved through a national network, as well as through the Swedish Inpatient and Outpatient Registers, which have a high level of coverage and accuracy [28, 29]. MGUS patients diagnosed from January 1, 1988 through December 31, 2013 were included.

For all included patients, we obtained information on sex, date of birth, and date of diagnosis where the diagnosis was made. For MGUS patients, information on M-protein type and concentration was collected, where this was available. For each MM and MGUS patient, four population-based control subjects matched by sex, year of birth, and county of residence were chosen randomly from the Swedish Register of Total Population database. The control subjects had to be alive and free of any preceding hematologic malignancy at the time of MM or MGUS diagnosis of the corresponding case.

We obtained information on occurrence and date of autoimmune disease in patients and in controls from the Swedish Inpatient Register from 1964 and onwards. The conditions included in the analyses were equivalent to previously published studies (Appendix 1) [20, 30].

Information on survival was gathered from the Swedish Cause of Death Register. End of follow-up was December 31, 2013.

Statistical analysis

xWe used the Kaplan-Meier method with log-rank test and regression models to compare outcome among patients and controls with and without autoimmune disease. Specifically, we calculated hazard ratios (HRs) and 95 % confidence intervals (CIs) with Cox proportional hazards models that were adjusted for age, year of diagnosis, and sex. In a sensitivity analysis, each MM or MGUS case was paired with a control, matching on age of diagnosis, and the risk for mortality was calculated using Cox proportional hazards model as stated above.

To avoid the possibility of autoimmune disease being discovered more often in cases than in controls due to the diagnostic work up of a plasma cell disorder, we excluded all autoimmune disease diagnosed less than 1 year prior to diagnosis of MM or MGUS. In order to investigate whether exposure time in addition to the presence of autoimmune disease had an impact on outcome, we included the duration of exposure in a separate model.

We performed analyses on seven specific autoimmune diseases previously found to increase the risk of MM and/or MGUS [20]; rheumatoid arthritis, pernicious anemia, chronic rheumatic heart disease, ulcerative colitis, polymyalgia rheumatica, giant cell arteritis, and psoriasis. Among MGUS patients, we also performed analyses on M-protein concentration (all isotypes combined) and by M-protein isotype where IgM MGUS was analyzed separately and IgG and IgA MGUS combined since IgM MGUS and non-IgM MGUS appear to be clinically distinct with regards to progression [31].

Results

A total of 8367 patients with MM and 18,768 patients with MGUS that were diagnosed from January 1, 2000 and from January 1, 1988 through December 31, 2013, respectively, were included in the study, as well as 33,577 matched control subjects for MM and 76,674 matched control subjects for MGUS (Table 1). The median age at diagnosis was 72 years for both MM and MGUS patients.

A history of autoimmune disease was found in 1378 MM (16 %) patients and in 4380 MM controls (13 %). Compared to MM patients with no history of autoimmune disease, patients with MM and a prior history of autoimmunity had a significantly increased risk of death (HR = 1.2, 95 % CI 1.2–1.3). The increased risk was similar in males (HR = 1.3, 95 % CI 1.1–1.4) and in females (HR = 1.2, 95 % CI 1.1–1.3). Compared to controls without prior autoimmune disease, MM controls with a history of autoimmunity had a significantly increased risk of death (HR = 1.8, 95 % CI 1.7–1.9) (Fig. 1, Table 2). In a sensitivity analysis, we excluded all individuals with a previous diagnosis of cancer, and found essentially the same results (data not shown).

A history of autoimmune disease was found in 4032 MGUS (21 %) patients and in 9046 MGUS controls (12 %). Compared to MGUS patients with no history of autoimmune disease, patients with MGUS and a prior history of autoimmunity had a significantly increased risk of death (HR = 1.4, 95 % CI 1.3–1.4). The results were the same for both females and males. Compared to controls without prior autoimmune disease, MGUS controls with prior autoimmunity had a significantly increased risk of death (HR = 1.7, 95 % CI 1.6–1.7) (Fig. 2, Table 2).

By a likelihood ratio test, the difference in effects of autoimmune disease in MM and MGUS patients compared to that in controls was statistically significant. The duration of exposure to autoimmune disease did not have a significant effect on survival when added to the model ($p = 0.20$ for MM and $p = 0.19$ for MGUS, respectively).

In a sensitivity analysis, where mortality was compared using age-matched MM or MGUS controls, the results were almost identical (data not shown).

Analyses by specific autoimmune disease

Of the included MM patients, 151 had a prior history of rheumatoid arthritis, 100 of pernicious anemia, 76 of chronic rheumatic heart disease, 52 of ulcerative colitis, 223 of polymyalgia rheumatica, 107 of psoriasis, and 58 of giant cell arteritis.

The increased risk of dying after ulcerative colitis was greater in MM patients (HR = 1.4, 95 % CI 1.0–1.9) than in controls (HR = 1.2, 95 % CI 0.9–1.7). For the other specific conditions analyzed, the excess mortality associated with a prior autoimmune disease was not different, or was lower, in MM patients compared to controls (Table 3).

Of the included MGUS patients, 665 had a prior history of rheumatoid arthritis, 149 of pernicious anemia, 203 of chronic rheumatic heart disease, 153 of ulcerative colitis, 817 of polymyalgia rheumatica, 336 of psoriasis, and 228

Table 1 Patient characteristics

	MM[a] patients	MM controls	MGUS[b] patients	MGUS controls
No[c]. in total	8367	33,577	18,768	76,674
Males no. (%)	4636 (55)	18,606 (55)	9765 (52)	39,928 (52)
Females no. (%)	3731 (45)	14,971 (45)	9003 (48)	36,746 (48)
Median age at diagnosis, years (range)	71 (31–97)		73 (30–101)	
No. with AI[d] (%)	1378 (16)	4380 (13)	4032 (21)	9046 (12)
Males (%)	688 (50)	2210 (51)	1990 (49)	4498 (50)
Females (%)	690 (50)	2170 (49)	2042 (51)	4548 (50)
Median age at diagnosis, years (range)	75 (32–98)		76 (31–101)	
Median age at AI diagnosis, years (range)	70 (16–97)	69 (7–97)	66 (10–96)	68 (10–99)
No. without AI (%)	6989 (84)	29,197 (87)	14,736 (79)	67,628 (88)
Males (%)	3948 (57)	16,396 (56)	7775 (53)	35,430 (52)
Females (%)	4202 (43)	12,801 (45)	8262 (47)	32,198 (47)
Median age at diagnosis, years (range)	70 (31–96)		71 (30–99)	

[a] *MM* multiple myeloma

[b] *MGUS* monoclonal gammopathy of undetermined significance
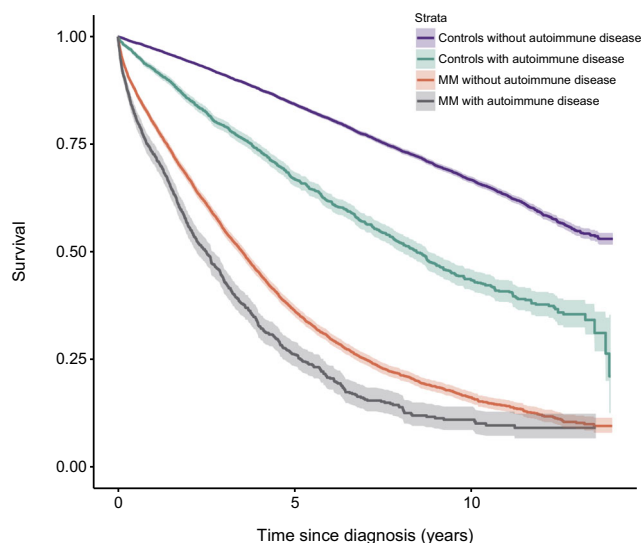
[c] *No.* number

[d] *AI* autoimmune disease

Fig. 1 Survival in patients with multiple myeloma (*MM*), with and without a personal history of autoimmune disease, compared to controls with and without a personal history of autoimmune disease

of giant cell arteritis. For all conditions analyzed, the excess mortality added by a prior autoimmune disease was lower in MGUS patients than the increased risk of dying in controls with a prior autoimmune disease (Table 3).

Analyses on MGUS patients by M-protein isotype and concentration

Information on M-protein isotype was available for 4210 individuals (22 %). The effect of a history of autoimmune disease on survival was not different between isotype IgA or IgG and isotype IgM.

Information on M-protein concentration was available for 3428 individuals (18 %). MGUS patients with a concentration of M-protein at diagnosis of 1.5 g/dL or more had a significantly higher risk of death (HR = 1.2, 95 % CI 1.0–1.4) compared to MGUS patients with a lower M-protein concentration. The interaction between autoimmune disease and concentration was not significant ($p = 0.28$).

Table 2 History of AI and survival in MM and MGUS

	MM[a]		MGUS[b]	
	HR[c]	95 % CI[d]	HR	95 % CI
Females				
Groups: MM/MGUS + AI[e] vs. MM/MGUS + no AI	1.2	1.1–1.3	1.4	1.3–1.5
	462/690		936/2042	
No. deaths/subjects in each group:	1947/3041		3415/6961	
Controls + AI vs. controls + no AI	1.9	1.7–2.0	1.7	1.6–1.8
No. deaths/subjects in each group:	719/2170		1722/4548	
	2679 /12,801		11,239/32,198	
Males				
MM/MGUS + AI vs. MM/MGUS + no AI	1.3	1.1–1.4	1.4	1.3–1.5
No. deaths/subjects in each group:	448/688		950/1990	
	2471/3948		4199/7775	
Controls + AI vs. controls + no AI	1.8	1.6–1.9	1.6	1.6–1.7
No. deaths/subjects in each group:	811/2210		1878/4498	
	3903/16,396		13,798/35,430	
Overall				
MM/MGUS + AI vs. MM/MGUS + no AI	1.2	1.2–1.3	1.4	1.3–1.4
No. deaths/subjects in each group:	910/1378		1886 /4032	
	4418/6989		7614/14,736	
Controls + AI vs. controls + no AI	1.8	1.7–1.9	1.7	1.6–1.7
No. deaths/subjects in each group:	1530 /4380		3600/9046	
	6582/29,197		25,037/67,628	

[a] *MM* multiple myeloma

[b] *MGUS* monoclonal gammopathy of undetermined significance

[c] *HR* hazard ratio

[d] *CI* confidence interval
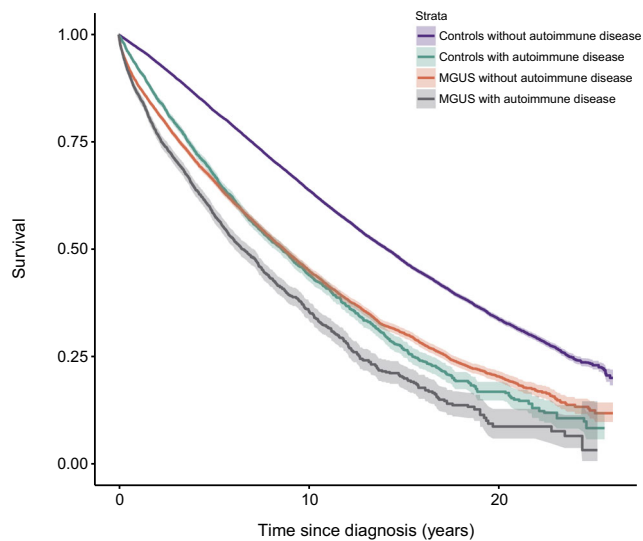
[e] *AI* autoimmune disease

Fig. 2 Survival in patients with monoclonal gammopathy of undetermined significance (*MGUS*), with and without a personal history of autoimmune disease, compared to controls with and without a personal history of autoimmune disease

Discussion

In this large population-based study including over 8000 MM patients, almost 19,000 MGUS patients, and their 110,000 matched control subjects, we found that a history of autoimmune disease was associated with a reduced survival in MM and MGUS patients. Furthermore, although based on smaller numbers, ulcerative colitis had a greater impact on mortality in MM than other autoimmune conditions. These novel findings suggest a more aggressive disease course in autoimmunity-associated plasma cell disorders and that more attention should be paid to comorbidity when predicting prognosis and tailoring therapy.

The finding that autoimmune disease is a predictor of worse survival in MM patients is an important finding, and contradicts results from a previous smaller study on autoimmune disease and MM survival where no effect on survival in MM was observed [22]. However, the abovementioned study was a cohort study on individuals with autoimmune disease, and captured only 457 cases of multiple myeloma. Our findings are consistent with autoimmune disorders being a predictor of poor survival in the population [23, 24], and expand on previous investigations showing autoimmune disease to have negative impact on survival in Hodgkin lymphoma and in certain digestive tract cancers [17, 25]. The intensity of medical treatments for MM patients requires careful consideration of risks and benefits, particularly if there are other co-existing serious illnesses. With an aging population, the number of MM patients has grown and will continue to grow in the years to come [32]. In MM patients, comorbid diseases may increase the risk of treatment-related complications. Also, MM

itself or its treatment may aggravate existing comorbid diseases, leading to lower performance status, decreased quality of life, and a shorter overall survival [33]. Despite the importance of considering comorbid diseases in the treatment and prognosis of MM, the literature on comorbidities in MM is limited and based on small series [34–38]. In these studies, survival is negatively influenced by increasing number of comorbid conditions. Approximately 80 % of MM patients have one or more comorbid conditions, and almost half have two or more [34–36]. There are few clinical trials where the elderly frail patients are included [39]; therefore, population-based studies are a valuable tool to estimate survival in a diverse MM population [32, 40–43].

In patients with MGUS, a history of autoimmune disease and its impact on survival have, to our knowledge, not been investigated previously. We found that MGUS patients with previous autoimmune disease had a significantly 1.4-fold increased risk of death. In patients with MGUS, the effect of a history of autoimmune disease on survival was not affected by isotype or by M-protein concentration at MGUS diagnosis. Previous investigators of cancer survival after autoimmune diseases have speculated that the underlying explanation may be poorer performance status or non-tolerance of therapy. However, this does not explain the decreased survival after autoimmune diseases in MGUS patients, who are asymptomatic by definition and do not receive therapy. Our findings of an increased risk of death in MGUS patients with a history of autoimmune disease thus suggest an unknown underlying factor which may impact the risk of death in MGUS patients with a prior autoimmune disease, and could also potentially shed light on the pathogenesis of MGUS. In addition, whether a prior autoimmune disease is also a risk factor for progression in MGUS needs to be clarified.

Considering autoimmune diseases are known to be more common in females than in males, we explored survival outcomes by sex to see if autoimmunity might be associated with different survival patterns for male and female patients with MM or MGUS; however, they were not.

Interestingly, a history of ulcerative colitis had a greater impact on survival in MM patients than a history of ulcerative colitis had in the general population, although the confidence intervals overlapped so the difference was not statistically significant. It is possible that therapy-related factors might have played a role. Another possibility is that individuals developing MM after ulcerative colitis are more likely to harbor additional poor prognostic factors.

Our study has several strengths, such as its large size and high-quality data from Sweden. The data is derived from a stable population with access to standardized medical health care during the entire study period, ensuring a generalizability of findings. The large study size has generated a high power, as shown in the narrow confidence intervals. Recall bias was ruled out due to the study design using nationwide registers.

Table 3 History of specific autoimmune conditions and survival in MM and MGUS (both genders)

	MM[a]			MGUS[b]		
	No. of MM patients with AI[c] (%)	HR[d]	95 % CI[e]	No. of MGUS patients with AI (%)	HR	95 % CI
Rheumatoid arthritis	151 (1.8)			665 (3.5)		
MM/MGUS + AI vs. MM/ MGUS + no AI	100/151	1.3	1.0–1.6	322/665	1.3	1.2–1.5
Controls + AI vs. controls + no AI	182/515	1.8	1.6–2.1	545/1334	1.8	1.7–2.0
Pernicious anemia	100 (1.2)			149 (0.8)		
MM/MGUS + AI vs. MM/ MGUS + no AI	70/100	1.2	1.0–1.5	90/149	1.5	1.2–1.9
Controls + AI vs. controls + no AI	62/127	2.1	1.7–2.7	211/328	2.0	1.7–2.3
Chronic rheumatic heart disease	76 (0.9)			203 (0.1)		
MM/MGUS + AI vs. MM/ MGUS + no AI	41/76	0.9	0.7–1.3	86/203	1.4	1.1–1.7
Controls + AI vs. controls + no AI	78/305	1.6	1.3–2.0	193/599	2.0	1.8–2.4
Ulcerative colitis	52 (0.6)			153 (0.8)		
MM/MGUS + AI vs. MM/ MGUS + no AI	33/52	1.4	1.0–1.9	47/153	1.1	0.8–1.5
Controls + AI vs. controls + no AI	38/191	1.2	0.9–1.7	87/327	1.6	1.3–2.0
Polymyalgia rheumatica	223 (2.7)			817 (4.4)		
MM/MGUS + AI vs. MM/ MGUS + no AI	146/223	1.0	0.8–1.2	343/817	1.0	0.9–1.2
Controls + AI vs. controls + no AI	227/603	1.5	1.4–1.8	600/1410	1.3	1.2–1.5
Giant cell arteritis	58 (0.7)			228 (1.2)		
MM/MGUS + AI vs. MM/ MGUS + no AI	38/58	0.8	0.6–1.1	92/228	1.0	0.8–1.2
Controls + AI vs. controls + no AI	53/150	1.4	1.1–1.8	141/300	1.4	1.1–1.6
Psoriasis	107 (1.3)			336 (1.8)		
MM/MGUS + AI vs. MM/ MGUS + no AI	51/107	0.9	0.7–1.2	103/336	1.3	1.0–1.6
Controls + AI vs. controls + no AI	98/507	1.3	1.1–1.6	203/824	1.4	1.2–1.6

[a] *MM* multiple myeloma

[b] *MGUS* monoclonal gammopathy of undetermined significance

[c] *AI* autoimmune disease

[d] *HR* hazard ratio

[e] *CI* confidence interval

Because of the large study size, patient-related information was only gathered through registers, and we were not able to validate individual medical records, and we did not have information on established risk factors such as genetic aberrations detectable by fluorescence in situ hybridization (FISH), both of which are limitations of our study. Also, this is a hypothesis-generating study, including many autoimmune diseases, and the findings on specific autoimmune diseases should be interpreted with caution. In our study, individuals with a history of autoimmune disease were older than those without. However, analyses were adjusted for age, and a sensitivity analysis where mortality was compared between cases and age-matched controls showed almost identical results. Thus, the age difference is unlikely to explain the difference in survival. The results for MGUS are cohort-based, and although large, it does not necessarily represent the true population since MGUS is an asymptomatic condition and the cohort was clinically based, not a screened population. The use of inpatient data could have led to under-ascertainment of less severe forms of autoimmune diseases. Importantly, the

autoimmune disease did not have to be the reason for admission, so all patients with autoimmune disease admitted for any reasons are included in our analysis. The prevalence of autoimmune disease was higher in MM (16 %) and MGUS (21 %) patients than in controls (12–13 %), which is consistent with previous findings of a significantly increased risk of MGUS and to some extent MM after autoimmune disease [20–22]. The overall high prevalence of autoimmunity in our study is surprising; however, a wide range of autoimmune disorders were included (Appendix 1) and although we were not able to validate individual medical records, since history of autoimmune diseases was assessed in the same way among MM and MGUS patients and matched controls, any under- or over-diagnosis should be non-differential.

In conclusion, our findings that a personal history of autoimmune disease has a negative impact on survival in MM and MGUS patients could be due to underlying common genetic factors, or that patients with a personal history of autoimmunity develop more severe forms of MM or MGUS as a result of either the autoimmune disease or its treatment, or cumulative comorbidity in the individual. Our findings suggest that more attention should be paid to comorbidity, such as autoimmune disease, as a prognostic factor in MM, and perhaps also in MGUS. Treatment options for MM are investigated today in randomized clinical trials which are subject to selection bias with strict inclusion and exclusion criteria, often with omission of elderly patients and patients with comorbidites. However, a majority of MM patients suffer from multiple other diseases, which may both affect survival and make patients unsuitable for certain treatments. Our findings raise interesting questions on the pathogenesis of MGUS, and highlight the importance of accounting for comorbidities such as autoimmune disease both for predicting prognosis and for tailoring therapy in patients with plasma cell dyscrasias.

Appendix

Table 4 Autoimmune conditions included in the study

Rheumatoid arthritis	Lupoid hepatitis
Systemic sclerosis	Celiac disease
Sjögren's syndrome	Granulomatosis with polyangiitis (Wegener's)
Systemic lupus erythematosus	Dressler syndrome
Polymyositis or dermatomyositis	Chronic rheumatic heart disease
Hashimoto thyroiditis	Multiple sclerosis
Grave's disease	Amyotrophic lateral sclerosis
Addison disease	Rheumatic fever
Pernicious anemia	Sarcoidosis
Autoimmune hemolytic anemia	Reiter disease
Immune thrombocytopenia	Crohn disease
Primary biliary cirrhosis	Ulcerative colitis
Discoid lupus erythematosus	Ankylosing spondylitis
Localized scleroderma	Polymyalgia rheumatica
Myasthenia gravis	Psoriasis
Autoimmune hepatitis	Behcet disease
Polyarteritis nodosa	Giant cell arteritis
Guillain-Barré syndrome	Vitiligo
Diabetes type 1	Aplastic anemia

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Original Investigation

The Role of Diagnosis and Clinical Follow-up of Monoclonal Gammopathy of Undetermined Significance on Survival in Multiple Myeloma

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IMPORTANCE Multiple myeloma (MM) is consistently preceded by the precursor state, monoclonal gammopathy of undetermined significance (MGUS). The average annual risk of progression from MGUS to multiple myeloma is 0.5% to 1.0%. Current guidelines suggest life-long clinical follow-up of individuals diagnosed as having MGUS depending on risk stratification. The impact of diagnosing and conducting clinical follow-up of MGUS on MM survival is unclear.

OBJECTIVE To estimate the impact of prior knowledge of MGUS diagnosis and comorbidities on MM survival.

DESIGN, SETTING, AND PARTICIPANTS We conducted a population-based study including all patients with MM (MM patients) diagnosed in Sweden (n = 14 798) from 1976 to 2005 (with follow-up until 2007); 394 (2.7%) had previously been diagnosed as having MGUS. Information on comorbidities was gathered for all patients. We calculated survival rates from the time of MM diagnosis, comparing patients with vs those without prior knowledge of MGUS. Using Cox proportional hazards models, we calculated hazard ratios (HRs) and 95% CIs for risk factors for death. χ^2 Tests were used to evaluate differences in comorbidities.

EXPOSURES Prior knowledge of MGUS among MM patients. In a subanalysis, monoclonal (M)-protein concentration and type were used as exposure.

MAIN OUTCOMES AND MEASURES Risk of death and comorbidities.

RESULTS Patients with MM with prior knowledge of MGUS had significantly (HR, 0.86; 95% CI, 0.77-0.96; $P < .01$) better overall survival (median survival, 2.8 years) than MM patients without prior knowledge of MGUS (median survival, 2.1 years), although MM patients with (vs without) prior knowledge of MGUS had more comorbidities ($P < .001$). Among MM patients with prior knowledge of MGUS, low M-protein concentration (<0.5 g/dL) at MGUS diagnosis was associated with poorer MM survival (HR, 1.86; 95% CI, 1.13-3.04; $P = .01$).

CONCLUSIONS AND RELEVANCE Patients with MM with prior knowledge of MGUS had better MM survival, suggesting that earlier treatment of MM leads to better survival. The observation that a low M-protein concentration at MGUS diagnosis was associated with poorer MM survival may reflect less frequent clinical follow-up. Our observations stress the importance of clinical follow-up in patients with MGUS, regardless of risk stratification.

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Multiple myeloma (MM) is a neoplastic disorder characterized by proliferation of plasma cells in the bone marrow, monoclonal (M)-protein in the blood and/or urine, and MM-related organ or tissue dysfunction.[1] Population-based studies have shown that survival in MM has improved significantly in recent years,[2-5] mainly owing to the introduction of novel agents (eg, thalidomide, bortezomib, lenalidomide, carfilzomib, pomalidomide), high-dose melphalan with autologous stem cell transplantation in younger patients, and improved supportive care.[2-5]

A recent prospective cancer screening trial including over 77 000 individuals followed for more than 10 years showed that MM is consistently preceded by a precursor state, monoclonal gammopathy of undetermined significance (MGUS).[6] Most MGUS cases, however, are never diagnosed. MGUS is characterized by a detectable M protein in persons without evidence for end-organ damage or other related plasma cell or lymphoproliferative disorders.[7] By screening, MGUS is detected in 2% to 3% of individuals 50 years or older and approximately 5% of persons 70 years or older.[8,9] However, only a small proportion of MGUS progresses to a malignant disorder[10]; in fact, the annual risk of progression to MM or other related disorders is, on average, 0.5% to 1%, with varying risks according to risk groups.[10-13] Current guidelines suggest, depending on the individual patient's clinical risk score, life-long monitoring of individuals with MGUS to detect progression to MM or related disorders.[11,14]

At this time, the impact of annual monitoring on the outcome of patients who eventually develop MM is unclear. Because MGUS is typically detected incidentally as part of medical workup for another cause,[15] the impact of comorbidity on survival in patients with MM (hereinafter, MM patients) with prior knowledge of MGUS is also unknown. To increase our understanding of the impact of prior knowledge of MGUS diagnosis and comorbidities on MM survival, we performed a large, population-based study using data on more than 14 000 MM patients diagnosed in Sweden from 1976 to 2005, with follow-up through 2007.

Methods

All residents of Sweden are, at birth or immigration, assigned a unique national registration number that is used in government-maintained nationwide health care and population registers, whereby medical record linkage is possible with a high degree of accuracy. Each individual's date of death is centrally registered in the Swedish Cause of Death Registry.

Since the mid-1950s, Sweden has provided universal medical health care for the entire population (currently approximately 9 million people). In contrast to many other countries, for example, the United States (where most hematologic patients are seen and treated primarily by physicians in private practice, outside hospitals), the Swedish health care system has a geographically defined referral structure for specialist assessments. Patients with hematologic disorders are typically diagnosed, treated, and followed clinically by physicians at hospital-based hematology or oncology cen-

At a Glance

- Almost 15 000 patients were diagnosed as having multiple myeloma (MM) in Sweden from 1976 to 2005.
- Three percent of patients had been clinically diagnosed as having MGUS prior to their MM diagnosis.
- Patients with prior knowledge of MGUS had significantly 14% (*P* < .01) better overall survival from MM (median, 2.8 years) than patients without prior knowledge of MGUS (median, 2.1 years).
- Patients with low M-protein (≤0.5 g/dL) at MGUS diagnosis had poorer survival from MM than patients who had higher M-protein concentration (0.5 to <3 g/dL) at MGUS diagnosis.
- Clinical follow-up in MGUS, regardless of risk stratification, may improve MM outcomes.

ters. These centers are affiliated with a few regional university hospitals, which offer inpatient hospital care to a defined primary catchment area in addition to being the hematology and oncology referral center for a larger health care region.

Since 1958, all physicians in Sweden have been required to report each case of cancer that they diagnose to the nationwide Swedish Cancer Register. The Register holds information on diagnosis, sex, date of birth, date of diagnosis, and area and hospital where the diagnosis was made, and has been reported to have more than 95% coverage for MM during the study period.[16] Information was gathered on all MM patients who were reported to the Swedish Cancer Registry from 1976 to 2005, with follow-up until 2007. Information on comorbidities was gathered from the Swedish Patient Registry, which captures information on individual patient-based discharge diagnoses and discharge listings from all inpatient care, with a very high coverage.[17] Information on date of death was gathered from the Cause of Death Registry.

Because MGUS is generally asymptomatic, it is usually an unexpected finding during a medical workup for another cause. In Sweden, when a clinician detects MGUS in a patient, he or she will typically consult with a hematology specialist at a regional hospital-based center, and, if needed, refer the patient for further workup, especially to rule out an underlying malignant neoplasm. These centers are affiliated with a hospital-based hematology and oncology centers.

The first population-based MGUS screening studies were initiated by Waldenström's group in Sweden in the early 1960s.[18] Indisputably, these early efforts have played an important role and facilitated an increasing awareness of MGUS among Swedish clinicians. In the present nationwide study, MGUS patients diagnosed between the late 1960s and the late 1970s were primarily diagnosed by Waldenström's group at Malmö University Hospital. During these years, diagnostic criteria were defined by the presence of an M protein in serum in the absence of an underlying lymphoproliferative malignant neoplasm.[18] From the early 1980s, efforts have been made, mainly influenced by Kyle's group at the Mayo Clinic,[19] to establish stringent criteria to distinguish MGUS from asymptomatic forms of myeloma and related disorders. MGUS is now defined by the presence of a monoclonal immunoglobulin level of less than 3 g/dL in serum; if bone marrow examination was

Table 1. Characteristics of 14 798 MM Patients With and Without Prior Knowledge of MGUS

Characteristic	Patients, No. (%)	
	MGUS-MM	MM
Total	394 (100)	14 404 (100)
Sex		
Male	189 (48.0)	7773 (54.0)
Female	205 (52.0)	6631 (46.0)
Median age at MM diagnosis, y		
All calendar periods	73	72
1976-1985	70	71
1986-1995	72	72
1996-2005	74	73
Calendar period at diagnosis		
1976-1985	26 (6.6)	4654 (32.3)
1986-1995	95 (21.1)	5198 (36.1)
1996-2005	273 (69.3)	4552 (31.6)
Isotype		
IgG	174 (44.2)	NA
IgA	95 (24.1)	NA
Unknown	125 (31.7)	NA
M-protein concentration, g/dL		
<0.5	24 (6.1)	NA
<1.5	120 (30.5)	NA
≥1.5	85 (21.3)	NA
≥2.5	11 (2.8)	NA

Abbreviations: MGUS-MM, multiple myeloma patients with prior knowledge of monoclonal gammopathy of undetermined significance; MM, multiple myeloma patients without prior knowledge of monoclonal gammopathy of undetermined significance; NA, not applicable.

performed, a plasma cell content of less than 10%; no evidence of other lymphoproliferative disorders; and the absence of clinical manifestations related to the monoclonal gammopathy.[7] These criteria are essentially the same as those used at Swedish hospitals during the study period.

Cohort

The following approaches were applied to establish a nationwide MGUS cohort: first, we retrieved information on all incident patients through our national network, which comprises all outpatient units, including all major regional hospital-based hematology/oncology centers in Sweden. For all MGUS patients, we obtained information on sex, date of birth, date of diagnosis, and region and unit where the diagnosis was made. When available, we also collected information on the MGUS isotype and concentration of the monoclonal spike at diagnosis. Second, we identified all MGUS patients who were reported in the Swedish Patient Registry, which captures information on individual patient-based inpatient and outpatients discharge diagnoses and discharge listings from all inpatient care, with a very high coverage.

Information on all MGUS patients from these 2 sources was merged into 1 master database. Using the nationwide Swedish Cancer Registry, which includes information on all incident cancers diagnosed since 1958 (including date of diagnosis and region and hospital where the diagnosis was made),[16]

we obtained data on all cancer diagnoses for all MGUS patients. To minimize the influence of misdiagnosis (eg, smoldering myeloma), MGUS patients with a lymphoproliferative malignant neoplasm diagnosed up to 6 months after MGUS were removed from the MGUS cohort. As an additional quality control measure, we removed any MGUS patient with a recorded preceding lymphoproliferative malignant neoplasm.[17]

To characterize patterns of underlying comorbidities among MM patients, using the Swedish Patient Registry,[17] we obtained all inpatient and outpatient discharge diagnoses for autoimmune diseases, infections, nonhematological malignant diseases, ischemic heart disease, heart failure, cerebrovascular diseases, chronic lung diseases, and renal diseases. This study was approved by the Regional Ethics Committee of Stockholm.

Statistical Analyses

Survival from time of MM diagnosis comparing patients with and without prior knowledge of non-IgM MGUS, diagnosed in the period of 1976 to 2005 with follow-up until 2007, was analyzed with Kaplan-Meier method. Risk factors for death were analyzed with a Cox proportional hazards model, wherein the hazards ratios (HRs) were estimated and 95% CIs calculated for the following variables: MM patients with prior knowledge of MGUS compared with MM patients without prior knowledge of MGUS, age at MM diagnosis, year of MM diagnosis, sex, immunologic isotype (IgG and IgA), as well as M-protein concentration. All calculations were adjusted for age of diagnosis, year of diagnosis, and sex. The cohort was divided into 3 equally large calendar periods: 1976 to 1985, 1986 to 1995, and 1996 to 2005.

We conducted extensive sensitivity analyses to exclude the effect of calendar time on the outcome. First, we conducted analyses stratified by calendar time, including only patients diagnosed during the last calendar period (1996-2005). Second, we conducted a nested case-control analysis in which all MM patients with prior knowledge of MGUS (cases) were matched (sex, year of MM of diagnosis ± 1 year, and age at MM diagnosis ± 1 year) with up to 4 MM patients without prior knowledge of MGUS (controls). All sensitivity analyses are provided in the eTables 1 through 3 in the Supplement. Third, we performed 2 analyses using cause-specific survival: one with MM as the primary cause of death and another using MM when listed as any underlying cause of death. Fourth, using Pearson product moment correlation coefficient, the correlation between the number of days from MGUS diagnosis until MM diagnosis and the age at diagnosis of MM was analyzed.

χ² Tests were used to test for differences in comorbidity patterns among MM patients with prior knowledge of MGUS vs those without prior knowledge. Statistical results were considered significant at P < .05. R 3.0.2 software was used for statistical analysis. Figures were drawn with the package ggplot2,[20] and models were fitted with the survival package.[21]

Results

The study cohort consisted of 14 798 patients diagnosed as having MM in Sweden between 1976 and 2005 and with fol-

Table 2. Hazard Ratios (HRs) and Median Survival of 14 798 MM Patients With and Without Prior Knowledge of MGUS

Patient Characteristic	HR (95% CI)	Median Survival (95% CI), y	P Value
MM[a]	1 [Reference]	2.14 (2.09-2.20)	<.01
MGUS-MM[a]	0.86 (0.77-0.96)	2.79 (2.56-3.25)	
M-protein concentration at MGUS diagnosis[b]			
<1.5 g/dL compared with ≥1.5 g/dL	1.01 (0.72-1.41)	NA	.99
<0.5 g/dL compared with ≥0.5-3.0 g/dL	1.86 (1.13-3.04)		.01
2.5-3.0 g/dL compared with <0.5 g/dL	0.79 (0.39-1.63)		.53
Isotype			
IgG	1 [Reference]	NA	.47
IgA	1.11 (0.84-1.47)		

Abbreviations: Ig, immunoglobulin; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; NA, not applicable.

[a] Adjusted for age at diagnosis, year of diagnosis, and sex.

[b] Hazard ratios of death with regard to monoclonal concentration and isotype at the time of MGUS diagnosis in MM. Adjusted for age at diagnosis, year of diagnosis, and sex.

low-up until 2007. A total of 394 MM patients had previously been diagnosed as having MGUS. Details of the study cohort are presented in **Table 1**. In MM patients with prior knowledge of MGUS vs those without prior knowledge, the median ages at MM diagnosis were 73 and 72 years, respectively (range, 19-101 years). Among MM patients with prior knowledge of MGUS, the median M-protein concentration at MGUS diagnosis was 1.2 g/dL (range, 0.1-2.9 g/dL).

The MM patients with prior knowledge of MGUS had significantly (HR, 0.86; 95% CI, 0.77-0.96) better survival (median survival, 2.8 years; 95% CI, 2.6-3.3 years) than those without prior knowledge (median survival, 2.1 years; 95% CI, 2.1-2.2 years) (**Table 2**). Survival of MM patients with prior knowledge of MGUS vs those without is shown in the **Figure**.

Among MM patients with prior knowledge of MGUS, an M-protein concentration of less than 0.5 g/dL at MGUS diagnosis was associated with poorer survival than having a concentration of 0.5 g/dL or greater (HR, 1.86; 95% CI, 1.13-3.04; P = .01) (**Table 2**). There was no difference in survival when monoclonal isotypes were compared (IgG vs IgA). The median time from MGUS to MM diagnosis was shorter for those MGUS patients with a higher M-protein concentration; for patients with an M-protein concentration of 0.5 g/dL or greater, the median time was 4.8 years, whereas it was 9.1 years for patients with M-protein concentrations of less than 0.5 g/dL.

In sensitivity analyses restricted to patients diagnosed as having MM after 1996, the results were essentially the same as the main model, with MM patients with prior knowledge of MGUS having significantly better survival than MM patients without prior knowledge of MGUS (HR, 0.81; 95% CI, 0.70-0.93). Similarly, in the sensitivity analysis based on a nested case-control design (see the Methods section), the results were similar to those of the main analysis (eTables 1-3 in the Supplement). Furthermore, in analysis using cause-specific survival (instead of overall survival) as the outcome, the risk of dying was lower in MM patients with prior knowledge of MGUS compared with MM patients without prior knowledge of MGUS; the HR was 0.75 (95% CI, 0.64-0.88) for patients with MM listed as a primary cause of death, and the HR was 0.79 (95% CI, 0.69-0.91) for patients with MM listed as an underlying cause of death. The correlation between the number of days from MGUS diagnosis until MM diagnosis and the age at diagnosis of MM was low (0.003) and nonsignificant (P = .94).

In models focusing on the impact of comorbidities at diagnosis of MM in relation to prior knowledge of MGUS, we found autoimmune diseases, infections, nonhematological malignant diseases, ischemic heart disease, heart failure, cerebrovascular diseases, and renal diseases to be significantly more common in MM patients with prior knowledge of MGUS (P < .001 for all comparisons) (**Table 3**). Patterns of comorbidity were similar independent of M-protein concentration at MGUS diagnosis (data not shown).

Discussion

The hypothesis that detection and follow-up of MGUS may influence survival in MM patients is unlikely to ever be tested in a prospective clinical study owing to the large sample size required, long follow-up time, and consequent extreme costs. Indeed, the current population-based study, including all patients diagnosed as having MM in Sweden from 1976 to 2005, linked with a nationwide clinical MGUS database, provides a unique opportunity to address this clinically important question. Based on a nationwide population-based cohort study including almost 15 000 MM patients, we found MM patients with prior knowledge of MGUS diagnosis to have better survival than patients diagnosed as having MM in the absence of prior MGUS knowledge. These findings were true even though MM patients with prior knowledge of MGUS (typically being detected incidentally as part of medical workup for another cause) had significantly higher prevalence of comorbidities than other patients. Quite counterintuitively, MM patients with prior knowledge of MGUS with low M-protein concentration (<0.5 g/dL) had significantly worse survival than those with higher M-protein concentrations (≥0.5 g/dL). We speculate that this may be reflective of current guidelines[11] suggesting less frequent monitoring of MGUS patients with lower M-protein concentrations. Our findings are striking and of clinical relevance because they demonstrate that, despite the lack of treatment options for patients with MGUS, the diagnosis and long-term follow-up of MGUS prolongs survival of patients with MM.

Current clinical guidelines suggest life-long monitoring of individuals diagnosed as having MGUS,[11,14] but how it influ-

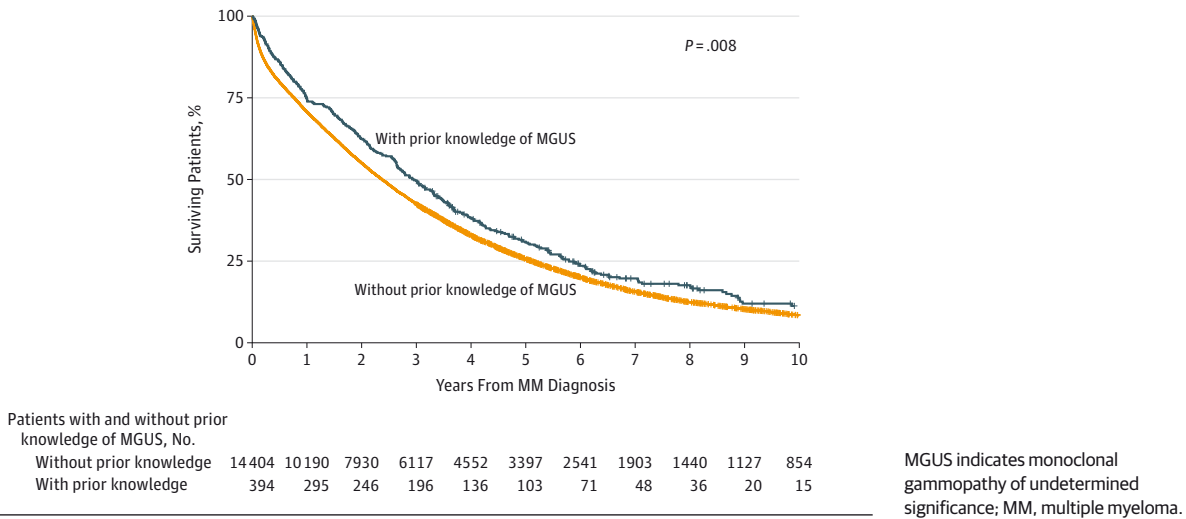**Figure. Survival Among MM Patients With and Without Prior Knowledge of MGUS**

Patients with and without prior
knowledge of MGUS, No.

Without prior knowledge	14 404	10 190	7930	6117	4552	3397	2541	1903	1440	1127	854
With prior knowledge	394	295	246	196	136	103	71	48	36	20	15

MGUS indicates monoclonal gammopathy of undetermined significance; MM, multiple myeloma.

Table 3. Comorbidities Among 14 798 MM Patients With and Without Prior Knowledge of MGUS

	At the Time of MM Diagnosis			At the Time of Death/End of Study Period		
Category[a]	MGUS-MM, No. (%)[b]	MM, No. (%)	P Value	MGUS-MM, No. (%)[b]	MM, No. (%)	P Value
Systemic autoimmune diseases	10 (2.5)	154 (1.1)	.01	13 (3.3)	241 (1.7)	.02
Organ-bound autoimmune diseases	34 (8.6)	740 (5.1)	.003	57 (14.5)	1534 (10.6)	.02
Autoimmune diseases without auto-antibodies	33 (8.4)	329 (2.3)	<.001	72 (18.3)	1552 (10.7)	<.001
Infections	110 (27.9)	2020 (14.0)	<.001	245 (62.2)	6643 (46.0)	<.001
Nonhematological malignant diseases	83 (21.1)	1548 (10.7)	<.001	NA	NA	NA
Ischemic heart disease	59 (15.0)	1241 (8.6)	<.001	95 (24.1)	2372 (16.4)	<.001
Heart failure	37 (9.4)	738 (5.1)	<.001	99 (25.1)	2952 (20.4)	.03
Cerebrovascular diseases	35 (8.9)	680 (4.7)	<.001	56 (14.2)	1549 (10.7)	.04
Chronic lung diseases	4 (1.0)	159 (1.1)	>.99	9 (2.3)	352 (2.4)	.97
Renal diseases	8 (2.0)	74 (0.5)	<.001	13 (3.3)	131 (0.9)	<.001

Abbreviations: MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; NA, not applicable.

[a] See eTable 1 in the Supplement for further information on each disease category.

[b] There were 394 patients (2.7%) with MM who were previously diagnosed as having MGUS.

ences outcomes remains largely unknown.[22] Interestingly, a recent study by a Spanish group[23] showed that treating high-risk smoldering myeloma resulted in improved progression-free and overall survival. We speculate that the reason for the prolonged survival observed in our study most likely reflects the fact that MGUS patients are evaluated more often for signs of MM progression and may be diagnosed and started on antimyeloma therapy at an earlier stage. This argues for early treatment approaches in MM[23] and raises the question of whether systematic screening for MGUS should be initiated. Alternatively, those with more aggressive disease may have a shorter duration of MGUS and are less likely to be diagnosed and identified during the MGUS state. Although, on a biological note, MM is consistently preceded by MGUS, most individuals are unaware of their MGUS diagnosis. This is due to the fact that MGUS typically is asymptomatic. Consequently, in the absence of population screening, most MM patients will be diagnosed owing to their symptoms rather than biochemi-

cal progression from MGUS to myeloma. Our results need to be confirmed by prospective studies.

Based on prior studies, the annual risk of progression from MGUS to MM is, on average, 0.5% to 1%.[10] However, recent risk models show a varying degree based on 4 risk factors: isotype, M-protein concentration, free light chain, and immunoparesis.[10,12] Interestingly, in our study, we found that among MM patients with a prior knowledge of MGUS, those individuals with an M-protein concentration of less than 0.5 g/dL at MGUS diagnosis had worse MM survival. Based on the fact that current guidelines suggest that low-risk (ie, a low concentration of M protein on serum protein electrophoresis test) MGUS require less frequent monitoring.[11] The reasons for this are unclear and need to be studied further. We have speculated that low-risk MGUS patients might be lost to follow-up or followed less intensely. Most low-risk patients will never develop MM, but according to our findings those who do develop MM seem to fare worse than high-risk MGUS patients

who develop MM. Our findings are consistent with those of a prior smaller study[22] of 116 MM patients seen at the Mayo Clinic between 1973 and 2004. That study showed that low-risk MGUS patients are more often diagnosed as having serious complications compared with high-risk MGUS patients. Although the risk of progression to MM is very low in individual patients, our findings suggest that life-long surveillance is important in MGUS patients independent of risk score.

As expected, we found that MM patients with prior knowledge of MGUS had significantly greater prevalence of comorbidities than other MM patients. These results most likely reflect the fact that MGUS is most often diagnosed during workup for an unrelated disease.[24] Previous studies have shown that comorbidity is a poor prognostic factor in the general population, with increasing mortality as number of comorbidities rises.[25,26] Despite our observation of a heavier burden of comorbidities, MM patients with previous knowledge of MGUS had significantly better survival than MM patients without prior knowledge of MGUS. Given this paradoxical difference, it can be argued that our study underestimates the true survival benefit of diagnosing and conducting clinical follow-up of MGUS. The impact of comorbidity in patients with MM and MGUS needs to be studied further.

Our study has several strengths. This is a register-based cohort study, which ensured a population-based setting and generalizability of the results. According to prior validation study, the Swedish Cancer Registry has a very high rate of completeness and diagnostic accuracy.[16] All information was registered before and independent of this study. In addition, all information was recorded prospectively in the database. As described in the Methods section, in Sweden, most individuals diagnosed as having an M protein are referred for further evaluation at specialized hematology centers. Based on the study design, we believe that the MGUS cohort in this investigation is quite representative of all individuals diagnosed as having MGUS and who subsequently developed MM in Sweden during the study period. Still, we cannot exclude the possibility that some general practitioners or other specialists diagnose MGUS without any further evaluation. Given the difference in the date of MGUS diagnosis, with most patients diagnosed in the last calendar period, we wanted to rule out the possibility that the findings were affected by superior survival in MM during that calendar period. Consequently, we performed extensive sensitivity analysis, including cause-specific survival, and the results were essentially the same. We therefore feel confident that the observed findings are robust. Our study also has some limitations. We did not have information from individual medical records. Therefore, we did not retrieve any information concerning treatment or other factors known to influence survival. Type and concentration of M proteins were available only for a proportion of the study cohort. In our study, individuals were identified as MM patients whenever they met the diagnostic criteria for MM. The proportion of patients diagnosed as having smoldering MM is unknown, and, because MGUS patients are typically diagnosed during workup for an unrelated condition, it is possible that they are more thoroughly investigated, and thus lead-time bias is a concern. However, median age at diagnosis is similar in both cohorts, which suggests minimal lead time bias. In addition, owing to lack of information on actual costs, we were unable to conduct cost-analyses designed to evaluate the financial burden of MGUS follow-up.

Conclusions

We found that despite a higher prevalence of comorbid conditions, MM patients with prior knowledge of MGUS have a survival superior to that of MM patients without prior knowledge. Furthermore, among MM patients with prior knowledge of MGUS, we found that a low M-protein concentration (<0.5 g/dL) was associated with shorter MM survival. This may be due to less intense monitoring in low-risk MGUS and needs to be studied further. Our results reflect the importance of life-long follow-up for individuals diagnosed as having MGUS, independent of risk score, and highlight the need for better risk models based on the biology of the disease. Patients should receive balanced information stressing not only the overall very low risk of progression to malignant neoplasm but also the symptoms that could signal such development and the need to consult their physician. The higher prevalence of comorbid conditions in MM patients with prior knowledge of MGUS supports the finding that MGUS most often is diagnosed during follow-up for unrelated conditions. Our findings raise the question whether screening for MGUS in the general population could translate into earlier detection and treatment of MM and lead to better MM survival.

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Monoclonal Gammopathy of Undetermined Significance and Risk of Arterial and Venous Thrombosis: Results from a Population-Based Study

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Running title: MGUS and thrombosis

Key words: monoclonal gammopathy of undetermined significance, population-based study, cohort study, arterial thrombosis, venous thrombosis, comorbidity

ABSTRACT

Background

Patients with multiple myeloma (MM) have an increased risk of venous and arterial thrombosis. There are also reports of an increased risk of thrombosis in its precursor condition monoclonal gammopathy of undetermined significance (MGUS). However, these studies have been performed on clinically established cohorts and are at risk of bias. In addition, the risk of thrombosis in light chain MGUS (LC-MGUS) has not been investigated.

Methods

We studied the longitudinal cohort of the AGES-Reykjavik Study, which includes 297 individuals with MGUS and 52 individuals with LC-MGUS that have been detected through screening. The outcome was first incidence of venous or arterial thrombosis, as diagnosis or as cause of death. Information on outcomes was provided through health care records with a median follow-up time of 8.8 years. Through logistic regression, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate history of thrombosis at study baseline. A Cox proportional hazards model was used to estimate hazard ratios (HRs) and 95% CIs for the risk of first incidence of thrombosis during follow-up.

Results

A history of any thrombosis was present in 30 (10.1%) of individuals with MGUS, 13 (25.0%) of individuals with LC-MGUS, and 642 (12.0%) of individuals without MGUS. When adjusting for age and sex, the risk of having had an arterial thrombosis at baseline was increased for LC-MGUS (OR = 2.0, 95% CI 1.03-3.8) but not for MGUS. No difference in history of venous thrombosis at baseline was detected in LC-MGUS or in MGUS. During a median follow-up time of 8.8 years, 80 (26.9%) of individuals with MGUS, 14 (26.9%) of individuals with LC-MGUS, and 1,343 (25.0%) of individuals without MGUS were diagnosed with a thrombosis. The crude risk of arterial, but not venous, thrombosis was increased in individuals with LC-MGUS (HR = 1.9, 95% CI 1.1-3.2). When adjusting for age, sex, and known risk factors, no statistically increased risk was found in MGUS or in LC-MGUS for arterial or venous thrombosis.

Conclusions

In this large, population-based, screening cohort study, we found no increased risk of arterial or venous thrombosis in MGUS, which opposes previous findings from clinically established cohorts. Individuals with LC-MGUS were more likely than individuals without MGUS to have a history of arterial thrombosis at baseline, and to experience an arterial thrombotic event during follow-up. To our knowledge, this is the first study to investigate risk of thrombosis in LC-MGUS. Future work is needed to better understand observed differences between studies and across populations, and to characterize the possible association between arterial thrombosis and LC-MGUS.

BACKGROUND

Multiple myeloma (MM) is a hematological malignancy where abnormal plasma cells proliferate and accumulate in the bone marrow.[1] All cases of MM are preceded by the asymptomatic condition monoclonal gammopathy of undetermined significance (MGUS).[2,3] MGUS is a premalignant condition defined by the presence of a monoclonal immunoglobulin (M-protein) in serum of less than 30 g/L, with less than 10% monoclonal plasma cells in the bone marrow, and absence of a lymphoproliferative disorder. The diagnosis requires the absence of hypercalcemia, renal insufficiency, anemia or skeletal lytic lesions (CRAB criteria), which can be attributed to an underlying plasma cell disorder.[1] MGUS is clinically relevant since the risk of progression to lymphoproliferative disorders is on average 1% per year.[4,5]

Since MGUS by its nature is asymptomatic and likely to remain undiscovered, information on etiology, comorbidity, and survival is limited. The prevalence of MGUS is estimated, based on a few screening studies as well as clinically established cohorts, to be approximately 2.4-3.5% in people aged 50 years or older.[6-9] Recently, light chain MGUS (LC-MGUS) was identified as a precursor condition leading to light-chain MM.[7,8] LC-MGUS is defined by an abnormal FLC ratio, with no expression of heavy chains, together with increased concentration of the involved light chain.[7] We recently proposed a new definition of LC-MGUS.(Paper I) The prevalence of LC-MGUS has been estimated to be 0.7 – 0.8%.[7,8]

Patients with MM have an increased risk of thrombosis, which has a negative impact on survival in these patients.[10,11] Some investigators have reported an increased risk in both venous and arterial thrombosis in individuals with MGUS, whereas others have detected no such risk.[10,12,13] However, previous studies have been performed on clinically established cohorts, and since MGUS is an asymptomatic conditions, the findings could be explained by the underlying comorbidities that lead to the detection of MGUS in the workup for other diseases.

The risk of thrombosis in LC-MGUS has previously not been investigated. Gregersen et al. found an increased risk of death from heart disease among individuals with MGUS in a clinically established cohort.[14] We recently showed, in a screened cohort, that individuals with MGUS and individuals with LC-MGUS have an increased risk of death from heart disease, and that the causes of death differ between the two conditions.(Paper II)

It is of clinical importance to investigate whether individuals with MGUS and LC-MGUS have an increased risk of thrombosis associated with the condition itself, or if previous results are due to the comorbidities likely to exist in clinically detected MGUS. An increased insight into the pathogenesis of such a risk could also shed light onto the management of thrombosis in MM. In this study we aimed to determine the association between arterial or venous thrombosis and MGUS and LC-MGUS in a screened, elderly population.

METHODS

Study Population

We performed a population-based cohort study. The group under study was the longitudinal cohort of the Age, Gene/Environment Susceptibility-Reykjavik Study (AGES-Reykjavik Study), which has been described previously.[15] Briefly, the Reykjavik Study is a longitudinal cohort study of men and women (30,795) born 1907-1934 in Iceland who were followed from 1967 onward in the Reykjavik Study by the Icelandic Heart Association. In 2002, 5,764 persons randomly chosen from survivors of the Reykjavik Study cohort were re-examined for the AGES-Reykjavik Study.

Participants in the AGES-Reykjavik Study examination in 2002-2006 completed a questionnaire, and underwent clinical examination as well as laboratory testing and radiological examinations. The participants are followed prospectively, and information on incidence of disease, date of death, and cause of death is collected annually through hospital, nursing home, and mortality records.

Blood samples from the participants in the AGES-Reykjavik Study were screened in 2012-2014 for monoclonal protein (M-protein) using serum protein electrophoresis (SPEP) and free light chain (FLC) analysis. MGUS was defined as a presence of M-protein band on SPEP, and those samples were also subjected to immunofixation (IFE). The definition of LC-MGUS was based on results from FLC analysis, and was defined as a pathological FLC ratio (<0.26 or >1.65) in combination with an increased concentration of more than 40.0 mg/L of the involved light chain. As described in a previous study, this screening yielded 300 individuals with MGUS and 52 individuals with LC-MGUS in the cohort.(Paper I)

Exclusion criteria were diagnosis of a lymphoproliferative disorder at baseline, missing consent form, and missing blood samples for SPEP, FLC and IFE analysis. A minimum follow-up time of ten days was required for inclusion in this study. Having a lymphoproliferative disorder as cause of death without a previous diagnosis of lymphoproliferative disorder was also an exclusion criterion, since it would be impossible to determine when the disease appeared and if it was really the true cause of death.

The outcome of interest was first incidence of venous or arterial thrombosis, including non-fatal and fatal events. Venous thrombosis was defined as pulmonary embolism, deep vein thrombosis, or other venous embolism (ICD codes I26, I80-I82). Arterial thrombosis was defined as acute coronary syndrome, ischemic stroke, or arterial embolism (ICD codes I20-I25, I63-I67, I74).

Information of previous occurrence of disease was available nine years back in time from study baseline. End of follow-up was March 2014. We had access to the first incidence of thrombosis for each individual.

The study was approved by the National Bioethics Committee of Iceland (VSN-00-063-V35), the Icelandic Data Protection Authority, the institutional review board for the NIH National Institute on Aging in the USA, and the Stockholm Reginal Ethics Review Board. Written informed consent was obtained from all participants.

Statistical Analysis

In a cross-sectional analysis, we assessed the presence of a history of thrombosis, as self-reported in the questionnaire or as ICD codes in health care records, at baseline. The difference in history of thrombosis was tested by Pearson's chi squared test and by a logistic regression model. A p-value of less than 0.05 was considered statistically significant. Analyses were made for a history of any thrombosis, and for arterial and venous thrombosis separately. In the logistic regression model, we calculated odds ratios (ORs) and 95% confidence intervals (CIs), both as crude estimates, and adjusted for age (as a continuous variable) and sex. In a multivariate model, ORs for arterial thrombosis were adjusted for known risk factors such as diabetes mellitus type II, hypertension (as a discrete variable), smoking (defined as previous-, current-, or never-smoker), family history of arterial thrombosis, and serum cholesterol level (as a continuous variable).[16-21] Similarly, ORs for venous thrombosis were adjusted for obesity (as measured by body mass index, BMI, continuous variable) and presence of any cancer (categorical variable, yes/no).[22-24]

Cox proportional hazards models were used to calculate hazard ratios (HR) and 95% confidence intervals (CI) for the risk of first incidence of thrombosis during follow-up. Participants were followed from baseline until time of thrombosis, death, or end of follow-up. The underlying time scale was time from study entry. Analyses were made for an incidence of any thrombosis, and for arterial and venous thrombosis separately. In the prospective analyses of arterial thrombosis, individuals with a history of arterial thrombosis at baseline were excluded, since they because of our data limitations were not at-risk for another event of arterial thrombosis during follow-up. Both crude HRs, as well as HRs adjusted for only age and sex, and HRs adjusted for several risk factors, were calculated. Similarly, in the prospective analyses of venous thrombosis, individuals with a history of venous thrombosis at baseline were excluded, and crude as well as adjusted HRs were calculated.

All calculations were performed using STATA version 13 (StataCorp 2013 Stata Statistical Software: Collage Station, TX, USA).

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RESULTS

From the original AGES-Reykjavik Study cohort of 5,764 individuals, 16 were excluded due to missing blood samples, one was excluded due to missing consent form, 22 were excluded due to lymphoproliferative disorder at baseline, one was excluded due to follow-up time less than ten days, and eight were excluded due to lymphoproliferative disorder as cause of death without a previous diagnosis of lymphoproliferative disorder. Overall, 5,716 individuals were included in the analyses, whereof 297 individuals with MGUS, 52 individuals with LC-MGUS, and 5,367 individuals with no MGUS (Table 1).

At study baseline, the median age of individuals with LC-MGUS (82 years) was higher than that of individuals with MGUS (78 years) or no MGUS (76 years), and male gender was more common among individuals with LC-MGUS. Information on MGUS isotype was available for all individuals with MGUS, whereas information on M-protein concentration was available for 54.2%.

Table 1. Characteristics of the study participants.

	MGUS[a]	LC-MGUS[b]	No MGUS
Total n[c] of cases (%)	297 (5.2)	52 (0.9)	5,367 (93.9)
Men, n (%)	158 (53.2)	34 (65.4)	2,222 (41.4)
Women, n (%)	139 (46.8)	18 (34.6)	3,145 (58.6)
Median age, years (range)	78 (67-93)	82 (69-96)	76 (66-98)
Age group, n (%)			
Less than 70 years	21 (7.1)	1 (1.9)	528 (9.8)
70-79 years	146 (49.2)	16 (30.8)	3,037 (56.6)
80-89 years	119 (40.0)	33 (63.5)	1,677 (31.2)
90 years and older	11 (3.7)	2 (3.8)	125 (2.3)
MGUS isotype, n (%)			
IgG	158 (53.2)	N.A.	N.A.[d]
IgA	27 (9.1)	N.A.	N.A.
IgM	79 (26.6)	N.A.	N.A.
IgD	1 (0.3)	N.A.	N.A.
Biclonal	32 (10.8)	N.A.	N.A.
M-protein concentration, n (%)			
>15.0 g/L	17 (5.7)	N.A.	N.A.
<15.0 g/L	145 (48.8)	N.A.	N.A.
Information missing	135 (45.5)	N.A.	N.A.
FLC[e] ratio			
0.26-1.65	167 (56.2)	0 (0.0)	N.A.
<0.26 / >1.65	130 (43.8)	52 (100.0)	N.A.

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]n: number, [d]N.A.: not applicable, [e]FLC: free light chain analysis.

Risk of Thrombosis at Baseline

A history of any thrombosis in the nine years prior to baseline was present in 30 (10.1%) of individuals with MGUS, 13 (25.0%) of individuals with LC-MGUS, and 642 (12.0%) of individuals without MGUS. The difference in history of thrombosis was statistically significant (p<0.01). Arterial thrombosis was more common than venous thrombosis in all groups (Table 2).

The risk of having had an arterial thrombosis at baseline was significantly increased for individuals with LC-MGUS (OR = 2.5, 95% CI 1.3-4.9), but not for individuals with MGUS. The risk remained increased in a model adjusted for age and sex (OR = 2.0, 1.03-3.8), but not when additional risk factors smoking, serum cholesterol, diabetes mellitus type II, hypertension, and a family history of thrombosis, were added to the model (OR = 1.9, 0.9-3.8).

No increased risk of venous thrombosis was found in individuals with MGUS or with LC-MGUS, compared to individuals without MGUS.

Risk of Thrombosis during Follow-up

During a median follow-up time of 8.8 years, 80 (26.9%) of all individuals with MGUS, 14 (26.9%) of all individuals with LC-MGUS, and 1,343 (25.0%) of all individuals without MGUS were diagnosed with any thrombosis (Table 3).

The risk of experiencing an arterial thrombosis was 1.9-fold increased for individuals with LC-MGUS (95% CI 1.1-3.2), but not for individuals with MGUS. When adjusted for age, sex, and risk factors for arterial thrombosis, the risk estimate was lower and not statistically significant (HR = 1.3, 0.7-1.3).

Individuals with MGUS did not have an increased risk of venous thrombosis in the prospective analysis, compared to individuals without MGUS. No individual with LC-MGUS experienced an event of venous thrombosis during follow-up.

Table 2. A history of thrombosis at baseline in individuals with MGUS and LC-MGUS, compared to individuals without MGUS.

| | MGUS[a] | | | | LC-MGUS[b] | | | | No MGUS | |
| | No.[c] | OR[d] (95% CI[e]) | | | No. | OR (95% CI) | | | No. | OR (95% CI) |
		Crude	Adjusted for age and sex*	Multivariate analysis[†]		Crude	Adjusted for age and sex	Multivariate analysis		
Any thrombosis	30 (10.1%)	0.8 (0.6-1.2)	0.7 (0.5-1.1)	0.8 (0.5-1.1)	13 (25.0%)	**2.5 (1.3-4.6)**	1.9 (1.00-3.6)	1.9 (0.94-3.7)	642 (12.0%)	1.00 (Ref.[f])
Arterial thrombosis	25 (8.4%)	0.8 (0.5-1.2)	0.7 (0.5-1.1)	0.7 (0.4-1.1)	12 (23.1%)	**2.5 (1.3-4.9)**	2.0 (1.03-3.8)	1.9 (0.93-3.8)	565 (10.5%)	1.00 (Ref.)
Venous thrombosis	6 (2.0%)	1.3 (0.6-3.0)	1.2 (0.5-2.8)	1.0 (0.4-2.5)	2 (3.9%)	2.5 (0.6-10.5)	2.0 (0.5-8.5)	2.0 (0.5-8.5)	84 (1.6%)	1.00 (Ref.)

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]No.: number of individuals, [d]OR: odds ratio, [e]CI: confidence interval, [f]Ref.: Reference. *Results adjusted for age and sex. [†]Results adjusted for age, sex, smoking, hypertension, cholesterol, diabetes mellitus type II, and family history of arterial thrombosis, and age, sex, body mass index, previous or current cancer, and family history of venous thrombosis, respectively.

Table 3. Risk of thrombosis in individuals with MGUS and LC-MGUS, compared to individuals without MGUS.

| | MGUS[a] | | | | LC-MGUS[b] | | | | No MGUS | |
| | No.[c] | HR[d] (95% CI[e]) | | | No. | HR (95% CI) | | | No. | HR (95% CI) |
		Crude	Adjusted for age and sex*	Multivariate analysis[†]		Crude	Adjusted for age and sex	Multivariate analysis		
Any thrombosis	80 (26.9%)	1.1 (0.9-1.4)	1.0 (0.8-1.3)	1.0 (0.8-1.3)	14 (26.9%)	1.5 (0.9-2.5)	1.1 (0.7-1.9)	1.1 (0.6-1.9)	1,343 (25.0%)	1.00 (Ref.[f])
Arterial thrombosis[††]	68 (25.0%)	1.1 (0.9-1.4)	1.0 (0.7-1.2)	0.9 (0.7-1.2)	13 (32.5%)	**1.9 (1.1-3.2)**	1.4 (0.8-2.4)	1.3 (0.7-2.3)	1,156 (24.1%)	1.00 (Ref.)
Venous thrombosis[**]	7 (2.4%)	0.9 (0.4-2.0)	0.9 (0.4-2.0)	0.9 (0.4-2.0)	0 (0.0%)	-	-	-	146 (2.8%)	1.00 (Ref.)

[a]MGUS: monoclonal gammopathy of undetermined significance, [b]LC-MGUS: light-chain monoclonal gammopathy of undetermined significance, [c]No.: number of individuals, [d]HR: hazard ratio, [e]CI: confidence interval, [f]Ref.: Reference. *Results adjusted for age and sex. [†]Results adjusted for age, sex, smoking, hypertension, cholesterol, diabetes mellitus type II, and family history of arterial thrombosis, and age, sex, body mass index, previous or current cancer, and family history of venous thrombosis, respectively. [††]Individuals with a previous arterial thrombosis excluded. [**]Individuals with a previous venous thrombosis excluded.

DISCUSSION

In this large, population-based study of more than 5,700 participants, we investigated for the first time in a screened cohort the risk of thrombosis in individuals with MGUS or LC-MGUS, and found no increased risk of arterial or venous thrombosis in MGUS. Furthermore, we demonstrate an elevated risk of arterial thrombosis in LC-MGUS. This indicates that LC-MGUS, but not MGUS, is associated with cardiovascular disease.

Our finding that individuals with MGUS are not at increased risk of neither arterial nor venous thrombosis is not in line with the findings of previous studies.[10,12] However, these previous studies have been performed on clinically established cohorts of MGUS patients. Since MGUS is an asymptomatic condition, the M-protein in these patients is typically found during work-up for unrelated conditions. Thus, the MGUS patients in the clinical cohorts often have several comorbidities,[25] and are not necessarily a representative sample of the individuals with MGUS in the population. It is plausible that previous findings of increased risk of arterial and venous thrombosis in these individuals may have been related to the underlying comorbidities, rather than an effect of the MGUS itself. Since MGUS in the individuals in our cohort was detected through screening, the results from our study are more probable to be reflective of the true risk of thrombosis MGUS.

We investigated the risk of venous and arterial thrombosis in LC-MGUS, and found no increased risk of venous thrombosis. Based on 52 individuals, we found that individuals with LC-MGUS were more likely to have a history of arterial thrombosis. When adjusting for several risk factors, the risk estimates of both the baseline analysis and the prospective analysis were elevated, but not statistically significant. Considering the increased crude estimates from both our baseline and prospective analyses on arterial thrombosis and LC-MGUS, it is plausible that our inability to gain statistical significance in the multivariate analysis is due to a power issue, stemming from small numbers and short follow-up time.

For individuals with LC-MGUS, the increased risk of having had a history of thrombosis at baseline was higher than the increased risk of experiencing a thrombotic event during follow-up. There are several possible explanations for this. In this data we only have access to the first incidence of arterial and venous thrombosis for every individual. Thus, for study subjects with an arterial thrombosis before baseline, any second incidence of arterial thrombosis during follow-up will go undetected. Thus, individuals with a history of arterial thrombosis were excluded in the prospective analysis, to prevent them from contributing person-time to the analysis without being at risk of the event of interest. It is possible that individuals with LC-MGUS and a high risk of arterial thrombosis tend to experience their thrombosis earlier in life, and are therefore, by the design of our study, prevented from experiencing another event later. Since we did not have access to information on treatment, another possibility is that some individuals with LC-MGUS experience events of thrombosis even earlier than nine years before baseline, and are protected from later thrombotic events through successful anticoagulation therapy.

The finding that the risks of thrombosis differ in MGUS and LC-MGUS is interesting, and in line with our recent study on survival and causes of death in MGUS and LC-MGUS (Paper II). We have demonstrated that individuals with LC-MGUS have an inferior survival, not only compared to individuals with no MGUS but also compared to individuals with

MGUS, and that the causes of death differ in LC-MGUS and MGUS. We also showed that individuals with LC-MGUS are at increased risk of death from heart disease. Taken together with the findings in this study, this suggests that individuals with LC-MGUS might have an increased risk of cardiovascular disease not observed in individuals with MGUS. This could be due to the FLC in individuals with LC-MGUS participating in the pathogenesis of cardiovascular disease. Furthermore, it is not necessarily the monoclonality of FLC that is the cause of disease, but it could also be the overload of FLC, regardless of the clonality, that causes disease. Elevated levels of polyclonal FLC have been associated with increased mortality as well as cardiovascular events.[26,27] Another possible explanation is a common etiology between cardiovascular disease and LC-MGUS.

Our study has several strengths; it is based on a large, population-based cohort of elderly people, who are well characterized with regard to extensive health-related factors and medical conditions. The screening approach, where MGUS status was determined through performing SPEP and FLC analysis on all participants, is an important strength adding to the validity of our study. Similarly, all analyses were performed by the same laboratory, the loss to follow-up in the cohort is non-existent, and information on outcomes were attained from high-quality registers with strict procedures. In our analysis we were able to adjust for important risk factors such as age, sex, obesity, family history, and laboratory findings.

However, some limitations need to be kept in mind when interpreting the results. Even though this is a screened study, bias might be present in the form of selective survival and participation. The median age in our cohort is rather high (77 years), possibly representing a selection of the population that is unusually healthy. Also, the group under study is an exclusively Northern European, Caucasian study group, and considering knowledge on MGUS variance across ethnic groups our findings may not be representative of everyone with MGUS.[6,28] We did not have the possibility to perform IFE on all samples that were normal on serum protein electrophoresis and fulfilled the criteria for LC-MGUS. Thus, the categories conventional MGUS and LC-MGUS might overlap in that some of the samples in LC-MGUS might have had a visible M-protein band on IFE and would then have been classified as conventional MGUS. Since MGUS is an asymptomatic condition, the M-protein detected through screening could have been present for any amount of time prior to the testing. We were not able to adjust for all known risk factors for thrombosis, such as for example hospitalization or surgery. Our results are based on only 52 individuals with LC-MGUS, and should be interpreted with caution. Furthermore, the information of previous diagnosis of thrombosis was only available nine years back in time from study baseline, and as mentioned, we only had access to information on first incidence of thrombosis.

In conclusion, we found no increased risk of venous or arterial thrombosis in MGUS subjects, and an increased risk of arterial, but not venous, thrombosis in LC-MGUS. These results suggest that the previously reported elevated risk of thrombosis in individuals with MGUS may be overestimated due to selection bias of MGUS in clinical cohorts. Furthermore, our findings imply that MGUS and LC-MGUS are two clinically different conditions, with different comorbidities and risk profiles. Attention should be directed towards further investigation of the association between LC-MGUS and arterial thrombosis.

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AUTHORSHIP CONTRIBUTIONS

EK Lindqvist, SY Kristinsson, O Landgren and SH Lund designed the study. G Eiriksdottir, V Gudnason, LJ Tauner and TB Harris obtained the data. R Costello and D Burton performed the serum analyses. M Björkholm, M Hultcrantz, N Korde and S Mailankody were involved in the designing of the study and in the management of the data. SH Lund and EK Lindqvist performed the statistical analyses. EK Lindqvist and SY Kristinsson wrote the report. All the authors were involved in the analyses and the interpretation of the results. All authors read, gave comments, and approved the final version of the manuscript. All the authors had full access to the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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