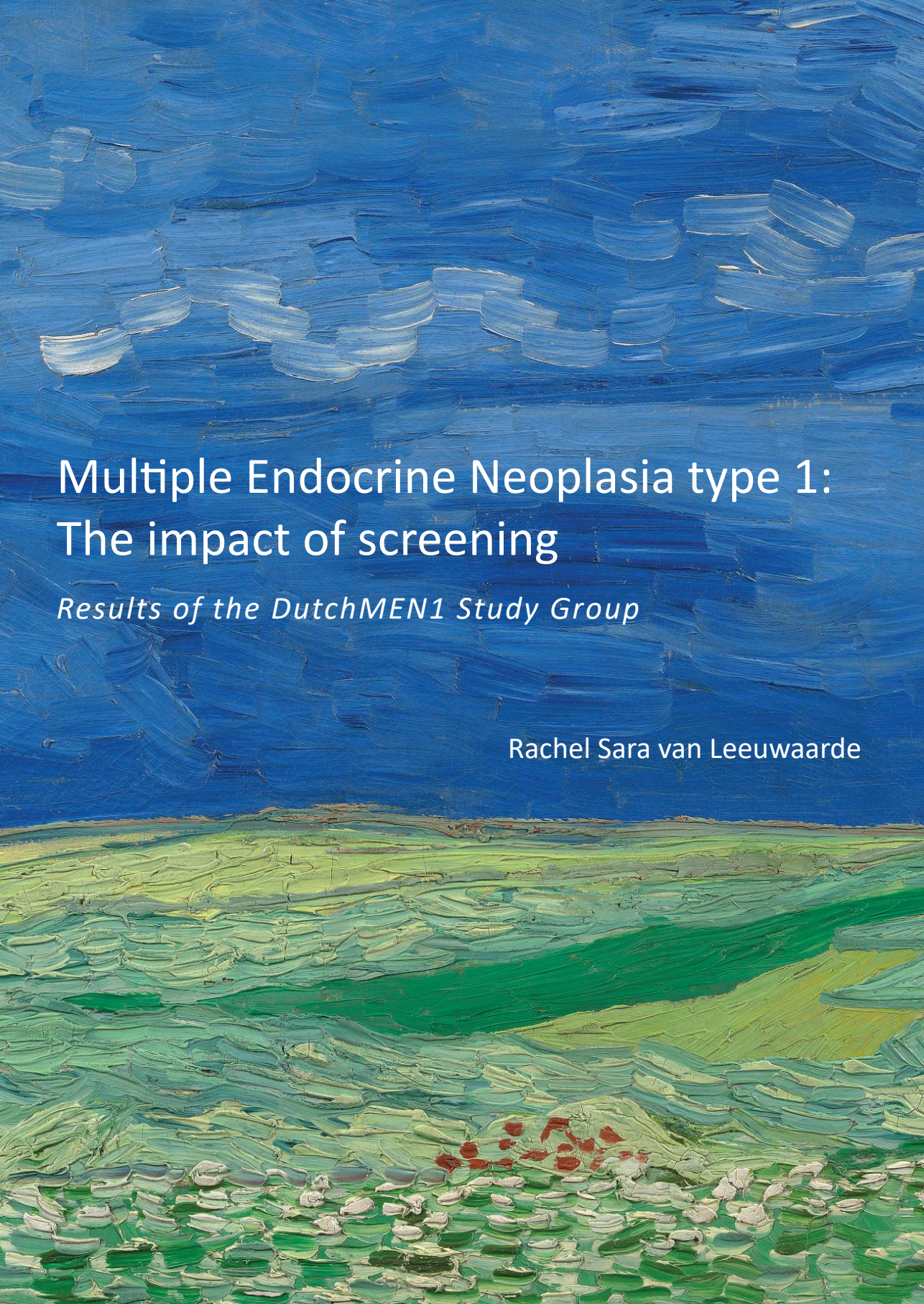

Multiple Endocrine Neoplasia type 1: The impact of screening

Results of the DutchMEN1 Study Group

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Multiple Endocrine Neoplasia type 1: The impact of screening

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Multipele Endocriene Neoplasie Type 1: De impact van screenen

Resultaten van de DutchMEN1 Study Group

(met een samenvatting in het Nederlands)

Proefschrift

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door

Rachel Sara van Leeuwaarde

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te Amsterdam

Promotoren Prof.dr. G.D. Valk
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Voor mijn vader

Contents

Chapter 1

General introduction and outline of the thesis

GENERAL INTRODUCTION

The first description of the simultaneous occurrence of multiple adenomas of endocrine glands was made by the Austrian pathologist Jakob Erdheim. Erdheim described a case of a growth hormone producing pituitary adenoma in association with parathyroid disease and thyroid nodules in 1903[1]. After fifty years, Underdahl[2] and colleagues reported a case series of eight patients with the combined occurrence of multiple endocrine tumors in which the parathyroid glands, the pituitary gland and the pancreas were affected. In 1954, one year later, Paul Wermer meticulously described four siblings and their father with primary hyperparathyroidism, ulcers, hypoglycaemia and pituitary tumors. In this report he proposed a genetic cause of the multiple endocrine tumors and suggested an autosomal dominant inheritance pattern[3]. This time, a new endocrine tumor syndrome, nowadays known as multiple endocrine neoplasia type 1, was born.

The next effort was to identify the involved chromosome and its specific genes. In 1988 the *MEN1* gene was mapped to chromosome 11q13 by linkage analysis[4]. Subsequently, in 1997 it was found that the *MEN1* gene consists of 10 exons and encoded a 610-amino acid protein, menin[5]. Eventually, in 1998 DNA analysis of the *MEN1* gene became available in the Netherlands.

Currently, the MEN1 syndrome is characterized by the classic triad of parathyroid, pituitary and (duodeno)pancreatic neuroendocrine tumors. Other encountered neoplasms are adrenocortical tumors, neuroendocrine tumors of the thymus and lungs, angiofibromas, collagenomas and lipomas. A recent association of breast cancer and MEN1 has further widened the landscape of MEN1[6].

The prevalence of MEN1 is estimated at 3-4/100,000, however, the age related penetrance of the three main manifestation is high. By the age of 33 years 50% of patients have developed their first manifestation. At an age of 80 years almost 100%, 90% and 80% have a primary hyperparathyroidism, dpNET and a pituitary tumor respectively. The mean age of death in the Dutch MEN1 cohort is 60 years (SD 12 years) of which 59% died due to a MEN1 related cause[7]. In comparison, the mean age of death in the general Dutch population in 2017 was 78 years (Centraal Bureau van de Statistiek).

In order to provide clinical guidance for physicians treating patients with MEN1, the clinical guidelines were developed. These guidelines were established by a self-assembled group of international leaders in the field of MEN1. Therefore, the guidelines mostly represent the views of these authors, rather than being evidence based guidelines. Obviously, this is not surprising given the rarity of the disease. Confronted by the complexity of the disease

and the need for sufficient high quality data, the DutchMEN study group was established in 2007.

THE DUTCHMEN STUDY GROUP

A nationwide collaboration, in which all eight academic hospitals of the Netherlands were represented, known as the DutchMEN study Group was established in 2008. Because patients with MEN1 are primarily treated in one of these tertiary referral centers, it was necessary to include all centers. The aim of this collaboration was to gain evidence based data by studying a representative unselected MEN1 sample. The nationwide nature of this study provided the highest sample size, which is reflected by a cohort of >90% of the Dutch MEN1 population. The cornerstone of this study group was a carefully designed longitudinal national database. The database was constructed based on pre-defined research questions and contains data from 1990 up to now. From the initiation of this study, the patient advocacy group was closely involved to guarantee the needs of the patients with MEN1. The retrospective database has now evolved into a prospective database accompanied by a biobank[8].

OUTLINE OF THE THESIS

Numerous scientific studies have improved our knowledge on the MEN1 syndrome over the years. Subsequently, we have shifted from diagnosing the disease when manifestations are already present to presymptomatic screening in patients with a proven *MEN1* germline mutation. Screening is a strategy to identify the possible presence of an as-yet-undiagnosed disease in individuals without signs or symptoms. The clinical practice guideline for MEN1 recommends a strict screening protocol from an early age[9]. The aim of screening is to prevent morbidity and mortality in patients with MEN1. Several MEN1 cohorts have revealed a reduced expectancy in comparison with the age-matched general population[7,10]. Nowadays, the main cause of death in the MEN1 population is metastasized neuroendocrine tumors[10–12]. In this light, screening assures an early diagnosis and subsequently minimizes premature mortality[13].

Familial screening for the *MEN1* gene is recommended when an index case is diagnosed with the disease. The autosomal dominant inheritance pattern gives rise to a 50% risk of carriership in first-degree relatives. This high risk with subsequent increased risk of morbidity, justifies screening in all first-degree relatives and other family members at risk.

Preferably, all family members should be screened in an early phase to prevent disease related morbidity and mortality. The most recent clinical guideline prescribes to start screening from the age of five years[9]. Naturally, screening is not necessary if no germline mutation is present, therefore these patients can be reassured and excluded from future screening. On the contrary, harbouring a germline mutation implies lifelong screening.

Underscoring the importance of an early diagnosis in individual family members, the aim of **chapter 2** was to determine the time between the diagnosis of *MEN1* in Dutch index cases and the subsequent *MEN1* diagnosis in other family members. The secondary aim was to determine the morbidity and mortality associated with this lag time. It was hypothesized that long lag times were associated with increased morbidity and possibly more mortality.

Current medical practice is moving towards personalized medicine as the preferred strategy in patient care. Patient characteristics that lead to higher risks for neuroendocrine tumors in MEN1 could result in intensified screening programs. Regarding the lifelong screening in patients with MEN1, a further intensified screening program, could lead to higher burden and concerns regarding the disease. Therefore, reporting new associations with the chance of an adjusted surveillance program should be done with caution. The National Institute of Health suggested an association between blood type O and pancreatic neuroendocrine tumors in MEN1[14]. Considering the possible adjustment on the neuroendocrine screening program by adding blood type as a prognostic factor, a validation study in a larger MEN1 cohort was undertaken. This effort underscored the necessity to obtain true prognostic markers for the development of neuroendocrine tumors in MEN1. The aim of **chapter 3** was to assess the association between blood type O and the occurrence and course of neuroendocrine tumors in patients with MEN1.

Screening of the Dutch MEN1 cohort according to the clinical guidelines and collecting data in the nationwide database has provided us with valuable information. Due to meticulously collected data, not yet known associations have become apparent. A relative risk for breast cancer of 2.83 (P<0.002) in women with MEN1 in comparison with the general population of the Netherlands was found. The mean age at diagnosis of breast cancer was 48 years (SD, 8.8 years) as compared with a median age of 61.2 years in the general Dutch population[6]. Menin as a co-regulator of the estrogen receptor α has been associated with breast cancer progression[15,16]. Loss of heterozygosity was revealed by DNA sequencing and confirmed by the reduction of more than 50% of the nuclear localization of menin[6]. Considering the impact of this finding for women with MEN1, understandable reluctance was postulated. In addition, the presumed association between blood type and neuroendocrine tumor occurrence learned that new associations should be approached with caution and a

critical view. In this respect, relevant questions arose whether adjustment for confounding factors as familial occurrence of breast cancer and other endocrine-related factors were considered. Furthermore, important questions on the consequence of screening of these women were posed[17]. Therefore, a validation study of these findings in larger study sample was performed. The initial aim of **chapter 4** was to assess the role of familial breast cancer risk, lifestyle, and endocrine-related risk factors in the higher risk for breast cancer in women with MEN1. The second aim was to formulate a recommendation on screening for daily clinical practice of MEN1 care.

Considering the significant morbidity and subsequent lifelong screening from an early age in patients seem at risk for an impaired quality of life (QOL). **Chapter 2** proposes a timely familial screening of the *MEN1* gene, which results in a burden of having the disease from a young age. Mutation carriers are subsequently exposed to an extensive lifelong screening programme. Novel findings and risk factors could lead to enhanced surveillance to guarantee more certainty in preventing morbidity. These factors could induce patients' worry about the disease and might lead to a decreased QOL. Therefore, a population based study in a large MEN1 cohort to assess this worry and QOL seemed at hand. In general, the impact of a disease is well represented by the QOL. Previous studies assessing the QOL in MEN1 populations describe an impaired QOL in comparison with the general population and a higher rate of depression[18,19]. These studies have assessed the QOL in selected populations, which are therefore prone for selection bias. The primary aim of **chapter 5** was to assess the health-related quality of life (HRQOL) in the Dutch MEN1 cohort in order to compare the HRQOL with the general Dutch population and to assess which variables were predicators for worse HRQOL. The secondary aim was to evaluate if the self-reported MEN1 manifestations were in line with the disease status as reported in the medical records and whether a discrepancy affected the HRQOL. The same cohort also filled out a cancer worry scale, which represents the fear of disease occurrence (FDO) in patients with MEN1. The primary aim of **chapter 6** was to evaluate MEN1-related FDO in patients themselves and for their family members with MEN1. The secondary aim was to assess the association of MEN1-related fear on QoL. In addition, we aimed to identify variables that were significantly related to MEN1-related fear.

Chapter 7 provides an overview of the medical advances in MEN1 regarding therapeutic approaches and management strategies from recent years.

Chapter 8 will discuss the findings of this thesis in light of our current MEN1 knowledge and daily clinical care. In this chapter our view for future studies and MEN related care will be elaborated.

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Chapter 2

Impact of delay in diagnosis in outcomes in MEN1: *Results from the Dutch MEN1 study group*

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Abstract

Objective

Identifying a germline mutation in the MEN1 gene in an index case has consequences for a whole family. Eligible family members should be offered genetic counseling and MEN1 mutation testing. Subsequently clinical screening of mutation carriers according to the guidelines should be initiated. We assessed if there is a lag time from MEN1 diagnosis of the index case to MEN1 diagnosis of family members. In addition we determined whether this lag time was associated with an increased morbidity and mortality risk.

Design

A cohort study was performed using the Dutch MEN1 database, including >90% of the Dutch MEN1 population >16 years (n=393).

Results

Fifty-eight MEN1 families were identified of whom 57 index cases and 247 non-index cases (n=304). The median lag time in MEN1 diagnosis of family members was 3.5 years (range 0-30). At the time of MEN1 diagnosis 30 (12.1%) non-index cases had a duodenopancreatic NET of whom 20% had metastases with a mean lag time of 10.9 years, in comparison with 7.1 years without metastases. Twenty-five (10.1%) non-index cases had a pituitary tumor of whom 80% had a microadenoma and 20% a macroadenoma, with mean lag times of 7.2 and 10.6 years respectively. Ninety-five (38.4%) non-index cases had a primary hyperparathyroidism with a mean lag time of 9.5 years in comparison with 7 patients without a hyperparathyroidism with a mean lag time of 3 years (*P*=0.005). Ten non-index cases died because of a MEN1 related cause that developed in or before the lag time.

Conclusion

There is a clinically relevant delay in MEN1 diagnosis in families because of a lag time between the diagnosis of an index case and the rest of the family. More emphasis should be placed on the conduct of proper counseling and genetic testing in all eligible family members.

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1) is a rare genetically inherited disease caused by a germline mutation in chromosome 11q13. It is inherited in an autosomal dominant pattern and therefore the risk of carriership in first degree relatives of MEN1 patients is 50%. MEN1 is characterized by the combined occurrence of (i) parathyroid hyperplasia or adenomas causing primary hyperparathyroidism (pHPT) (ii) neuro-endocrine tumors (NETs) of the pancreas and duodenum (dpNET) (iii) pituitary tumors (PIT) (iv) NET of the stomach, thymus and lung, and (v) adrenal hyperplasia or adenomas. Mortality is mostly related to thymic NETs and duodeno-pancreatic NETs[1] (dpNETs). The prevalence of MEN1 is estimated at 3-4/100,000, which underscores the rarity of the disease[2].

The MEN1 diagnosis in an individual is established if one of the following three criteria is met: the presence of two or more primary MEN1 related endocrine tumors: (i.e. pHPT, dpNET, and PIT), the occurrence of one of the MEN1-associated tumors in a first-degree relative of a patient with a clinical diagnosis of MEN1; and identification of a germline MEN1 mutation[3]. In case patients with sporadically occurring tumors are suspected for MEN1, their MEN1 risk can be calculated[4]. However, considering the very low prevalence of the disease, a doctor's delay in recognizing MEN1 in patients with apparently sporadically occurring MEN1 related tumors seems obvious. The lag time between index diagnosis and diagnosis of family members could be considered another type of delay.

The consequences of both types of delay in diagnosis can be deleterious. In several MEN1 cohorts a significantly reduced life expectancy in patients with MEN1 was described in comparison with the age matched general population. Causes of death were often directly related to MEN1[4–6]. Earlier diagnosis may result in a decrease of premature mortality[4]. Periodic screening for mutation carriers has been proposed to reduce morbidity and mortality, because manifestations are discovered in an often presymptomatic phase and treatment can be initiated in time to prevent further progression[3,7]. Genetic screening leads to less morbidity in comparison to patients with a clinical MEN1 diagnosis[8,9].

The current clinical practice guidelines recommend that patients with MEN1 and their first degree family members should be offered genetic testing from the age of 5 years. Individuals with a genetic predisposition for developing MEN1 related tumors should be offered periodic clinical screening to detect manifestation in a presymptomatic stage[3]. Therefore, genetic counseling and mutation analysis of family members at risk of carrying a MEN1 mutation is of utmost importance. However, genetic testing of the entire family of MEN1 patients is not always performed because not all family members are in contact with the index case, the physician is not allowed to contact family members directly because

of ethical considerations and some patients simply refuse genetic counseling. Potentially, presymptomatic MEN1 mutation carriers can therefore be unaware of their mutational status for a long time. This raises the question whether MEN1 related morbidity and mortality could be reduced in families if genetic counseling and testing in the whole family is immediately performed at time of diagnosis of the index case.

The primary aim of this nationwide study was to determine the time between the diagnosis of MEN1 in Dutch index cases and the subsequent MEN1 diagnosis in other family members. The secondary aim was to determine the morbidity and mortality associated with this lag time.

PATIENTS AND METHODS

Patients

In this analysis, all patients from the national MEN1 cohort of the Dutch MEN1 study group (DMSG) were included. Patients were diagnosed according to the recently updated clinical practice guidelines[3], if they were aged 16 years or older and treated at one of the Dutch University Medical Centers (UMCs). In each UMC, MEN1 patients were identified by a standard identification procedure using the hospital diagnosis databases. This longitudinal database with 24 years of follow-up includes >90% of the total Dutch MEN1 population. Clinical and demographic data were collected by medical record review in a standardized manner using predefined definitions[11,12]. Data of all identified patients were collected from every quarter of every available year of follow-up during the period 1990-2014. Family relationships/trees were documented in the medical records at the department of internal medicine or clinical genetics. Patients were eligible for the present analysis if they had a confirmed *MEN1* mutation and had at least one family member with an identical *MEN1* mutation. Patients were considered an 'index' cases if they were the first to be diagnosed with MEN1 within their family. Non-index cases were patients diagnosed with MEN1 as the result of a previous MEN1 diagnosis of a family member. If patients received a definite MEN1 diagnosis not as a result of family screening, although other family members were already diagnosed with MEN1, these patients were also considered index cases because they were not diagnosed as a result of family screening. MEN1 patients without family members with a MEN1 diagnosis were not included in this analysis.

Before 1998 the diagnosis of MEN1 was based on clinical criteria; after 1998, patients were mainly diagnosed by direct mutation testing.

The study protocol was approved by the medical ethical committees of all UMCs in The Netherlands. Detailed information on the DMSG database methods was described previously[11].

Statistical analysis

Lag time from index diagnosis

The lag time elapsing between the date of the MEN1 diagnosis of the index case and the date of MEN1 diagnosis in a non-index case (either by genetic testing or clinical diagnosis) in the same family was defined as "lag time" in this study. In a family with more than one index case, the first chronological index case was used to calculate the lag time. For determining the morbidity that arose during the lag time, non-index cases were only included in the analysis if the manifestation of interest was present or not and the lag time could be calculated at the moment of diagnosis. In 2001, the clinical guideline for diagnosis and therapy of MEN type 1 and type 2 was published[13], and in 2007, the DMSG was founded, a collaboration focused on improving MEN1 research and care in all eight UMCs in The Netherlands. To assess the influence of these changes in the care of patients, the lag time was analyzed per period.

The mean with ± SD or median with range was calculated for analysis of the descriptive data. Continuous variables were analyzed by using independent sample t test or Mann-Whitney U test. Dichotomous variables were compared with logistic regression. Lag time was used as a continuous variable and defined in years. Because of non-normal distribution logarithmic transformation of the lag time in years was performed.

Generalized linear mixed model analysis was applied to adjust for clustering within families. Possible confounding was assessed for age. All analyses were conducted using SPSS 21.0 and R Studio version 0.99.441.

RESULTS

Study population

In the period 1990-2014, a total of 393 MEN1 patients were included in the DMSG database, and 58 different MEN1 families with at least two family members were identified. In all families, a MEN1 germline mutation was confirmed, except in one family. The largest family consisted of 25 members with MEN1, and the smallest of two members. In six families, there was more than one index case; the second index case was a family member from

another side of the family. Fifty-seven index cases and 247 non-index cases were eligible for the present analysis (n=304). The index case was diagnosed in 25 (43%) families before mutation testing was available in 1998 and in 33 (57%) families thereafter. Patients who had no family members with MEN1 (n = 89) were not included in this analysis. A total of 57 (18.8%) patients were diagnosed solely because of the presence of two or more clinical manifestations (index cases) without other family members being diagnosed with MEN1. Non-index cases were diagnosed either in a presymptomatic phase by genetic testing (n=132) or because of a MEN1-related tumor and a (first-degree) family member with a MEN1 diagnosis (n=115).

Lag time from index diagnosis and morbidity at time of diagnosis

The median lag time from MEN1 diagnosis of the index cases to the MEN1 diagnosis of their individual family members was 3.5 (range, 0-30) years. The lag time before 1998 was 8 (range, 0-30) years, and from 1998-2001 it was 2.6 (range, 0-15.5) years (P<0.001) (Table 2.1). The median lag time from 1998-2001 was longer than the period from 2001-2007, which was 1.4 (range, 0-7.75) years (P<0.001). The lag time after 2007, the year the DMSG was founded, was 0.75 years (P=0.119) (Table 2.1). Only one family, consisting of one index case and two relatives, was diagnosed after the publication of the revised MEN1 management guidelines in 2012. There was no lag time in this family.

A total of 95 (38.4%) non-index cases had pHPT at the moment of the diagnosis of MEN1, and only seven (2.8%) non-index cases had no pHPT. The age-adjusted mean lag times were 9.5 (SD, 8.8) and 3 (SD, 4.1) years, respectively (P=0.035). Data on pHPT were not

Table 2.1. Median lag time from index cases to family members

Moment diagnosis of index case	Median (in years)	Min, max	*P*
Total	3.5	0, 30	
<1998	8	0, 30	0.001
1998-2001	2.6	0, 15.5	
1998-2001	2.6	0, 15.5	0.004
2001-2007	1.4	0, 7.75	
2001-2007	1.4	0, 7.75	0.119
>2007	0.75	0, 5.75	

Mann-Whitney U test, time in years.
1998: Start genetic testing *MEN1* gene in the Netherlands.
2001: Publication of the Guideline for Diagnosis and Treatment of MEN1 and MEN2.
2007: Initiation Dutch MEN1 study group.

available in 13 patients. Twenty-five (10.1%) non-index cases had a PIT at the moment of MEN1 diagnosis, with a mean lag time of 7.9 (SD, 8.7) years. There was no PIT present at the moment of diagnosis in 181 (73.3%) non-index cases, and in 41 non-index cases the PIT status was not known. Twenty patients had a microadenoma with a mean lag time adjusted for age of 7.2 (SD, 8.7) years, and five had a macroadenoma with a mean lag time of 10.6 years (*P*=0.0834). The mean lag time for pituitary macroadenomas with compression of the optic chiasm was 19.9 (range, 19.3-20.5) years, but there were only two family members with chiasmic compression.

Thirty (12.1%) non-index cases had a dpNET with a mean lag time of 7.9 years (SD, 8.5) years. There was no dpNET present at the moment of diagnosis in 188 (76.1%) non-index cases, and in 29 non-index cases the dpNET status was not known. The mean lag time between dpNET smaller and larger than 20 millimeters was not statistically different (*P*=0.831): 8.2 years (SD, 8.9) and 7 (SD, 7.5) respectively. The mean lag time was 10.9 years (SD, 12) years in non-index cases (n=6) with a metastasized dpNET.

Generalized mixed model analyses were applied to adjust for clustering in families, but the logistic regression models without random slopes or intercepts were the models with the best fit in all analyses, according to Akaike's Information Criterion.

Mortality

Ten patients (4%), nine men and one woman, died due to a MEN1 related cause that might have developed before or within in the lag time (Table 2.2). The manifestation that eventually lead to death was diagnosed at time of MEN1 diagnosis and not before the MEN1 diagnosis.

Index cases vs. non-index cases

The mean age of MEN1 diagnosis of the index cases was higher than the age of diagnosis of the non-index case: 42 years and 34 years respectively (*P*=0.001).

Manifestations at time of MEN1 diagnosis

There were no manifestations present at the moment of the MEN1 diagnosis of the six patients less than 10 years of age. In the 10- to 20-year age group, 12 (20.3%) of the 59 non-index cases had a pHPT, three (5.1%) had a PIT, and three (5.1%) had a dpNET. dpNETs in this age group were smaller than 20 mm, and no metastases were present at the time of diagnosis. In all other age groups, one or more manifestations were present

Table 2.2. Mortality in non-index cases

Cases	Gender	Age at diagnosis	Cause of death	Delay**
1.	Male	63	Metastasized dNET	2
2.	Male	41	Metastasized thymus NET	2.3
3.	Male	40	Metastasized thymus NET	2.5
4.	Male	55	Metastasized pNET	6
5.	Male	75	Metastasized pNET	6.3
6.	Male	52	Metastasized NET	10
7.	Female	59	Bleeding ZES*	15.5
8.	Male	46	Metastasized thymus NET	19.3
9.	Male	61	Metastasized pNET	20.5
10.	Male	74	Metastasized pNET	2.3

* Zollinger-Ellison Syndrome.
** Diagnostic delay from index case in years.

at the moment of MEN1 diagnosis (Table 2.3). Fifty-three family members had more than one manifestation at the time of diagnosis. Fifteen family members had more than two manifestations at diagnosis.

A total of 39 family members without a MEN1 diagnosis were under medical care for a pHPT while a family member was already diagnosed with MEN1. Three family members were treated for a dpNET and two for a PIT with a confirmed MEN1 diagnosis in a family member. Another three patients had more than one related MEN1 manifestation and a family member with MEN1, but were not identified as having MEN1.

Table 2.3. Manifestations at time of diagnosis according to age in non-index cases

Age	pHPT	dpNET	PIT	Total
<10	0	0	0	6
11–20	12	3	3	59
21–30	19	3	6	48
31–40	27	4	7	45
41–50	18	8	5	35
51–60	13	8	3	25
>60	12	6	4	21

DISCUSSION

MEN1 is not only a diagnosis for an individual patient but also has implications for the whole family. If one family member has a proven mutation in the MEN1 gene, preferably all eligible family members should undergo mutation analysis. In our Dutch MEN1 population, there is a clinically relevant delay in MEN1 diagnosis in families from the moment the index patient is diagnosed with MEN1. The mean lag time in families has significantly decreased since the start of genetic screening in 1998, which can be considered a landmark in the diagnosis of MEN1. Publication of the international guideline in 2001 has led to a further significant decrease in lag time. A decrease of lag time is also seen after 2007, the year the Dutch MEN1 study group was founded. After publication of the revised MEN1 management guidelines in 2012, only one new MEN1 family was diagnosed. In this family there was no lag time. A longer follow-up will reveal whether there is a significant difference between lag times before and after the publication of the revised guidelines. The morbidity in family members, when a lag time is present, ranges from dpNET with metastases, pituitary macroadenomas and presence of multiple MEN1 manifestations. Ten patients died because of a manifestation which might have developed within or before the lag time from the index case. These findings suggest that morbidity and mortality can be reduced if more emphasis is placed on genetic counseling and testing of the whole family at the time the index case is diagnosed.

Strengths

To our knowledge, this is the first time the lag time in MEN1 diagnosis from the diagnosis of the index case to diagnosis of other family members has been investigated, as well as the morbidity arising from this lag time. This is therefore a novel way of addressing diagnostic delay in MEN1 patients, which reveals serious clinical consequences. A major strength of this study is the DMSG database, which consists of >90% of all MEN1 patients in The Netherlands. This high coverage of MEN1 patients minimizes the occurrence of selection bias. Furthermore, the database contains data from 1990 to 2014 collected every quarter of every year. The long follow-up and high density of the data make this database very suitable and reliable for calculating the lag time to diagnosis of the individual manifestations. All genetic MEN1 analyses were performed at one central location in The Netherlands; therefore, the chance of missing genetic analyses or families is minimal. This database also allowed us to make family trees of all families to identify index cases and their family members. The lag time was calculated according to the present guideline that recommends that MEN1 germline mutation testing should be offered to first-degree relatives of MEN1 patients at the age of 5 years.

Limitations

It is questionable whether the manifestations developed in the period of the lag time from diagnosis of the index case or whether the manifestations were present before MEN1 was diagnosed in the index cases. However, even if manifestations were present before theMEN1diagnosis of the index case, one can expect that the manifestations progressed in the lag time. In this view, lag time is still relevant.

Comparison with previous literature

In previous studies, it is acknowledged that early diagnosis of MEN1 reduces morbidity and mortality. Genetic testing and periodical clinical screening may lead to a better clinical outcome[6,9,10].

In one study the age of diagnosis in index cases is significantly higher than in family members, 47.5 vs. 38.5 (P<0.001) respectively[14]. We confirmed this finding in our study; however, the ages in both patient groups were lower in our study, namely 42 vs. 34 years (P=0.001), probably reflecting differences in case mix because we report the results of a true national database including >90% of the total Dutch MEN1 population above the age of 16 years.

Clinical implications

Considering the morbidity and mortality that arise in the lag time, our results imply that all family members of MEN1 patients should be counseled and offered mutation analysis as soon as possible from the moment the index case is diagnosed. A timely start of regular screening in accordance to the guidelines is of equal importance. The guideline recommends to start screening from the age of 5 years, based on the presentation of a pituitary macroadenoma in a child at the age of 5 and pHPT in another child of 5 years of age[15,16]. In our cohort the six patients younger than 10 years did not have any manifestation at diagnosis. The first manifestations were diagnosed in the age group of 10-20 years. Considering the low prevalence of manifestations under the age of 10, an informed decision should be made weighing the risk and benefits to start screening at such a young age.

Remarkable are the number of patients in care for a MEN1 related manifestation without a MEN1 diagnosis while a family member is already diagnosed with MEN1. This indicates that physicians are often unaware of the possibility of MEN1 causing endocrine diseases and the importance of the family history[4]. Evidence based clinical guidelines can improve awareness and knowledge, but also offer guidance for clinical practice. After publication

of the clinical guideline in 2001 a significant decrease in lag time has been observed in the Netherlands. The implementation and adherence to the guideline has thus been successful. Although not statistically significant, the collaboration of all academic hospitals in the DMSG in 2007 has led to a further decrease of the lag time (1.4 years vs 0.75 years). The publication of the guideline and this collaboration have contributed to the awareness of MEN1 by organizing meetings, improving education, and working together in patient care and research. Combining guidelines with educational interventions and making guidelines easy to understand are two important aspects in enhancing the use of guidelines in primary care[17]. Primary care providers have expressed that a lack of education and the challenge of keeping up with the guidelines made them uncertain about guidelines, diagnosis, and treatment. This is especially challenging for a rare disease such as MEN1 because the prevalence in primary care is very low. Meeting with academic mentors to discuss clinical questions and reinforce the guidelines could improve the use of the guidelines[18]. Relatives with lag times in this study were receiving both specialist and in primary care.

Relatives and their physicians may been not have been aware of the presence of MEN1 in their family. Index cases may feel the burden of bringing bad news to the family and consider this as an obstacle. On the other hand, social consequences such as employment and insurance issues could make informed family members reluctant in genetic screening[19]. A relevant issue is that the index case might not know all family members, especially if the family is big and not living in the same area. This is illustrated by the finding that in 6 families more than 1 index case was identified. Apparently the second diagnosed index case was not aware of the MEN1 diagnosis of the first index case in the family. These families were relatively big with more than 10 family members with MEN1. One can expect that the whole family is at least twice as big and contact between different family members differs.

Ethical considerations as an individual's right not to know their genetic predisposition as well as their privacy are matters of concern[19]. Some authors propose that close family members should receive written information about their risk, even without the consent of the affected MEN1 relatives[19,20]. Considering the morbidity and mortality associated with a delay of MEN1 diagnosis in families, at least this should initiate the discussion with ethical policy makers.

An extension of doctors delay: Lag time from index diagnosis

In the literature, until now more emphasis has been on the lag time between the appearance of the first sign, symptom, or manifestation to the diagnosis of MEN1. This is the so-called doctor's delay. These lag times vary from 7.6 to 17.2 years[7,9,21]. Proposed reasons for this

delay are the lack of acknowledgement of the MEN1 syndrome and the insufficient sharing of medical information about the patients among medical practitioners[14]. This is especially relevant for index cases because they are diagnosed solely on clinical grounds. However, family members (non-index cases) outnumber the index cases and therefore, in our view, more emphasis should be placed on immediate genetic counseling and testing of eligible family members after diagnosis of the index case. There are clinically relevant manifestations when there is lag time; 30 patients had a pNET, of which seven patients already had metastases. The mean lag time for a microadenoma, a macroadenoma and chiasm compression increases for each stadium, which was 8, 10.6 and 19.9 years, respectively. The difference in lag time was not statistically significant because of the low prevalence of subjects, but a concordant increase could be observed. In conclusion, immediate genetic testing of family members of MEN1 patients and prompt clinical screening according to our MEN1 guidelines will prevent morbidity and mortality and improve long-term outcome in MEN1 patients.

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Chapter 3

No association of blood type O with neuroendocrine tumors in multiple endocrine neoplasia type 1

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Abstract

Context

An association between ABO blood type and the development of cancer, in particular, pancreatic cancer, has been reported in the literature. An association between blood type O and neuroendocrine tumors in multiple endocrine neoplasia type 1 (MEN1) patients was recently suggested. Therefore, blood type O was proposed as an additional factor to personalize screening criteria for neuroendocrine tumors in MEN1 patients.

Objective

The aim of this study was to assess the association between blood type O and the occurrence of neuroendocrine tumors in the national Dutch MEN1 cohort.

Design

Cohort study using the Dutch National MEN1 database, which includes >90% of the Dutch MEN1 population. Demographic and clinical data were analyzed by blood type. Chi-square tests and Fisher exact tests were used to determine the association between blood type O and occurrence of neuroendocrine tumors. A cumulative incidence analysis (Gray's test) was performed to assess the equality of cumulative incidence of neuroendocrine tumors in blood type groups, taking death as a competing risk into account.

Results

ABO blood type of 200 of 322 MEN1 patients was known. Demographic and clinical characteristics were similar amongst blood type O and non-O type cohorts. The occurrence of neuroendocrine tumors of the lung, thymus, pancreas and the gastrointestinal tract was equally distributed across the blood type O and non-O type cohorts (Grays's test for equality; P=0.72). Furthermore, we found no association between blood type O and the occurrence of metastatic disease or survival.

Conclusions

An association between blood type O and the occurrence of neuroendocrine tumors in MEN1 patients was not confirmed. Addition of the blood type to screening and surveillance practice seems for this reason not of additional value for identifying MEN1 patients at risk for the development of neuroendocrine tumors, metastatic disease or a shortened survival.

INTRODUCTION

ABO blood type system classifies human blood based on the presence or absence of the antigens A and B carried on the surface of erythrocytes. During the past decades, several studies assessed the relationship between ABO blood type and risk of cancer of the gastrointestinal (GI) tract. Blood type O was found to be associated with a decreased risk of pancreatic adenocarcinoma[1-3]. However, studies on the association between ABO blood type and colon- and gastric cancer showed conflicting results[1,4-6]. Blood group antigens are not only ex- pressed on the surface of erythrocytes but also on other tissues throughout the body including malignancies of the GI tract[7-13]. Two studies showed a relationship be- tween blood typeOand the prevalence of neuroendocrine tumors in Multiple Endocrine Neoplasia type 1 (MEN1) and Von Hippel-Lindau disease[14,15].

MEN1 is an autosomal dominant inherited endocrine tumor syndrome and is characterized by the development of neuroendocrine tumors, parathyroid hyperplasia or adenomas, pituitary adenomas and adrenal gland adenomas[16]. In a cohort of 105 MEN1 patients, a significant association was found between blood type O and neuroendocrine tumors of the lung, thymus, pancreas and gastrointestinal tract. A possible addition of blood type criterion to the current screening and surveillance practices of MEN1 patients was therefore proposed[14]. Additions to the current neuroendocrine screening program are valuable because prognostic factors for neuroendocrine tumor development in MEN1 patients remain largely unknown[17]. So far, the association between blood type O and neuroendocrine tumors was not confirmed in a smaller study of 62 MEN1 patients[18]. Therefore, a validation study in a larger population-based cohort is required. The aim of the current study was to assess the association between blood type O and the occurrence and course of neuroendocrine tumors in MEN1 patients.

PATIENTS AND METHODS

Study design

In this analysis, patients were selected from the Dutch national MEN1 database of the DutchMEN1 Study Group (DMSG). All MEN1 patients diagnosed according to the recently updated clinical practice guidelines, aged 16 years and older, treated at one of the Dutch University Medical Centers (UMCs), were included in the database[17]. In each UMC, MEN1 patients were identified by a standard identification procedure using the hospital diagnosis databases. This longitudinal database with 24 years of follow-up includes >90% of the

total Dutch MEN1 population[19]. Clinical and demographic data were collected by medical record review in a standardized manner using predefined definitions. Data of all identified patients were collected from every quarter of every available year of follow-up during the period 1990 to 2010. The study protocol was approved by the medical ethical committees of all UMCs in The Netherlands. Detailed information on the DMSG database methods were described previously[20].

Patient selection and outcome definition

Patients were included when the blood type was known. Preoperative blood type screening is routinely performed with major surgery in the Netherlands. For minor surgery, such as a parathyroidectomy blood type screening is not routinely done. The reference standard for the presence of a pancreas, duodenum, stomach or lung neuroendocrine tumor was the outcome of pathology examination. If pathology was not available, only neuroendocrine tumors diagnosed on imaging and confirmed at least once on consecutive imaging studies, irrespective of imaging modality, were considered as positive. Used imaging modalities were magnetic resonance imaging (MRI), computed tomography (CT) and endoscopic (EUS) ultrasound[20]. Patients with a thymic neuroendocrine tumor were diagnosed based on the results of a pathology examination[21]. The reference standard for metastatic disease was defined as metastases confirmed by pathological examination (metastases in liver, lymph nodes, and peritoneum) or metastases identified on MRI, CT, or EUS examination (metastases in liver, lymph nodes, bones, peritoneum).

Statistical analysis

Based on blood type, patients were stratified into groups: individual blood types (A, AB, B, and O), and blood type O vs. non-O type. Baseline characteristics of blood type O and non-O type groups were compared using χ^2 tests and independent sample t-tests. The Kruskal-Wallis test was used to compare disease burden, in terms of the number of tumor sites identified. To assess the statistical significance of blood type O as a prognostic factor for neuroendocrine tumors, a cumulative incidence analysis was performed using Gray's test. By calculating the cumulative incidence function, death as a competing risk factor was taken into account. In this analysis, blood type was considered a lifelong exposure. Finally, the blood type distribution of the DMSG cohort was compared to the blood type distribution of the general population in the Netherlands using a Fisher's exact 4 X 2 contingency table. Data are presented as mean ± SD, median (range) or number (percentage) as specified. Analyzes were conducted using SPSS 22.0, R 3.0.3 and In-Silico Online (http://in-silico.

net/tools/statistics/fisher_exact_test) statistical software. *P*-values less than 0.05 were considered as statistically significant.

RESULTS

Blood type data of 200 (62%) patients were available from the DMSG cohort of 322 MEN1 patients. Within the group of known blood types, a total of 136 (68%) patients were diagnosed with a neuroendocrine tumor, at a mean age of 42 years and a median follow-up of 10.8 years. Of these 136 patients, 38 (28%) patients developed more than one neuroendocrine tumor in the course of follow-up. Neuroendocrine tumors were found in five organs, duodenum (n=17), lung (n=29), pancreas (n=121), stomach (n=8), and thymus

Table 3.1. Demographics and clinical characteristics by blood type

	Total (n=200)	**Non-O (n=120)**	**O (n=80)**	***P***
Age at diagnosis (yr)	41.6±14.0	42.6±13.8	40.0±14.3	0.31
Median follow-up in quarters of a year	43 [1-84]	37 [1-84]	49 [5-84]	0.05
Sex (%)				
Male	40	38	43	0.48
Female	60	62	57	
Neuroendocrine tumor (n, %)				
Yes	136 (68)	83 (69)	53 (66)	0.67
No	64 (32)	37 (31)	27 (34)	
Neuroendocrine tumor location (n, %)[a]				
Duodenum	17 (9)	11 (9)	6 (8)	0.69
Lung	29 (15)	19 (16)	10 (13)	
Pancreas	121 (61)	72 (60)	49 (61)	
Stomach	8 (4)	7 (6)	1 (1)	
Thymus	10 (5)	5 (4)	5 (6)	
Tumor metastasis (n, %)				
Yes	45 (23)	24 (20)	21 (26)	0.30
No	155 (77)	96 (80)	59 (74)	
Location of metastasis (n, %)				
Lymph node	31 (16)	17 (14)	14 (18)	0.34
Liver	19 (10)	12 (10)	7 (9)	
Other	14 (7)	7 (6)	7 (9)	
Rhesus factor (n, %)				
Positive	164 (82)	96 (80)	68 (85)	0.37
Negative	36 (18)	24 (20)	12 (15)	

[a] Prevalence data of the DMSG MEN1 cohort between 1990-2010. A total of 136 patients had a neuroendocrine tumor. A total of 38 patients had multiple neuroendocrine tumors. Of the 136 patients with a neuroendocrine tumor, 45 develop metastatic disease.

Table 3.2. Distribution of blood type (ABO) in patients with MEN1, with neuroendocrine tumors of the duodenum, lung, pancreas, stomach or thymus

Neuroendocrine tumor	n	Type A	Type AB	Type B	Type O	*P*
Yes	136	67	6	10	53	0.87[a]
No	64	28	4	5	27	

[a] Calculated by χ^2 2 x 2 contingency table.

Table 3.3. Comparison of blood types in the United States, The Netherlands, the NIH MEN1 cohort and the DMSG MEN1 cohort

Population	n	Type A	Type AB	Type B	Type O	*P*
United States (14)	307,212,123	42.9%	4.0%	10.0%	44.0%	0.02[b]
NIH MEN1 cohort (14)	105	22.9%	5.7%	8.6%	62.9%	
The Netherlands[a]	16,909,701	42%	3%	8%	47%	0.70[b]
DMSG MEN1 cohort	200	48%	5.0%	8.0%	40%	

[a] 'Blood types in the Netherlands' http://www.sanquin.nl/en/donate-blood/about-blood/blood-stocks/ Retrieved 02/01/2015.
[b] Calculated by Fisher's exact 4 x 2 contingency table.

(n=10). Forty-five (23%) patients developed metastatic disease, and 31 patients died (16%). Demographics and clinical characteristics of blood type O and non-O type patients did not differ (Table 3.1). The prevalence of patients with a neuroendocrine tumor was equal in the blood type O and non-O type patient groups (66% vs. 69%, respectively *P*=0.67) (Table 3.1). The prevalence of neuroendocrine tumors to the other blood types of the ABO system were also equally distributed (*P*=0.87) (Table 3.2). Considering death as a competing risk factor, the cumulative incidence function for neuroendocrine tumors was not significantly different in the blood type O group in comparison with the non-O group (Figure 3.1) (*P*=0.72). Finally, we compared the blood type distribution in the DMSG cohort with the general population of the Netherlands (Table 3.3)[14]. In the DMSG cohort 48% of the patients had blood type A, 5.0% blood type AB, 8.0% type B and 40% type O. The distribution of blood types did not differ significantly to the general Dutch population (*P*=0.70).

DISCUSSION

In the present study, we found no relation between blood type O and the development of neuroendocrine tumors, metastatic disease or survival in MEN1 patients. There was, furthermore, no association between other ABO blood types the prevalence neuroendocrine tumors.

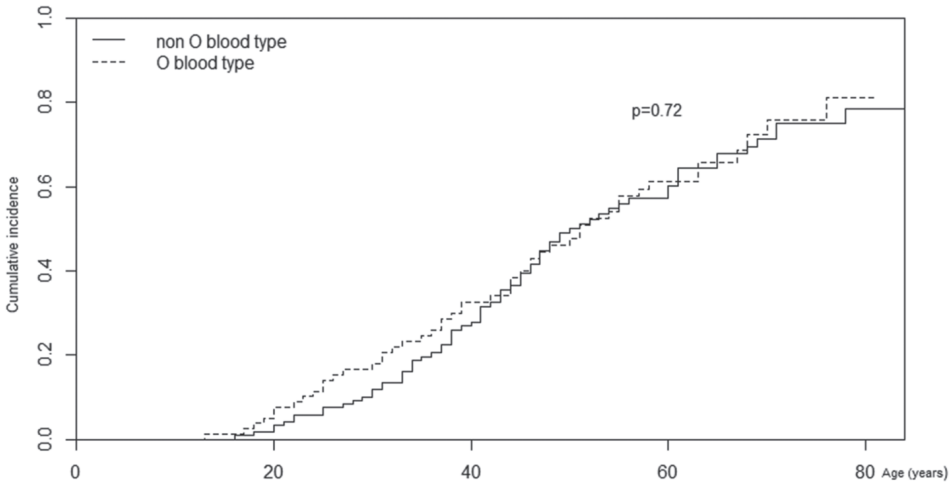

Figure 3.1. Age related penetrance of neuroendocrine tumors in MEN1 patients with non O blood type vs O blood type.

The human ABO genes are located on chromosome 9 and have three main allele forms: A, B, and O. Alleles A and B encode a glycosyltransferase, a functional enzyme that adds a terminal α-N-acetylgalactosamine and α-D-galactose to the H antigen, creating the A and B antigen respectively. These A and B antigens on the extracellular surface of the red blood cell membranes and anti-B or anti-A antibodies in the serum determine ABO blood groups. The O allele encodes a non-functional enzyme and has both anti-A and anti-B antibodies in the serum. Blood type AB has both antigens but no antibodies in the serum[22,23]. A and B blood group antigens are also expressed on epithelial cells other than red blood cells, such as the gastrointestinal, bronchopulmonary, and urogenital tracts[24]. It is hypothesized that ABO antigens have a significant role in intracellular adhesion and membrane signaling, processes that are necessary for the progression and spread of malignant cells[24]. Furthermore, the protective function of A and B antibodies in patients with blood type O against tumor cell progression is speculated[25]. There is an indication that modifications in blood group antigens occur during pancreatic tumor genesis. Blood group antigens have an altered expression in pancreatic cancer cells compared to normal pancreatic cells[8,26,27]. However, the question is whether there is a similar expression of blood group antigens in neuroendocrine tumors as in carcinomas. The expression of blood type substance A, B and H have been compared between rectal neuroendocrine tumors and rectal adenocarcinomas. In this study no difference was found, indicating that the same mechanisms apply both to carcinomas and neuroendocrine tumors[28].

Most epidemiologic and molecular studies showing an association between blood type and cancer occurrence found a favorable relationship between blood type O carriers in comparison with non-O type carriers. Investigators hypothesized that the glycosyltransferase modification occurs only in blood types A and B because blood type O lacks a functional glycosyltransferase. Another hypothesis could be the presence of blood group antibodies in serum that act as a defense system. Blood type O has both antibodies, which could in this view, lead to a decreased incidence of cancer. Epidemiologic studies show a trend towards fewer tumors in blood type O carriers, in comparison to non-O type carriers. In our opinion, this is only confirmed for pancreas adenocarcinomas in which several large epidemiologic studies have been conducted[1,3,29-33].

To our knowledge, our current study population of 200 MEN1 patients with determined blood types is the largest studied MEN1 population until now. The previous cohort in which 105 MEN1 patients were included, a significant predominance of blood type O patients with neuroendocrine tumors was found. This study reported a higher prevalence of blood type O (76.1%), in patients with a neuroendocrine tumor in comparison with those who did not have a neuroendocrine tumor (52.5%; P=0.01)[14]. These results were not confirmed in our study with a respective prevalence of patients with a neuroendocrine tumor of 66% in the O blood type group and 69% non-O blood type group (P=0.67). Furthermore, a cumulative incidence analysis showed an equal distribution of neuroendocrine tumors across the blood type O and non-O type cohorts (P=0.72). A smaller study of 62 patients could also not confirm the association between blood type and neuroendocrine tumors in MEN1 patients[18]. Moreover, in the initial study no neuroendocrine tumors in blood type AB patients were found further supporting the assumption that blood type O was associated the occurrence of neuroendocrine tumors[14]. However, in our population 6 of 10 patients with blood type AB blood developed a neuroendocrine tumor.

If A and B antibodies have a protective role, one can hypothesize that differences in ABO antibody levels might explain the discrepancy in outcomes of our study compared with the previously published studies[14,18,25]. If so, there must be a different mechanism causing variations in antibody levels between the populations which seems unlikely. However, this is speculative and would require further research.

Differences in the origin of the studied populations might be another explanation for the contradictory results[34]. In the previous report, 80% of the patients with a known blood type and a neuroendocrine tumor was Caucasian[14]. Also in the Netherlands, 88% of the general population is Caucasian, which also applies for the Dutch MEN1 cohort[35,36]. Therefore, differences in the origin of the studied populations seems not to explain the results.

In our studied cohort the relative large amount of patients with a neuroendocrine tumor (68%) can be explained by the fact we only included patients with a known blood type. In most cases, the blood type data was available because these patients underwent major (neuroendocrine tumor) surgery. Therefore, we studied a sub-population of MEN1 patients with a high likelihood of a neuroendocrine tumor. However, the DMSG cohort is a population-based cohort, which covers almost the entire MEN1 population from the Netherlands in which >90% of patients are followed. From this cohort, 62% of the patients was included, this amount of patients is larger compared to the previous report[14].

A major strength of this study is the true population-based database, reducing the chance of selection bias of the total population to a minimum[19]. Furthermore we investigated the relation between blood type and the occurrence of neuroendocrine tumors further with a competing risk analysis, in addition to previous research. This analysis takes death as competing risk factor into account. The inclusion of only patients with a known blood type and, therefore, a selection of patients with a high likelihood of a neuroendocrine tumor, could be a limitation of this study design. However, the chance of finding an association between a blood type and the occurrence of a neuroendocrine tumor is relative high in this population. Furthermore, we expect no selection of a particular blood type in the studied population blood since the distribution of blood types did not differ from the distribution of the general Dutch population.

In summary, in our population-based study of MEN1 patients with a known blood type, blood type O was not associated with an increased risk of neuroendocrine tumors. At this time, blood type screening seems for this reason not of additional value for identifying patients at higher risk for the development of neuroendocrine tumors and metastatic disease.

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Chapter 4

MEN1-dependent breast cancer: indication for early screening? *Results from the Dutch MEN1 study group*

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Abstract

Objective

Multiple Endocrine Neoplasia type 1 (MEN1) is associated with an early onset elevated breast cancer risk. This finding potentially has implications for breast cancer screening for women with MEN1, and therefore it is necessary to assess whether other risk factors are involved to identify those at greatest risk.

Design

A cross-sectional case control study was performed using the Dutch MEN1 cohort, including >90% of the Dutch MEN1 population. All women with a confirmed *MEN1* mutation received a questionnaire regarding family history and breast cancer-related endocrine and general cancer risk factors.

Results

A total of 138 of 165 (84%) eligible women with *MEN1* completed the questionnaire. Eleven of the 138 women had breast cancer. Another 34 relatives with breast cancer were identified in the families of the included women, of whom 11 were obligate *MEN1* carriers, 14 had no *MEN1* mutation and 9 had an unknown *MEN1* status. The median age at breast cancer diagnosis of women with *MEN1* (n=22) was 45 years (range, 30 to 80 years) in comparison with 57.5 years (range, 40-85 years) in female relatives without MEN1 (n=14) (*P*=0.03) and 61.2 years in the Dutch reference population. Known endocrine risk factors and general risk factors were not different for women with and without breast cancer.

Conclusion

The increased breast cancer risk in *MEN1* carriers was not related to other known breast cancer risk factors or familial cancer history, and therefore breast cancer surveillance from the age of 40 years for all women with the *MEN1* is justifiable.

INTRODUCTION

Carrying a mutation in a gene that gives rise to an increased breast cancer risk leads to distress in patients and may necessitate breast cancer screening from a younger age. Recently, our group reported that the *MEN1* gene predisposes for early-onset breast cancer in female carriers. Multiple endocrine neoplasia type 1 (MEN1)-related breast tumors showed loss of the wild- type MEN1 allele, suggesting a cell-autonomous and MEN1 gene–dependent tumorigenic mechanism[1]. This was further strengthened by the observation that silencing of the *MEN1* gene results in proliferative gene expression changes in primary mammary luminal progenitor cells in human breast tissue[2]. Considering the impact on patients and the potential need of changing the current guidelines regarding early breast cancer surveillance, further research is indispensable to identify potential additional risk factors which might have influenced this result[3].

A mutation in the *MEN1* tumor-suppressor gene leads to MEN1, which is classically characterized by the combined occurrence of parathyroid adenomas, pituitary adenomas, and duodenopancreatic neuroendocrine tumors[4,5]. The prevalence of MEN1 is 3 to 4/100,000, which underlines the rarity of the disease[6]. Performing association studies for a rare disease in this population is therefore challenging, and cautiousness in formulating an advice on breast cancer surveillance should be regarded.

The relative risk for breast cancer for women with MEN1 of 2.83[1] categorizes the *MEN1* gene as a moderate risk factor for breast cancer[7]. Women with an increased breast cancer risk may benefit from intensified screening from an earlier age and possibly at shorter intervals than women at average risk[8]. Screening is associated with a reduction in breast cancer mortality of ~35% to 70% in different European studies[9–11]. However, breast cancer surveillance also gives rise to a risk of false-positive findings, resulting in unnecessary biopsies, especially when screening starts from a younger age[12,13]. When weighing the potential benefits and harms of screening, the important question arises from which age and interval women with MEN1 should be screened.

The mean age at breast cancer diagnosis was 48 years for the Dutch MEN1 population and 51 years for three validation cohorts[1]. This is 10 years earlier than the median age of 61.2 years at breast cancer diagnosis for women in the general Dutch population and underlines the young breast cancer onset in women with MEN1.

In formulating advise, known breast cancer risk factors such as lifestyle factors[14,15], endocrine-related risk factors[16], and a high occurrence of breast cancer in the family should be considered. These risk factors should be considered, which is challenging considering

given the small sample size of the cohorts of patients with MEN1 as a consequence of the rarity of the disease. Nevertheless, the elevated relative risk and younger age of onset justify further research. Therefore, the initial aim of this study was to assess the role of familial breast cancer risk, lifestyle, and endocrine-related risk factors in the higher risk for breast cancer in women with MEN1. The second aim was to formulate a recommendation on screening for daily clinical practice of MEN1 care.

METHODS

Study design and patients

Female patients were selected for this study from the Dutch MEN1 study group (DMSG) database. This longitudinal database includes >90% of all Dutch patients with MEN1, aged 16 years and older at the end of 2013, treated at one of the Dutch University Medical Centers between 1990 and 2013. Women with a *MEN1* mutation or a clinical MEN1 diagnosis from a family with a confirmed *MEN1* mutation were eligible for this study. We performed a cross-sectional case control study from April 2015 to October 2016 in which eligible women were invited to complete a questionnaire.

All women who participated in the study provided written informed consent. The Medical Ethical Committees of all University Medical Centers in the Netherlands confirmed that the Medical Research Involving Human Subjects Act (WMO) did not apply for this study, and therefore an official approval was not required under WMO.

Questionnaire

The questionnaire was completed by hand or on line. The questionnaire addressed the following topics.

Medical history

Respondents were asked whether they had breast cancer and when this was diagnosed.

Family history

Participants were asked to fill out which family members had non-MEN1- related cancer and whether these family members were *MEN1* carriers. This was specifically asked for their first- and second-degree relatives.

Lifestyle factors

Current and past smoking status were assessed by inquiring the years and type of smoking (cigarettes/cigars). Regarding alcohol consumption, we asked about alcohol consumption per decade, start of alcohol consumption, and quantity of consumption. Weight and history of weight change were determined. Height was self-reported and used to calculate the Body Mass Index.

Endocrine-related factors

Women were asked to complete questions about age at menarche, oral contraceptive use, pregnancies, breast feeding and hormone replacement.

Family history

We constructed pedigrees for each participant/respondent. Accuracy of the family relationships and medical data of the relatives was verified through the pedigrees of the MEN1 families that are documented in the medical records at the department of internal medicine or clinical genetics[17]. The pedigrees focused on MEN1 and breast cancer. Participants filled out which family members had breast cancer and if those family members were diagnosed with MEN1.

Age at breast cancer diagnosis

To compare the age at breast cancer diagnosis of our cohort with the age of breast cancer occurrence of the general Dutch population, data were retrieved from the Netherlands Cancer Registry, which is hosted by the Netherlands Comprehensive Cancer Organisation. The age of breast cancer onset of women with MEN1 and their female relatives without MEN1 was assessed to study if an early age of breast cancer onset was a familial predisposition or exclusively related to MEN1. The age of breast cancer occurrence of family members who were not included in our DMSG database was reported by the respondents. These family members were non-MEN1 carriers, *MEN1* carriers not living in the Netherlands or deceased (otherwise these patients would have been included in the database).

Statistical analysis

Descriptive statistics were used to characterize the study population, to determine the age of breast cancer onset, and to examine the difference between women with and without breast cancer. For this cross-sectional case control study, women with MEN1 were cases, and women

with MEN1 but without breast cancer were used as controls. Although this is a case-control study, odds ratios are not calculated because prevalent cases are presented wherein the odds ratio is not representative for the risk ratio. Independent sample t tests or Mann-Whitney U tests for continuous variables and the χ^2 tests or Fisher's exact tests for categorical variables compared potential risk factors between women with and without breast cancer.

RESULTS

Response rate

A total of 210 women with MEN1 were identified of whom 45 (21.4%) were ineligible for inclusion because they only had two MEN1-related major manifestations, but none had a confirmed *MEN1* mutation or family members with a *MEN1* mutation, were not in follow up at time of data collection, or had died. Of the 165 eligible women, three refused to participate, eight women could not be reached and three women died during data collection. Of the remaining 151 women, a total of 138 completed the questionnaire, giving a response rate of 84% (Figure 4.1).

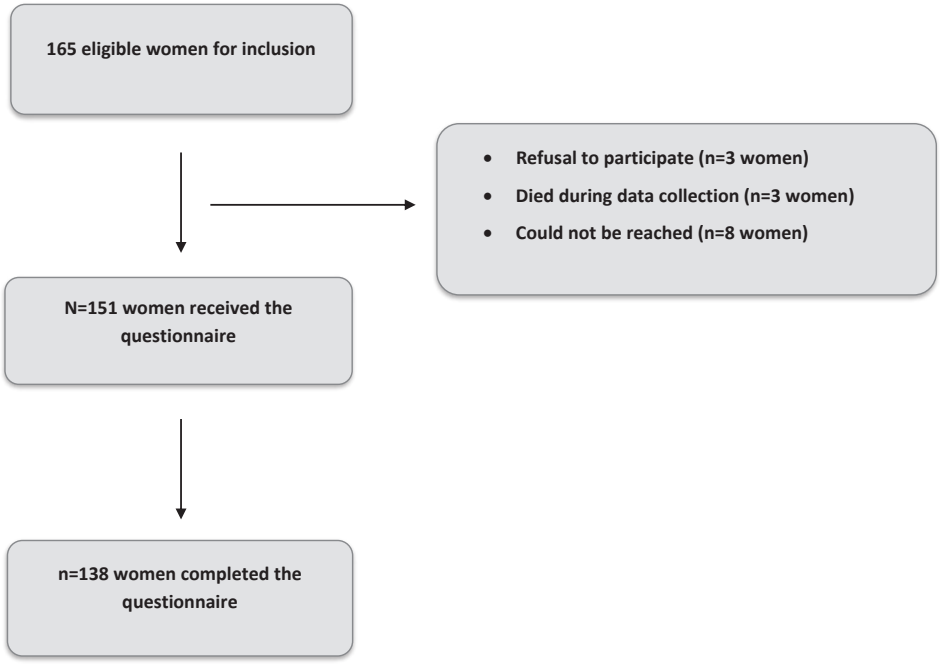

Figure 4.1. Flowchart study population.

Familial breast cancer

Respondents were from a total of 64 families, with a median of two (range, 1 to 12 respondents) per family. Eleven respondents with breast cancer and MEN1 were from 10 different families. According to the Dutch guidelines, additional BRCA1/2 and CHEK1 mutation analyses were performed in a family with two cases, of whom both mutations were negative. Through the family histories of all the respondents, 11 additional obligate MEN1 carriers with breast cancer from 11 families were identified. A total of 21 (33%) families had patients with breast cancer and MEN1. Sixteen (73%) women with breast cancer and MEN1 had no family members with breast cancer. Five women had a single family member with MEN1 and breast cancer. In two different families, there were two first-degree relatives with MEN1 and breast cancer. In one of these families, CHEK2 and BRCA were tested and found to be negative. In one family, there were two second-degree family members, one with breast cancer and MEN1 and one with breast cancer at the age of 80 years for whom the MEN1 status was unknown. One family member with breast cancer, identified by the questionnaire, had BRCA1 but was not a carrier of the *MEN1* gene.

Comparison of risk factors between respondents with and without breast cancer

Although there were some small differences between the groups, no significant and clinically relevant differences in breast cancer risk factors between the respondents with and without breast cancer were observed (Table 4.1). All women were white and none of the cases had a prolactinoma.

Age at breast cancer diagnosis

Eleven respondents with a confirmed MEN1 gene mutation had been diagnosed with breast cancer and were included in the DutchMEN database. The breast cancer diagnosis was based on pathology reports. The median age at diagnosis was 44 years (range, 34 to 64 years). Another 34 women with breast cancer were identified through the family history of the respondents. Eleven of those women were obligate MEN1 carriers, 14 had breast cancer but did not have MEN1, and nine had an unknown MEN1 status. The median age at breast cancer diagnosis of the total of 22 women with MEN1 (confirmed *MEN1* gene mutation and obligate carriers) was 45 years (range, 30 to 80 years), in comparison with a median age of breast cancer onset of 57.5 years (range, 40 to 85 years) (P=0.03) in the relatives who did not have a MEN1 mutation. The mean age at breast cancer diagnosis in the Dutch population was 61.2 years from 2009 to 2013[18]. Five out of 35 women with MEN1 having a negative MEN1 mutation test had breast cancer, resembling the chance of breast cancer for women

Table 4.1. Comparison of general and reproductive/endocrine factors in MEN1 women with breast cancer and without breast cancer

	Breast cancer (n=11)	No breast cancer (n=127)	*P*
Age at menarche	13 (12-14)	13 (9-18)	0.6
Parity	2.0 (0-3)	2.0 (0-7)	1.0
Nulliparity	36%	33%	1.0
OAC before age 20[a]	50%	72%	0.2
Duration OAC (years)[a]	11 (2-26)	10 (1-36)	0.9
Age at menarche	13 (12-14)	13 (9-18)	0.6
Duration breast feeding (months)[b]	5 (0-12)	3 (0-52)	0.4
Age at first birth	26 (20-31)	27 (19-40)	0.5
BMI at inclusion	23 (17-32)	24 (19-41)	0.3
BMI at age 18	20.8 (18-25)	21 (16-29)	0.8
Smoking ever*	55%	41%	0.4
Smoking – age at start	16 (14-25)	16 (12-29)	0.4
Duration smoking[c]	11 (2-29)	16 (4-49)	0.3
Age at first alcohol use	18 (16-20)	16 (12-50)	0.1
Never alcohol	18%	20%	0.6
CAT scans (thoracic)	2 (1-9)	2 (1-14)	1.0

Data are presented as median ± range or percentage. *P*-values are derived from χ^2 or Fisher's exact tests and independent-sample t-test or Mann–Whitney U-test.
[a] OAC = oral anticonception.
[b] Total duration of breastfeeding in months.
[c] Time in years.
* Smoking ever = more than 1 cigarette per day >1 year.

from the general Dutch population. One of those women had a prolactinoma before breast cancer diagnosis. The median age of breast cancer in those patients was 60 years (range, 48 to 69 years), which is in line with the women in the general Dutch population.

DISCUSSION

In the current study, based on a valid representation of all known Dutch MEN1 families corresponding to a response rate of 84%, predisposing general and reproductive risk factors were equally present in women with MEN1 with and without breast cancer. In the majority of cases, familial breast cancer was not present. By identifying 11 additional MEN1-related breast cancer cases, we confirmed that that the age of breast cancer onset is significantly lower in women with MEN1 than women without MEN1.

Familial breast cancer risk can be modified by other risk factors, such as age at menarche and menopause, age at first child birth, parity, oral contraception use, breast-feeding, alcohol consumption and smoking[19–25]. Patients with breast cancer could have had more predisposing factors for breast cancer than control subjects[16]. However, Table 4.1 reflects that there are no major differences between cases and control subjects that predispose for breast cancer. Small, non-significant differences between cases and control subjects found for breastfeeding, in which cases breastfed 2 months longer than control subjects. Each year of breastfeeding significantly reduces the breast cancer risk by 4.3%, in addition to the risk associated with each birth[26]. Furthermore, a higher percentage of cases ever smoked, but the reported duration of smoking was longer in control subjects. Cases started smoking at a younger age than control subjects. An increased breast cancer risk has been found in women who smoke between menarche and first full-term pregnancy[27,28]. Frequent radiation exposure by surveillance with computer-assisted tomography (CAT) scans as part of MEN1 screening could potentially increase the breast cancer risk. In this respect, CAT scans can be considered a confounder, and therefore the frequency of CAT scans was compared for women with and without breast cancer. This number was equal for both groups and therefore this risk seems marginal.

The age of breast cancer diagnosis of mutation-negative women with MEN1 was not different from the general Dutch population. This outcome confirms the findings of a previous study in which mutation-positive MEN1 patients had a different phenotype when compared with mutation negative patients[29].

Limitations

The small sample size of this study is a limitation, especially in finding significant differences in risk factors between women with and without breast cancer. Only large effects can be detected with a small sample size, which is reflected by the limited power. However, to our knowledge, this is, to date, the largest cohort in which this topic has been studied, and we aimed for identifying strong risk factors that may modify the relation between MEN1 and breast cancer. In line with the retrospective nature of this study, other low- and moderate-penetrance breast cancer genes, such as CHEK2, were not tested in all respondents with breast cancer because it was not the standard of care at time of genetic counseling. This can be considered another limitation. The guidelines regarding genetic testing in women with breast cancer were revised in the Netherlands in 2014, and since then CHEK2 has been routinely tested. According to the guidelines, women included in the current study who were eligible for additional testing for the CHEK2 mutation were tested and did not have this mutation. The rationale for CHEK2 mutation testing is the occurrence of the

CHEK2*1100delC mutation in 1% of the general Dutch population and in 5% of women with breast cancer[30]. At this time, genetic testing for other low- and moderate-penetrance breast cancer genes is not the standard of care.

Another potential concern is the recall bias, which refers to systematically overreporting or under-reporting of exposure to risk factors in women with breast cancer in comparison to women without breast cancer. Previous case-control studies assessing the association between risk factors and breast cancer have concluded that there was minimal recall bias in reporting alcohol consumption and physical activity[31,32].

Strengths

A major strength of this study is the population-based DMSG database, which consists of 90% of the total Dutch MEN1 population. The occurrence of selection bias is therefore minimized by the high coverage of patients with MEN1. Subsequently, the high response rate of 84% contributes to the reliability and validity of the study results. This is the largest MEN1 cohort in which this topic has been studied, which makes the data unique. The women who were identified by the questionnaire were not included in the database because they either had died or did not live in the Netherlands. This confirms the rigorous manner of identifying of all patients with MEN1 who are currently under medical care in the Netherlands by the DMSG database.

Familial breast cancer risk is an important risk factor which is reflected by its inclusion in different screening assessment tools such as Claus, BOADICEA and Tyrer-Cusick models in estimating breast cancer risk[33–35]. Familial breast cancer and MEN1 carriership were assessed thoroughly in the questionnaire, and the accuracy was verified by pedigrees present at the department of internal medicine and genetics. Because more family members of one family filled out the questionnaire, the accuracy of the family trees could be checked. The accuracy of family histories provided by women on reporting breast cancer family history is generally reliable[36]. By comparing the age of breast cancer onset in non-MEN1 female relatives to women with MEN1, the optimal controls were created to adjust for another familial risk factor, which can be considered a major strength.

Breast cancer surveillance in women with MEN1

The purpose of breast cancer screening is to reduce breast cancer-specific mortality and the incidence of advanced breast cancer. The incidence and mortality risk of breast cancer in the Netherlands were 172/100,000 and 35.5/100,000 in 2014, respectively. On average,

there is a relative reduction of 50% in mortality from breast cancer in women who attend mammographic screening[9–11].

In formulating advice on breast cancer screening, treatment-related morbidity and the harms of screening are considered. The national breast cancer screening program in the Netherlands consists of biennial screening mammography for women aged 50 to74 years[37]. This is in line with most European countries and the recommendation of the U.S. Preventive Services Task Force[38,39]. Considering the median age of 45 for breast cancer diagnosis in women with MEN1, the question arises whether the current screening program applies for this population. For women aged 40 to 74 years, with an average breast cancer risk, there is evidence that screening by mammography reduces breast cancer mortality, but there is also considerable harm in this group due to diagnosis and treatment of noninvasive breast cancer that would not have become life threatening or to clinical attention in the woman's lifetime in the absence of screening. False-positive results as a consequence of overdiagnosis leads to unnecessary and invasive follow-up[13].

Mandelblatt *et al.*[12] studied the harms and benefits of eight different screening strategies by using different simulation models and found that annual screening from the age of 40 years in women with a twofold to fourfold increase in breast cancer risk had similar or even more favorable harm/benefit ratios as biennial screening of women with average risk from 50 to 74 years of age. This seems directly applicable for women with MEN1 with a relative risk of 2.8 and a median age at diagnosis of 45 years. However, as part of surveillance for MEN1, women with MEN1 undergo a stringent screening program to detect MEN1-related tumors. The addition of another screening modality will increase the burden. Moreover, the tumors can be characterized as prognostically "favorable" because the majority of tumors of initial cases were luminal-type breast cancers. Only one woman had a triple-negative breast tumor[1]. Considering this, one might question if women with MEN1 should be screened from an earlier age. Interestingly 60% of women with breast cancer were premenopausal at time of breast cancer diagnosis, which is in line with the younger age at diagnosis.

Clinical implications: *to screen or not to screen?*

The findings of the current study highlight the need for adaptation of the clinical guidelines regarding breast cancer screening. The small population, and consequently the limited power, make it difficult to formulate a strong recommendation. However, in this extended cohort and in three independent international cohorts[1], the younger age at breast cancer diagnosis has been confirmed. This early age of breast cancer onset, which is at least 12

years earlier than the general population, can therefore not be neglected. In addition, based on the results, there is no indication that breast cancer was caused by other risk factors or familial risk. A suggested age to start screening is from the age of 40 years biennially. This is almost 10 years before the mean age of breast cancer in our cohort and in concordance with the Dutch screening program that starts at the age of 50 years, which is 10 years before the mean age of breast cancer in the general Dutch population. In our view, a biennial screening program from the age of 40 is justifiable because the majority of breast tumors were of luminal type and can therefore be considered prognostically favorable[1]. In addition, the burden of an annual screening program can therefore be avoided. Large international collaborations are needed to assess the effect of breast cancer screening in women with MEN1 for whom the prevention of progressed breast cancer by early diagnosis is weighed against the potential harms as a consequence of overdiagnosis and unnecessary invasive follow-up.

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Chapter 5

Quality of Life in Multiple Endocrine Neoplasia type 1: *Results from the DutchMEN Study Group*

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Abstract

Objective

Multiple Endocrine Neoplasia type 1 (MEN1) is a hereditary endocrine tumor syndrome characterized by the triad of primary hyperparathyroidism (pHPT), duodenopancreatic neuroendocrine tumors (dpNETs) and pituitary tumors. Patients are confronted with substantial morbidity and are consequently at risk for an impaired quality of life (QOL). Meticulous assessment of QOL and associated factors in a representative population is needed to understand the full spectrum of the burden of the disease.

Design

A cross-sectional study was performed using the national Dutch MEN1 cohort. Patients with a confirmed MEN1 mutation received the SF-36 Health Related Quality of Life questionnaire and questions regarding sociodemographic and medical history.

Results

A total of 227 of 285 (80%) eligible MEN1 patients returned the questionnaires. Health related QOL scores (HRQOL) in MEN1 patients were significantly lower for the majority of subscales of the SF-36 in comparison with the general Dutch population. The most consistent predictor for HRQOL was employment status, followed by the presence of a pituitary tumor. 16% of patients harbouring a pNET and 29% of patients with a pituitary tumors according to the medical records, reported that they were unaware of such a tumor. These subgroups of patients had several significant better QOL scores than patients who were aware of their pNET or pituitary tumors.

Conclusions

Patients with MEN1 have impaired QOL in comparison with the general Dutch population warranting special attention within routine care. For daily practice, physicians should be aware of their patients' impaired QOL and of the impact of unemployment on QOL.

INTRODUCTION

Multiple Endocrine Neoplasia type 1 (MEN1) is a hereditary disease characterized by the classic triad of primary hyperparathyroidism (pHPT), pancreatic neuroendocrine tumors (pNETs) and pituitary tumors[1,2]. Other encountered neoplasms are neuroendocrine tumors of thymic, bronchial or gastric origin, adrenal tumors and smooth muscle, skin and subcutaneous tumors. Recently, it was reported that females with MEN1 have an almost three times higher risk for breast cancer at a 15 years younger age, which underlines the complexity and severity of the disease[3,4].

The penetrance of the disease is high, especially for pHPT, duodenopancreatic NETs (dpNETs) and pituitary tumors, respectively 100%, 80% and 70%, with the first manifestations occurring in childhood, further contributing to the burden of the disease for *MEN1* mutation carriers[5,6]. Confronted by this knowledge, accompanied by significant morbidity and early mortality, nowadays mostly arising from dpNETs, MEN1 patients are at risk for impaired quality of life[7,8].

Recently it was reported that MEN1 patients have a high fear of MEN1 related tumor recurrence[7]. Studies assessing the health related quality of life (HR) of patients with MEN1 indicate an impaired HRQOL, with a higher rate of depression for patients with a high burden of disease and treatment[8]. It is also suggested that HRQOL in MEN1 patients is worse than HRQOL in the general population[8,9]. Unfortunately, due to the manner of recruiting the participants, the studied populations were often prone for selection bias. Since, these studies recruited in hospital patients or via patients' support groups supposedly patients in need of medical or peer support were included. Therefore the reported QOL might not be generalizable to the total MEN1 patient population. In addition, in several reports medical information was assessed by self-reporting and there was no access to the medical records to retrieve the actual medical disease status. Therefore, it was unclear whether the reported disease status of patients was accurate[8,10]. In this respect it is also not known whether these patients were actual genetically proven *MEN1* patients or so called 'phenocopies', who have been reported to be a different disease entity with a more indolent disease course[5].

The Dutch MEN1 Study Group (DMSG) has meticulously registered the Dutch MEN1 population in a national database. This database contains data from 1990 up to present, collected every quarter of every year. The design of long follow-up and high density of the data allows for an accurate representation of disease status of all patients. This high coverage also minimizes the occurrence of a selected study sample[11].

The primary aim of this study was to assess HRQOL in the Dutch MEN1 cohort in order to compare the HRQOL with the general Dutch population and to assess which variables were predicators for worse HRQOL. The secondary aim was to evaluate if the self-reported MEN1 manifestations were in line with the disease status as reported in the medical records and whether a discrepancy affected the HRQOL.

METHODS

Study population

Participants were recruited from the Dutch MEN1 cohort. This MEN1 cohort is established by the Dutch MEN1 Study Group (DMSG). Participants were retrieved from the DMSG database. This longitudinal database includes >90% of all Dutch MEN1 patients, aged 16 years and older at the end of 2017, treated at one of the Dutch University Medical Centers (UMCs) between 1990 up to present[11]. Patients were eligible for the present study if they had a confirmed *MEN1* mutation. Demographic and clinical data such as MEN1 related medical history were retrieved from this database.

Disease manifestations

Primary HPT was defined as hypercalcaemia combined with elevated or inappropriately non-suppressed parathyroid hormone levels in two consecutive measurements. The presence of a pNET was confirmed according to the outcome of pathology examination. If pathology was not available, pNET presence was based on MRI, computed tomography (CT) or endoscopic ultrasound, which had to be confirmed at least once by consecutive imaging studies. The absence of a pNET also had to be confirmed on a minimum of two subsequent imaging studies during follow-up. The reference standard for the presence of pituitary tumors was (1) pathology or (2) consecutive radiological examination demonstrating a pituitary tumor. Details for the reference standard of pHPT, pNET and pituitary tumors have been described previously[12–14].

Study design

A cross sectional study was conducted in which eligible patients were invited to complete a questionnaire from April 2015 till December 2016. After two weeks, a reminder email was sent to the participants. The questionnaire could be completed by hand or as a web-based questionnaire. All participants provided written informed consent.

Questionnaires

Sociodemographic data such as education and employment were obtained.

Disease status. Participants had to complete questions on the presence of their own history of pHPT, pituitary tumors and pNETs. In addition they were asked if they were operated or received other treatment for these manifestations. If they were operated the exact year of surgery was asked.

Health-related quality of life. Health-related QOL was assessed using the Short-Form 36 (SF-36) Health Survey composed of eight multi-item scales assessing physical functioning, role limitations due to physical health problems and emotional problems, bodily pain, general health perceptions, vitality, social functioning, and general mental health. Scale scores range from 0 to 100, with higher scores indicating better levels of functioning and well-being. Cronbach's α for the SF-36 scales ranged from .84 (social functioning) to .93 (physical pain). Only general health perception had a low Cronbach's α (.55), which will therefore be of low significance.

The normative data on the SF-36 Health Survey were derived from the general population of the Netherlands[15].

Statistical analysis

Descriptive statistics were applied to characterize the study population. Univariate analyses (Independent sample T-test/Mann Whitney U test, Chi-Square test/Fisher's exact test, Pearsons correlation) were used when appropriate and to evaluate which MEN1 related manifestations and sociodemographic variables were associated with HRQOL.

A multivariable analysis adjusted for age and gender was carried out using multiple linear regression to assess which patient characteristics were associated with HRQOL. Collinearity was tested using variance inflation factors (VIF). In the linear models, none of the VIF values were greater than 1.6, suggesting that collinearity was not a problem.

Analyses were conducted using SPSS 22.0. *P*-values less than 0.05 were considered as statistically significant.

RESULTS

Response rate

Between 2015 and 2017, a total of 285 patients (120 men and 165 women) were eligible for inclusion, of which 252 (102 men and 150 women) received the questionnaire. Thirty-three patients could not be reached. The questionnaire was completed by 227 individuals (84 (70%) men and 143 (87%) women), resulting in a total response rate of 80%.

Population characteristics

The mean age of the study population was 47 (SD ±15) years (Table 5.1). All patients had a confirmed *MEN1* mutation, or one or more MEN1 related manifestations and a first-degree relative with MEN1. Eighty-three percent of patients reported to ever had a pPHT. More than half of the patients reported to ever have had a pNET (55%) and 38% of patients had a self-reported pituitary tumors.

Table 5.1. Baseline characteristics (N=227)

Gender	
Female	143 (63%)
Male	84 (37%)
Age (mean, SD)	47 (15)
Education[a]	
Primary school	6 (3%)
Secundary school	149 (65%)
College or university	66 (29%)
Employment (age < 65 years)	
Yes	154 (80%)
No	39 (20%)
Index case	
Yes	51 (23%)
No	173 (76%)
Presymptomatic diagnosis	
Yes	98 (44%)
No	125 (56%)
Years since MEN1 diagnosis	
<5 years	27 (12%)
≥5 years	190 (84%)

Health Related Quality of Life

HRQOL scores in MEN1 patients, adjusted for age and gender, were significantly lower for the majority of subscales of the Health Related Quality of Life Short Form 36 in comparison with the general Dutch population (Figure 5.1). The subscales general health perceptions and vitality were 0.5 SD lower, which can be considered as a clinically relevant difference. The only scale that was comparable with the general population was the physical functioning scale.

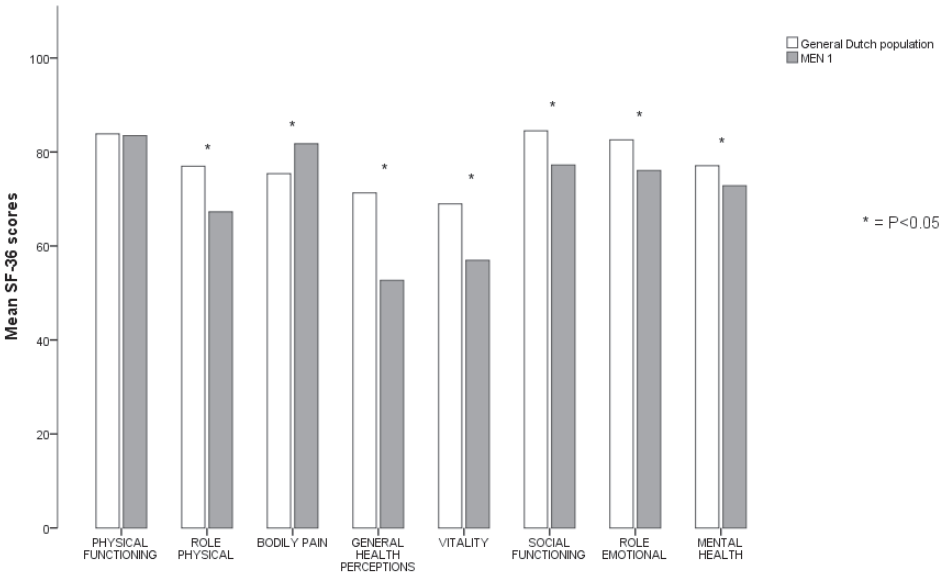

Figure 5.1. Mean SF 36 scores of MEN1 patients versus the general Dutch population.

Self-reported manifestations versus manifestations according to the medical records

Only one (3%) of thirty-one patients who, according to the medical records, did not have a pHPT reported to have a pHPT. A total of seven (4%) of 192 patients with pHPT according to their medical record, were unaware of having a pHPT. Twenty-three (16%) of the total of 140 patients who according to the medical record had a pNET reported that they did not have and never had a pNET. The median size of the pNET of these patients was 8.5 millimetres [IQR 4,13 9] compared to 13 millimetres [IQR 9,17] in patients who accurately reported to have a pNET. One of those patients even had pancreatic surgery for a pNET.

Six (5%) of the 122 patients who according to the medical records did not have and never had a pNET, reported to have a pNET. Twenty-nine (29%) of 101 patients who ever had

a pituitary tumor according to the medical records, reported that they did not have and never had a pituitary tumor. The median size of the pituitary tumors in this group was 5 millimetres [IQR 4,6] compared to 5 millimetres [IQR 3,7] in patients who accurately reported to have a pituitary tumor. One of those patients underwent a transsphenoidal resection for a pituitary tumor. Four (5%) of 85 patients, who never had a pituitary tumor, thought they did have a pituitary tumor.

Patients who reported not to have a pNET, but had a pNET according to the medical records, had slightly better HRQOL scores in comparison with patients who reported to ever had a pNET. This difference was significant for the emotional functioning score, with mean scores of 85.9 and 65.4 respectively (CI -35.4, -5.6) (*P*=0.03).

Patient who ever had a pituitary tumor diagnosis as reported in their medical records, but reported not to have a pituitary tumor, had better HRQOL scores than patients who reported to have or had a PIT (Figure 5.2). This difference was significant for physical role (*P*=0.01), emotional functioning (*P*=0.02) and mental health (*P*=0.02) subscale.

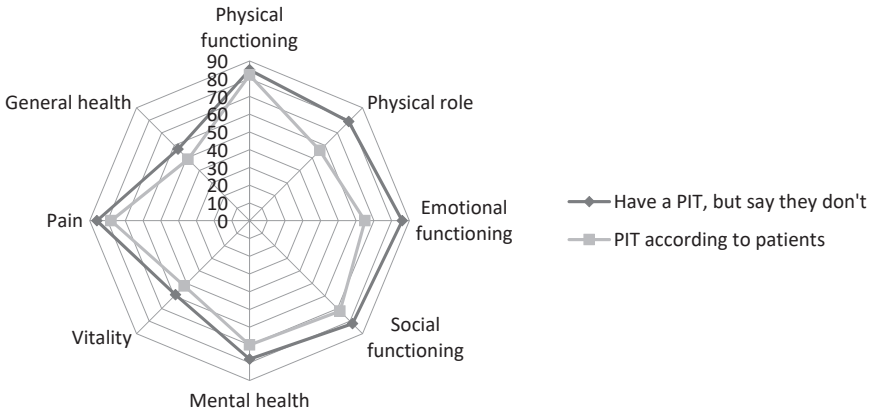

Figure 5.2. HR-QOL scores of patients with a pituitary tumor in comparison with patients who do not think they have a PIT, but they do according to the medical data.
Physical role (*P*=0.01), Emotional functioning (*P*=0.02) and mental health (*P*=0.02).

Univariate analysis

In univariate analyses age, years since MEN1 diagnosis, education level, pituitary tumor, index cases and employment were significantly related to two or more subscales of the Health Related Quality of Life Short Form 36 (supplementary data).

Multivariable analysis

Multivariable analyses including age, the presence of a pNET, pHPT, pituitary tumor, years of diagnosis, education level, index case, employment (yes/no) and presymptomatic diagnosis (yes/no), being an index case, a pituitary tumor diagnosis and being employed were associated with HRQOL. Employment was the only predictor that was significantly related to all subscales (supplementary data).

Employment status

A subgroup analysis of patients before the retirement age was conducted since employment appeared to be such a crucial factor for HRQOL. Therefore, the group was divided according to employment status: employed (n=154) and unemployed (n=39) (Table 5.2). Age at completion of the questionnaire and education level was significantly different between the active and inactive group. The mean age of patients in the employed group was almost ten years younger than the unemployed group, 42 years and 51 respectively (*P*=0.01). Patients with a college or university degree (n=61) had the highest percentage of employment (95%, n=58) in comparison with patients with only primary education (n=1 who is unemployed) or secondary education (n=126, of whom 95 are employed (75%) (*P*=0.01). Odds ratios and 95% CI of univariate and multivariable analyses of active versus inactive employment status are presented in Table 5.2.

The multivariable analysis of patients below 65 years of age with the same variables as reported earlier showed the same outcomes, concluding that employment remained a crucial predictor for HRQOL in patients with MEN1 (Table 5.2).

DISCUSSION

In this large nationwide study including a representative sample of MEN1 patients, quality of life was significantly and clinically relevantly impaired in comparison with the general Dutch population. A worse QOL in MEN1 patients was suggested in previous studies with either selected or small MEN1 populations[8,9].

An in depth analysis revealed that the most consistent predictor for HRQOL was employment status, followed by the presence of a pituitary tumor. Employment was a robust predictor for HRQOL across all HRQOL subscales. Employment has proven to be beneficial for health in general[16], therefore not being able to work can have a significant effect on the QOL. The percentage of unemployment was 20% in this population. Unemployment in

Table 5.2. Characteristics of MEN1 patients of working age and independent variables of employment

	All patients (n=196)	Inactive employment status (n=39)	Active employment status (n=154)	Univariate analysis			Multivariable analysis		
				OR	95% CI	*P*	OR	95% CI	*P*
Age	46 [19,64]	52 [19,64]	44 [20,64]	0.2	0.1, 0.5	<0.01	0.9	0.9, 0.99	0.02
Male	73	11 (15%)	62 (85%)	0.6	0.3, 1.3	0.2			
Female	120	28 (23%)	92 (77%)						
Education level	2	2 (100%)	0 (0%)	8.1	2.4, 27.2	0.001	0.1	0.04, 0.6	
Primary	126	31(25%)	95 (75%)						
Secundary	61	3 (5%)	58 (95%)						
Tertiary									0.005
pNET according to patients	105	24 (23%)	81 (77%)	0.7	0.3, 1.4	0.3			
PIT according to patients	77	18(23%)	59 (77%)	0.7	0.4, 1.5	0.4			
pHPT according to patients	157	37 (24%)	120 (76%)	0.2	0.04, 0.8	0.03	5.2	0.6, 46.5	0.14
Years of diagnosis	12 [0,46]	13 [4,46]	12 [0,32]	1.0	0.91, 0.99	0.03	1.0	0.9, 1.0	0.3
Presymptomatic	86	9 (10%)	77 (90%)	3.5	1.5, 7.8	0.003	0.7	0.2, 2.4	0.6
Index case	47	16(34%)	31 (66%)	0.4	0.2, 0.8	0.01	2.4	0.8, 7.0	0.1

the Netherlands was approximately 5.5% during the time of this study, revealing a high percentage of unemployment in MEN1 patients.

An online survey in 153 MEN1 patients who are part of the American MEN support group revealed that this group experienced significant financial burden and unemployment, which were both correlated to worse QOL[17]. A similar effect of employment on QOL is observed in different malignancies[18]. Both, losing a job because of health issues and being a long-term cancer survivor are risk factors for lower QOL[19]. Cancer survivors who continue to work have a better health and QOL than patients who are not able to work[20]. Since cancer survivorship is associated with unemployment, this group of patients is at risk of not returning to work[21]. In our study, an older age and lower levels of education were associated with unemployment. This is in line with previous studies assessing predictors of unemployment of cancer survivors[22]. Quality of life and return to work seem to benefit from rehabilitation programmes[23]. Multidisciplinary interventions that combine vocational counselling, patient education/counselling and physical exercises showed higher return to work rates than care as usual[21]. Intervention studies assessing a similar rehabilitation programme in MEN1 patients would be helpful to gain more insight in the value of these programmes and to develop a MEN1 specific multidisciplinary reactivation programme.

Remarkably, in a substantial proportion of patients, there was a discrepancy between the self-reporting of patients of having a pNET or pituitary tumor and the disease status as reported in their medical records. In sixteen and 29% of patients who reported not to have a pNET or pituitary tumor, the medical records showed the presence of these manifestations. Patients could not have been informed properly, could have forgotten their own disease status or misunderstood the questions. Interestingly, patients who were not aware of having a pNET or a pituitary tumor had a better HRQOL compared to patients who were aware of their disease status. When compared with patients having a pNET, the differences of HRQOL between those who were aware compared to those unaware, was worse in patients having a PIT. This suggests that in particular the knowledge of patients that they have a pituitary tumor played a significant role in the QOL.

This finding also suggests that ignorance can be bliss in this particular group of patients. However, withholding information regarding disease status is ethically not allowed. Most MEN1 related tumors remain stable and asymptomatic, and require no treatment. In these cases, the major burden is the knowledge about the disease status without a direct clinical consequence. This requires specific coping strategies in dealing with lifelong disease burden with an insecure outcome necessitating specific attention.

Limitations

The cross sectional design of the study does not allow assessing the QOL in the course of the disease. A longitudinal study would give insight in the variability of QOL in the course of MEN1 related therapies and surveillance. The heterogeneity of MEN1, with its variable disease prevalence, various treatment options and strict follow up regimen sets the ideal basis for a longitudinal QOL study.

Strengths

A major strength is the high response rate of 80% of this study contributing to the validity and generalizability of the study. Another major strength is the availability of the DMSG longitudinal MEN1 database comprising of an extensive clinical dataset. Data filled out by the respondents could therefore be cross-referenced from this database.

Clinical implications

Because of the reduced QOL, special attention of care providers for this aspect should be routine in the regular care for patients with MEN1. Integrating structured QOL assessments to the surveillance program will provide more insight into the perceived burden of patients with MEN1 and the QOL in the course of the disease. This will ultimately contribute to improving the quality of this aspect of MEN1 patient care.

Patient counselling on the natural course of the disease and possible treatment options are imperative to provide insight in the disease. Tumors have malignant potential, but genetic testing to pursue early diagnosis and subsequent surveillance has decreased morbidity and mortality[24]. This information might assure patients to adhere to the current screening protocols and be assured that an early diagnosis will lead to an improved overall outcome.

Physicians should be especially aware of the impact of the relationship between unemployment and QOL. Unemployed patients should be considered as high-risk patients for worse QOL. Multidimensional rehabilitation programmes might be helpful in returning to work and hereby improving QOL.

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Supplementary data: Univariate and multivariate analyses per SF-36 subscales

	Univariate analysis			Multivariable analysis		
Physical functioning	**B (unstandardized)**	**95% CI**	***P***	**B (unstandardized)**	**95% CI**	***P***
Employment	22.9	16.8, 29.0	<0.001	20.1	12.6, 27.5	<0.001
Age	-0.5	-0.72, 0.34	<0.001	-0.05	-4.2, 4.1	0.98
Years of MEN1 diagnosis	-0.6	-0.9, -0.2	0.001	-0.3	-0.7, 0.03	0.07
Presymptomatic diagnosis	6.3	0.3, 12.3	0.04	-3.6	-10.5, 3.2	0.3
Index case	-6.8	-13.9, 0.24	0.06	-6.8	-14.6, 0.9	0.8
Education level	10.6	4.5, 16.6	0.001	4.9	-1.0, 10.7	0.12
Pituitary tumor	-2.6	-8.9, 3.7	0.4			
pNET	-3.3	-9.4, 2.8	0.3			
pHPT	-6.1	-14.3, 2.2	0.2			

	Univariate analysis			Multivariable analysis		
Social functioning	**B (unstandardized)**	**95% CI**	***P***	**B (unstandardized)**	**95% CI**	***P***
Employment	12.6	5.3, 19.9	0.01	11.8	4.6, 19.0	0.002
Age	-0.18	-0.4, 0.1	0.1			
Years of MEN1 diagnosis	-0.2	-0.6, 0.2	0.3			
Presymptomatic diagnosis	4.7	-2.1, 11.4	0.2			
Index case	-10.0	-17.9, -2.1	0.01	-7.1	-15.0, 0.7	0.08
Education level	5.3	-1.5, 12.0	0.1			
Pituitary tumor	-7.9	-14.7, -1.0	0.02	-6.7	-13.4, 0.04	0.05
pNET	-1.5	-8.3, 5.2	0.7			
pHPT	-6.2	-15.3, 2.9	0.2			

	Univariate analysis			Multivariable analysis		
Role physical functioning	**B (unstandardized)**	**95% CI**	***P***	**B (unstandardized)**	**95% CI**	***P***
Employment	24.1	12.0, 36.3	<0.001	62.1	46.6, 77.7	<0.001
Age	-0.3	-0.7, 0.04	0.08			
Years of MEN1 diagnosis	-0.8	-1.4, -0.1	0.03	-0.3	-0.9, 0.4	0.4
Presymptomatic diagnosis	8.9	-2.4, 20.2	0.1			
Index case	-11.6	-24.9, 1.8	0.09			
Education level	6.4	-5.0, 17.8	0.3			
Pituitary tumor	-18.0	-29.3, -6.6	0.002	-19.1	-30.3, -7.8	0.001
pNET	-8.0	-19.3, 3.4	0.2			
pHPT	-11.2	-26.4, 4.1	0.2			

	Univariate analysis			Multivariable analysis		
Vitality	**B (unstandardized)**	**95% CI**	***P***	**B (unstandardized)**	**95% CI**	***P***
Employment	10.4	3.8, 17.0	<0.001	10.1	3.6, 16.6	0.002
Age	-0.1	-0.3, 0.11	0.4			
Years of MEN1 diagnosis	-0.3	-0.6, 0.08	0.13			
Presymptomatic diagnosis	4.6	-1.5, 10.7	0.14			
Index case	-2.9	-10.1, 4.3	0.4			
Education level	3.6	-2.5, 9.7	0.3			
Pituitary tumor	-8.6	-14.7, -2.5	0.006	-8.3	-14.3, -2.3	0.007
pNET	-4.8	-10.9, 1.2	0.1			
pHPT	-6.7	-14.9, 1.4	0.1			

	Univariate analysis			Multivariable analysis		
Mental health	**B (unstandardized)**	**95% CI**	***P***	**B (unstandardized)**	**95% CI**	***P***
Employment	8.5	3.2- 13.8	0.002	8.3	3.1, 13.6	0.002
Age	-0.09	-0.3, 0.08	0.3			
Years of MEN1 diagnosis	0.03	-0.3, 0.8	0.8			
Presymptomatic diagnosis	4.3	-0.6, 0.2	0.09			
Index case	-3.9	-9.8, 1.9	0.18			
Education level	1.8	-3.1, 6.7	0.5			
Pituitary tumor	-5.0	-10.0, -0.07	0.05	-4.8	-9.6, 0.06	0.05
pNET	-0.9	-5.8, 4.0	0.7			
pHPT	-5.6	-12.2, 0.9	0.09			

	Univariate analysis			Multivariable analysis		
Emotional functioning	**B (unstandardized)**	**95% CI**	***P***	**B (unstandardized)**	**95% CI**	***P***
Employment	0.6	-1.9, 21.1	0.1			
Age	0.2	-0.1, 0.5	0.2			
Years of MEN1 diagnosis	0.4	-0.2, 1.0	0.2			
Presymptomatic diagnosis	5.4	-5.0, 15.9	0.3			
Index case	-18.0	-30.2, -5.9	0.004	-15.0	-27.2, -2.8	0.02
Education level	-0.7	-11.1, 9.8	0.9			
Pituitary tumor	-16.9	-27.3, -6.5	0.002	-14.2	-24.8, -3.7	0.008
pNET	2.0	-8.4, 12.4	0.7			
pHPT	-5.3	-19.3, 8.7	0.5			

	Univariate analysis			Multivariable analysis		
Bodily pain	**B (unstandardized)**	**95% CI**	***P***	**B (unstandardized)**	**95% CI**	***P***
Employment	13.1	7.1, 19.2	<0.001	11.4	3.8, 18.9	0.003
Age	-0.3	-0.5, -0.1	0.001	-0.4	-4.4, 3.5	0.8
Years of MEN1 diagnosis	-0.2	-0.5, 0.2	0.3			
Presymptomatic diagnosis	1.3	-4.5, 7.0	0.7			
Index case	-2.8	-9.7, 4.0	0.4			
Education level	6.0	0.3, 11.7	0.04	2.6	-3.3, 8.5	0.4
Pituitary tumor	-5.3	-11.1, 0.5	0.08			
pNET	-2.3	-8.3, 3.2	0.4			
pHPT	-1.6	-9.3, 6.1	0.7			

	Univariate analysis			Multivariable analysis		
General health	**B (unstandardized)**	**95% CI**	***P***	**B (unstandardized)**	**95% CI**	***P***
Employment	12.3	6.3, 18.3	<0.001	12.1	5.6, 18.6	<0.001
Age	-0.2	-0.4, -0.1	0.05			
Years of MEN1 diagnosis	54.9	49.6, 60.2	<0.001	0.1	-0.2, 0.4	0.6
Presymptomatic diagnosis	4.6	-1.0, 10.2	0.2			
Index case	-4.2	-10.8, 2.4	0.2			
Education level	3.7	-1.9, 0.3	0.2			
Pituitary tumor	-6.1	-11.8, -0.4	0.04	-5.6	-11.4, 0.2	0.06
pNET	-4.9	-10.4, 0.7	0.09			
pHPT	-9.0	-16.5, -1.5	0.02	-5.1	-13.2, 3.0	0.2

Chapter 6

High fear of disease occurrence is associated with low quality of life in patients with Multiple Endocrine Neoplasia type 1 (MEN1): *Results from the Dutch MEN1 Study Group*

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Abstract

Objective

Multiple Endocrine Neoplasia type 1 (MEN1) is a hereditary disease characterized by a high risk of developing primary hyperparathyroidism, duodenopancreatic neuroendocrine tumors, and pituitary tumors (PITs). It is unclear if having MEN1 leads to psychological distress because of fear of disease occurrence (FDO), thereby potentially affecting quality of life.

Design

A cross-sectional study was performed using the Dutch MEN1 cohort. All patients received the Cancer Worry Scale (a score ≥14 reflects high FDO), the Medical Outcomes Study 36-item Short- Form Health Survey (SF-36), and questions on sociodemographic and medical history.

Results

A total of 227 of 285 (80%) eligible patients with MEN1 completed the questionnaire. The mean (±6 standard deviation) age was 47±15 years. Overall, patients experienced an FDO of 15.1±4.7, with 58%of patients having a score ≥14. This is higher than reported in previous studies assessing fear of cancer recurrence in different cancer populations (31% to 52%). Adjusted for age and sex, the FDO score was negatively associated with almost all SF-36 subscales. In multivariable analysis, the diagnosis of a PIT, a pancreatic neuroendocrine tumor, and not being employed were associated with FDO (P<0.05). Patients had higher FDO scores for their family members than for themselves.

Conclusion

The majority of patients with MEN1 have FDO for themselves and even more for their relatives. This psychological distress is associated with a lower health-related quality of life. Therefore, in the medical care for MEN1, emphasis should also be placed on FDO and quality of life.

INTRODUCTION

Multiple Endocrine Neoplasia (MEN) type 1 is a hereditary disease with an autosomal dominant inheritance pattern caused by a germline mutation on chromosome 11q13[1]. MEN1 is characterized by a life time risk of developing primary hyperparathyroidism (pHPT) of almost 100%, a lifetime risk of developing duodenopancreatic neuroendocrine tumors (dpNETs) of >80% and a lifetime risk of developing pituitary tumors (PITs) of 70%[1–4]. The mean ages at diagnosis for the three main manifestations, pHPT, dpNET and PIT, in the Dutch MEN1 population are 36, 41 and 40 years, respectively[4]. Other neoplasms, such as adrenal tumors; neuroendocrine tumors of gastric, bronchial or thymic origin; skin and subcutaneous tumors; smooth muscle tumors; and, as recently discovered, breast cancer, can occur during the course of the disease[5,6]. These manifestations cause significant morbidity; dpNETs and thymic neuroendocrine tumors lead to premature death[4,7,8]. The average life expectancy in the Dutch MEN1 population is 73 years, which is 10 years shorter than the general Dutch population[4]. The young age at which *MEN1* mutation carriers are confronted with a MEN1-related disease, makes lifelong screening and intensive monitoring indispensable for early tumor detection to prevent morbidity and mortality[9].

A diagnosis of MEN1 has a considerable impact on an individual and might lead to psychological distress and worry about disease occurrence. Considering the autosomal inheritance pattern, theoretically 50% of family members are *MEN1* carriers. For individuals with *MEN1*, this implies that 50% of their children potentially have *MEN1*. As for other autosomal-dominant hereditary diseases, the stress caused by MEN1 could extend to fear of disease-occurrence (FDO) in family members.

Fear of cancer occurrence has been studied in other hereditary cancer syndromes with the same inheritance pattern as MEN1[10–12]. Patients with Von Hippel Lindau disease (VHL) reported frequent concerns about developing a VHL-related tumors in themselves or in a family member with VHL[11]. Moreover, more patients with Li-Fraumeni syndrome expressed greater concerns about cancer occurrence in family members than about the chance of developing cancer themselves[10]. The quality of life (QOL) of patients with Li-Fraumeni and VHL syndrome was comparable to an age- and sex-matched reference group from the general population[10], with the exception that VHL patients had a significantly lower score for general health[11]. Few studies have addressed the QOL in MEN1 patients, and these studies had a limited or selected study population[13,14]. These limitations reflect the difficulty in performing research in rare tumor syndromes, such as MEN1, which has a prevalence of 3 to 4 per 100,000[1]. This highlights the need for QOL studies in an unselected MEN1 population with an adequate sample size to assess the impact of MEN1 on patients and to determine whether there is a need for more psychological support.

To address this important aspect for patients with MEN1, the primary aim of this study was to evaluate MEN1-related FDO in patients themselves and for their family members with MEN1. The secondary aim was to assess the association of MEN1-related fear on health related QOL. In addition, we aimed to identify variables that were significantly related to MEN1-related fear.

METHODS

Study population

Patients were selected from the Dutch MEN1 study group database. This longitudinal database includes >90% of all Dutch patients with MEN1, aged 16 years and older at the end of 2013, treated at one of the Dutch University Medical Centers between 1990 and 2013. Patients were eligible for the current study if they had a confirmed MEN1 mutation. Demographic and clinical data (*e.g.*, age, sex, and MEN1-related medical history) were retrieved from this database.

Study design

A cross-sectional study was performed from April 2015 to December 2016 in which all eligible patients were invited to complete a questionnaire. After 2 weeks, a reminder e-mail was sent to the participants. The questionnaire could be completed by hand or as a web-based questionnaire. All patients with MEN1 who participated in the study provided written informed consent.

Questionnaires

Sociodemographic data (*e.g.*, marital status, offspring, education, and employment) were obtained.

MEN1-related fear

MEN1-related FDO was assessed with an eight-item questionnaire adapted from the Cancer Worry Scale (CWS)[15,16]. The eight items of the CWS are rated on a four-point Likert scale ranging from “never” to “almost always”. The total sumscore ranges from 8 to 32, with higher scores indicating more frequent worries about cancer. Cronbach α in this study was 0.89. A diagnostic cut-off score of 14 or higher (sensitivity, 77%; specificity, 81%) indicates severe FDO[16]. The CWS has previously been used in different hereditary

tumor syndrome populations, which included healthy subjects (non- carriers), mutation carriers with disease manifestations, and mutation carriers at risk. These studies showed that the CWS in these populations is a valid tool with high internal consistency[10–12]. Five similar questions regarding FDO in family members were added to the original scale. These questions were from the original scale but addressed fear of family members instead of fear of the patients themselves. These additional questions did not affect the outcome of the original FDO score because they were used in a separate analysis. Cronbach α for these additional questions was 0.87, which reflects a high internal consistency of the questions.

Perceived risk

Respondents were asked to report their perceived risk of developing an (additional) tumor compared with that of an 'average person in the Dutch population' of their age (item adapted from Lerman *et al.*)[15]. Response categories ranged from 'lower' to 'much higher' on a five-point scale.

Health-related quality of life

Health-related QOL was assessed with the Medical Out- comes Study 36-item Short-Form Health Survey (SF-36) composed of eight multi-item scales assessing physical functioning, role limitations due to physical health problems and emotional problems, bodily pain, general health perceptions, vitality, social functioning, and general mental health. Scale scores range from 0 to 100, with higher scores indicating better levels of functioning and well-being. Cronbach α for the SF-36 scales ranged from 0.84 (social functioning) to 0.93 (physical pain). Only general health perception had a low Cronbach α (0.55), which is therefore of low significance.

Statistical analysis

Descriptive statistics were applied to characterize the study population. Univariate analyses (independent sample t-test, χ^2 test/Fisher exact test, Pearsons correlation) were used to evaluate which MEN1-related manifestations and sociodemographic variables were associated with cancer worry and Health-related QOL.

To compare FDO of the patients for themselves with FDO in first-degree relatives we performed the Wilcoxon signed rank test. Binary logistic regression was performed to assess the association between low (<14) and high (≥14) FDO scores with the eight different SF-36 subscales adjusted for age and sex. A multivariable analysis was carried out using multiple linear regression to assess which patient characteristics were associated with

FDO. Collinearity was tested using variance inflation factors. In the linear models, none of the variance inflation factors were >1.20, suggesting that collinearity was not a problem. Multiple imputation was used for missing data in the CWS.

Analyses were conducted using SPSS 22.0. *P*-values <0.05 were considered as statistically significant.

RESULTS

Response rate

A total of 285 patients (120 men and 165 women) were eligible for inclusion, of whom 252 (102 men and 150 women) received the questionnaire. The questionnaire was completed by 227 individuals [84 (70%) men and 143 (87%) women], resulting in a total response rate of 80% (Figure 6.1).

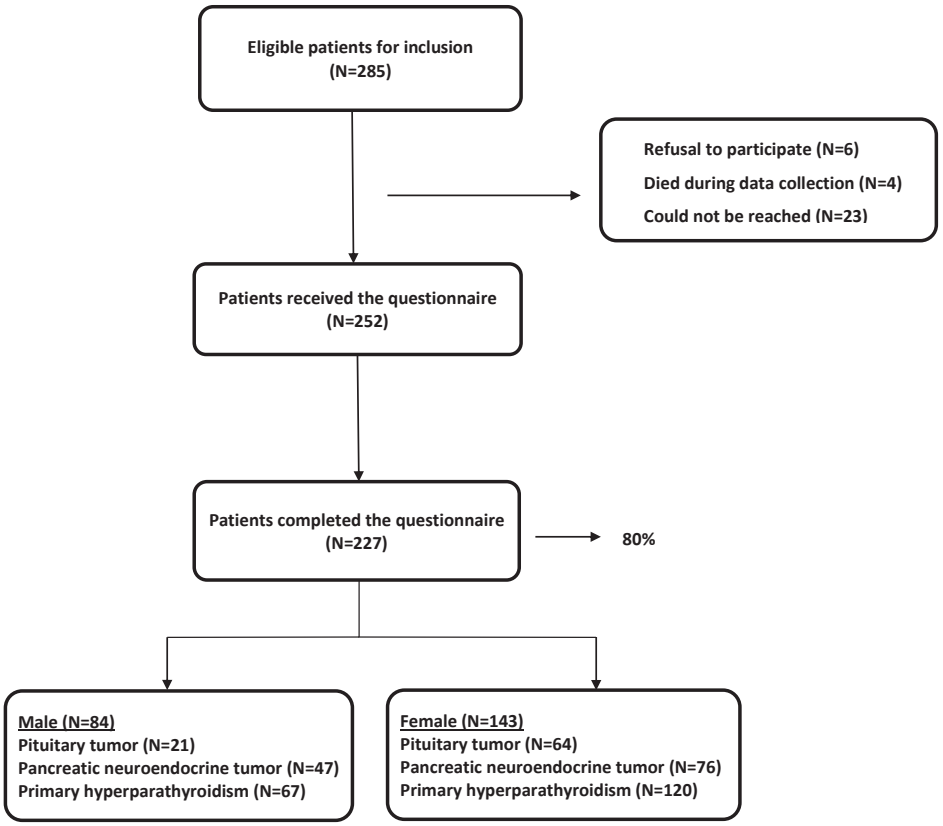

Figure 6.1. Flowchart study population.

Sample characteristics

The mean [± standard deviation (SD)] age of the study population was 47 SD ±15 years. All patients had a confirmed *MEN1* mutation or one or more MEN1 related manifestations and a first-degree relative with MEN1. Eighty-three percent of patients had ever had pPHT. More than half of the patients had ever had a pancreatic neuroendocrine tumor (pNET) (55%), and 38% of patients had ever had a PIT (Table 6.1).

Table 6.1. Baseline characteristics (N=227)

Gender	
Female	143 (63%)
Male	84 (37%)
Age (mean, SD)	47 (15)
Education[a]	
Primary school	6 (3%)
High school	149 (65%)
College or university	66 (29%)
Employment	
Yes	159 (70%)
No	64 (28%)
Children	
Yes	139 (61%)
No	69 (30%)
Unknown	19 (8%)
Index case	
Yes	51 (23%)
No	173 (76%)
Presymptomatic diagnosis	
Yes	98 (44%)
No	125 (56%)
Years since MEN1 diagnosis	
<5 years	27 (12%)
≥5 years	190 (84%)
Primary hyperparathyroidism	
Yes	187 (83%)
No	38 (17%)
Pituitary tumor	
Yes	85 (38%)
No	139 (62%)
Pancreatic neuroendocrine tumor	
Yes	123 (55%)
No	101 (45%)

Fear of disease occurrence

Patients with MEN1 had a mean (±SD) FDO score of 15.1±4.7, which can be considered as a high level of fear, in comparison with other types of cancer (Figure 6.2). The same CWS (consisting of the original eight questions) was used in this comparison with the other cross-sectional studies. A total of 58% of patients with MEN1 had an FDO score ≥14.

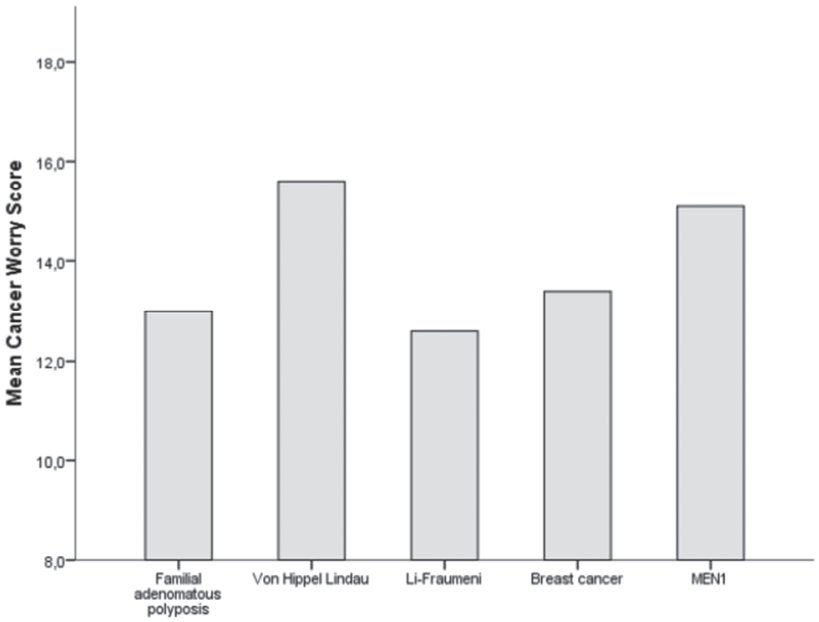

Figure 6.2. Fear of cancer recurrence scores in different (hereditary) tumor syndromes.

Fear of disease occurrence in relation to Quality of Life

Adjusted for age and sex, the FDO score was significantly associated with all scales of the SF-36. Table 6.2 shows the mean (SD) SF-36 health-related QOL scores stratified by high or low FDO. Patients with high FDO showed lower QOL on all subscales, except for the physical functioning subscale (P=0.05), compared with patients with low FDO.

Manifestations and fear of disease occurrence

Patients who had a pNET had higher FDO than patients without a pNET, with scores of 15.6±4.8 and 14.4±4.5, respectively (P<0.01). Having pHPT also led to a slightly higher FDO than never having had pHPT (15.2±4.6 versus 14.4±5.0, respectively; P=0.15). Patients with a PIT had more fear than patients without a PIT, with FDO scores of 16.2±4.5 and 14.4±4.7 (P<0.01).

Table 6.2. Means and standard deviations of the Short Form 36 Health Related Quality of Life scale stratified by age and gender

SF-36	Low Fear of disease occurrence <14 n=90 Mean (SD)	High Fear of disease occurrence <14 n=120 Mean (SD)	*P*
Physical functioning	87.3 (18.7)	80.4 (24.6)	0.05
Role functioning	80.6 (35.3)	56.7 (43.2)	<0.001
Emotional functioning	91.7 (22.8)	63.8 (42.6)	<0.001
Social functioning	85.7 (20.3)	70.5 (25.7)	<0.001
Mental health	80.3 (13.5)	66.8 (18.6)	<0.001
Vitality	64.6 (21.0)	51.2 (21.5)	<0.001
Bodily pain	88.8 (14.7)	76.1 (23.2)	<0.001
General health	51.6 (15.7)	47.3 (21.8)	0.11

Mann Whitney U test.

A total of 41 patients had small incidentalomas (<1 cm) not requiring intervention (medication or surgery), 12 patients had undergone surgery, and 32 patients were on medication (hormonal supplementation because of pituitary insufficiency, dopamine agonists, or somatostatin analogs). The FDO values were 15.9±4.4, 15.4±4.7, and 17.9±4.9, respectively.

Patients who were employed had a FDO of 14.8±4.3, in comparison with an FDO of 15.6±5.4 for unemployed patients (P<0.01). Presymptomatic patients (*e.g.*, patients who did not have an MEN1-related manifestation at time of diagnosis) had an FDO of 15.5±4.7, in comparison with an FDOof 14.7±4.6 in non-presymptomatic patients (P<0.01). There were no differences in FDO for sex, age, and education level.

Multivariable analysis

In a multivariable analysis including age, sex, pNET, pHPT, PIT, number of MEN1-related manifestations, employment (yes/no) and presymptomatic diagnosis (yes/no), the diagnosis of a pituitary tumors, a pNET, and being unemployed were associated with FDO (P<0.05).

Multiple MEN1 manifestations

An increasing number of manifestations led to more FDO (P=0.02 for trend). The FDO was lower between respondents without a manifestation (13.8±5.4; n=24) and patients who were already affected by the disease with one or more manifestations (15.2±4.6; P<0.01). Respondents without or with one manifestation had scores of 13.8±5.4 and

13.7±4.0, respectively. Patients with two or three manifestations had scores of 15.6±4.5 and 16.1±4.0), respectively.

Fear of disease occurrence in family members

On five different items of the FDO scale, patients had significantly more fear for their family members than for themselves. However, the effect sizes ranged from 0.10 to 0.22 (Cohen *d*), which can be considered a small-to-medium effect (Table 6.3).

Disease perception and fear of disease occurrence

Respondents were asked whether they believed they had an equal, slightly higher, moderately higher, or severely higher chance of developing a MEN1-related tumor in the next 10 years in comparison with the general Dutch population. Thirty-nine percent of patients responded that they had an equal or slightly higher chance, and 40% considered their chances as being much higher. Patients who considered themselves to have a severely higher chance had the greatest FDO. Patients who considered themselves to have an equal chance had the lowest FDO (Table 6.4).

Table 6.3. Comparison of Fear of disease occurrence in patients themselves and in their family members

	r	*P*
Regular thoughts about tumor recurrence (self vs. family)	-0.15	0.02
Thoughts about tumor recurrence have influenced mood	-0.14	0.03
Thoughts about tumor recurrence interfered in daily activities	-0.10	0.14
How concerned are you about recurrence of a tumor?	-0.22	<0.01
How often have you worried about tumor recurrence	-0.22	<0.01
Is this worry a problem for you?	-0.20	<0.01

Wilcoxon signed-rank test (*r* using Cohen criteria of .1 = small effect, .3 = medium effect, .5 = large effect).

Table 6.4. Fear of disease occurrence and the chance of MEN1 related tumor development in the next ten years in comparison with the general population

	Fear of disease occurrence	N
Equal chance	11.9	33 (15%)
Slightly higher chance	12.8	53 (24%)
Moderately higher chance	14.7	46 (21%)
Severely higher chance	17.7	90 (40%)

Kruskal-Wallis *P*<0.01, df 3.

DISCUSSION

In this study, we focused on FDO in patients and their family members in a large and representative cohort of patients with MEN1. The high FDO that was found in this study can be interpreted as a substantial fear of further implications of the disease. High fear can be characterized as chronic worry, excessive monitoring for signs of disease occurrence, seeking medical reassurance, avoidance of disease reminders, intrusive thoughts and images about occurrence, and difficulties planning for the future[17–22].

Patients with high FDO had lower scores on the SF-36 scale, indicating a lower QOL. In addition, an increase in the of number of manifestations of MEN1 was directly correlated to higher FDO scores.

FDO has been derived from fear of cancer recurrence, which has been a topic of increasing interest in recent years[23–26]. Fear of cancer recurrence has been defined as "fear, worry or concern relating to the possibility that cancer will come back or progress"[27]. Because some MEN1 patients with MEN1 have not experienced a MEN1 manifestation, 'recurrence' was replaced by 'occurrence', and 'cancer' was replaced by 'disease' because pHPT and PITs are not recognized as cancer.

The FDO score was high in comparison with other types of cancer. The outcome of 15.1 is comparable with patients with the VHL syndrome (15.6)[11]. Fear in patients with breast cancer for example was lower with an average score of 13.4[16]. This reflects the major impact of MEN1 beacuse fear in breast cancer patients is generally considered high. Patients with Li-Fraumeni and Familial adenomatous polyposis also have lower scores compared with patients with MEN1 (13.9 and 12.4, respectively)[10,12] (Figure 6.2).

Patients who considered their chance of occurrence of a MEN1-related tumor as comparable with the general population had less FDO than respondents considering themselves as having a severely higher chance. Regarding the high age-related penetrance of the disease, patients with a higher number of manifestations had more FDO.

In this study, the diagnosis of a PIT, pNET and being unemployed were related to elevated FDO. pNETs lead to the most morbidity and mortality in MEN1, and therefore it seems obvious that patients with a pNET diagnosis have greater FDO[4,7,28–30]. However, it is interesting that patients with a PIT diagnosis have more FDO since PITs in MEN1 are generally benign and have a slow growth rate. In addition, the majority of patients with PITs are successfully managed by pharmacological treatment or by a watchful waiting strategy[31,32]. Patients who had an operation in the past had the lowest FDO, whereas patients with a current PIT had greater FDO. This suggests that present disease status is

more relevant than past severe pituitary disease. This finding could suggest that patients were not aware of the indolent course of PITs or that the knowledge of having a tumor near their brain is a high burden. Adequate patient information on the expected course of individual MEN1 manifestations is therefore mandatory.

Unemployment was associated with FDO, which is in line with previous research. Employment status in general has proved to be beneficial for health and in particular mental health[33]. Cancer survivors who continued working had better health and QOL than those who are not able to work[34].

Age was not associated with FDO, although a younger age has been indicated to be a predictor for elevated FDO in other studies[19,20,25]. In MEN1 there is an age-related effect, with young patients with MEN1 generally having fewer manifestations[4]. This could explain why young age is not related to high FDO in this cohort.

Patients were more worried about their family members than about themselves. This underlines the necessity of an integral familial approach in identifying FDO and in supporting of the family as a whole.

Limitations

A limitation of this study is its cross-sectional design that did not allow assessing changes of FDO or QOL over time. In hereditary tumor syndromes, FDO could vary in time, and it would be interesting to study the course of FDO in MEN1.

MEN1-related fear was assessed by an adapted CWS that has not been validated in an MEN1 population. However, the CWS has been validated in breast cancer[16] and has been used in other hereditary syndromes with similar inheritance patterns such as VHL[11], Li-Fraumeni[10] and familial adenomatous polyposis[12].

Another limitation is that the questions regarding FDO in patients themselves in comparison with their family members have not been validated in other studies. Because there was a trend toward greater FDO for family members, this could be relevant for other hereditary diseases and necessitates further research.

Strengths

All respondents are from the population-based DMSG database, which consists of >90% of the total Dutch MEN1 population. The high response rate of 80% contributes to the generalizability and validity of the study results, which can be considered a major strength.

This response rate is much higher than other similar studies[10,12,16,17,24,35]. This is the largest MEN1 cohort in which FDO associated with QOL has been studied, which makes the data unique. In accordance with recent findings, only *MEN1* mutation-positive patients were included. *MEN1* mutation-negative patients have a different phenotype, a different clinical course, and no family members with a *MEN1* mutation, and therefore we excluded these patients to prevent heterogeneity[4].

Clinical implications

The high percentage of patients with MEN1 (58%) with a high FDO highlights the need for more attention and support for aspects of fear and worry regarding the disease. This topic has been largely neglected, which is incomprehensible considering the results regarding FDO and its impact on QOL.

MEN1 is a diagnosis that often affects multiple family members, and therefore the high FDO for patients' family members requires that regular follow-up visits include addressing worries about relatives with MEN1-related problems, and psychosocial support should be provided when needed. A study in women with an increased risk of breast cancer who expressed a high level of unmet need in support showed that women were mostly interested in attending a support group where they could participate in discussions and receive more information[36]. Identifying the need for support in patients with MEN1 would be the first step toward interventions to reduce fear and improve QOL, especially because the prevalence rate for high FDO is higher in patients with MEN1 [58%, in comparison to 52% in patients with gastrointestinal stromal tumors[35], 38% in patients with colorectal tumors[37], 36% in patients with prostate cancer[24] and 31% of breast cancer patients[16]].

A psychological intervention has shown efficacy to reduce fear in patients with curable breast and colorectal cancer and melanoma. Similar interventions could be beneficial for selected patients with MEN1 after gaining more knowledge on FDO and performing evidence-based interventional studies in patients with MEN1[38].

In summary, there is FDO in patients with MEN1, which is associated with a lower QOL. Future studies should focus on interventions that improve QOL and hereby improve care and subsequently the QOL of patients with MEN1.

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Chapter 7

The future: Advances in therapeutic approach and management strategies for MEN1

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Abstract

Multiple endocrine neoplasia type 1 is a rare autosomal inherited disorder associated with a high risk for patients to simultaneously develop tumours of the parathyroid glands, duodeno-pancreatic neuroendocrine tumors and tumors of the anterior pituitary gland. Early identification of MEN1 in patients enables presymptomatic screening of manifestations which makes timely interventions possible with the intention to prevent morbidity and mortality. Causes of death nowadays have shifted towards local or metastatic progression of malignant neuro endocrine tumors. In early cohorts, complications like peptic ulcers in gastrinoma, renal failure in hyperparathyroidism, hypoglycemia and acute hypercalcemia were the primary cause of early mortality. Improved medical treatments of these complications led to a significantly improved life expectancy. The MEN1 landscape is still evolving, considering the finding of breast cancer as a new MEN1-related manifestation and ongoing publications on follow up and medical care for patients with MEN1. This review aims at summarizing the most recent insights into the follow-up and medical care for patients with MEN1 and identifying the gaps for future research.

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN 1) (OMIM 131100) is a rare autosomal inherited disorder associated with a high risk for patients to simultaneously develop tumours of the parathyroid glands, duodeno-pancreatic neuroendocrine tumors (NETs) and tumors of the anterior pituitary gland[1]. Patients with MEN 1 are also at risk of developing adrenal tumors and NETs of lung, thymus and stomach, lipomas, angiofibromas and collagenomas[2]. Recently, females with MEN1 were found to have a 2-3 times higher risk for breast cancer at a younger age as compared with the general population[3,4].

Nowadays, in approximately 90% of patients a germline mutation of the MEN1 gene located on chromosome 11q13 is found[5–8]. Early identification of MEN 1 in patients enables presymptomatic screening of manifestations which makes timely interventions possible with the intention to prevent morbidity and mortality[9]. The guidelines for clinical practice which were originally published in 2001 and updated in 2012 led to more clarity for medical professionals how to take care of patients with MEN1. In the guidelines, it is advised to perform MEN1 mutation analysis in the offspring of patients carrying the MEN1 mutation already at the young age of five years[10,11].

Survival and cause of death in MEN1 patients have dramatically changed over the past decades. In early cohorts, complications like peptic ulcers in gastrinoma, renal failure in hyperparathyroidism, hypoglycemia and acute hypercalcemia were the primary cause of early mortality in MEN1 patients[12–14]. With improved medical treatments these complications have become rare and life expectancy has significantly improved. Notwithstanding, approximately two thirds of MEN1 patients still die from MEN1 related causes in the late stage of the disease. Cause of death nowadays has shifted towards local or metastatic progression of malignant NETs. In recent years, studies based on larger MEN1 cohorts have sought for further evidence to support the clinical practice guidelines with regard to follow up and interventions to ultimately improve the prognosis of patients with MEN1. The present review aims at summarizing the most recent insights into the follow-up and medical care for patients with MEN1 and identifying the gaps for future research.

GENETIC TESTING OF MEN1 IN INDEX CASES

Endocrine diseases associated with MEN1 such as primary hyperparathyroidism (pHPT) and pituitary tumors (PIT) are relatively common in the general population. Moreover, an increasing number of incidentalomas in endocrine organs is found on imaging studies[15–17]. In patients who are not from known MEN1 families, apparently sporadically occurring

tumors might actually be caused by a not yet identified *MEN1* mutation. It is important to timely identify index cases, because subsequent early detection of MEN1 related tumors is associated with improved outcomes and survival[5,18,19]. In addition, the presence of a MEN1 mutation also has important implications for family members of the index case[9].

The clinical practice guidelines present a consensus recommendation when to screen potential index cases for *MEN1* mutation[11,20]. Genetic screening for index cases is advised when clinical criteria for diagnosing MEN1 are met or when there is high suspicion for (atypical) MEN1. High suspicion for MEN1 which is defined as: parathyroid adenoma below the age of 30 years (or multigland parathyroid disease at any age); gastrinoma, or multiple pancreatic NET at any age; or individuals who have two or more MEN1-associated tumors that are not part of the classical triad of parathyroid, pancreatic islet, and anterior pituitary tumors (e.g. parathyroid tumor plus adrenal tumor)[11,20].

Several studies have raised concerns that these recommendations might be too conservative, resulting in a delay in the diagnosis of index cases[21–25]. Therefore, risk factors for *MEN1* mutation in potential index cases were recently assessed in the Dutch and Swedish population[21,26]. The results showed that clinicians in both the Netherlands and Sweden already frequently referred patients for genetic counseling and testing for MEN1 who did not meet the criteria for genetic testing as provided by the current practice guidelines (64% and 81% of individuals tested in both cohorts respectively). Mutations were also identified in patients who did not fulfill the suggested criteria for mutation analysis. Altogether, a mutation was identified in 15.9% and 13.2% of the Dutch and Swedish cohort respectively. The main risk factors for a MEN1 mutation were: recurrent or multiglandular primary hyperparathyroidism (odds ratio [OR] 162.40); non-recurrent hyperparathyroidism (OR 25.78); pancreatic and duodenal NET (OR 17.94); pituitary tumor (OR 4.71); NET of stomach, thymus, or bronchus (OR 25.84); and positive family history (up to third degree relatives) for any neuro-endocrine tumor (OR 4.53). Interestingly, in the current practice guidelines family history other than family members with proven MEN1, is not included to assess the risk for MEN1. The study confirmed that the risk for having a MEN1 mutation decreases with increasing age of first manifestation. A clinical prediction model for estimating the risk for a MEN1 mutation in the individual patient was formulated, that can be used in genetic counseling when considering testing for MEN1[26].

FAMILIAL MANAGEMENT

Since the identification of the *MEN1* gene in 1997, familial screening of the *MEN1* gene in eligible family members after identification of the index case, has become possible. The autosomal dominant inheritance pattern leads to a 50% risk of *MEN1* carriership for first-degree family members[1]. The current clinical practice guidelines recommend to offer mutational analysis for first-degree relatives of known *MEN1* mutation carriers. Performing genetic testing in family members will identify MEN1 carriers that require screening for early tumor detection and treatment. Family members who do not harbour the MEN1 mutation will not undergo unnecessary screening and are secured from future worry of tumor development[11]. Due to genetic testing, family members have an earlier MEN1 diagnosis in comparison with the index case, with mean ages at diagnosis of respectively 42 and 34 years in the Dutch MEN1 cohort[9]. This age difference was confirmed in an Italian multicenter study with an age difference of 47 and 36.5 years at MEN1 diagnosis in index cases and family members[27]. However, there is no current advise on timing of familial screening. One study advocates for timely genetic screening of family members after MEN1 diagnosis of the index case. In this study the median lag time between diagnosis of a family member was 3.5 years, with a maximum lag time of 30 years. At the time of MEN1 diagnosis in family members of the index cases, patients with metastases had a longer lag time compared with patients without metastases. Non-index cases with a pituitary tumor at the time of MEN1 diagnosis with a macroadenoma also had a longer lag time compared with patients with a microadenoma. Ten non-index cases died because of a MEN1-related cause that developed during or before the lag time. These findings stretch the need for prompt genetic screening in all eligible family members[9].

MANAGEMENT FOR PATIENTS WITHOUT A CONFIRMED MEN1 MUTATION

Traditionally, a *MEN1* gene mutation was not identified in up to 25% of MEN1 patients who meet the clinical criteria for the diagnosis of MEN1[11]. With recent new techniques such as multiplex ligation-dependent probe amplification (MLPA) new mutations of the *MEN1* gene are uncovered which increases the sensitivity of genetic analysis[28,29]. Sensitivity is expected to further increase by the introduction of next generation sequencing techniques. Despite these new techniques, a *MEN1* mutation is not found in approximately 10% of patients with a clinical diagnosis of MEN1, a phenomenon also referred to as 'phenocopies'[30].

In the past few years a discussion arose whether these patients are correctly diagnosed as having MEN1. Research was initiated to identify other genes that might cause a MEN1-like phenotype[31–33]. Newly found mutations in the cyclin-dependent kinase inhibitor (*CDNK1B*) are of particular importance. Mutations in the *CDNK1B* gene caused, both in experimental animal studies and observational studies, a syndrome of parathyroid and anterior pituitary tumors[34]. Patients with a *CDNK1B* gene mutation have a clinical course different from patients with *MEN1* mutations, and have a lower risk to develop the pancreatic neuro-endocrine tumors (pNET). For this reason, after identifying these mutations, a new endocrine tumor syndrome was referred to as MENX or, more recently, MEN4[34].

In a recent nationwide study, major differences in the clinical course were found between 30 mutation negative MEN1 patients and 293 mutation positive patients[5]. Only one of the mutation negative patients appeared to have a *CDKN1B* mutation confirming the rarity of this mutation. The median age for developing the first main MEN1 manifestation was ten years later in mutation negative patients and a third primary MEN1 manifestation did not occur in this patient group. In addition, those patients hardly ever developed other associated tumors. Median survival in mutation positive patients was estimated at 73.0 years compared to 84.0 years in mutation negative patients. These results suggest that, instead of having the MEN1 syndrome, many mutation negative patients have a syndrome that is caused by a yet unknown genetic predisposition or co-incidentally have two sporadically occurring endocrine tumors. Consequently, systematic follow-up for early detection of endocrine tumors according to the MEN1 screening protocol appeared to be not necessary for most mutation negative patients.

PRIMARY HYPERPARATHYROIDISM

Primary hyperparathyroidism (pHPT) is the hallmark disease of MEN1. With a prevalence of around 90%[5,11,14] [5,11,14] it is the most common manifestation and often the first clinical feature of MEN1[19]. It is also responsible for most MEN-related surgeries[35,36].

Since presymptomatic screening for MEN1 became possible in 1997 and patients could be identified at an early age before symptoms occurred, more insight is gained about the clinical manifestations of MEN1 in children. In an Italian study prevalence of pHPT in children and adolescents was 50%. In this study 11/22 children (median age 12) were asymptomatic at the end of the study. Eleven of the twelve children that did develop a manifestation of MEN1 were diagnosed with pHPT[37]. Another study shows that of the children and adolescents that develop MEN1-related disease before the age of 21 (n=160),

75% (n=122) has pHPT. Most cases of pHPT before the age of 21 occur after the age of 10, are asymptomatic and detected by biochemical screening. However 9-14% presented with urolithiasis and 22-30% of the patients underwent parathyroid surgery before the age of 21 either because of symptoms our because of the height of serum calcium (>2.75 mmol/L)[38].

Due to the genetic background of MEN1-related pHPT, patients are younger at diagnosis, more often have multiglandular disease and there is an equal gender distribution compared to patients with sporadic pHPT. Anecdotally parathyroid carcinomas are described in MEN1, but this remains rare[39–42].

Although MEN1 patients often have lower parathyroid hormone (PTH) and calcium levels compared to patients with sporadic pHPT, early and severe bone involvement as well as more frequent renal complications have been reported[43]. Even after surgical intervention one study showed that bone recovery was better in patients with sporadic pHPT in patients with MEN1 after one year follow up. Risk of bone and renal complications is higher in patients with uncontrolled hyperparathyroidism[44].

MEN1 patients without pHPT are monitored with annual calcium and PTH measurements[11]. If the diagnosis pHPT is established and the decision to proceed with surgical treatment is made, preoperative localisation studies seem to be of little added value, since a bilateral neck exploration is the surgical procedure of choice[11,45]. One study showed that preoperative localisation studies for primary parathyroidectomy in MEN1 may only alter the surgical approach in 7% of the cases, which the authors deemed insufficient to recommend this on a routine basis[46]. When surgery is considered for persistent or recurrent pHPT, localisation studies are necessary to guide surgical approach, with ultrasound and sestamibi being the most sensitive conventional imaging studies[47]. Fluorine-18 fluorocholine PET-CT should be considered when conventional imaging studies are inconclusive[48].

The optimal timing of surgical intervention is under debate and should be evaluated individually. With severe hypercalcemia and symptomatic pHPT the indication for surgery is obvious. However, when hypercalcemia is mild and the diagnosis is made by presymptomatic screening in young patients the optimal timing is less clear. Early surgery can be more difficult because glands are only minimally enlarged, which might predispose the patient to recurrence and reoperation at an early age. On the other hand, longstanding elevated PTH might predispose the patient to more severe bone disease[49].

The cornerstone in treatment of pHPT is surgery. There is still a debate on the most effective type of surgery depending on the number of parathyroids which are surgically removed[50–52]. In this time of shared decision making, patient may weight risks and benefits of the

extensiveness of surgery differently than doctors do and might opt for more conservative approaches with clear understanding of failure risks.

One of the most important challenges after initial parathyroidectomy is managing the frequently occurring postoperative hypocalcaemia. This might be severe and symptomatic requiring extended hospital stay for i.v. calcium, but also the milder cases which can be managed by administering oral active vitamin D (alfacalcidol or calcitriol) and calcium require careful and frequent monitoring. Often medication can be tapered and stopped but this may take over one year[50].

If surgery is not feasible because of inoperability of the patient, patient refusal or the inability to demonstrate the source of persistent pHPT, the calcimimetic agent cinacalcet can be used. Cinacalcet is registered for the use in patients with pHPT who, though meeting the criteria for surgery, cannot be operated. Small series have shown that Cinacalcet is effective to achieve reductions in serum calcium in MEN1 patients with (recurrent) pHPT[53–56]. However, results of long-term use in MEN1 patients are still lacking.

DUODENOPANCREATIC NEUROENDOCRINE TUMORS

Neuroendocrine tumors of the pancreas (pNETs) are the second most frequent occurring tumors among patients with MEN1[19,57–60]. The median age of patients at pNET diagnosis is around the forth decade but tumors can already occur in childhood[38,57]. At the age of 80 years the penetrance of pNETs is over 80% and metastatic pNET is the most important cause of MEN1-related mortality[5,8,61,62]. Pancreatic NETs can either lead to a clinical syndrome because they are hormonally active (functional) or can be non-functional. The most frequent occurring functional tumors secrete gastrin or insulin, respectively leading to a high gastric acid secretion (Zollinger-Ellison syndrome) and hypoglycemia. Gastrin secreting NETs are mostly located submucosally in the duodenum and then often occur as multiple tumors[63–65]. Rates of pancreatic gastrinomas are only 0-18% in series that include immunohistochemistry in the classification of pNETs[66–69]. Insulinomas occur in about 2-24% of patients[6,59,70,71]. Glucagonomas, glucagon-secreting tumors, occur in less than 3% of patients with MEN1[72,73]. Vipomas, ViP-sectreting tumors have been reported in a few patients with MEN1[74].

In the clinical practice guidelines it is suggested to annually screen for pNETs using plasma hormonal measurements and by imaging to identify tumors for timely interventions with the aim to prevent morbidity and mortality because of metastasized disease[11,75]. For screening for gastrinomas fasting plasma gastrin appears to be useful to identify those patients who

have to undergo further imaging to confirm and localize the gastrinoma[11]. In patients with complaints of hypoglycemia, a 72 hour fast is the cornerstone of the diagnosis of an insulinoma[11]. The diagnostic accuracy of the tumor markers glucagon, pancreas polypeptide and chromogranin A which up until now are recommended in the clinical guidelines, even if measured in combination, turned out to be too low, making these measurements unsuitable for the annual screening for pNETs[76,77]. In addition to plasma hormonal assessments, yearly radiological imaging studies are advised[11]. However, one must keep in mind the life-time cumulative radiation exposure when CT scans are used especially for young patients[78]. Therefore, expert centers preferably use MRI[11,79]. Endoscopic ultrasound is more sensitive for identifying small tumors compared with CT scan and MRI[80–82]. However, by using EUS mainly the smaller tumors are identified which are generally of no clinical consequences[83]. When taking the slow growth rate of MEN1-related non-functioning-pNETs less than two centimeters into account, one might consider a less frequent radiological surveillance schedule once tumors appear to be stable in size[84]. The recently introduced imaging using ^{68}Gallium-somatostatin receptor positron emission tomography (PET) seems to be suitable for identifying small tumors and metastasized disease. In addition 18F-FDG PET/CT appeared to identify tumors of increased malignant potential. However, the clinical utility of PET imaging is not yet clear and more studies are needed[85–88].

The optimal treatment for single non-metastasized functioning pNET is surgery since this offers the highest chance for definitive curative therapy. However, non-functioning pNETs are the most frequent type and the pancreas usually harbors multiple tumors[61,89]. Functioning pNETs occur mostly in combination with other pNETs making the decision which tumor should be removed difficult. In addition, especially small (<2 cm) non-functioning-pNETs detected through periodical screening pose a challenge for the physician. Although pNETs have an indolent course, these tumors can metastasize[61]. To prevent metastasized disease, the current clinical practice guideline suggests follow-up for NF-pNETs smaller than one centimeter unless tumors exhibit significant growth and to consider surgery for larger tumours[11]. However, since MEN1 related pNETs are often multiple and occur throughout the lives of patients this strategy leads to multiple operations. In addition, pancreatic surgery in patients with MEN1 is associated with a high rate of short- and long-term complications[90]. Recent evidence from the Dutch and French cohorts pointed out that a conservative approach for tumors up to 2 cm doesn't lead to a higher chance of metastasis for patients[91,92]. Therefore, a watchful waiting strategy for patients with pNETs smaller than 2 centimeter appears to avoid major surgery without losing oncological safety.

Reducing acid output in patients with gastrinomas can be achieved with proton pump inhibitors. Since the introduction of these agents, the Zollinger-Ellison syndrome is no

longer the main reason for premature MEN1 related death. At this time, evidence for medical therapy from RCT's or controlled studies of sufficient size and methodological quality for the effectiveness of agents to prevent growth or metastatic behavior of small pNETs in patients with MEN1, such as somatostatin analogues, is not available. There is also no scientific evidence available for treatment for advanced MEN1 related pNETs. Trials of systemic antitumor therapies and other treatment modalities such as Peptide Radionuclide Receptor Therapy (PRRT) and loco regional therapy of metastasis included mainly patients with sporadically occurring tumors.

At this moment, no known clinical characteristics can predict the growth of individual tumors, which hampers tailored patient care. Therefore, treatment decisions regarding pNETs in MEN1 should be discussed in multidisciplinary tumor boards with special MEN1 expertise, and are currently based on "simple" clinical characteristics such as tumor size and growth[11,93]. However, prediction of tumor behavior for individual patients is not possible. Since liver metastasis caused by pNETs are an important reason for premature death of MEN 1 patients, future research should focus on identifying driving factors for tumor behavior as well as the identification of those patients at risk for future liver metastases[62]. Future research should therefore be based on the earlier recognized need of circulating multianalyte biomarkers and the clinical use of miRNA and circulating tumors cells that would allow for accurate characterization of the evolution of these tumors[94].

PITUITARY TUMOURS

Pituitary tumours (PIT) are the third most common MEN1 manifestation with a reported prevalence of 20-65%[12,13,95–97]. The median age for development of PIT is around the fourth decade, although cases as young as five years of age have been described[27,57,97,98] In general, these tumors are mostly benign, but can cause significant morbidity. Clinical symptoms depend on the type and presence of hormonal hypersecretion, the presence of hypopituitarism, and the size of pituitary tumors. Pituitary macroadenoma can cause ophthalmologic symptoms, especially visual impairment because of compression of the optic chiasm[99,100]. Symptoms related to hormone secretion comprise reduced fertility, amenorrhea, galactorrhea in women with prolactinoma and reduced fertility and impotence in men with prolactinoma. Other hormone secretion related manifestations include Cushing's disease and acromegaly caused by corticotroph and somatotroph adenomas, respectively.

Pharmacological treatment of PIT depend on the type of hormone secretion. Treatment for prolactinomas is by dopamine agonists; while somatotroph adenomas can be medically

treated by somatostatin analogs and the newer growth hormone receptor antagonist Pegvisomant[10,11,97].

MEN1-associated PIT were considered more aggressive than sporadic PIT, and more often unresponsive to medical treatment (especially in prolactinomas) necessitating earlier surgery[11,95,96,102]. For this reason screening for PIT was introduced in the clinical practice guidelines for MEN1[11].

According to the current clinical practice guideline screening for PIT is performed by annual testing of prolactin and IGF-1, and Magnetic Resonance Imaging (MRI) every three years[11]. Intensive radiological screening appears to reveal pituitary incidentalomas, which significance is still largely unknown[97]. Incidental microadenomas are reported in up to 10% of normal population[16,17,103]. In a recent study, the impact of screening for PIT among MEN1 patients was evaluated. PIT was diagnosed in approximately 40% of the MEN1 patients, of whom 50% were diagnosed by MEN1-related screening. The incidence of PIT in the screening program was 34 per 1,000 patient years[97]. Almost 50% of pituitary tumors diagnosed during screening, were non- functioning microadenomas. Only very few microadenomas showed minimal growth and the prolactinomas responded very well to medical treatment[97].

These findings were confirmed in a recent Italian cohort in which 178 (44.0%) patients developed PIT. In 56 patients PIT was the first MEN1 manifestation. In patients in whom a PIT was diagnosed in the course of follow up, both small microadenomas (63%) and non-functioning tumors (20.2%) were found. Most patients were successfully managed by pharmacological treatment (57.3%) or a watchful waiting strategy (25.3%)[27].

In conclusion, in contrast to earlier studies, more recent studies on PIT in MEN1 patients show that these tumors usually respond well to medical treatment regimes, in line with PIT occurring in the general population. The benefits of frequent screening for PIT by imaging seems questionable since this mainly results in the detection of incidentalomas that do not require treatment. Non-functioning microadenoma in patients with MEN1 can be treated according to the same guidelines as sporadic incidentalomas of the pituitary gland[103].

THYMIC NEUROENDOCRINE TUMOURS

Prevalence of thymic NET among MEN1 patients is relativaly low, and reported between 2.8-8.0%[19,104–107]. Most cohorts report that thymic NET occur predominantly in men with a mean age around the fifth decade[104,106–111] in contrast with previous studies, a Japanese

study reported a relatively high percentage (36%) of women in their cohort of MEN1 patients with a thymic NET[112].

Despite the low prevalence, thymic NET has become increasingly important in the epidemiology of MEN1. Thymic NETs are one of the most important causes of MEN1-related mortality, second to metastasized pancreatic NET[5,8,14]. In a study from the French Groupe d'etude des Tumeurs Endocrines (GTE), malignant thymic NET was the manifestation with the highest risk of mortality among MEN1 patients[14].

Thymic NET is usually asymptomatic until the late stage of the disease, and neuroendocrine tumor markers are generally not elevated[104,106,109]. Therefore radiological screening every one to two years by CT or MRI scan is currently adviced[11]. However, up to now, it is unclear if this intensive radiological screening is frequent enough to diagnose the often aggressively behaving thymic NET at an early stage to lead to a survival benefit. At the other hand, the total MEN1 population is exposed to intensive radiological screening for timely diagnosing a thymic NET in very few patients in every year of follow up[113]. Because of its aggressive behavior, prophylactic surgery of the thymus is recommended by several authors[11]. At present, prophylactic thymectomy is usually performed through a cervical incision at the time of parathyroid surgery. In the Dutch cohort none of the 97 patients who underwent prophylactic surgery of the thymus developed a thymic NET during a median follow-up of 8 years (range 0-40 years), and a median age of 47 years (range 20-78 years) at the end of follow-up[108]. However, a cervical thymectomy is often not complete and sporadic cases of thymic malignancies after a prophylactic cervical thymectomy have been reported[111,114,115]. Thymic NET are primarily treated by surgery. Evidence for both (neo) adjuvant and palliative chemotherapy in thymic NET are scarce and often not specific for MEN1 patients. Chemotherapeutic treatment that has been used for thymic NET include cisplatin, etoposide and 5 fluorouracil[116,117]. Somatostatin analogs might improve symptoms and are associated with tumor regression in some cases[118].

PULMONARY ENDOCRINE TUMOURS

Prevalence of pulmonary NET is reported between 1.4-13.3%, with a higher incidence of lung NET since the introduction of radiological screening for thymic and lung NET[14,19,108,119]. The prognosis of lung NET is generally favourable and mortality from lung NET is sporadic, in which some series report no mortality after more than 10 years of follow-up[14,107,108,119–121]. Pulmonary NETs are mainly stable tumors[108,121]. Tumor diameter of pulmonary NETs increased by only 17% per year (doubling time 4.5 years). Doubling time in male patients appeared to be higher than in female patients (2.5 vs 5.5 years)[108].

Up to now, the treatment of pulmonary NET has primarily been surgical. However, there is no evident survival benefit from surgery in these indolent tumors[108]. Recent findings might reveal potential new mechanism for pharmacological control for growth of pulmonary NETs. In a cohort of pulmonary NETs occurring in the general population with a somatic mutation of the *MEN1* gene in the tumour, a correlation was found between MEN1 mutations and the overexpression of human epidermal growth factors receptors (HERs)[122]. If expression of HERs are elevated in MEN1 patients, has not yet been confirmed, but this finding suggests that HER inhibitors might have a potential for clinical use in pulmonary NET. A new class of anti-cancer drugs that might be promising in treatment of pulmonary NETs are inhibitors of epigenetic pathways. In a recent in-vitro study such epigenetic pathway inhibitors demonstrated to be very promising in decreasing proliferation of NET[123].

ADRENAL TUMORS

Since early publications adrenal involvement has been described in. The incidence of adrenal involvement varies from 5% in early series to 73% in more recent studies[19,57–59,124–127]. The majority of adrenal lesions are non-functional and include cortical adenomas, hyperplasia, multiple adenomas, nodular hyperplasia or cysts. Bilateral hyperplasia is also commonly described[124,125].

ACTH-independent Cushing's syndrome and primary hyperaldosteronism are the most encountered clinical syndrome in the presence of an adrenal lesion and cortical hyperfunction[124–126]. Pheochromocytomas are reported in patients with MEN1, but remain rare[125,126]. Adrenocortical carcinomas (ACC) are described in several series and seem to occur more frequent in patients with MEN1 than in a group of sporadic adrenal tumors. In one study ACC was present in 13.8% of adrenal lesions[124], which is line with other studies[126,128]. Sporadically hyperandrogenemia occurs in association with ACC.

The age at diagnosis of adrenal tumors in most series is in the fifth decade[59,124,126,128], but adrenal involvement has also been described under the age of 20 years[38].

Adrenal lesions are usually identified through CT, MRI or endoscopy in the course of follow up of screening of MEN1-related manifestations. The clinical guideline suggests annual screening when adrenal lesions are present due to a prevalence of 13% of ACC in MEN1 patients with adrenal lesions reported in one study[11,124]. Biochemical testing should be undertaken when an adrenal lesion larger than 1 cm is present or in symptomatic patients with signs of hormonal overproduction. Biochemical investigation consists of a low-dose

dexamethasone suppression test, plasma renin and aldosterone concentrations, plasma or urinary catecholamines and/or metanephrines[11].

The majority of patients will only undergo regular follow-up with imaging studies. Since ACC was found to be more prevalent in patients with MEN1 and has a weak, but evident impact on mortality due to aggressive tumors[14,124,125,128] management of adrenal lesion should be in line with this finding. Indications for adrenal surgery are: adrenal lesions with a diameter greater than 4 cm; lesions with atypical or suspect radiological features; or a progressive lesion over a 6-month interval adrenal tumors[11,124,125].

BREAST CANCER

In 2014, breast cancer was identified as a MEN1 manifestation. In the Dutch MEN1 population, the relative risk for breast cancer was 2.83 in females with *MEN1*. In addition, breast tumors from MEN1 patients showed loss of heterozygosity (LOH) at the *MEN1* locus. This clinical observation was validated in three independent MEN1 cohorts from France, Australia and the United States[3]. Further research did not demonstrate that other endocrine risk factors or general risk factors were associated with the increased risk and confirmed that MEN1-related breast cancer is diagnosed at an average age of 48 years, which is significantly younger compared with the general population[4].

The *MEN1* gene product, menin, appears to have a dual role in breast tumorigenesis. In accordance with the observations in female MEN1 patients, genetic loss of function MEN1 mouse models show increased incidence of both *in situ* and invasive mammary cancer[129]. However, in sporadic breast cancer menin seems to have a proliferative function. In breast cancer cell lines, menin is a co-activator of the estrogen receptor alpha, a critical driver in approximately 70% of sporadic breast cancer cases. Menin has been reported to be involved in resistance to endocrine therapy[130,131].

In addition to the LOH in a subset of samples, expression of menin was reduced in 80% of MEN1-related breast cancer samples. In contrast, in only 5% of sporadic breast cancer samples no menin was found by immunostaining[3]. Silencing of the *MEN1* gene in primary human mammary luminal progenitor cells did reveal an anti-proliferative role for menin, further supporting distinct roles in sporadic versus MEN1-related breast cancer[132].

Currently, there is no guideline regarding breast cancer screening in MEN1 patients. A recent report addressing this issue formulated an advise based on the increased risk, the early age of breast cancer onset and the absence of other breast cancer risk factors or familial

risk in females with breast cancer and MEN1[4]. Since the majority of MEN1-related breast tumors were of the luminal type, which is prognostically favourable, screening biennially from the age of 40 is considered justifiable. This advice results from the mean age of breast cancer in the different MEN1 cohorts and is in concordance with a study assessing the harms and benefits of different screening strategies[4]. Annual screening from the age of 40 years in women with a twofold to fourfold increase in breast cancer risk was found to have similar or even more favorable harm/benefit ratios as biennial screening of women with average- risk from 50 to 74 years of age, which seems directly applicable for women with MEN1 with a relative risk of 2.83. International collaborations should be initiated now to assess the effect of breast cancer screening in females with MEN1 in which the prevention of advanced breast cancer by early diagnosis is weighed against the potential harms as a consequence of overdiagnosis and unnecessary invasive follow up[4].

FUTURE CHALLENGES AND CONSIDERATIONS

Considering the recent update of the clinical guidelines, ongoing MEN1 publications and the finding of breast cancer as a new MEN1-related manifestation, one can conclude that the MEN1 landscape is still evolving. However, there are some challenges in addressing underexposed topics, increasing population sizes by constructing national MEN1 registries, international collaborations and working towards individualized MEN1 care.

Quality of life / psychosocial aspects

A fundamental, but up to now, underexposed topic remains the quality of life and the psychosocial impact of MEN1. The often young age at diagnosis and subsequent life-long screening with inevitable treatments, might lead to psychological distress and perished quality of life. One study reported a mean number of 3.2 surgical treatments for a MEN1 patient and 61% of the patients had 3-7 surgeries[35]. Moreover, MEN1 is not solely a disease effecting one individual, but the autosomal dominant inheritance pattern gives rise to a theoretical carrier ship of 50% of family members. In other hereditary cancer syndromes with a similar inheritance patter, such as Li-Fraumeni, patients worried more about affected family members than about themselves. The degree of cancer worry was already considerable in those patients and warrants for more emphasis and care for patients with high levels of worry and psychological distress[133].

Up to now one single center study addressed the quality of life in patients with MEN1. In comparison with the general Swedish population, MEN1 patients reported significantly

lower levels of General Health and Social Functioning on the Health Related Quality of life Short Form 36[35]. Another study addressed the quality of Life in MEN1 patients after pancreatoduodenal surgery. Global quality of life scores showed no difference from the general population, but interestingly MEN1 patients had more financial difficulties caused by their physical condition and medical treatment[134]. These studies give more insight in the impact of MEN1 and stretch out the need for more studies focusing on this topic.

Personalized MEN1 care

Current guidelines for clinical care provide an excellent clinical guidance for diagnosis, screening and treatment of MEN1 related tumors[10,11]. However, guidelines are population based and only limitedly suitable for personalized care, which in general comes down to the physician and his team of experts. Future research should ideally focus on biomarkers for early diagnosis and importantly predictors of disease progression. In case of MEN1 these markers should differentiate between the various MEN1 related manifestations, which can be considered challenging. Circulating multianalyte biomarkers, the clinical use of miRNA and circulating tumors cells are promising novel tools to accurately characterize the evolution of these tumors in the future[94]. Reducing the number of imaging studies, especially CT scans, would be a major improvement in the follow up.

National registries and international collaborations

Performing research of the highest level of scientific evidence in a rare disease such as MEN1 remains a challenge due to the low incidence and prevalence of the disease. The limited number patients and low occurrence disease specific events complicates performing randomized controlled trials. Therefore, cohort studies, as next best level of evidence are regularly performed to answer relevant MEN1 related research questions. To achieve the most optimal population size in order to gain more scientific power, nationwide cohort studies are indispensible. Recently, results from an Italian nationwide cohort study were published, which included data from 14 referral centers from 12 different Italian cities[27]. Other European countries with national MEN1 databases are the Group d'etude des Tumeurs Endocrine in France and the Dutch MEN1 Study Group (DMSG) in The Netherlands. These cohorts comprise respectively 924 and 393 MEN1 patients in their national registries[9,38].

To gain more insight in the natural course of the disease, improve management strategies and work towards more targeted treatment, efforts to build and maintain these national registries seem at hand. Ultimately, international collaboration based on these national

research groups can be formed which will lead to larger MEN1 populations and hereby improved scientific possibilities which will lead to better care for the individual patient. Patient advocacy groups should be part of the national study groups since these parties represent the MEN1 patients and are closely involved in MEN1 patient care. In conclusion, collaborations on national and international levels will improve our knowledge and hereby the management for patients with MEN1.

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Chapter 8

General discussion and future directions

INTRODUCTION

The updated clinical practice guideline for MEN1 was much welcomed by physicians treating patients with MEN1 in 2012[1]. Clinicians confronted with diagnostic and treatment dilemmas were offered guidance in their daily practice in dealing with this particular group of patients. The guidelines were developed by expert leaders in the field who so-called 'self-assembled' themselves and made a tremendous effort to provide their colleagues worldwide with tools for the management of patients with MEN1.

To provide insight in the quality of the guideline, recommendations and assessment of quality of evidence were graded according to the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) system[2]. However, still some crucial recommendations were formed based on single case reports; such as the age to start periodical screening from the age of five years. This recommendation is based on a case of a five-year-old boy with a pituitary macroadenoma from a family with MEN1[3]. The age at which screening is considered no longer beneficial has not been formulated. This underscores the rare occurrence of the disease and the paucity of data that is relied on.

In line with the rarity of the disease, large randomized trials are lacking. Ideally risk-benefit analyses combined with financial aspects should be assessed in order to provide the most beneficial screening strategy. National surveillance programs for different cancer types such as breast cancer and cervical cancer are subject to national laws and regulations that arise from governmental authorities. Up to now, MEN1, with a prevalence of 2-3 per 100,000[4], has not been of high priority for policymakers. The high quality of national screening programs accompanied by review by independent experts is therefore a less feasible prospect in the field of MEN1. However, lifelong screening for MEN1 related tumors is nowadays the cornerstone in the management of patients with MEN1. For a significant number of patients this is in a presymptomatic phase, without patients having a detectable manifestation.

Despite the rarity of the disease, several established MEN1 research groups frequently publish data on interesting findings. Some findings have direct impact on the screening of individual manifestations and subsequently impact the patients. Awareness regarding the validity of these findings is therefore justified.

Screening is pivotal in managing MEN1; this chapter will discuss the merits and pitfalls of screening in MEN1 in accordance with the results of this thesis.

CONSIDERATIONS AND PERSPECTIVES IN SCREENING IN MEN1

The clinical guideline provides an extensive screening program. However, the authors state that the adherence to the guideline is subject to the clinical judgement and local resources. It is suggested to start screening from the age of five years with biochemical testing and a MRI of the pituitary gland. The first should be performed annually and the latter once every three years. Annual abdominal magnetic resonance imaging (MRI), computer-assisted tomography (CT) or endoscopic ultrasound (EUS) are suggested before the age of ten years. In addition a CT or MRI of the thorax should be performed every 1 or 2 years. Naturally, this is a framework on which care for MEN1 patients and their families can be build upon, but should also be considered a starting point for further research[1].

Table 8.1. Suggested biochemical and radiological screening in individuals at high risk of developing MEN1[1]

Tumor	Age to begin (yr)	Biochemical test (plasma or serum) annually	Imaging test (time interval)
Parathyroid	8	Calcium, PTH	None
Pancreatic NET			
Gastrinoma	20	Gastrin (± gastric pH)	None
Insulinoma	5	Fasting glucose, insulin	None
Other pancreatic NET	<10	Chromogranin-A; pancreatic polypeptide, glucagon, VIP	MRI, CT, or EUS (annually)
Anterior pituitary	5	Prolactin, IGF-I	MRI (every 3 yr)
Adrenal	<10	None unless symptoms or signs of functioning tumor and/or tumor >1 cm are identified on imaging	MRI or CT (annually with pancreatic imaging)
Thymic and bronchial carcinoid	15	None	CT or MRI (every 1-2 yr)

For example, according to the guidelines, mutation positive and mutation negative patients should undergo a uniform screening programme based on the assumption that these groups of patients are comparable. A genetic diagnosis is based on a confirmation of a *MEN1* germline mutation. A clinical diagnosis is made by the occurrence of two or more MEN1 associated tumors irrespective the outcome of mutation testing. In an earlier comparative analysis of these two groups, a completely different phenotype and clinical course was found and the median survival was better in mutation-negative patients compared to mutation-positive patients, with a median survival of 73 years in comparison with 87

years (P=0.001)[5]. These results confirm a different disease entity and highlight the need for further epidemiological research in population-based patient cohorts.

The current guideline provides a clear recommendation to offer genetic counselling in all first-degree relatives when MEN1 is diagnosed in a family based on the assumption that earlier identification and subsequent screening for manifestations lead to a better prognosis. This is irrespective of the psychological burden of the knowledge of having the disease and the intensity of the screening program. **Chapter 2** therefore provides more insight in the morbidity and mortality in association with the lag times from diagnosis of the index case and subsequent family members. A significant shortening of lag time is observed after introduction of genetic testing for *MEN1* in the Netherlands in 1998. Before and after 1998 lag times were 8 years and 2.6 years, respectively (P<0.002). A further reduction to 1.4 years was seen after the publication of the first international guideline of MEN1. After 2007 the time to diagnosis in family members is less than one year. It could be concluded that the introduction of mutation screening and subsequent implementation of the guidelines was successful in shortening the lag time. With respect to the effectiveness of the early screening, in general, in individuals with longer lag times more morbidity was seen. The mean lag time between the identification of the mutation in an index case and the subsequent genetic diagnosis in family members for patients with microadenomas compared with macroadenomas was 7.2 and 10.6 years respectively. The lag time difference between patients with metastasized and non-metastasized pancreatic neuroendocrine tumors (pNETs) was 8.2 versus 10.9 years. However, the standard deviations were quite large indicating a considerable spread around the means, indicating that there were individuals with relatively short lag times who already had metastasized disease at diagnosis. This can also be seen in the presented mortality data. Two patients around the age of 40 had lag times of approximately two years and died because of metastasized thymus neuroendocrine tumor. Thymus neuroendocrine tumors in men with MEN1 have an unfavourable course[6]; an earlier diagnosis in these cases could have changed the course and outcome of the disease. Two patients with a lag time of two years died due to a metastasized pNET. In cases with a metastasized pNET it is questionable whether a two-year earlier diagnosis could have prevented death, because the natural course of pNETs in MEN1 is characterized by a low growth tendency[7]. It is plausible that the pNETs were present for many years, nonetheless, this remains speculative. Moreover, in the period of their death, treatment options such as Peptide Receptor Radionuclide Therapy were not available, which could also have contributed to an earlier death.

From **chapter 2** we can conclude that an early diagnosis in relatives is imperative and that the guidelines can be compulsory in their recommendation by adding a time period in which

familial screening should be undertaken. A feasible time in which a whole family should be screened for this germline mutation could be one year. Obviously it should be taken into account that physicians are not allowed to approach family members themselves, and therefore an important task for the clinical genetics and treating physician is to provide adequate information.

NOVEL FINDINGS AND ITS EFFECT ON SCREENING

Worldwide there are several established MEN1 research groups such as the Group d'étude des Tumeurs Endocrines from France, the MEN1 cohort from Sweden, Tasmania, Firenze and the National Institute of Health (NIH) in the United States. Nowadays, the Dutch MEN1 Study Group (DMSG) has manifested itself as one of the leading MEN1 groups. Novel data from all the mentioned groups are considered of sufficient quality and considerable importance. Reasonably, data published from these cohorts has a significant chance of being implemented in the guidelines. The results from **chapter 3** show that an assumed association between blood type and the occurrence of neuroendocrine tumors in MEN1 was not correct. The NIH initially proposed that data as blood type could be a useful addition to the current screening and surveillance program[8]. The authors acknowledged that validation studies were warranted before implementation in the guideline. Results from our larger and more representative Dutch MEN1 cohort, contradicted the previous assumptions by the NIH. There was no association between blood type O and neuroendocrine tumors in the Dutch MEN1 cohort. An explanation for this difference could be a selected MEN1 population in the NIH with a high occurrence of blood type O. The NIH is a tertiary referral center to which patients generally are referred with an already diagnosed tumor. In comparison, the Dutch MEN1 population is based on a population based cohort with >90% of the Dutch MEN1 population. The NIH cohort presented regular survival plots without adjusting for death as a competing risk. This difference in conclusion underscores the importance of validation studies before drawing conclusions that result in adjusted screening strategies with the risk of an increased burden for patients.

In this respect, the guideline proposes to assess chromogranin A (CgA), pancreatic polypeptide (PP) and glucagon in screening for pNETs. These tumor markers had not been validated for MEN1, but were incorporated in the guideline. The diagnostic accuracy of the proposed tumor markers was therefore assessed by the DMSG. The areas under the curve (AUC) were 0.48 [95% CI 0.35-0.61], 0.58 [95% CI 0.46-0.70] and 0.64 [95% CI 0.50-0.77], for CgA, PP and glucagon respectively[9]. Considering the very low diagnostic value of these markers, it is questionable whether these markers should be assessed during screening.

An AUC around 0.5 is considered as a low diagnostic accuracy and therefore not useful. These findings were replicated in another cohort and it is expected that the assessment of tumor markers for screening for pNETs will be removed from the guidelines.

The finding of the DMSG in 2014 of the association between breast cancer and MEN1[10] was received with a considerable amount of scepticism[11]. In line with the previous findings this scepticism was appropriate, because the consequence of such a conclusion would have a major impact on women with *MEN1*. Therefore, to further examine the association between breast cancer and *MEN1*, a more in depth analysis in a larger cohort was undertaken. The first step was to assess whether other familial factors could have led to the higher breast cancer occurrence in these women. All women with *MEN1* from the Dutch cohort were approached to report the occurrence of breast cancer in their families. The high response rate of 84% contributed to the validity of this study. Because more women from a family filled out the questionnaire, the data could be crosschecked. **Chapter 4** presents the results of this conduct, which points out that breast cancer occurred at the age of 57.5 years in women without MEN1 and 45 years in women with MEN1 (P=0.03). Another eleven obligate MEN1 carriers with breast cancer were identified by this assessment. The incidence of breast cancer is increasing over the last decades with a current incidence of 14,864/100, 000 of invasive breast cancer yearly in the Netherlands (IKNL). Evidently, women with MEN1 would also have this relatively high risk, however, the age difference between the women with and without MEN1 is striking. The age of breast cancer in women without MEN1 is in line with the general population. This assumes that other factors are at play.

Other predisposing factors could be genetic breast cancer mutations or unfavourable reproductive factors. *BRCA* and *CHEK2* mutations were checked in one family with two first-degree relatives, and found negative. According to the Dutch guidelines the other women were not eligible for additional gene testing. Women who had breast cancer had no more hormonal predisposing factors than women without breast cancer. All these factors point out to a *MEN1* gene dependent tumorigenic mechanism leading to breast cancer. Menin, the protein product of the *MEN1* gene, has a role in regulating the estrogen receptor and subsequently promotes the proliferation of breast cancer in sporadic breast cancer[12]. Breast cancer tissue of MEN1 patients showed a loss of heterozygosity and a reduced menin expression which indicated that *MEN1* mutations are involved in breast cancer development[10].

This thorough assessment and assumption that breast cancer is associated with the MEN1 gene demanded an advise on a personalized breast cancer surveillance in these women. The Dutch breast cancer surveillance guideline suggests annual surveillance in women with a moderate breast cancer risk from the age of 40 years (IKNL). This is line with a harms

and benefits analysis of eight different screenings strategies by using different simulation models. Surveillance from the age of 40 has similar of more favourable harm/benefit ratio as biennial screening of women with an average-risk from 50 to 74 years of age[13].

Considering the luminal type of breast cancer with a prognostically favourable disease course, which holds for the majority of breast cancers cases in MEN1, a biennial surveillance program seems justifiable. Evidence is lacking on the preferred imaging study. The Dutch guideline prescribes mammographic imaging for this moderate risk group. MRI might be another option because breast tissue of younger women is generally more dense. Therefore, mammography might be less well interpreted. Mammographic imaging is considered as painful and will lead to more radiation in a population that is already screened by imaging from a young age. In this respect, MRI might be the preferred imaging study.

THE PSYCHOSOCIAL IMPACT OF SCREENING IN MEN1

Harbouring a *MEN1* germline mutation has lifelong implications. Screening for a MEN1 related manifestation is a major part of the disease. Naturally, numerous factors in a chronic disease can contribute to a decreased quality of life.

Quality of life remains a subjective phenomenon. In general, it is characterized by the degree an individual is healthy, comfortable, able to participate in the society and enjoy life events. In MEN1, one can expect that one's health is impaired by the disease itself, supported by the high penetrance of the disease[5]. Moreover, patients might be less comfortable because there is a constant threat for themselves and *MEN1* carriers in their family. Additionally, due to the morbidity caused by the disease, patients might not able to participate in important activities.

Chapter 5 reveals that the QOL in patients with MEN1 is indeed impaired in comparison with the general population. This was expected since the morbidity is significant[14,15]. Factors leading to a decreased QOL are being an index case, the presence of a pituitary tumor and being unemployed. An index case is the first in a family with a MEN1 diagnosis and most likely to have more MEN1 related manifestations. The index case could also feel responsible for the presence of the disease in the whole family. **Chapter 6** shows that patients have more fear about disease occurrence in their family members than in themselves. Fear of disease occurrence is significantly related to QOL. Therefore, fear seems a crucial factor in patients with MEN1. **Chapter 5** underlines this by revealing that MEN1 patients have a relatively high fear in comparison with other tumor types, such as breast cancer, Li-Fraumeni syndrome, Familial Adenomatosis polyposis and Von Hippel Lindau disease. Only patients

with Von Hippel Lindau disease had more fear of disease occurrence than patients with MEN1. A priori, one would not expect that this fear would be that significant. **Chapter 5** also reveals that the more aware patients are of the disease, the more fear they encounter. More fear leads to a decreased QOL warranting that physicians should be alert about the possible presence of this fear. The presence of a pituitary tumor and the association with QOL is an interesting finding. Patients who had a pituitary tumor according to their medical record but reported **not** to have a pituitary tumor had a better QOL in comparison with patients who reported to have a pituitary tumor. The knowledge of having a manifestation seems an important factor here and therefore ignorance in this respect can truly be bliss.

Unemployment was an independent factor leading to less QOL. It was not clear whether the disease itself led to unemployment in this group. Employment is considered a virtue and not being able to work is generally associated with less QOL. This indicates that universal aspects apply for the MEN1 population. By screening, patients are constantly confronted with the disease and its consequences, which may lead to fear and diminished QOL. Unfortunately, not screening MEN1 patients may lead to significant morbidity and mortality. This can be considered a challenge for physicians and researchers in the field of MEN1, but more so, serve as a starting point for future studies and perspectives we can build on.

CONCLUSION AND FUTURE DIRECTIONS

This thesis underlines the importance of screening, but also acknowledges the considerable impact it has on patients. Major novel findings should be addressed with an open, but critical attitude and should preferably be validated in other cohorts. This stretches out the need for collaboration between the different international cohorts.

In current practice, personalized medicine is widely propagated. Apparently, this seems preeminent in patients with MEN1. Particularly this group of patients is in need of a personalized screening and management. Unfortunately, no genotype-phenotype relation has been described[16,17]. Therefore, at genetic diagnosis, no classification or screening program according to genotype can be made. However, an exception should be made for mutation-negative patients who should have a less strict surveillance programme[5].

In this line, a recent study of a subgroup of patients with a missense mutation showed faster growth rates for small pancreatic neuroendocrine tumors[7]. In the contrary, another study showed a more aggressive pNET phenotype in nonsense/frameshift mutation[18]. This seems conflicting, but also hopeful, trends are observed which might be validated in larger cohorts. Future studies should focus on assessing these trends and possible associations.

The need for biomarkers of proper diagnostic accuracy is urgent. Most biomarkers are not useful in screening for pNETs in patients with MEN1[9]. Recently, there is substantial attention for a new biomarker panel for NETs based on circulating transcripts analyses, the so-called NETest. This test is a blood-derived multianalyte assay which measures the gene expression of 51 circulating NET marker genes simultaneously by q-PCR[19,20]. At this time, the NETest does not seem suitable as a screening test because it has low specificity. However, it does have a superior sensitivity over chromogranin A[21]. A blood based test that predicts the presence of an aggressive NET in an early stage or which predicts the natural course of NETs in MEN1 would be a future merit. Insecurity about the future might lead to more fear and reduced QOL. Developing novel markers and tests that lead to personalized screenings strategies could potentially have beneficial effects on patients' comfort and wellbeing.

First and foremost, patient should be involved in future study needs and aspects. Clinical research should primarily be patient driven. For this thesis, patients were involved from the early beginnings.

The high response rate of the studies in this thesis is a reflection of this close involvement. All patients should be informed about research from an early stage. Ideally all patients should grant permission to easily inform and include them for future studies. Reporting the results to the patients should be done on a regular basis. Meetings should be organized with focus on the patients and with high gratitude towards them. After all, without their efforts many studies would not be possible.

A future aim is to study the QOL in a longitudinal manner in which patients fill out the questionnaires in a structured fashion as part of their care visits to provide the physicians and care givers more insight in their well being. The first aim should be to gain more insight in patients' QOL and the second aim is to gather data in longitudinal manner to observe changes in QOL.

In conclusion, rare tumor syndromes are of little priority on the agendas of policymakers. However, over the last years, MEN1 has shown to be a model for sporadic diseases and therefore as a valuable asset in negotiations. Alongside the patient advocacy groups, physicians should place more effort on convincing the government on national and European level that care for rare diseases are as important as more common diseases. As caretakers for this vulnerable group of patients, a responsibility lies in putting this on the appropriate agendas. Efforts should be made to establish international collaborations for validation cohorts, but also to gain more power in research for rare diseases. Physicians should learn more from non-medical fields. As doctors we are used to our established

structures, but a versatile attitude and willingness to continuously grow and learn from our patients and beyond, will benefit our medical work and eventually our patients.

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Chapter 9

Summary in English
Nederlandse samenvatting

SUMMARY IN ENGLISH

Background

The MEN1 syndrome is a hereditary disease characterized by the simultaneous occurrence of parathyroid, pituitary and (duodeno)pancreatic neuroendocrine tumors (dpNETs). MEN1 also predisposes for adrenocortical tumors, neuroendocrine tumors of the thymus and lungs, angiofibromas, collagenomas and lipomas[1]. Additionally, breast cancer has recently been identified as a MEN1 related manifestation[2].

A MEN1 diagnosis is established if either a *MEN1* germline mutation is identified, two or more of the three main manifestations are present (parathyroid tumors, pituitary tumors and dpNETs) or in the presence of a MEN1 related tumor and first-degree relative with MEN1[3].

The prevalence of MEN1 is estimated at 3-4/100,000 with a high age-related penetrance of the three main manifestations[4].

To provide clinical guidance for physicians treating patients with MEN1, the clinical guidelines were developed. The clinical practice guideline for MEN1 recommends a strict screening protocol from an early age[3]. The first part of this thesis elaborates on familial screening and the consequence of novel findings with regards to screening. The second part describes the impact of having the MEN1 syndrome and subsequent screening of the disease and its manifestations.

Chapter 2 reveals the morbidity and mortality arising from lag times from diagnosis of the index case and subsequent family members. A trend was observed in which individuals with longer lag times had more morbidity. The mean lag time between microadenomas and macroadenomas was 7.2 and 10.6 years, respectively. The difference between non-metastasized tumors smaller than 20 mm and metastasized dpNETs was 8.2 versus 10.9 years. Mortality occurred in primarily metastasized disease with considerable lag times. The mean lag time in MEN1 diagnosis of family members was 3.5 years (range, 0-30 years). In recent years this lag time has been reduced to less than one year.

From **chapter 2** we can conclude that an early diagnosis in relatives is imperative and will lead to less morbidity. In this chapter it is suggested to screen the whole family within one year after a germline mutation is identified in the index case.

The National Institute of Health previously proposed that blood type O was associated with a higher occurrence of neuroendocrine tumors in MEN1 and that blood type could be a useful addition to the current screening and surveillance program[5].

Chapter 3 shows that there was no association between blood type O and neuroendocrine tumors in the nationwide Dutch MEN1 cohort. After an assessment in our larger population based MEN1 cohort and adjusting for death as a competing risk factor, the previous assumptions by the NIH were contradicted. Addition of the blood type to the screening program therefore seemed not of additional value for identifying MEN1 patients at risk for the development of neuroendocrine tumors, metastatic disease or a shortened survival.

A recent study revealed an association between *MEN1* and an early-onset elevated relative breast cancer risk of 2.83. **Chapter 4** presents the results of a study assessing whether other risk factors were associated with this higher risk. The analysis in a larger MEN1 cohort revealed that breast cancer occurred at the age of 57.5 years in women without MEN1 and 45 years in women with MEN1 (P=0.03). In the previous study, ten women with MEN1 and breast cancer were presented. The current analysis revealed another eleven obligate MEN1 carriers with breast cancer.

BRCA and *CHEK2* mutations were checked in women who were eligible for additional gene testing. These mutations were not found. Women who had breast cancer had no more hormonal predisposing factors than women without breast cancer. Based on these results a suggestion for breast cancer surveillance in these women was made. Surveillance from the age of 40 seemed most appropriate. This is in line with the finding that women with a moderate relative risk for breast cancer had similar or more favourable harm/benefit ratio than women with an average-risk who had biennial screening from 50 to 74 years of age[6].

A biennial surveillance program was deemed justifiable considering the luminal type of breast cancer with a prognostically favourable disease course, which holds for the majority of breast cancers cases in MEN1.

A MEN1 diagnosis holds lifelong implications. Screening for a MEN1 related manifestation is a major part of the disease. Evidently, numerous factors in a chronic disease can contribute to a decreased quality of life (QOL).

Chapter 5 reveals that the QOL in patients with MEN1 is impaired in comparison with the general population. Factors leading to a decreased QOL are being an index case, the presence of a pituitary tumor and being unemployed. Unemployment was an independent factor leading to a diminished QOL. An index case is the first case in a family with a MEN1 diagnosis and most likely to have more MEN1 related manifestations.

A striking feature was that patients who had a pituitary tumor but reported **not** to have a pituitary tumor had a better QOL in comparison with patients who reported to have a

pituitary tumor. The knowledge of having a manifestation seems an important factor and therefore ignorance in this respect can truly be bliss.

Chapter 6 shows that patients have more fear about disease occurrence in their family members than in themselves. Fear of disease occurrence is significantly related to QOL. The more aware patients are of the disease, the more fear they encounter. Since more fear leads to decreased QOL, physicians should be alert about the possible presence of this fear. MEN1 patients have a relatively high fear in comparison with other tumor types, such as breast cancer, Li-Fraumeni syndrome, Familial Adenomatosis polyposis and Von Hippel Lindau disease. Only patients with Von Hippel Lindau disease had more fear of disease occurrence than MEN1 patients.

Chapter 7 discusses the overall medical advances in therapeutic strategies and management approaches in MEN1.

In the general discussion, **chapter 8**, the main findings of this thesis are discussed and future directions are proposed.

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NEDERLANDSE SAMENVATTING (VOOR NIET INGEWIJDEN)

Het MEN1 syndroom – de inleiding

Multipele Endocriene Neoplasie type 1 ("MEN1") is een zeldzaam erfelijk tumorsyndroom. Het kenmerkt zich door het gelijktijdig voorkomen van verschillende endocriene (hormoonvormende) tumoren. De drie karakteristieke MEN1 tumoren zijn (i) adenomen (goedaardige tumoren) van de bijschildklieren, (ii) neuroendocriene tumoren van de twaalfvingerige darm en alvleesklier, en (iii) tumoren van de hypofyse. Deze worden ook wel de drie hoofdmanifestaties genoemd. Er zijn echter ook andere tumoren die regelmatig gezien worden binnen het MEN1 syndroom zoals: bijniertumoren, neuroendocriene tumoren van de longen en thymus en lipomen.

De zeldzaamheid van de ziekte uit zich in een lage prevalentie. Ongeveer 3-4 per 100.000 mensen heeft dit syndroom. De kans dat een van de tumoren zich in het leven openbaart bij mensen met MEN1 syndroom is hoog. Op 80-jarige leeftijd heeft bijna 100% een bijschildklieradenoom gehad, 90% een neuroendocriene tumor van de twaalfvingerige darm of pancreas en 80% heeft een hypofysetumor gehad.

Het MEN1 syndroom is een erfelijke ziekte. Dit houdt in dat een afwijking in het DNA deze ziekte veroorzaakt. Indien een persoon drager is van het MEN1 gen, dan is er sprake van het MEN1 syndroom. Dit kan worden vastgesteld door DNA-diagnostiek. Als een van de ouders het MEN1 gen draagt, dan is er bij ieder kind 50% kans dat deze ook drager is van dat gen en dus ook het MEN1 syndroom heeft.

Een MEN1 diagnose wordt gesteld op basis van de volgende criteria:

1. een aangetoonde DNA afwijking passend bij het MEN1 syndroom
2. twee van de drie hoofdmanifestaties van het MEN1 syndroom
3. een MEN1 gerelateerde tumor en een eerstegraads familielid met het MEN1 syndroom

De DutchMEN1 Study Group

De DutchMEN1 Study Group (DMSG) is opgericht in 2008 om meer inzicht te krijgen in het ziektebeloop van het MEN1 syndroom. Alle acht academische ziekenhuizen in Nederland zijn vertegenwoordigd in de DMSG. Meer dan 95% van de patiënten met het MEN1 syndroom in Nederland worden behandeld in een academisch ziekenhuis. Hierdoor bestaat deze studiepopulatie uit bijna alle MEN1 patiënten in Nederland. De data die verkregen wordt uit ons onderzoek is derhalve gebaseerd op de volledige populatie, hetgeen bias (vertekening) door selectie uitsluit.

De MEN1 richtlijn voor zorgverleners

Ter leidraad voor de screening en behandeling van patiënten met het MEN1 syndroom, is een internationale MEN1 richtlijn ontwikkeld voor artsen die MEN1 patiënten begeleiden. Deze richtlijn is grotendeels gebaseerd op zogenaamd 'expert opinion' en weinig op gedegen wetenschappelijk onderzoek. Er is derhalve een behoefte aan wetenschappelijke studies van goede kwaliteit.

Dit proefschrift

Dit proefschrift behandelt verschillende facetten van het screenen van patiënten met het MEN1 syndroom. Er wordt inzicht gegeven hoe in Nederland de familiescreening is verlopen en tot welke gevolgen een vertraging in deze screening heeft geleid. Vervolgens worden de consequenties van nieuwe bevindingen voor de screening binnen het MEN1 syndroom uitgelicht. Tenslotte wordt de impact van het hebben van het MEN1 syndroom op de kwaliteit van leven behandeld en de angst die hiermee gepaard gaat.

Hoofdstuk 2 geeft inzage in de familiescreening in Nederlandse families. De MEN1 richtlijn schrijft voor om eerstegraads familieleden zo spoedig mogelijk te testen op het MEN1 gen om de ziekte tijdig op te sporen en de ziektelast te beperken. De gemiddelde tijd vanaf de index patiënt (de eerste persoon in de familie waarbij MEN1 is gediagnostiseerd) tot een volgend familielid is 3.5 jaar met een spreiding van 0 tot 30 jaar. Hierbij moet de kanttekening worden geplaatst dat het pas in 1998 mogelijk werd om in Nederland DNA onderzoek te doen naar het MEN1 gen. Voor 1998 was de gemiddelde tijd tot screenen van een familielid 8 jaar. Na 1998 was de gemiddelde tijd 2.6 jaar. Na 2007 zien we dat het screenen van familieleden binnen 1 jaar plaatsvindt.

In dit hoofdstuk laten we zien dat een lange vertraging leidt tot meer ziektelast. Dit proefschrift laat zien dat voor tumoren van de hypofyse kleiner dan 1 cm, de gemiddelde vertragingstijd 7.2 jaar is. Echter, de gemiddelde vertragingstijd voor het ontwikkelen van hypofysetumoren groter dan 1 cm is 10.9 jaar. De gemiddelde vertragingstijd tot niet uitgezaaide neuroendocriene tumoren van het pancreas en duodenum was 7.1 jaar, in vergelijking met 10.9 jaar bij uitgezaaide ziekte.

Er zijn 10 patiënten overleden ten gevolge van een MEN1 gerelateerd tumor die ontstaan is in de periode van de vertraging. Deze cijfers tonen aan dat het belangrijk is om familieleden zo snel mogelijk te screenen op het MEN1 syndroom, als deze ziekte wordt gevonden in een familie. In dit proefschrift wordt voorgesteld om een hele familie binnen 1 jaar te screenen na diagnose van het eerste familielid. Een dilemma hierin is dat een behandelend

arts de familie zelf niet kan inlichten, vanwege de bestaande geheimhoudingsplicht van de arts. Deze verantwoordelijkheid ligt daarom bij de patiënt zelf. Goede voorlichting en begeleiding van de index patiënt hierin is een belangrijke taak voor de arts.

Nieuwe bevindingen kunnen leiden tot verandering in de MEN1 richtlijn. De National Institute of Health (NIH), een vooraanstaand instituut en ziekenhuis in de Verenigde Staten, publiceerde dat het hebben van bloedgroep O een risicofactor is voor het ontwikkelen van een neuroendocriene tumor bij patiënten met het MEN1 syndroom. Dit zou betekenen dat patiënten met bloedgroep O een grotere kans hebben op het krijgen van een neuroendocriene tumor. **Hoofdstuk 3** laat zien dat in de Nederlandse MEN1 patiëntengroep, bestaande uit 200 patiënten, neuroendocriene tumoren gelijkmatig verdeeld waren over patiënten met bloedgroep O en niet-bloedgroep O. Er was ook geen sprake van ernstigere, uitgezaaide ziekte bij patiënten met bloedgroep O. De bloedgroepverdeling van de NIH groep was afwijkend van de bloedgroepverdeling in de algemene bevolking in Verenigde Staten. Naar verhouding was de groep met bloedgroep A veel kleiner, hetgeen het verschil kan verklaren. De Nederlandse patiëntengroep (N=200) was tweemaal zo groot als de Amerikaanse groep (N=105). Op basis van onze studie ontkrachten we de resultaten van voorgaand onderzoek en kunnen we concluderen dat bloedgroep O niet leidt tot een hoger risico op neuroendocriene tumoren.

Een andere nieuwe bevinding is het vaker voorkomen van borstkanker bij vrouwen met het MEN1 syndroom. Recent onderzoek liet zien dat vrouwen met het MEN1 syndroom gemiddeld 15 jaar eerder en een 2.8 maal grotere kans hebben op het krijgen van borstkanker in vergelijking met de Nederlandse vrouwelijke bevolking. Deze bevinding stuitte op kritiek omdat borstkanker ook veroorzaakt kan worden door andere borstkankergenen. Daarnaast kunnen ook hormonale factoren een belangrijke rol spelen in de ontwikkeling van borstkanker.

In **hoofdstuk 4** presenteren we de resultaten van een onderzoek in een grotere groep patiënten met het MEN1 syndroom dan eerder gepubliceerd. Hieruit blijkt dat er geen andere borstkankergenen zorgden voor het verhoogde risico. Ook andere hormonale risicofactoren waren niet vaker aanwezig bij de vrouwen met borstkanker in vergelijking met de vrouwen zonder borstkanker. De leeftijd bleek in een groep van 22 vrouwen met borstkanker en MEN1 gemiddeld 45 jaar, met een spreiding van 30-80 jaar, in vergelijking met een gemiddelde leeftijd van 58 jaar in vrouwelijke familieleden met borstkanker zonder het MEN1 syndroom. Gezien de jonge leeftijd en het vaker voorkomen van borstkanker bij vrouwen met het MEN1 syndroom, is besloten om vanaf de leeftijd van 40 jaar tweejaarlijks te screenen op borstkanker. De tumoren waren van het hormoongevoelig type, hetgeen

gunstiger is dan hormoonongevoelige tumoren. Er is daarnaast geen enkele vrouw met het MEN1 syndroom overleden ten gevolge van borstkanker. Om deze redenen is besloten om vrouwen met MEN1 tweejaarlijks en niet jaarlijks te screenen op borstkanker tot de leeftijd van 50 jaar. Vanaf 50 jarige leeftijd participeren ze in het reguliere bevolkingsonderzoek.

Het volgende deel van dit proefschrift gaat over de impact van het MEN1 syndroom op de patiënten. Het MEN1 syndroom vereist levenslange screening op de verschillende MEN1 gerelateerde aandoeningen. Deze screening begint al vanaf 5 jarige leeftijd. Als er een MEN1 gerelateerde tumor wordt geconstateerd dan wordt de screening intensiever. De kans op het ontstaan van de hoofdmanifestaties is zeer groot, met een reële kans op een operatie. MEN1 patiënten zijn daarom vaak jaarlijks (of vaker) in het ziekenhuis of in contact met hun behandeld arts.

Daarnaast is het MEN1 een syndroom dat de hele familie raakt. Er zijn vaak familieleden die ook het MEN1 syndroom hebben en mogelijk zijn overleden ten gevolge van de ziekte.

Hoofdstuk 5 laat zien dat (in de groep van 227 patiënten) de kwaliteit van leven bij patiënten met het MEN1 syndroom slechter is dan de normale Nederlandse bevolking. Patiënten die werkloos zijn hebben consequent een slechtere kwaliteit van leven dan de patiënten die werken. Daarnaast blijkt dat patiënten met een hypofysetumor en de index patiënten een slechtere kwaliteit van leven hebben. Er bleek een verschil te zijn tussen mensen die aangaven geen tumor te hebben, maar volgens de medische gegevens deze wel hebben. 16% bleek een neuroendocriene tumor te hebben, maar was hiervan niet op de hoogte. Daarnaast bleek 29% van de patiënten een hypofysetumor te hebben zonder hiervan op de hoogte te zijn. Patiënten die niet op de hoogte waren van een aanwezige hypofysetumor bleken een betere kwaliteit van leven te hebben dan patiënten die het wel wisten.

Hoofdstuk 6 laat zien dat patiënten een aanzienlijke angst hebben die gerelateerd is aan het MEN1 syndroom. In vergelijking met andere tumorsyndromen blijkt dit in het geval van patiënten met het MEN1 syndroom bovengemiddeld hoog te zijn. De angst bleek significant gerelateerd te zijn aan de kwaliteit van leven. Hoe meer angst, des te slechter is de kwaliteit van leven. De aanwezigheid van een neuroendocriene tumor, een hypofysetumor en werkloosheid bleken allen gerelateerd aan meer angst omtrent de ziekte. Hoe meer MEN1 gerelateerde manifestaties de patiënten hadden, des te meer angst was aanwezig. De patiënten hadden daarnaast meer angst omtrent hun kinderen met het MEN1 syndroom dan voor zichzelf. Patiënten met een reëel ziekte-inzicht omtrent het MEN1 syndroom ervaren de meeste angst. Zoals ook uit **hoofdstuk 5** blijkt, een goed ziekte-inzicht met de bijhorende implicaties, leidt tot een slechter welbevinden en meer angst gerelateerd aan de ziekte. In deze zin is het geluk letterlijk met de onwetenden.

Chapter 10

List of publications
Curriculum Vitae

LIST OF PUBLICATIONS

- van Treijen MJC, Korse CM, **van Leeuwaarde RS**, Saveur LJ, Vriens MR, Verbeek WHM, Tesselaar MET, Valk GD. Blood Transcript Profiling for the Detection of Neuroendocrine Tumors: Results of a Large Independent Prospective Blinded Validation Study. *Front. Endocrinol.* 2018 Dec 4.
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CURRICULUM VITAE

Rachel Sara van Leeuwaarde was born on the 15th of April in 1982. Physically she was born in Amsterdam, but her heart also belonged to Surinam, which is where both her parents were born and a part of her family and friends live. With her Jewish and Surinamese background, she fitted right in at the Vossius Gymnasium in Amsterdam, where she went to high school from 1994 until 2000. During these years, she decided that she wanted to become a doctor. She was also interested in music, culture and reading. That combination made Rachel a broadly developed woman after finishing the Vossius Gymnasium.

In 2000, she applied for medical school. She was eliminated by lottery. However, that did not stop her from chasing her dream to become a doctor. From 2000 until 2001 she studied pharmacy in Utrecht and obtained her first-year diploma (*propedeuse*). In 2001, she was admitted by decentralized selection for medical school at the VU (Vrije Universiteit) medical center in Amsterdam.

During medical school, besides studying, she had a broad interest and was involved in other activities, such as chairing the interns (*co-assistenten*) at the VU medical center and working on scientific projects.

As she was interested and wanted to be involved in modelling the medical education of interns of the VU medical center, she became chair of the interns from 2006 until 2007, where she amongst others represented the interns in order to improve the programme interns.

For her scientific internship she combined the two great passions in her life, Surinam and medicine. She performed a nationwide analysis of the incidence and outcome of breast cancer in Surinam during 1994-2003 under supervision of dr. Anneke Westermann.

In 2007 she obtained her Master's degree Medicine (Cum Laude) at the VU medical center in Amsterdam.

Shortly after her graduation, Rachel was admitted to the internal medicine residency programme (*arts in opleiding tot specialist*) at the University Medical Center of Utrecht (UMCU), under supervision of prof. dr. Douwe Biesma. The first years of her education programme, Rachel worked in the Gelre Hospital in Apeldoorn where she worked under

supervision of dr. Cees Schaar. In this period she initially developed an interest in oncology. After returning to the University Medical she worked under supervision of prof. dr. Margriet Schneider.

Again, Rachel was very involved in the education of the residents and she became the chair of the internal residents in 2012.

During her outpatient clinic rotation, Rachel first came into contact with the specialism of endocrinology. She was very much attracted by the approach in endocrinology, whereby there is focus on understanding the physiological processes by way of logically analyzing such processes.

However, Rachel remained interested in both oncology and endocrinology. She decided to become a PhD candidate and started her PhD in the field of endocrine oncology, specifically focused on the MEN1 syndrome, under supervision of prof. dr. Gerlof Valk and prof. dr. Menno Vriens. She found this syndrome particularly interesting as this syndrome involves both oncology and endocrinology. In 2017 she finished her residency programme under supervision of prof. dr. Karin Kaasjager.

Currently, Rachel is working in the joint endocrine cancer center (*gezamenlijk neuro-endocriene tumoren (NET) centrum*) of the UMC Utrecht and the Antoni van Leeuwenhoek hospital, where she works as an endocrinologist as part of the multi-disciplinary team of specialists. In this team she focuses on both clinical and scientific work.

Rachel is a very loyal, genuine, caring, open, curious, positive spirit. She is genuinely interested in the feelings and interests of others. This is also reflected in the subject matter of her thesis, which is amongst others focused on the broader wellbeing of her patients.

Rachel is married to Niven Mehra and lives in Utrecht.

Sara van Mourik
Rachel Imambaks

Amsterdam, February 2019

Chapter 11

Postscriptum

POSTSCRIPTUM

Promoveren doe je niet alleen. Het was een voorrecht om dit promotietraject te mogen doorlopen en een groot geluk om deel te kunnen uitmaken van een inspirerende onderzoeksgroep.

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Mijn vrienden, *l'chaim.*

Lieve Niven, *at last.* ♪.

Papa, deze is voor jou