Hereditary colorectal cancer syndromes

Epidemiological studies on Peutz-Jeghers syndrome & Lynch syndrome

Margot van Lier

Colofon

ISBN 978-94-6169-031-9

Financial support for printing this thesis was kindly given by Zambon Nederland BV, ABBOTT Immunology, Tramedico, Dr. Falk Pharma Benelux BV, Bayer BV, Merck Sharp & Dohme BV, PENTAX Nederland BV, Gerrit Blaauw fonds, J.E. Jurriaanse Stichting, de Nederlandse Vereniging voor Gastroenterologie, the department of Gastroenterology and Hepatology, Erasmus University Medical Center, Rotterdam, and the Erasmus University, Rotterdam

Lay out: Optima Grafische Communicatie, Rotterdam, The Netherlands
Printed by: Optima Grafische Communicatie, Rotterdam, The Netherlands
Coverdesign, -concept and -illustration: Stan W.H. van Lier
http://www.stanvanlier.nl
info@stanvanlier.nl

Hereditary colorectal cancer syndromes: Epidemiological studies on Peutz-Jeghers syndrome & Lynch syndrome

Erfelijke colorectaal kanker syndromen: Epidemiologische studies naar het Peutz-Jeghers syndroom en het Lynch syndroom

Proefschrift

ter verkrijging van de graad van doctor
aan de Erasmus Universiteit Rotterdam
op gezag van de rector magnificus Prof.dr. H.G. Schmidt
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op 25 maart 2011 om 11.30 uur

door

Margot Gerarda Franka van Lier
geboren te Nijmegen

PROMOTIECOMMISSIE

Promotoren:	Prof.dr. E.J. Kuipers
	Prof.dr. E.W. Steyerberg
Overige leden:	Prof.dr. M.J. Bruno
	Prof.dr. J.F. Lange
	Prof.dr. E.M.H. Mathus-Vliegen
Copromotoren:	Dr. M.E. van Leerdam
	Dr. A. Wagner

CONTENTS

1 Introduction and outline of the thesis

INTRODUCTION

Colorectal cancer (CRC) is the second most common malignancy among women after breast cancer, and the third most common malignancy among men after lung and prostate cancer in the European Union.[1] In the Netherlands, approximately 10000 cases are diagnosed each year.[2] CRC is moreover associated with high mortality and ranks second to lung cancer as a cause of cancer-related mortality in Europe.[1] CRC results from both genetic and environmental factors. Genetic factors are a major cause of disease in approximately 20% of CRC cases, with a spectrum ranging from ill-defined familial aggregation without a detectable disease-causing mutation (classified as familial CRC), to well-defined autosomal dominant inherited syndromes.[3,4]

The well-defined inherited cancer susceptibility syndromes are responsible for approximately 5% of all CRC's.[5] These hereditary cancers represent a significant proportion of all CRC burden, especially since these cancers are often diagnosed at a young age. Hereditary CRC syndromes can be divided into syndromes with and without gastrointestinal polyposis (Table 1). The polyposis syndromes can be further subdivided according to the histology of the polyps; being either hamartomatous or adenomatous polyps. Lynch syndrome, responsible for approximately 3% of all CRCs, is the most common form of hereditary CRC, followed by familial adenomatous polyposis, accounting for nearly 1% of CRC cases. The hamartomatous polyposis syndromes including Peutz-Jeghers syndrome, are rare and together account for less than 1% of all CRCs.[6,7]

Recognition of hereditary CRC syndromes is of utmost importance in order to provide adequate counseling and surveillance to patients and family members at risk. New insights into clinical as well as molecular and genetic characteristics are important to decrease morbidity and mortality associated with these syndromes. Hereditary CRC syndromes that could earlier only be defined on the basis of clinical features and occurrence in pedigrees, can now be

Table 1. Hereditary colorectal cancer syndromes.[8,9]

Syndrome	Gene
Non-polyposis	
Lynch syndrome (formerly known as HNPCC)	MMR genes
Polyposis	
Adenomatous polyposis	
- Familial adenomatous polyposis (FAP) / Attenuated Familial adenomatous polyposis (aFAP)	*APC*
- MUTYH-associated polyposis (MAP)	*MYH* (biallelic)
Hamartomatous polyposis	
- Peutz-Jeghers syndrome	*STK11* (*LKB1*)
- Juvenile polyposis	*SMAD4, PTEN*
- Cowden syndrome	*PTEN*

MMR = Mismatch repair genes, including *MLH1*, *MSH2*, *MSH6* and *PMS2*.
HNPCC = Hereditary non-polyposis colorectal cancer.

defined on the basis of molecular and genetic characteristics.[10] Hereby, dedicated treatment and surveillance can be offered to patients and affected family members. On the other hand, non-carriers can be reassured and dismissed from burdensome surveillance programs.

This thesis will focus on two of the abovementioned syndromes: Peutz-Jeghers syndrome and Lynch syndrome. These two genetic cancer susceptibility syndromes are associated with an elevated gastrointestinal cancer risk concerning predominantly CRC, as well as an elevated risk for extra-gastrointestinal malignancies

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant inherited disorder characterized by gastrointestinal hamartomas and mucocutaneous pigmentations.[11,12] Jan Peutz, a Dutch physician, was the first to recognize the combination of intestinal polyposis, mucocutaneous pigmentation, and heredity in 1921.[11] The hamartomas may cause complications already early in life, including anaemia, bleeding, and intussusception. In 1998 it was discovered that germline mutations in the *STK11* gene (Serine Threonine Kinase 11) cause PJS.[13,14] The *STK11* gene, also known as *LKB1* gene, has been designated as a tumor suppressor gene.[15] Indeed, it has been recognized that PJS is associated with an increased risk for the development of gastrointestinal and extra-gastrointestinal malignancies. However, as PJS is a rare disorder, it has not been studied as extensively as other hereditary cancer syndromes. The clinical management of PJS patients is thus hampered by a lack of detailed epidemiological and clinical data. Accurate data on for example the intussusception and cancer risk are missing. In order to gain more insight into PJS and to improve counseling and surveillance of PJS patients, we evaluated a large and unique cohort of Dutch PJS patients. We determined cancer, mortality and intussusception risks, and we furthermore studied quality of life, genetic test uptake and decisions regarding family planning.

Lynch syndrome (LS) is another and more common autosomal dominant inherited disorder, not only responsible for 3% of all CRCs[6,16], but also for 2% of all endometrial cancers.[17] The cancers are generally diagnosed at a young age and multiple synchronous or metachronous malignancies occur in 30% of the patients.[5,18,19] In addition, LS is associated with an increased risk for the development of other malignancies including carcinomas of the small intestine, stomach, pancreas and biliary tract, ovaries, brain, upper urinary tract, and skin. LS is caused by germline mutations in one of the mismatch repair genes, *MLH1*, *MSH2*, *MSH6*, and *PMS2*. These mutations lead to microsatellite instability (MSI) in tumor DNA. MSI is the molecular hallmark of LS, and can be detected in more than 90% of all LS associated cancers.[20]

Previous studies have provided many data on LS, including data on LS-associated cancer risks, the value of colonoscopic surveillance as well as molecular and genetic characteristics. However, one of the most challenging problems regarding LS is its early detection. Early detection of LS is of great importance, particularly in pre-symptomatic mutation carriers but also for symptomatic mutation carriers (i.e. patients with CRC) considering the high risk of metachronous malignancies, since colonoscopic surveillance has proven to reduce CRC morbidity and mortality by 65-70%.[21-23] Furthermore, prophylactic surgery may prevent

endometrial and ovarian carcinoma effectively.[24] Yet, early detection of LS remains challenging as the syndrome lacks a pre-morbid phenotype, like the mucocutaneous pigmentations in PJS. The first manifestation in many previously healthy LS patients is the presence of an advanced cancer. Our studies on LS therefore predominantly focus on the detection of the syndrome in order to improve early detection and thereby the outcome of LS families.

OUTLINE OF THE THESIS

Part I: Peutz-Jeghers syndrome

The first part of this thesis focuses on Peutz-Jeghers Syndrome (PJS). As mentioned before, PJS is difficult to assess since it concerns a rare disorder and so far few detailed data about PJS have been available. However, detailed data on cancer and intussusception risk are relevant for the development of surveillance recommendations and adequate counseling of PJS patients.

Therefore we firstly conducted a review of the literature (**Chapter 2**) to evaluate reported cancer risks associated with PJS, and used these data to develop a surveillance recommendation. This literature review showed high cumulative and relative cancer risks, but there was a rather wide range in reported cancer risk estimates. Consequently, we performed a large cohort study to investigate cumulative and relative cancer risks as well as the mortality risk in a Dutch PJS population (**Chapter 3**).

We also assessed the intussusception risk among Dutch PJS patients (**Chapter 4**). The intussusceptions are caused by the gastrointestinal polyps and lead to considerable morbidity. Balloon-assisted enteroscopy nowadays allows enteroscopic removal of small-bowel polyps and can theoretically prevent intussusceptions. As balloon-assisted enteroscopy may therefore play a role in the surveillance of PJS patients, we evaluated its therapeutic efficacy and safety for detection and treatment of small-bowel polyps in PJS patients (**Chapter 5**).

Because PJS is a burdensome disorder, we assessed the quality of life and psychological distress in PJS patients compared to the general population (**Chapter 6**). Simultaneously, we assessed predictors for genetic testing in PJS patients and the influence of PJS on their desire to have children, as well as their attitudes towards prenatal diagnosis and preimplantation genetic diagnosis (**Chapter 7**).

Part II: Lynch syndrome

The second part of this thesis concerns Lynch syndrome (LS). LS has been studied more extensively than PJS, and detailed epidemiological data as well as data regarding molecular and genetic characteristics are available. However, nowadays one of the most important

challenges is early detection of the syndrome. Many attempts have been made to improve the detection of LS. These attempts as well as the current approach to diagnose LS, including molecular analyses on tumor DNA of patients fulfilling certain clinical criteria, are described in detail in this thesis (**Chapter 8**).

The most widely accepted approach for the identification of LS patients occurs nowadays on the basis of the revised Bethesda Guidelines. These guidelines have been developed to select patients whose tumors should be analyzed for MSI to make underlying LS more or less likely. The revised Bethesda Guidelines are based on family history, age at cancer diagnosis, number of LS-associated carcinomas and certain histological tumor features. We evaluated the implementation of the revised Bethesda Guidelines into clinical practice (**Chapter 9**).

As the implementation of the revised Bethesda Guidelines into clinical practice was very poor, we concluded that the detection of LS is suboptimal. Therefore we performed a large population-based prospective study to determine whether further improvement of LS detection can be obtained by routine performance of molecular analyses in CRCs and endometrial cancers of patients up to the age of 70 years (**Chapters 10 and 11**).

Finally, **Chapter 12** presents an overview and general discussion of this thesis.

REFERENCES

1. Ferlay J, Autier P, Boniol M, Heanue M, Colombet M, Boyle P. Estimates of the cancer incidence and mortality in Europe in 2006. Ann Oncol 2007;18(3):581-592.
2. www.ikcnet.nl. 2010.
3. Ramsoekh D, van Leerdam ME, Wagner A, Kuipers EJ. Review article: Detection and management of hereditary non-polyposis colorectal cancer (Lynch syndrome). Aliment Pharmacol Ther 2007; 26 Suppl 2:101-111.
4. Rustgi AK. The genetics of hereditary colon cancer. Genes Dev 2007;21(20):2525-2538.
5. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003;348(10):919-932.
6. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338(21):1481-1487.
7. Burt R, Neklason DW. Genetic testing for inherited colon cancer. Gastroenterology 2005;128(6): 1696-1716.
8. Vasen HF. Clinical diagnosis and management of hereditary colorectal cancer syndromes. J Clin Oncol 2000;18(21 Suppl):81S-92S.
9. Allen BA, Terdiman JP. Hereditary polyposis syndromes and hereditary non-polyposis colorectal cancer. Best Pract Res Clin Gastroenterol 2003;17(2):237-258.
10. Lynch HT, Lynch JF, Lynch PM, Attard T. Hereditary colorectal cancer syndromes: molecular genetics, genetic counseling, diagnosis and management. Fam Cancer 2008;7(1):27-39.
11. Peutz JLA. Over een zeer merkwaardige, gecombineerde familiaire polyposis van de slijmliezen van den tractus intestinalis met die van de neuskeelholte en gepaard met eigenaardige pigmentaties van huid en slijmvliezen. Ned Maandschr v Geneesk 1921;10:134-146.
12. McGarrity TJ, Kulin HE, Zaino RJ. Peutz-Jeghers syndrome. Am J Gastroenterol 2000;95(3):596-604.
13. Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 1998;391(6663):184-187.
14. Jenne DE, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat Genet 1998;18(1):38-43.
15. Hezel AF, Bardeesy N. LKB1; linking cell structure and tumor suppression. Oncogene 2008;27(55): 6908-6919.
16. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol 2008;26(35):5783-5788.
17. Hampel H, Frankel W, Panescu J, Lockman J, Sotamaa K, Fix D, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res 2006;66(15):7810-7817.
18. Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM, et al. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. Gastroenterology 1993;104(5):1535-1549.
19. Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. Cancer 1993;71(3):677-685.
20. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58(22):5248-5257.

21. Jarvinen HJ, Aarnio M, Mustonen H, ktan-Collan K, Aaltonen LA, Peltomaki P, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000;118(5):829-834.
22. de Jong AE, Hendriks YM, Kleibeuker JH, de Boer SY, Cats A, Griffioen G, et al. Decrease in mortality in Lynch syndrome families because of surveillance. Gastroenterology 2006;130(3):665-671.
23. Stupart DA, Goldberg PA, Algar U, Ramesar R. Surveillance colonoscopy improves survival in a cohort of subjects with a single mismatch repair gene mutation. Colorectal Dis 2009;11(2):126-130.
24. Schmeler KM, Sun CC, Bodurka DC, White KG, Soliman PT, Uyei AR, et al. Prophylactic bilateral salpingo-oophorectomy compared with surveillance in women with BRCA mutations. Obstet Gynecol 2006;108:515-520.

2

High cancer risk in Peutz-Jeghers syndrome: A systematic review and surveillance recommendations

Am J Gastroenterol. 2010;105(6):1258-1264

M.G.F. van Lier[1]
A. Wagner[2]
E.M.H. Mathus-Vliegen[3]
E.J. Kuipers[1,4]
E.W. Steyerberg[5]
M.E. van Leerdam[1]

Departments of Gastroenterology and Hepatology[1], Clinical genetics[2], Internal Medicine[4] and Public Health[5], Erasmus University Medical Center, Rotterdam, the Netherlands. Department of Gastroenterology and Hepatology[3], Academic Medical Center, Amsterdam, the Netherlands.

ABSTRACT

Background: Peutz-Jeghers syndrome (PJS) is an autosomal dominant inherited disorder associated with an increased cancer risk. Surveillance and patient management are however hampered by a wide range in cancer risk estimates. We therefore performed a systematic review to assess cancer risks in PJS patients and used these data to develop a surveillance recommendation.

Methods: A systematic PubMed search was performed up to February 2009 and all original articles dealing with PJS patients with confirmed cancer diagnoses were included. Data considering cancer frequencies, mean ages at cancer diagnosis, relative risks and cumulative risks were collected.

Results: Twenty-one original articles, 20 cohort studies and one meta-analysis, fulfilled the inclusion criteria. The cohort studies showed some overlap in patient population and included a total of 1644 patients; 349 of them developed 384 malignancies at an average age of 42 years. The most common malignancy was colorectal cancer, followed by breast cancer, small-bowel, gastric and pancreatic cancer. The reported life time risk for any cancer varied between 37 and 93% with relative risks ranging from 9.9 to 18 in comparison with the general population. Age-related cumulative risks were given for any cancer, gastrointestinal cancer, gynaecological cancer, colorectal, pancreatic and lung cancer.

Conclusions: PJS patients are markedly at risk for several malignancies, in particular gastrointestinal cancers and breast cancer. Based on these elevated risks a surveillance recommendation is developed to detect malignancies in an early phase and to remove polyps that may be premalignant and may cause complications, in order to improve outcome.

INTRODUCTION

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant inherited disorder, characterized by gastrointestinal hamartomas and mucocutaneous pigmentations. The incidence has been estimated between 1:8,300 and 1:200,000 births.[1-4] Jan Peutz, a Dutch physician, was the first to recognize the combination of intestinal polyposis, mucocutaneous pigmentation and heredity in 1921.[5] Thereafter, Jeghers published a description of the syndrome in 1949[6], leading to the eponym "Peutz-Jeghers syndrome".[7]

In 1998 investigators discovered that germline mutations in the *STK11* gene (Serine Threonine Kinase 11, also known as *LKB1*-gene) cause PJS.[8,9] *STK11* is a serine threonine kinase localized on chromosome19p13.3 and is designated as a tumor suppressor gene.[10] Genetic testing for clinical practice is widely available, and with the currently available techniques, an *STK11* germline mutation can be found in approximately 80% of clinically affected PJS families.[11] Nevertheless, a second gene locus might still exist.[12,13]

Although the mechanism of carcinogenesis remains debatable, PJS patients carry a considerably increased risk for the development of both gastrointestinal and extra-gastrointestinal malignancies, as summarised in a previous meta-analysis.[14] Several surveillance recommendations have been published.[2,4,15-22] However, the clinical management of patients is still hampered by the wide range in reported cancer risk estimates and in our clinical practice cancer risks seem lower than reported in the meta-analysis.[14] Recently, international collaborations have led to publications on larger cohorts of PJS patients with a focus on their increased cancer risks.[23,24] We therefore reviewed literature to assess the risk and onset of malignancies in PJS patients. Based on this risk profile we developed a Dutch surveillance recommendation in collaboration with a national working group.

METHODS

We performed a systematic search on PubMed until February 2009, to identify all English and Dutch literature under the MESH headings and texts words of *"Peutz–Jeghers syndrome or Peutz"* and *"neoplasms or neoplasm* or cancer or tumour* or tumor or tumors or carcinom*"*. One reviewer (MGFvL) inspected the title and abstract of each electronic citation to identify those manuscripts suitable for this review. The full texts were obtained, and an extensive manual search was conducted using references from all retrieved reports and review articles.

Cohort studies and meta-analyses reporting cancer risks in PJS were considered eligible, and case reports, review articles and editorials were excluded. Original manuscripts were included regardless of their research question, if cancer risks could be estimated in patients with Peutz-Jeghers syndrome by fulfilling the following inclusion-criteria: 1) PJS diagnosis (either on the basis of clinical criteria or an *STK11* mutation), and 2) confirmation of cancer diagnoses.

The quality of the included articles was assessed by evaluating the diagnosis of PJS (based on either clinical criteria such as family history, hamartomas, small-bowel polyposis and pigmentations, or based on an *STK11* mutation), and the diagnosis of cancer (e.g. by histological confirmation). Two reviewers (MGFvL and AW) abstracted detailed data from the articles that fulfilled our inclusion criteria, and discrepancies were resolved by consensus of the study group. Data extracted included diagnosis of PJS, number of included PJS patients, number of PJS families, sex, age at the end of follow-up, cancer diagnosis, age at cancer diagnosis, outcome measures such as relative cancer risks and cumulative cancer risks, and study design and location of study. We pooled data in order to calculate cancer frequencies, mean ages at cancer diagnosis, relative risks and cumulative risks. We registered those cases in which several publications derived from the same data set. Overlap in patient population was assumed if patients from a single medical center were included in more than one article.

RESULTS

Our search through PubMed identified 1049 articles. This search in combination with an extensive manual search yielded 21 original articles that met the inclusion criteria.[3,14,23-41] These studies, 20 cohort studies and 1 meta-analysis[14], were published between 1975 and 2007. There was considerable overlap in patient populations, caused by two large collaborative studies[23,24] and the meta-analysis.[14] Despite the overlap, we chose to report all studies meeting the inclusion criteria since the smaller cohort studies reported on different outcome measures or contained more detailed data than the large collaborative studies and the meta-analysis.

The definitions for PJS and the methods to confirm the diagnosis of cancer varied between the publications, and *STK11* mutation-analysis had been performed in only 10 of the 21 studies. In the 20 cohort studies a total of 1644 patient were evaluated, and 349 of them developed 384 malignancies, at an average age of 42 years. In Table 1 the absolute number of diagnosed cancer cases and the average ages at cancer diagnosis are shown (excluding the meta-analysis). The most frequently reported cancers were colorectal cancer (n = 80) and breast cancer (n = 59), followed by small-bowel, stomach and pancreatic cancer.

Between the studies there was some variation in outcome measures. Relative cancer risks were reported in only four publications, summarized in Table 2.[14,32,35,39] In these four studies the relative risk of any cancer varied between 9.9 and 18. In addition, the relative risk of any cancer could be calculated from two collaborative studies, and these relative risks at age 60 were 7.3 and 4.8 compared to the general population.[23,24] Relative risks (RR) of cancer at specific sites were reported in only one study.[14] Compared to the general population the relative risks were significantly increased for the following malignancies; small intestinal (RR520), gastric (RR213), pancreatic (RR132), colorectal (RR84), ovarian (RR27), lung (RR17), endometrial (RR16) and breast cancer (RR15). In a previous study Giardiello and colleagues

Table 1. Reported cancers and age at diagnosis in 1644 PJS patients from 20 cohort studies.[3,23-41]

Cancer	No. cancers	Mean Age in years
Gastrointestinal	*198*	*42 (n=69)*
Colorectum	80	43 (n=23)
Small intestine (incl. duodenum)	41	37 (n=18)
Stomach	35	40 (n=14)
Esophagus	3	33 (n=1)
Pancreas	32	52 (n=12)
Biliary tract	7	32 (n=1)
Extra-gastrointestinal		
Breast	59	44 (n=23)
Uterus	10	43 (n=1)
Ovary	16	35 (n=8) (incl. 1 Sertoli tumor at age 6)
Cervix	14	36 (n=5)
Testes	3	6 (n=1)
Lung	25	47 (n=8)
Other*	44	45 (n=9)
Unknown	15	50 (n=7)
Total	*384*	*42 (n=131)*

* Other includes: multiple myeloma, leukaemia, thyroid, prostate, liver, gallbladder, kidney, adrenal, nasopharyngeal, bone and skin cancer.

N.B. - Hearle et al. '06 and Lim et al. '04: Gastro-esophageal cancers classified as gastric cancer.[23,28]
- Scott et al. '02: Bowel cancer classified as colorectal cancer.[30]
- Utsunomiya et al. '75: Cancer deaths instead of cancer incidence.[3]
- Spigelman et al. '89: Ovarian cancers include 1 adnexal carcinoma.[37]

found a similar relative risk for pancreatic cancer of 132. They also defined the relative risks for any cancer according to age; the relative cancer risk was 5 for PJS patients < 40 years and 23 for patients ≥ 40 years.[39] In one study relative risks of cancer mortality were determined on the basis of 66 PJS patients.[37] The relative risk of death from any cancer was 9 (95% CI 4.2 - 17.3), and the relative risk of gastrointestinal cancer death was 13 (95% CI 2.7 - 38). In another

Table 2. Relative cancer risks.

Site	RR	Reference(s)
Any cancer	9.9 - 18	[35,32,14,39]
Males	6.2 - 22	[35,39]
Females	16 - 18.5	[39,35]
GI cancer	50.5	[35]
Males	30.3	[35]
Females	150.9	[35]
Gynaecological cancer & breast cancer (females)	20.3	[35]

RR = Relative risk

GI = Gastrointestinal (colorectal, small intestinal, gastric, and esophageal cancer)

study standardized mortality ratio's were determined on the basis of 70 PJS patients, and by the age of 65 years the standardized mortality ratio for all cancers was 9.9 (95% CI 0.4 - 20.4) and 24.8 (95% CI 0.7 - 63.6) for gastrointestinal cancer.[29]

Cumulative risks for any cancer were calculated in 6 studies up to age 60, 65 or 70 (Table 3).[14,23,24,28,29,34] The lowest cumulative cancer risk was reported to be 37% (95% CI 21 - 61) at the age 65[29], although the same authors reported a cumulative risk for any cancer in PJS at the age of 70 years of 81% in a large collaborative study.[28] The percentage of 37% was based on 70 clinical PJS-patients regardless of their *STK11* mutation status. When only *STK11* mutation carriers were taken into account, the cumulative cancer risk was higher; 47% (95% CI 27 - 73) at the age of 65 years.[29] However, in a larger study cumulative cancer risks were evaluated in patients with and without an *STK11* mutation, and there was no statistically significant difference between the two groups.[23]

Four studies reported age-related cumulative cancer risks (any cancer, gastrointestinal cancer and gynaecological cancer), as shown graphically in Figure 1.[23,24,28,34] Cumulative risks for breast cancer ranged from 5 to 8% at age 40, increasing to 45% at 70 years.[23,24,28] Two studies reported age-related cumulative risks for colorectal cancer, pancreatic cancer, and lung cancer, graphically shown in Figure 2.[23,28] One study evaluated differences in cumulative risks between males and females for any cancer, showing that at the age of 70 years males and females carry similar risks for the development of a malignancy (55% and 59%, respectively).[24]

Table 3. Cumulative cancer risks (approaching life time risks).

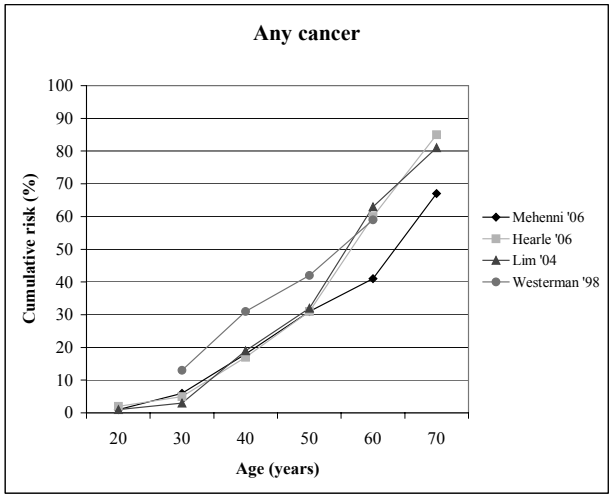
Site cancer	Age (yrs)	CR	Reference(s)
Any cancer	60 - 70	37 - 93%	29,34,24,28,23,14
GI cancer	60 - 70	38 - 66%	34,23,24,28
Gynaecological cancer	60 - 70	13 - 18%	28,23
Per origin			
Stomach	65	29%	14
Small-bowel	65	13%	14
Colorectum	65	39 - 39%	14,23
Pancreas	65 - 70	11 - 36%	23,14
Lung	65 - 70	7 - 17%	28,14,23
Breast	60 - 70	32 - 54%	28,23,14
Uterus	65	9%	14
Ovary	65	21%	14
Cervix	65	10%	14
Testes	65	9%	14

CR = Cumulative risk

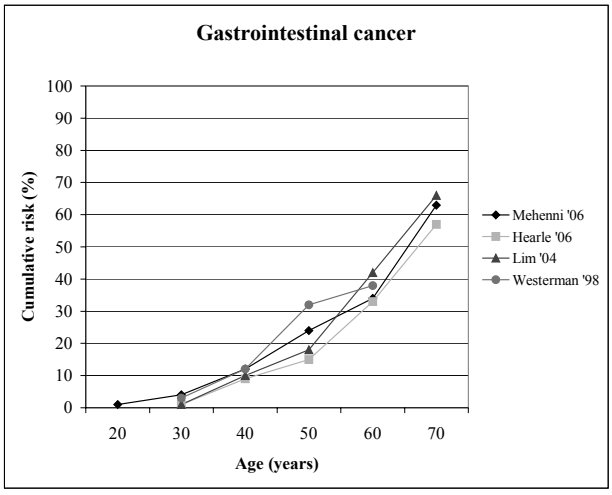
GI = Gastrointestinal (colorectal, small intestinal, gastric, esophageal and pancreatic cancer)

N.B. Westerman et al. '98: GI cancer does not include pancreatic cancer.[34]

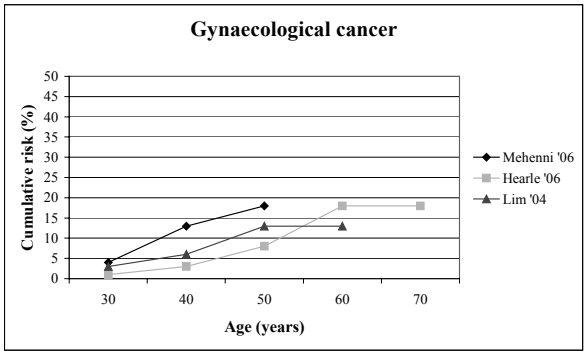
Figure 1. Cumulative cancer risks according to age.



A. Cumulative risks any cancer according to age.[23,24,28,34]

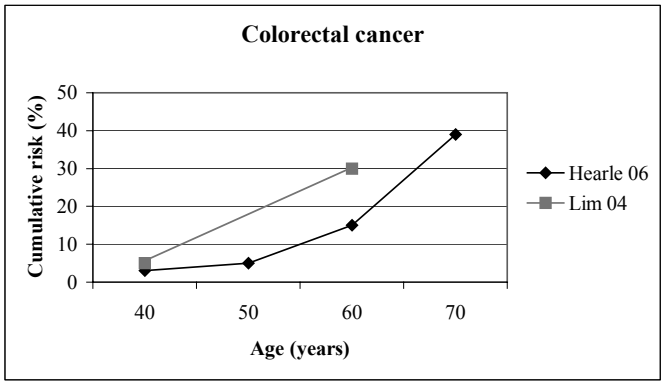


B. Cumulative risks gastrointestinal cancers according to age.[23,24,28,34]

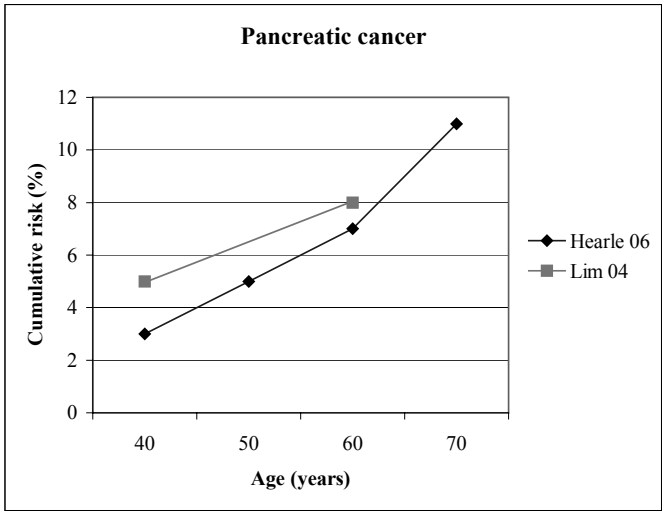


C. Cumulative risks gynaecological cancers according to age.[23,24,28]

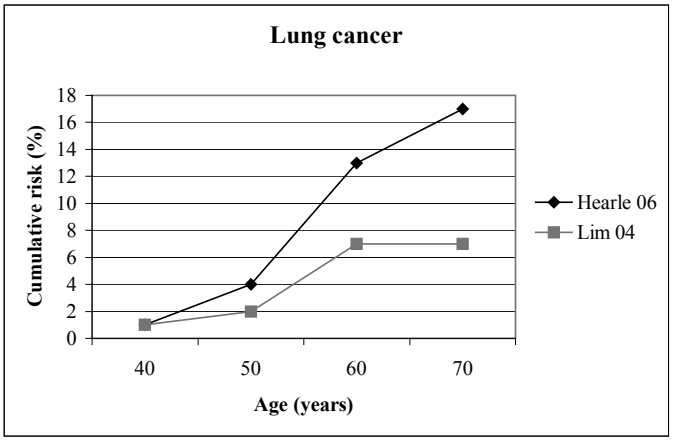
Figure 2. Cumulative cancer risks according to age and origin.



A. Cumulative colorectal cancer risks according to age.[23,24]



B. Cumulative pancreatic cancer risks according to age.[23,24]



C. Cumulative lung cancer risks according to age.[23,24]

DISCUSSION

This systematic review confirms that PJS patients carry a high cancer risk already at a young age[14,25,29], which is consistent with the identification of the *STK11* gene as a tumor suppressor gene.[10] Twenty cohort studies reported on 1644 patients; 349 of them developed 384 malignancies at an average age of 42 years. The overall risk was most markedly increased for colorectal, breast, small-bowel, gastric and pancreatic cancer. There was overlap in patient populations due to two large collaborative studies.[23,24] This might have overestimated the cancer risks. However, when we excluded the cohorts already represented in two collaborative studies, we found similar results (757 different patients; 148 of them developed 163 malignancies at an average age of 42 years). Since the exclusion of overlapping studies led to loss of interesting data, we chose to report on all studies fulfilling the inclusion criteria.

The relative cancer risks varied between 4.8 and 18 compared to the general population, with life time cumulative cancer risks up to 93%. The upper limit of these relative risks approached the high relative cancer risk reported in the meta-analysis published in 2000, and the upper limit of cumulative risks (93%) was derived from Giardiello's meta-analysis.[14] Although the largest included cohort study showed no statistically significant difference in cumulative cancer risk between patients with and without an *STK11* mutation[23], the cancer risk did seem higher for *STK11* mutation carriers compared to patients without a mutation in another study.[29] In the future, it would be interesting to gain more insight in genotype-phenotype correlations and to investigate whether differences in *STK11* mutation types are related to cancer proneness.

There are some limitations to this systematic review that need to be addressed. First of all, the included studies may be hampered by selection bias; only patients with the most severe phenotypes might have been included, thereby overestimating cancer risk. Yet, the patients described in the cohort studies were selected systematically and were not recruited because of cancer in the proband; only one proband presented with cancer at the first consultation.[36] Also referral bias might have led to overestimation of cancer risks. Only patients with a severe phenotypic expression of the disease (including cancers) might have been referred to specialised centres who subsequently report their data. On the other hand, cancer risks may have been underestimated since cancer risks partly depend on the duration of follow-up; some studies reported on relatively young patients at the end of follow-up in whom cancer may still develop.[27] Other studies displayed no data on the age of the included patients or the duration of follow-up.[3,23,24] Finally there were some difficulties in pooling data since different definitions and different endpoints were used. For example, pancreatic cancer was considered as extra-gastrointestinal cancer in one study[34] but as gastrointestinal cancer in other studies.[23,28]

Assessment of the cancer risk in PJS is difficult for several other reasons; the true incidence of PJS is not known and some cases with an uncomplicated syndrome (e.g. patients without cancer) remain unpublished (publication bias). Furthermore, pseudocarcinomatous invasion

of epithelial cells into the muscularis propria and serosa may be mistaken for an invasive carcinoma, overestimating cancer incidence.[42,43] Pseudo-invasion can be distinguished from invasive carcinoma by the lack of cytological atypia. This phenomenon occurs predominantly in the small-bowel since it is caused by torsion and infarction of the polyps during bowel obstruction; pseudo-invasion was observed in approximately 10% of small-bowel polyps in one study.[44]

Nevertheless, cancer risks in PJS patients are very high and come close to other high-risk conditions in which surveillance has been recommended. The upper confidence limit of the breast cancer risk in PJS has for example been shown to be as high as the breast cancer risk in patients with *BRCA1* or *BRCA2* mutations.[28] The high cancer risks justify surveillance of PJS patients. However, the optimal surveillance strategy remains to be established, and the wide spectrum of PJS-associated cancers as well as other complications caused by the polyposis such as intussusception, have to be taken into account.

Based on risks of intussusception[45,46] and other polyp-related complications such as bleeding or anaemia early in life, and based on the increased cancer risks later in life described in this review, we proposed a new Dutch surveillance recommendation in collaboration with a national working group (Table 4). In this working group, gastroenterologists, internists, clinical geneticists, paediatricians and gynaecologists from the Netherlands are represented. The recommendation was developed on the basis of the literature reviewed here and clinical experience, and solely reflects expert-opinion since no controlled trials have been published on the effectiveness of surveillance in PJS. With respect to uncontrolled data, German investigators recently reported that a similar surveillance strategy as proposed by us, led to early detection of 50% of all cancers (5/10) diagnosed in 31 PJS patients.[47]

New surveillance and treatment techniques such as video capsule endoscopy (VCE), MRI enteroclysis and double balloon enteroscopy (DBE) that have become widely available are incorporated into this new surveillance recommendation. This is the main difference between the surveillance recommendations proposed here compared to previously published surveillance guidelines.[2,4,15-22] It has been shown that VCE and/or MRI are good alternatives to small-bowel-follow-through for the detection of small-bowel polyps[48,49], and that DBE is clinically useful and safe for therapy of small-bowel polyps in PJS patients.[50]

Another difference between the recommendations presented here and the guideline published by Giardiello and colleagues in 2006[2,4,15-22], the latest guideline in print, is that we advocate to start small-bowel surveillance at a more regular basis already at a young age (starting at age 10 with 2-3 year intervals, compared to a starting age of 18 years and a baseline examination at age 8). It is generally accepted that surveillance for gastrointestinal cancer is not indicated before the age of 20-25 years.[28] Yet, we recommend small intestinal surveillance starting at a younger age in view of the morbidity caused by the hamartomas.[45,46] "Benign" complications of the polyps such as bleeding and intussusception predominate the first three decades of life, whereas malignant complications become more common there-

Table 4. Dutch surveillance recommendations for PJS patients.

Examination*	Starting age	Interval
History, physical examination (including palpation testis) and haemoglobin analysis	10 years	1 years (paediatrician)
Video capsule endoscopy (VCE) and/or MRI-enteroclysis‡	10 years	2-3 years
Gastroduodenoscopy	20 years	2-5 years (depending on findings)
Colonoscopy	25-30 years	2-5 years (depending on findings)
MRI & endoscopic ultrasonography (EUS) pancreas	30 years	1 year, only in a prospective ongoing trial[61]
Breast exam & breast MRI Mammography & breast MRI	25 years 30 years	1 year 1 year§
Pelvic exam, cervical smear, transvaginal ultrasonography and CA-125.	25-30 years	1 year

* Earlier and/or more frequently in symptomatic patients / if clinically indicated.

‡ If VCE shows polyps it's recommended to perform an MRI-enteroclysis to determine the exact localisation and size if the polyps. Polyps > 1 cm in diameter are an indication for double balloon enteroscopy (DBE) with polypectomy.
In addition, we recommend intra-operative enteroscopy with polyp removal in every indicated laparotomy, to avoid re-laparotomies. If surgery is indicated a laparoscopic approach is preferred when possible.

§ Mammography and MRI alternately performed every six months.

after.[3] By the removal of large polyps, bleeding and intussusception might be prevented. There is no consensus on the management of small-bowel polyps. Generally polypectomy has been recommended for polyps > 1 - 1.5 cm and symptomatic small-bowel polyps.[2,22,51,52] Furthermore, we propose colonoscopic surveillance from a later starting age than Giardiello and colleagues[2,4,15-22] (30 years versus 18 years, respectively), since the colorectal cancer risk is low under the age of 30 years (Figure 2).

A point of discussion is whether the malignancies in the gastro-intestinal tract originate from the hamartomas or from coexisting adenomas or normal mucosa.[53,54] The location of the gastrointestinal malignancies in PJS patients did not always correlate with the location of the hamartomatous polyps.[54] However, a metastasizing duodenal carcinoma arising in a hamartoma was first reported in 1965[55], and ever since several studies have reported a hamartoma-adenoma-carcinoma sequence.[36,56-58] The latter suggests that endoscopic polyp-removal could potentially decrease the risk for malignancies. To answer the question whether or not hamartomas are pre-malignant, further basal research is required and prospective studies should demonstrate whether or not the incidence of gastrointestinal malignancies decreases with endoscopic polypectomy. For now, the mechanism of carcinogenesis remains unknown and the primary aim of cancer surveillance is the early detection of malignancies thereby improving outcome, and perhaps removal of premalignant polyps decreasing the gastrointestinal cancer risk.

Pancreatic screening seems promising[59-61], but it is in the Netherlands nowadays only performed in light of an ongoing prospective trial since there are still many unanswered questions regarding pancreatic screening.[61] These include whether early detection of (precursor) lesions leads to an improved patient outcome, and also focus on the best way to manage detected lesions. In contrast, the beneficial effect of breast cancer surveillance in high risk individuals has been established. Since the breast cancer risk in PJS approaches the breast cancer risk in patients with *BRCA1* or *BRCA2* mutations, the breast cancer surveillance is similar.[62]

In addition to ovarian carcinomas, mucinous neoplasms of the ovary and ovarian sex-cord tumors with annular tubules (SCTATs) occur frequently in women with PJS.[63,64] The latter may cause sexual precocity and infertility and are generally considered benign but may become malignant. The gynecological surveillance recommendation is therefore also directed at early detection of these lesions. Although the risk for a testicular tumor was not established in this review, annual testicular palpation is recommended in boys since testicular Sertoli cell tumors occur more frequently in PJS and may cause precocious puberty and gynaecomasty.[65] Annual physical examination of children and haemoglobin analysis may furthermore reveal anemia, raising suspicion of gastrointestinal hamartomas.

In conclusion, PJS patients carry a markedly elevated cancer risk, concerning mainly gastrointestinal carcinomas and breast cancer. However, cancer risks may be lower than in a previously published meta-analysis.[14] Although the benefits of surveillance remain to be established, surveillance seems justified and therefore we made surveillance and treatment recommendations. The effect of such a surveillance program on the cancer incidence, survival as well as the cost-effectiveness will have to be established in prospective trials.

ACKNOWLEDGEMENTS

We would like to thank all the other members of the Dutch Peutz-Jeghers Working Group, responsible for the development of the surveillance recommendations published here: Dr. M.A.M.J. Jacobs, gastroenterologist, VU Medical Center, Amsterdam; Dr. C.M.F. Kneepkens, paediatric gastroenterologist, VU Medical Center, Amsterdam; Dr. J.J. Koornstra, gastroenterologist, UMCG, Groningen; Dr. M.A.C. Lubsen-Brandsma, gynaecologist, Academic Medical Center, Amsterdam; Dr. F.H. Menko, clinical geneticist, VU Medical Center, Amsterdam; M. Pelger, Foundation for the Detection of Hereditary Tumors, Leiden; H.M. Ploeger department of clinical genetics, VU Medical Center, Amsterdam; Dr. H.F.A. Vasen, internist LUMC, Leiden and director Foundation for the Detection of Hereditary Tumors, Leiden.

REFERENCES

1. Finan MC, Ray MK. Gastrointestinal polyposis syndromes. Dermatol Clin 1989;7(3):419-434.
2. Allen BA, Terdiman JP. Hereditary polyposis syndromes and hereditary non-polyposis colorectal cancer. Best Pract Res Clin Gastroenterol 2003;17(2):237-258.
3. Utsunomiya J, Gocho H, Miyanaga T, Hamaguchi E, Kashimure A. Peutz-Jeghers syndrome: its natural course and management. Johns Hopkins Med J 1975;136(2):71-82.
4. Lindor NM, Greene MH. The concise handbook of family cancer syndromes. Mayo Familial Cancer Program. J Natl Cancer Inst 1998;90(14):1039-1071.
5. Peutz JLA. Over een zeer merkwaardige, gecombineerde familiaire polyposis van de slijmliezen van den tractus intestinalis met die van de neuskeelholte en gepaard met eigenaardige pigmentaties van huid en slijmvliezen. Ned Maandschr v Geneesk 1921;10:134-146.
6. Jeghers H, McKusick VA, Katz KH. Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits; a syndrome of diagnostic significance. N Engl J Med 1949;241(26):1031-1036.
7. Bruwer A, Bargen JA, Kierland RR. Surface pigmentation and generalized intestinal polyposis; (Peutz-Jeghers syndrome). Proc Staff Meet Mayo Clin 1954;29(6):168-171.
8. Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 1998;391(6663):184-187.
9. Jenne DE, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat Genet 1998;18(1):38-43.
10. Hezel AF, Bardeesy N. LKB1; linking cell structure and tumor suppression. Oncogene 2008;27(55): 6908-6919.
11. Volikos E, Robinson J, Aittomaki K, Mecklin JP, Jarvinen H, Westerman AM, et al. LKB1 exonic and whole gene deletions are a common cause of Peutz-Jeghers syndrome. J Med Genet 2006; 43(5):e18.
12. Mehenni H, Gehrig C, Nezu J, Oku A, Shimane M, Rossier C, et al. Loss of LKB1 kinase activity in Peutz-Jeghers syndrome, and evidence for allelic and locus heterogeneity. Am J Hum Genet 1998; 63(6):1641-1650.
13. Hearle N, Lucassen A, Wang R, Lim W, Ross F, Wheeler R, et al. Mapping of a translocation breakpoint in a Peutz-Jeghers hamartoma to the putative PJS locus at 19q13.4 and mutation analysis of candidate genes in polyp and STK11-negative PJS cases. Genes Chromosomes Cancer 2004;41(2): 163-169.
14. Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119(6):1447-1453.
15. Tomlinson IP, Houlston RS. Peutz-Jeghers syndrome. J Med Genet 1997;34(12):1007-1011.
16. Burt RW. Colon cancer screening. Gastroenterology 2000;119(3):837-853.
17. The Johns Hopkins guide for patients and families: Peutz-Jeghers syndrome. Baltimore: Johns Hopkins University. 2001.
18. McGrath DR, Spigelman AD. Preventive measures in Peutz-Jeghers syndrome. Fam Cancer 2001; 1(2):121-125.
19. Dunlop MG. Guidance on gastrointestinal surveillance for hereditary non-polyposis colorectal cancer, familial adenomatous polypolis, juvenile polyposis, and Peutz-Jeghers syndrome. Gut 2002;51 Suppl 5:V21-V27.
20. Hinds R, Philp C, Hyer W, Fell JM. Complications of childhood Peutz-Jeghers syndrome: implications for pediatric screening. J Pediatr Gastroenterol Nutr 2004;39(2):219-220.

21. McGarrity TJ, Amos C. Peutz-Jeghers syndrome: clinicopathology and molecular alterations. Cell Mol Life Sci 2006;63(18):2135-2144.
22. Giardiello FM, Trimbath JD. Peutz-Jeghers Syndrome and Management Recommendations. Clinical Gastroenterology and Hepatology 2006;4(4):408-415.
23. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 2006;12(10):3209-3215.
24. Mehenni H, Resta N, Park JG, Miyaki M, Guanti G, Costanza MC. Cancer risks in LKB1 germline mutation carriers. Gut 2006;55(7):984-990.
25. Mehenni H, Resta N, Guanti G, Mota-Vieira L, Lerner A, Peyman M, et al. Molecular and clinical characteristics in 46 families affected with Peutz-Jeghers syndrome. Dig Dis Sci 2007;52(8):1924-1933.
26. Schumacher V, Vogel T, Leube B, Driemel C, Goecke T, Moslein G, et al. STK11 genotyping and cancer risk in Peutz-Jeghers syndrome. J Med Genet 2005;42(5):428-435.
27. Amos CI, Keitheri-Cheteri MB, Sabripour M, Wei C, McGarrity TJ, Seldin MF, et al. Genotype-phenotype correlations in Peutz-Jeghers syndrome. J Med Genet 2004;41(5):327-333.
28. Lim W, Olschwang S, Keller JJ, Westerman AM, Menko FH, Boardman LA, et al. Relative frequency and morphology of cancers in STK11 mutation carriers. Gastroenterology 2004;126(7):1788-1794.
29. Lim W, Hearle N, Shah B, Murday V, Hodgson SV, Lucassen A, et al. Further observations on LKB1/STK11 status and cancer risk in Peutz-Jeghers syndrome. Br J Cancer 2003;89(2):308-313.
30. Scott RJ, Crooks R, Meldrum CJ, Thomas L, Smith CJ, Mowat D, et al. Mutation analysis of the STK11/LKB1 gene and clinical characteristics of an Australian series of Peutz-Jeghers syndrome patients. Clin Genet 2002;62(4):282-287.
31. Olschwang S, Boisson C, Thomas G. Peutz-Jeghers families unlinked to STK11/LKB1 gene mutations are highly predisposed to primitive biliary adenocarcinoma. J Med Genet 2001;38(6):356-360.
32. Choi HS, Park YJ, Youk EG, Yoon KA, Ku JL, Kim NK, et al. Clinical characteristics of Peutz-Jeghers syndrome in Korean polyposis patients. Int J Colorectal Dis 2000;15(1):35-38.
33. Westerman AM, Entius MM, de Baar E, Boor PP, Koole R, van Velthuysen ML, et al. Peutz-Jeghers syndrome: 78-year follow-up of the original family. Lancet 1999;353(9160):1211-1215.
34. Westerman AM, Entius MM, van Velthuysen M, Coebergh JW, Lindhout D, Offerhaus G, et al. Cancer risk in Peutz–Jeghers syndrome (abstract). Eur J Hepatogastroenterol 1998;10:A42.
35. Boardman LA, Thibodeau SN, Schaid DJ, Lindor NM, McDonnell SK, Burgart LJ, et al. Increased risk for cancer in patients with the Peutz-Jeghers syndrome. Ann Intern Med 1998;128(11):896-899.
36. Hizawa K, Iida M, Matsumoto T, Kohrogi N, Kinoshita H, Yao T, et al. Cancer in Peutz-Jeghers syndrome. Cancer 1993;72(9):2777-2781.
37. Spigelman AD, Murday V, Phillips RK. Cancer and the Peutz-Jeghers syndrome. Gut 1989;30(11):1588-1590.
38. Foley TR, McGarrity TJ, Abt AB. Peutz-Jeghers syndrome: a clinicopathologic survey of the "Harrisburg family" with a 49-year follow-up. Gastroenterology 1988;95(6):1535-1540.
39. Giardiello FM, Welsh SB, Hamilton SR, Offerhaus GJ, Gittelsohn AM, Booker SV, et al. Increased risk of cancer in the Peutz-Jeghers syndrome. N Engl J Med 1987;316(24):1511-1514.
40. Burdick D, Prior JT. Peutz-Jeghers syndrome. A clinicopathologic study of a large family with a 27-year follow-up. Cancer 1982;50(10):2139-2146.
41. Linos DA, Dozois RR, Dahlin DC, Bartholomew LG. Does Peutz-Jeghers syndrome predispose to gastrointestinal malignancy? A later look. Arch Surg 1981;116(9):1182-1184.

42. Westerman AM, van Velthuysen ML, Bac DJ, Schouten WR, Wilson JH. Malignancy in Peutz-Jeghers syndrome? The pitfall of pseudo-invasion. J Clin Gastroenterol 1997;25(1):387-390.
43. Petersen VC, Sheehan AL, Bryan RL, Armstrong CP, Shepherd NA. Misplacement of dysplastic epithelium in Peutz-Jeghers polyps: The ultimate diagnostic pitfall? American Journal of Surgical Pathology 2000;24(1):34-39.
44. Shepherd NA, Bussey HJ, Jass JR. Epithelial misplacement in Peutz-Jeghers polyps. A diagnostic pitfall. Am J Surg Pathol 1987;11(10):743-749.
45. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, et al. STK11 status and intussusception risk in Peutz-Jeghers syndrome. J Med Genet 2006;43(8):e41.
46. van Lier MG, Wagner A, Westerman AM, de Rooij FW, Wilson JH, Kuipers EJ, et al. High cumulative risk of intussusceptions in patients with Peutz-Jeghers syndrome (abstract). Gastroenterology 2009;136(5, Supplement 1):A-264.
47. Salloch H, Reinacher-Schick A, Schulmann K, Pox C, Willert J, Tannapfel A, et al. Truncating mutations in Peutz-Jeghers syndrome are associated with more polyps, surgical interventions and cancers. Int J Colorectal Dis 2010;25(1):97-107.
48. Caspari R, von Falkenhausen M, Krautmacher C, Schild H, Heller J, Sauerbruch T. Comparison of capsule endoscopy and magnetic resonance imaging for the detection of polyps of the small intestine in patients with familial adenomatous polyposis or with Peutz-Jeghers' syndrome. Endoscopy 2004;36(12):1054-1059.
49. Brown G, Fraser C, Schofield G, Taylor S, Bartram C, Phillips R, et al. Video capsule endoscopy in peutz-jeghers syndrome: a blinded comparison with barium follow-through for detection of small-bowel polyps. Endoscopy 2006;38(4):385-390.
50. Gao H, van Lier MG, Poley JW, Kuipers EJ, van Leerdam ME, Mensink P. Treatment and surveillance of small bowel polyps using double balloon enteroscopy in patients with Peutz-Jeghers Syndrome (abstract). Gastrointestinal Endoscopy 2009;69(5):AB204.
51. Choi HS, Park YJ, Park JG. Peutz-Jeghers syndrome: a new understanding. J Korean Med Sci 1999; 14(1):2-7.
52. McGarrity TJ, Kulin HE, Zaino RJ. Peutz-Jeghers syndrome. Am J Gastroenterol 2000;95(3):596-604.
53. Hizawa K, Iida M, Matsumoto T, Kohrogi N, Yao T, Fujishima M. Neoplastic transformation arising in Peutz-Jeghers polyposis. Dis Colon Rectum 1993;36(10):953-957.
54. Dozois RR, Judd ES, Dahlin DC, Bartholomew LG. The Peutz-Jeghers syndrome. Is there a predisposition to the development of intestinal malignancy? Arch Surg 1969;98(4):509-517.
55. Williams JP, Knudsen A. Peutz-Jeghers Syndrome with Metastasizing Duodenal Carcinoma. Gut 1965;6:179-184.
56. Perzin KH, Bridge MF. Adenomatous and carcinomatous changes in hamartomatous polyps of the small intestine (Peutz-Jeghers syndrome): report of a case and review of the literature. Cancer 1982;49(5):971-983.
57. Gruber SB, Entius MM, Petersen GM, Laken SJ, Longo PA, Boyer R, et al. Pathogenesis of adenocarcinoma in Peutz-Jeghers syndrome. Cancer Res 1998;58(23):5267-5270.
58. Wang ZJ, Ellis I, Zauber P, Iwama T, Marchese C, Talbot I, et al. Allelic imbalance at the LKB1 (STK11) locus in tumours from patients with Peutz-Jeghers' syndrome provides evidence for a hamartoma-(adenoma)-carcinoma sequence. J Pathol 1999;188(1):9-13.
59. Brentnall TA. Cancer surveillance of patients from familial pancreatic cancer kindreds. Medical Clinics of North America 2000;84(3):707-718.

60. Canto MI, Goggins M, Hruban RH, Petersen GM, Giardiello FM, Yeo C, et al. Screening for early pancreatic neoplasia in high-risk individuals: a prospective controlled study. Clin Gastroenterol Hepatol 2006;4(6):766-781.
61. Poley JW, Kluijt I, Gouma DJ, Harinck F, Wagner A, Aalfs C, et al. The Yield of First-Time Endoscopic Ultrasonography in Screening Individuals at a High Risk of Developing Pancreatic Cancer. Am J Gastroenterol 2009;104(9):2175-2181.
62. Burke W, Daly M, Garber J, Botkin J, Kahn MJ, Lynch P, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. II. BRCA1 and BRCA2. Cancer Genetics Studies Consortium. JAMA 1997;277(12):997-1003.
63. Scully RE. Sex cord tumor with annular tubules a distinctive ovarian tumor of the Peutz-Jeghers syndrome. Cancer 1970;25(5):1107-1121.
64. Hemminki A. The molecular basis and clinical aspects of Peutz-Jeghers syndrome. Cell Mol Life Sci 1999;55(5):735-750.
65. Young S, Gooneratne S, Straus FH, Zeller WP, Bulun SE, Rosenthal IM. Feminizing Sertoli cell tumors in boys with Peutz-Jeghers syndrome. Am J Surg Pathol 1995;19(1):50-58.

3

High cancer risk and increased mortality in patients with Peutz-Jeghers syndrome

Gut 2011;60(2):141-147

M.G. F. van Lier[1]
A.M. Westerman[2]
A. Wagner[3]
C.W.N. Looman[4]
J.H.P. Wilson[2]
F.W.M. de Rooij[2]
V.E.P.P. Lemmens[5]
E.J. Kuipers[1,2]
E.M.H. Mathus-Vliegen[6]
M.E. van Leerdam[1]

Departments of Gastroenterology and Hepatology[1], Internal Medicine[2], Clinical Genetics[3], and Public Health[4], Erasmus MC, University Medical Center, Rotterdam, the Netherlands. Department of Research[5], Comprehensive Cancer Centre South, Eindhoven, the Netherlands. Department of Gastroenterology and Hepatology[6], Academic Medical Center, Amsterdam, the Netherlands.

ABSTRACT

Background: Peutz-Jeghers syndrome (PJS) is associated with an increased cancer risk. As the determination of optimal surveillance strategies is hampered by wide ranges in cancer risk estimates and lack of data on cancer-related mortality, we assessed cancer risks and mortality in a large cohort of PJS patients.

Methods: Dutch PJS patients were included in this cohort study. Patients were followed prospectively between January 1995 and July 2009, and clinical data from the period before 1995 were collected retrospectively. Data were obtained by interview and chart-review. Cumulative cancer risks were calculated by Kaplan-Meier analysis and relative cancer and mortality risks by Poisson regression analysis.

Results: We included 133 PJS patients (48% males) from 54 families, contributing 5004 person-years of follow-up. Forty-nine cancers were diagnosed in 42 patients (32%), including 25 gastrointestinal (GI) cancers. The median age at first cancer diagnosis was 45 years. The cumulative cancer risk was 20% at age 40 (GI cancer 12%), increasing to 76% at age 70 (GI cancer 51%). Cumulative cancer risks were higher for females than for males (p=0.005). The relative cancer risk was higher in PJS patients than in the general population (HR 8.96; 95%CI 6.46-12.42), and higher among female (HR 20.40; 95%CI 13.43-30.99) than among male patients (HR 4.76; 95%CI 2.82-8.04). Forty-two patients had died at a median age of 45 years, including 28 cancer-related deaths (67%). Mortality was increased in our cohort compared to the general population (HR 3.50; 95%CI 2.57-4.75).

Conclusions: PJS patients carry high cancer risks, leading to increased mortality. The malignancies occur particularly in the GI tract and develop at young age. These results justify surveillance in order to detect malignancies in an early phase to improve outcome.

INTRODUCTION

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant inherited disorder characterized by gastrointestinal hamartomatous polyposis and mucocutaneous pigmentations.[1] Germline mutations in the *STK11* gene (Serine Threonine Kinase 11, also known as *LKB1* gene) cause PJS and genetic testing is now widely available.[2,3] With the currently available techniques a pathogenic *STK11* germline mutation can be detected in 80-94% of families with the PJS phenotype.[4,5]

It has been recognized that PJS is associated with an increased risk for the development of gastrointestinal (GI) and extra-GI malignancies such as breast cancer and gynaecological carcinomas. The malignancies associated with PJS have been reported to occur at a young age[6,7], which is consistent with the identification of the *STK11* gene as a tumor suppressor gene.[8] Besides, the spectrum of PJS includes the frequent occurrence of tumors that are considered to be benign or with low malignant potential such as Sertoli tumors of the testis and certain sex cord tumors of the ovary.[9,10]

Since PJS is a rare disorder, it is difficult to assess cancer risks properly. So far, only large collaborative studies evaluating heterogeneous groups of patients[11-13] or small cohort studies[14-21] have been conducted, leading to a wide range in reported cancer risk estimates.[7] The wide range in risk estimates hampers the development of optimal surveillance strategies and adequate counseling of PJS patients. In addition, data on the mortality in PJS patients are lacking. Therefore the aim of this study was to evaluate a large homogenous cohort of PJS patients in order to determine 1) cumulative cancer risks, 2) the relative cancer risk compared to the general population, and 3) the mortality rate in PJS patients compared to the general population.

METHODS

All PJS patients from two Dutch academic hospitals were included in this cohort study between 1995 and July 2009. After informed consent, we included patients without selection for medical history. All patients had a definite diagnosis of PJS, defined by diagnostic criteria recommended by the World Health Organization (Table 1)[22], a proven *STK11* mutation, or both. The study was approved by the Institutional Review Board of both participating hospitals.

Patient information was obtained by interview and chart-review. The following data were collected per patient: date of birth, establishment of PJS diagnosis, *STK11* mutation-status, family history of PJS, diagnosis and characteristics of cancer(s) and date and cause of death. Cancer characteristics that were recorded included date of diagnosis, tumor type and origin, tumor invasion (carcinoma in situ (CIS), or invasive carcinoma), as well as data on confirma-

Table 1. Diagnostic criteria for PJS recommended by the World Health Organization.[22]

A) *Positive* family history of PJS, and
1. Any number of histologically confirmed PJS polyps*, or
2. Characteristic, prominent, mucocutaneous pigmentation.
B) *Negative* family history of PJS, and
1. Three or more histologically confirmed PJS polyps*, or
2. Any number of histologically confirmed PJS polyps* and characteristic, prominent, mucocutaneous pigmentation.

* Histology PJS polyps: A central core of smooth muscle that shows tree-like branching, covered by the mucosa native to the region which is heaped into folds producing a villous pattern.

tion (anamnestic, medical record, or histology), and presentation (surveillance or complaints / other). Cause of death was classified as cancer-related, intussusception-related or other. Patients were followed prospectively between January 1995 and July 2009, and clinical data from the period before 1995 were collected retrospectively. In addition, family pedigrees were traced backwards and laterally as far as possible, and data of deceased family members fulfilling the diagnostic criteria for PJS were also collected.

Statistical analyses

Data were analyzed using the SPSS 15.0 statistical software and the R-2.6.0 statistical package for Windows. Cumulative age-specific cancer risks were calculated with the Kaplan-Meier method and the Cox Proportional Hazards Model. Cumulative cancer risks were determined for any cancer (overall cancer risk) and GI cancer. The following cancers were classified as GI cancer; colorectal, small intestinal (including ampullary cancers), stomach, esophageal, pancreatic and biliary cancers, as well as adenocarcinomas from the digestive tract (not otherwise specified). In case of multiple primary tumors only the first malignancy contributed to the calculation of the overall cumulative cancer risk. Second primary malignancies were included in the analysis of the cumulative GI cancer risk, yet in patients with two GI cancers only the first event contributed.

The relative cancer risk, i.e. the ratio of cancer risk in the Dutch PJS cohort compared to the cancer risk in the Dutch general population, was calculated for the period between 1960 and 2009 by Poisson regression analysis. Although PJS patients can be considered at risk for cancer (and death) from birth, person-years at risk were calculated from the age of 5 years until the date of cancer diagnosis, date of death, date of last contact or the closing date of the study. We calculated person-years at risk from the age of 5, since establishment of the PJS diagnosis before that age can be difficult. As a result, recording of events (death or cancer incidence) in the first years of life is incomplete, as reflected by the fact that there was no perinatal mortality in our cohort. To adjust for possible differences in associated cancer risk between males and females, different age groups, and different time periods, the number of person-years were calculated according to sex and subdivided into 5-year age groups and 5-year calendar periods. Gender, age, calendar period and tumor-specific cancer incidence

rates of the general Dutch population were derived from the Comprehensive Cancer Centre South (1960-2007). These data are representative for the Netherlands.[23] Incidence rates for 2007 were assumed to be representative for 2008 and 2009. For the calculation of the relative cancer risk, only the first event contributed in case of multiple primary tumors. Since our cohort was too small to calculate relative cancer risks according to tumor origin (i.e. GI cancer), we determined the distribution of tumor origin according to age in the PJS cohort and compared this to the distribution in the general population by the Fisher's exact test (not adjusted for calendar period). For this comparison, age was categorized according to 25th and 75th percentile of age at first cancer in the PJS cohort: 5-34 years, 35-54 and 55 or older. Cancers diagnosed in the PJS cohort before 1960 were not included in these analyses as data on cancer incidence in the general population were not available for the period before 1960. Gynaecological cancer was defined as cancer originating in the cervix, uterus, and adnexa / ovaries.

Mortality in the PJS cohort was compared to mortality in the general population for the period between 1880 and 2009 by Poisson regression analysis in a similar manner as described above. Data on mortality in the general Dutch population between 1880 and 2006 were derived from the Human Mortality Database (University of California, Berkeley, USA, and Max Planck Institute for Demographic Research, Germany).[24] The number of deaths in the general population in 2006 was assumed to be representative for 2007, 2008 and 2009. We calculated overall relative cancer and mortality risks (hazard ratio's, HR), and also compared relative cancer and mortality risks between males and females, between the period before and after 1970, and between individuals younger and older than 45 years by adding interaction terms.

RESULTS

Study population

A total of 133 PJS patients were included, contributing to a total of 5004 person-years of follow-up (including 1400 person-years of prospective follow-up). These included 64 males (2687 person-years) and 69 females (2317 person-years). At the closing date of the study, 2 patients (1%) had been lost to follow-up, 42 patients (32%) had died at a median age of 45 (range 3-76 years), and the median age of the 89 patients (67%) still alive was 34 years. Baseline characteristics of the 133 included patients are shown in Table 2. They came from 54 different families, including the original Peutz kindred.[1,25] Probands generally came under medical attention because of the combination of pigmentations and complications of polyposis, predominantly abdominal pain. None of the probands presented with cancer at the first consultation. One hundred patients in the cohort had a family history of PJS, and the number of patients affected with PJS per family ranged from 2-24 (median 3, including

Table 2. Characteristics of the PJS cohort (n = 133).

Gender	
Male	64 (48%)
Female	69 (52%)
Age at the end of follow-up	
Median age (range) patients alive (n = 89)	34 (4-75) years
Median age (range) patients deceased (n = 42)	45 (3-76) years
Patients lost to follow-up (n = 2)	Not applicable
Family history	
Familial PJS	100 (75%)
Sporadic PJS	23 (17%)
Family history unknown	10 (8%)
DNA mutation analysis	80 (60%)
***STK11* mutation carrier**	77 / 80 (96%)
Cancer	42 (32%)

proband). *STK11* mutation analysis was performed in 80 patients (60%), and a pathogenic germline mutation was detected in 77 patients (96% of patients tested). A total of 81 patients underwent some kind of surveillance.

Cancer spectrum

Forty-nine cancers were diagnosed in 42 of the 133 included patients (32%), including 7 patients diagnosed with 2 primary carcinomas. The characteristics of the 49 malignancies, including 25 GI cancers, 6 gynaecological cancers and 6 breast cancers, are shown in Table 3. The GI cancers included 7 colorectal, 6 small intestinal, 4 gastric, 3 pancreatic and 2 biliary cancers as well as 3 adenocarcinomas from the digestive tract not further specified (Table 4). The median age at first cancer diagnosis was 45 years (range 15-76 years), and the median age at first GI cancer diagnosis was 42 years (range 15-76 years). There was no difference in cancer incidence between index cases (probands) and their relatives ($p = 0.46$), or between patients from the two participating hospitals ($p = 0.52$). Nineteen of the 42 patients diagnosed with cancer participated in a surveillance program; in 6 of the 42 patients (14%) the malignancy was discovered during surveillance, including 3 carcinomas in situ (1 colorectal and 2 gastric lesions). Twelve of the 19 patients under surveillance developed cancer in an organ not being under surveillance (i.e. lung cancer), and one male patient undergoing surveillance of the GI tract was diagnosed with liver metastasis of an adenocarcinoma of unknown primary origin. Another 2 cancers, both located outside the GI tract and diagnosed in 1960 and 1964 respectively, were solely based on anamnestic data and could not be confirmed histologically or by a medical report.

Table 3. Characteristics of the cancers observed in the PJS cohort.

Sex	Age diagn.	Year diagn.	Site / type of cancer	No. cancer	Confir-mation	Presen-tation
F [§]	15	1977	Jejunal adenocarcinoma	1	HI	C/O
F	16	1998	Malignant Sertoli cell tumor ovary	1 [a]	HI	C/O
M [§]	16	1962	Small intestinal adenocarcinoma	1 [b]	HI	C/O
F [*]	26	2008	Gastric adenocarcinoma	2 [a]	HI	SV
M	29	1992	Liposarcoma	1 [c]	HI	C/O
F	30	2005	Melanoma	1	MR	C/O
F	30	1999	Ovarian small cell carcinoma	1	HI	C/O
M	30	1979	Gastric adenocarcinoma	1	HI	C/O
M [*]	30	2004	Colorectal adenocarcinoma	1	HI	SV
M	31	1947 [‡]	Adenocarcinoma digestive tract	1	MR [1,25,59]	C/O
M	33	1953 [‡]	Seminoma of testis	1	HI	C/O
F	35	1994	Cervical adenoma malignum	1	HI	SV
F	35	1987	Pancreatic adenocarcinoma	1	HI	C/O
M	35	1998	Adenocarcinoma digestive tract	1	HI	C/O
F	36	1997	Adenocarcinoma digestive tract	1	HI	C/O
F	37	2004	Malignant Sertoli cell tumor ovary	1	HI	C/O
F	37	1996	Colorectal adenocarcinoma	1 [d]	HI	SV
F	40	1937 [‡]	Colorectal adenocarcinoma	1	MR [1,25,59]	C/O
M	41	2000	Ampullary adenocarcinoma	1	HI	C/O
M	43	1975	Colorectal adenocarcinoma	1	HI	C/O
F	45	2005	Broncheo-alveolar carcinoma	1	HI	C/O
F	45	1996	Broncheo-alveolar carcinoma	1	HI	C/O
F	45	1960	Cervical carcinoma	1	AN	C/O
M	45	2008	Pancreatic acinar cell carcinoma	2 [c]	HI	C/O
F	46	1944 [‡]	Breast carcinoma	1	MR [1,25,59]	C/O
F	48	2002	Pulmonary adenocarcinoma	1	HI	C/O
M	48	2001	Non-Hodgkin lymphoma (B-cell)	1 [e]	HI	C/O
F	49	2009	Breast carcinoma	2 [d]	HI	C/O
F [*]	49	2008	Gastric adenocarcinoma	1	HI	SV
F	51	1998	Melanoma	1	MR	C/O
F	51	1977	Squamous cell carcinoma of paranasal sinus	1	HI	C/O
M	51	2004	Pulmonary adenocarcinoma	1	HI	C/O
M	52	2001	Adenocarcinoma, unknown primary origin	1	HI	C/O
F	53	1964	Breast carcinoma	1	AN	C/O
M	53	2006	Ampullary adenocarcinoma	2 [e]	HI	C/O
M	54	2004	Ampullary adenocarcinoma	1	HI	C/O
M	54	1980	Jejunal adenocarcinoma	1	MR	C/O
F	55	2009	Breast carcinoma	1	MR	C/O

Table 3 (continued)

Sex	Age diagn.	Year diagn.	Site / type of cancer	No. cancer	Confir-mation	Presen-tation
M	57	2006	Biliary adenocarcinoma	1	HI	C/O
M	57	2004	Adenocarcinoma, unknown primary origin	2 [b]	HI	C/O
F	61	2006	Breast carcinoma	1	HI	SV
F	61	1996	Breast carcinoma	1	HI	C/O
F	61	1988	Colorectal adenocarcinoma	1 [f]	HI	C/O
M	61	1933 [‡]	Gastric adenocarcinoma	1	MR [1,25,59]	C/O
M	64	1996	Colorectal adenocarcinoma	1 [g]	HI	C/O
M	66	1998	Pancreatic adenocarcinoma	2 [g]	HI	C/O
F	72	1999	Cervical adenoma malignum	2 [f]	HI	C/O
F	73	2008	Biliary adenocarcinoma	1	HI	C/O
M	76	1937 [‡]	Colorectal adenocarcinoma	1	MR [1,25,59]	C/O

diagn. = cancer diagnosis, No = number, M = Male, F = Female, HI = Histology, MR = Medical record, AN = Anamnestic, SV = Surveillance, C/O = Complaints / Other.
[*] = Carcinoma in situ, [§] = No revision to rule out pseudo-invasion, [‡] = Diagnosed before 1960, hence not included in relative cancer risk analysis.
[1,25,59] = malignancies confirmed by previous reports, see references.
[a–g] = patients with two primary cancers: [a] = same patient, [b] = same patient, [c] = same patient, [d] = same patient, [e] = same patient, [f] = same patient, [g] = same patient.

Table 4. Cancers in the PJS cohort (n = 49) according to origin.

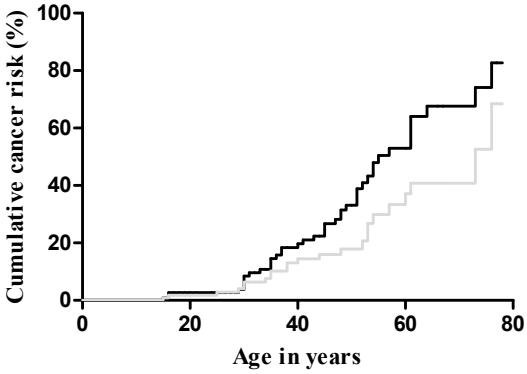
Origin	Number
Colorectal cancer	7
Small intestinal cancer	6
Gastric cancer	4
Pancreatic cancer	3
Biliary cancer	2
Adenocarcinoma digestive tract not further specified	3
Breast cancer	6
Cervical cancer	3
Ovarian cancer	3
Testis cancer	1
Lung cancer	4
Adenocarcinoma unknown primary origin	2
Other[*]	5

[*] Including 2 melanomas, 1 liposarcoma, 1 Non-Hodgkin lymphoma (B-cell), 1 squamous cell carcinoma of paranasal sinus.

Cumulative cancer risk

The Kaplan-Meier estimate for the cumulative cancer risk was 20% ±5% at age 40 (GI cancer 12% ±4%), 36% ±6% at age 50 (GI cancer 21% ±5%), 58% ±7% at age 60 (GI cancer 35% ±8%), and 76% ±8% at age 70 (GI cancer 51% ±10%) (Figure 1). The cumulative cancer risk was higher for females than males with PJS (HR 5.14; 95%CI 1.63 - 16.2, p = 0.005), but univariate analysis (log-rank) showed no difference in cumulative GI cancer risk between males and females (p = 0.845). There was no significant difference in cumulative cancer risk between sporadic PJS cases and patients with a family history of PJS (HR 0.56; 95%CI 0.17 - 1.85, p = 0.34), nor did mutation status affect cancer risk (HR 1.01; 95%CI 0.11 - 9.03, p = 0.99).

Figure 1. Cumulative cancer risks according to age.



Black line: Cumulative risk for any cancer.
Gray line: Cumulative risk for gastrointestinal cancer.

Relative cancer risk

Poisson regression analysis showed that the overall relative cancer risk was significantly higher in PJS patients than in the general population (HR 8.96; 95%CI 6.46 - 12.42, p < 0.001), with a higher risk (p < 0.001) in females (HR 20.40; 95%CI 13.43 - 30.99) than in males with PJS (HR 4.76; 95%CI 2.82 - 8.04) as compared to the female and male general population, respectively. There was no relative cancer risk difference (p = 0.28) between the period before (HR 16.50; 95%CI 5.32 - 51.14) and after 1970 (HR 8.60; 95%CI 6.11 - 12.10), but there was a trend (p = 0.08) for a higher relative cancer risk in PJS patients < 45 years (HR 12.88; 95%CI 7.89 - 21.02) than in PJS patients ≥ 45 years (HR 7.20; 95%CI 4.65 - 11.16). Table 5 demonstrates that GI cancers account for a significantly larger proportion of all cancers in our PJS cohort than in the general population, especially among males and younger patients.

Table 5. Proportional distribution of tumor origin according to age in PJS patients compared to the general population.

Tumor	Age (years)	PJS cohort % (absolute numbers)	General population	p-value
GI cancer (♂ + ♀)	All ages	48.8% (21/43)	22.7%	< 0.001
	5-34	55.5% (5/9)*	5%*	< 0.001*
	35-54	45.8% (11/24)	15.3%	< 0.001
	55+	50% (5/10)	25.3%	0.073
GI cancer (♂)	All ages	72.2% (13/18)	23.5%	< 0.001
	5-34	75% (3/4)	5.9%	0.001
	35-54	70% (7/10)	23.6%	0.002
	55+	75% (3/4)	24.2%	0.046
GI cancer (♀)	All ages	32% (8/25)	21.7%	0.214
	5-34	40% (2/5)	4.2%	0.017
	35-54	28.6% (4/14)	10.3%	0.048
	55+	33.3% (2/6)	26.9%	0.663
Gynaecological Cancer (♀)	All ages	24% (6/25)	11.5%	0.051
	5-34	40% (2/5)	13.8%	0.144
	35-54	21.4% (3/14)	12.6%	0.406
	55+	16.7% (1/6)	11.1%	0.505
Breast cancer (♀)	All ages	20% (5/25)	32.7%	0.174
	5-34	0% (0/5)	22.1%	0.593
	35-54	14.3% (2/14)	48.1%	0.014
	55+	50% (3/6)	28%	0.359

* i.e. 55.5% of cancers in the PJS cohort (♂+♀) aged 5-34 years concerned GI cancers (5 GI cancers among 9 cancers diagnosed in this subgroup = 55.5%), whereas this was 5% in the general population (♂+♀) aged 5-34 years (p < 0.001).
Note: Cancers diagnosed in the PJS cohort before 1960 were not included in these analyses.

Mortality

The cause of death of the 42 deceased patients is depicted in Table 6. Most patients died as a result of cancer (67%) or bowel intussusception (19%). All intussusception-related deaths occurred before 1970. The median age at death was 45 years (range 3-76 years). The youngest

Table 6. Mortality in the PJS cohort: cause of death and relative mortality risk.

Cause of death (n=42)		
Cancer	n = 28 (67%)	
Bowel intussusception	n = 8 (19%)	
Other	n = 3	
Unknown	n = 3	
Relative mortality risk:		
HR (PJS -vs- general population)	3.50 (95%CI 2.57 - 4.75)	
- HR ♂ PJS	2.81 (95%CI 1.85 - 4.26)	p = 0.076
- HR ♀ PJS	4.89 (95%CI 3.12 - 7.66)	
- HR ≥ 1970	3.44 (95%CI 3.35 - 5.06)	p = 0.90
- HR < 1970	3.59 (95%CI 2.16 - 5.95)	
- HR ≥ 45 years	4.17 (95%CI 2.75 - 6.34)	p = 0.26
- HR < 45 years	2.94 (95%CI 1.88 - 4.61)	

HR = Hazard ratio, 95%CI = 95% confidence interval

7 deceased patients died as a result of an acute intussusception at ages between 3 and 20 years, and the youngest age at cancer-related death was 30 years. There was a clear excess mortality risk of 250% in the PJS cohort compared to the general population (HR 3.50; 95%CI 2.57 - 4.75, $p < 0.001$). Table 6 shows that the mortality excess tended to be higher (borderline significance, $p = 0.076$) among female than male PJS patients. There was no relative mortality risk difference between the period before and after 1970 or between PJS patients < 45 years and ≥ 45 years, as compared to the general population.

DISCUSSION

This prospective cohort study demonstrates that PJS patients carry a markedly elevated cancer risk at young age, concerning primarily cancers in the GI tract. The life time cumulative cancer risk is more than 76% in PJS patients, and higher in females than in males, but independent of family history and *STK11* mutation status. The relative cancer risk adjusted for age, sex and calendar period is nearly 10 times higher than in the general population, and the relative cancer risk was most pronounced in females suffering from PJS. The fact that cancer risks are higher in female PJS patients than in male patients can largely be explained by the additional risk of breast cancer and gynaecological cancers, as there was no difference in cumulative GI cancer risk between males and females. In addition to the elevated cancer risk we also demonstrated an increased mortality in PJS patients compared to the general population, with a trend for a larger mortality excess among female PJS patients than in males with PJS. The increased mortality can in part be explained by the elevated cancer risk. Apart from cancer, acute bowel intussusception was, at least before 1970, another important cause of death. Our results suggest that surveillance of PJS patients may prolong life expectancy and improve outcome of these patients, by early detection of carcinomas and timely removal of hamartomas in order to prevent intussusception.

The presented elevated cancer risk is in line with previous reports on the increased cancer risk associated with PJS.[6,11-21,26-32] The cumulative cancer risks in relation to age were similar in our study as in a previous study[12,13,28], i.e. 20 versus 17% at age 40, 36 versus 31% at age 50, 58 versus 60% at age 60 and 76 versus 85% at the age of 70 years. However, in general the relative and cumulative cancer risks in our study are lower than the previously reported relative cancer risk up to 18 and life time cumulative cancer risk up to 93%.[6,12,13,15,19,28] This might be explained by differences in period of follow-up or the use of other inclusion criteria. In the present study patients were included on the basis of the WHO criteria, whereas most other studies used clinical criteria as described by Giardiello and colleagues.[15]

Our study has some important advantages over the previously performed studies. We describe a unique large pedigree-based cohort of Dutch patients with thorough case ascertainment and a substantial prospective period of follow-up. In contrast, previous large

studies concern a meta-analysis (with a similar number of person-years of follow-up as in our study)[6] and multinational collaborations, retrospectively evaluating heterogeneous PJS cohorts.[12,13,28] Collaborative studies may introduce bias in calculating relative cancer risks since cancer incidences vary between countries, whereas we compared Dutch PJS patients exposed to similar environmental factors, to the Dutch general population. Another advantage of our study is that the relative cancer risks calculated have not only been adjusted for age and sex like in the previous studies, but also for calendar period. Moreover, this is the first study to specifically report on mortality and cause of death among PJS patients.

However, this study also has some limitations. First of all, selection bias may have resulted in overestimated cancer risks, as particularly patients with most severe phenotypes may have been included. Nevertheless, patients were included systematically regardless of their medical history to minimize this form of bias, and there was no difference in cancer incidence between probands and relatives making ascertainment bias less likely. In addition, referral bias might have led to overestimation of cancer risks, if only patients with a severe course of the disease (including intussusception and cancer) have been referred to our specialised centres. Furthermore, detailed data on surveillance in our cohort are missing since there has not been a nation-wide implemented surveillance strategy for PJS patients in the Netherlands until recently. One might postulate that surveillance could have increased the cancer incidence in our population, as 6 (12%) of the 49 cancers in our cohort, including 3 intramucosal neoplasias, were diagnosed during surveillance in asymptomatic patients. Yet, the development of a symptomatic invasive carcinoma would have been a matter of time in these young patients (26, 30, 35, 37, 49 and 61 years old, respectively), and therefore these cases were included in our risk analyses.

Another pitfall in the calculation of the cancer risk in PJS is a phenomenon called pseudo-invasion. Pseudo-invasion is epithelial displacement through the muscularis mucosae, mimicking an invading carcinoma.[33-35] This phenomenon has been observed in approximately 10% of small-bowel polyps in one study, and can be distinguished from invasive carcinoma by the lack of cytological atypia.[36] On histological revision, we found one case of pseudo-invasion of the small-bowel in our cohort, which has been excluded from further cancer-risk analyses.[33] However, no tissue was available from two small intestinal cancers (Table 3), thus pseudo-invasion could not be ruled out in these two cases.

The exact mechanism of carcinogenesis in PJS remains to be established. One unresolved question, important in light of surveillance, is whether the malignancies in the stomach, small intestine and colorectum originate from the hamartomas or from coexisting adenomas or otherwise normal appearing mucosa.[37,38] Although several studies have reported a hamartoma-adenoma-carcinoma sequence[17,39,40], other facts contradict this theory. For example, the number of polyps decreases with advancing age, whereas the cancer incidence increases with advancing age.[6,32,41] Furthermore, the location of GI cancers in PJS patients does not always correlate with the location of the hamartomas[38], as we also demonstrated in

this series; more colorectal cancers (n=7) were diagnosed than small intestinal cancers (n=6), whereas the small intestine is the preferential localisation for the hamartomas.

To answer the question whether or not hamartomas are pre-malignant and to gain more insight into PJS-related carcinogenesis, further basal research is required. Although it has been suggested that a second gene locus responsible for PJS might exist[42,43], we believe that future research should focus on the *STK11* gene function since *STK11* mutations can already be detected in more than 90% of patients (96% in these series) with new available techniques.[4,5] The development of hamartomas and malignancies might be independent stromal and epithelial processes[8], which complicates the elucidation of the molecular mechanisms underlying *STK11*-associated carcinogenesis. The exact role of *STK11* in the carcinogenic pathway is still unclear, but up-regulation of mTOR signaling seems to be an important step as mTOR inhibitors have been shown to reduce tumor burden in mouse models.[44,45] Elucidating the molecular background of cancer susceptibility in PJS patients might reveal therapeutic options.

Nevertheless, the cancer risks observed in our study are very high and come close to other high-risk conditions for which surveillance has been recommended.[46,47] Although the wide tumor spectrum in PJS makes screening for cancer a difficult task in the individual patient, the high cancer risks justify surveillance of PJS patients. Several surveillance recommendations for PJS have previously been published.[48-58] On the basis of the results presented here,

Table 7. Surveillance recommendations for PJS patients.[7]

Examination*	Starting age	Interval
History, physical examination (including palpation testis) and haemoglobin analysis	10 years	1 years (paediatrician)
Video capsule endoscopy (VCE) and/or MRI-enteroclysis‡	10 years	2-3 years
Gastroduodenoscopy	20 years	2-5 years (depending on findings)
Colonoscopy	25-30 years	2-5 years (depending on findings)
MRI & endoscopic ultrasonography (EUS) pancreas	30 years	1 year, only in a prospective ongoing trial.[60]
Breast exam & breast MRI Mammography & breast MRI	25 years 30 years	1 year 1 year§
Pelvic exam, cervical smear, transvaginal ultrasonography and CA-125.	25-30 years	1 year

* Earlier and/or more frequently in symptomatic patients / if clinically indicated.

‡ If VCE shows polyps it's recommended to perform an MRI-enteroclysis to determine the exact localisation and size if the polyps. Polyps > 1 cm in diameter are an indication for double balloon enteroscopy (DBE) with polypectomy.
In addition, we recommend intra-operative enteroscopy with polyp removal in every indicated laparotomy, to avoid re-laparotomies. If surgery is indicated a laparoscopic approach is preferred when possible.

§ Mammography and MRI alternately performed every six months.

our previous review on the elevated cancer risk in PJS[7], and the elevated intussusception risk in PJS patients (submitted), we have also formulated a surveillance recommendation in collaboration with a national working group (Table 7).[7] The main differences between this surveillance recommendation and previously published guidelines have been discussed earlier.[7] Our surveillance recommendation is solely based on expert opinion, since no controlled trials have been published on the effectiveness of surveillance in PJS. The optimal surveillance strategy remains to be established in prospective trials.

In conclusion, PJS patients carry a markedly elevated cancer risk with cancers in particular affecting the GI tract. Female patients are furthermore at high risk for the development of gynaecological tumors and breast cancer. The increased cancer risk, combined with the elevated intestinal intussusception risk, leads to an increased mortality. Although the benefits of surveillance remain to be established, surveillance seems justified. The effect of surveillance on the cancer and intussusception incidence, outcome and survival, as well as cost-effectiveness, will have to be established in prospective trials.

ACKNOWLEDGMENTS

The authors would like to thank Drs. F. Santegoets, Erasmus MC University Medical Center, for his help in the database development.

REFERENCES

1. Peutz JLA. Over een zeer merkwaardige, gecombineerde familiaire polyposis van de slijmliezen van den tractus intestinalis met die van de neuskeelholte en gepaard met eigenaardige pigmentaties van huid en slijmvliezen. Ned Maandschr v Geneesk 1921;10:134-146.
2. Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 1998;391(6663):184-187.
3. Jenne DE, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat Genet 1998;18(1):38-43.
4. Volikos E, Robinson J, Aittomaki K, Mecklin JP, Jarvinen H, Westerman AM, et al. LKB1 exonic and whole gene deletions are a common cause of Peutz-Jeghers syndrome. J Med Genet 2006; 43(5):e18.
5. Aretz S, Stienen D, Uhlhaas S, Loff S, Back W, Pagenstecher C, et al. High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome. Hum Mutat 2005;26(6):513-519.
6. Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119(6):1447-1453.
7. van Lier MG, Wagner A, Mathus-Vliegen EM, Kuipers EJ, Steyerberg EW, van Leerdam ME. High Cancer Risk in Peutz-Jeghers Syndrome: A Systematic Review and Surveillance Recommendations. Am J Gastroenterol 2010;105(6):1258-1264.
8. Hezel AF, Bardeesy N. LKB1; linking cell structure and tumor suppression. Oncogene 2008;27(55): 6908-6919.
9. Scully RE. Sex cord tumor with annular tubules a distinctive ovarian tumor of the Peutz-Jeghers syndrome. Cancer 1970;25(5):1107-1121.
10. Young S, Gooneratne S, Straus FH, Zeller WP, Bulun SE, Rosenthal IM. Feminizing Sertoli cell tumors in boys with Peutz-Jeghers syndrome. Am J Surg Pathol 1995;19(1):50-58.
11. Lim W, Hearle N, Shah B, Murday V, Hodgson SV, Lucassen A, et al. Further observations on LKB1/ STK11 status and cancer risk in Peutz-Jeghers syndrome. Br J Cancer 2003;89(2):308-313.
12. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 2006;12(10):3209-3215.
13. Mehenni H, Resta N, Park JG, Miyaki M, Guanti G, Costanza MC. Cancer risks in LKB1 germline mutation carriers. Gut 2006;55(7):984-990.
14. Linos DA, Dozois RR, Dahlin DC, Bartholomew LG. Does Peutz-Jeghers syndrome predispose to gastrointestinal malignancy? A later look. Arch Surg 1981;116(9):1182-1184.
15. Giardiello FM, Welsh SB, Hamilton SR, Offerhaus GJ, Gittelsohn AM, Booker SV, et al. Increased risk of cancer in the Peutz-Jeghers syndrome. N Engl J Med 1987;316(24):1511-1514.
16. Spigelman AD, Murday V, Phillips RK. Cancer and the Peutz-Jeghers syndrome. Gut 1989;30(11): 1588-1590.
17. Hizawa K, Iida M, Matsumoto T, Kohrogi N, Kinoshita H, Yao T, et al. Cancer in Peutz-Jeghers syndrome. Cancer 1993;72(9):2777-2781.
18. Boardman LA, Thibodeau SN, Schaid DJ, Lindor NM, McDonnell SK, Burgart LJ, et al. Increased risk for cancer in patients with the Peutz-Jeghers syndrome. Ann Intern Med 1998;128(11):896-899.
19. Choi HS, Park YJ, Youk EG, Yoon KA, Ku JL, Kim NK, et al. Clinical characteristics of Peutz-Jeghers syndrome in Korean polyposis patients. Int J Colorectal Dis 2000;15(1):35-38.
20. Scott RJ, Crooks R, Meldrum CJ, Thomas L, Smith CJ, Mowat D, et al. Mutation analysis of the STK11/LKB1 gene and clinical characteristics of an Australian series of Peutz-Jeghers syndrome patients. Clin Genet 2002;62(4):282-287.

21. Amos CI, Keitheri-Cheteri MB, Sabripour M, Wei C, McGarrity TJ, Seldin MF, et al. Genotype-phenotype correlations in Peutz-Jeghers syndrome. J Med Genet 2004;41(5):327-333.
22. Aaltonen LA, Jarvinen H, Gruber SB, Billaud M, Jass JR. World Health Organization Classification of Tumours. Pathology and Genetics. Tumours of the Digestive System. Lyon: IARC Press; 2000.
23. www.ikcnet.nl. 2010.
24. www.mortality.org. 2010.
25. Westerman AM, Entius MM, de Baar E, Boor PP, Koole R, van Velthuysen ML, et al. Peutz-Jeghers syndrome: 78-year follow-up of the original family. Lancet 1999;353(9160):1211-1215.
26. Mehenni H, Resta N, Guanti G, Mota-Vieira L, Lerner A, Peyman M, et al. Molecular and clinical characteristics in 46 families affected with Peutz-Jeghers syndrome. Dig Dis Sci 2007;52(8):1924-1933.
27. Schumacher V, Vogel T, Leube B, Driemel C, Goecke T, Moslein G, et al. STK11 genotyping and cancer risk in Peutz-Jeghers syndrome. J Med Genet 2005;42(5):428-435.
28. Lim W, Olschwang S, Keller JJ, Westerman AM, Menko FH, Boardman LA, et al. Relative frequency and morphology of cancers in STK11 mutation carriers. Gastroenterology 2004;126(7):1788-1794.
29. Olschwang S, Boisson C, Thomas G. Peutz-Jeghers families unlinked to STK11/LKB1 gene mutations are highly predisposed to primitive biliary adenocarcinoma. J Med Genet 2001;38(6):356-360.
30. Foley TR, McGarrity TJ, Abt AB. Peutz-Jeghers syndrome: a clinicopathologic survey of the "Harrisburg family" with a 49-year follow-up. Gastroenterology 1988;95(6):1535-1540.
31. Burdick D, Prior JT. Peutz-Jeghers syndrome. A clinicopathologic study of a large family with a 27-year follow-up. Cancer 1982;50(10):2139-2146.
32. Utsunomiya J, Gocho H, Miyanaga T, Hamaguchi E, Kashimure A. Peutz-Jeghers syndrome: its natural course and management. Johns Hopkins Med J 1975;136(2):71-82.
33. Westerman AM, van Velthuysen ML, Bac DJ, Schouten WR, Wilson JH. Malignancy in Peutz-Jeghers syndrome? The pitfall of pseudo-invasion. J Clin Gastroenterol 1997;25(1):387-390.
34. Petersen VC, Sheehan AL, Bryan RL, Armstrong CP, Shepherd NA. Misplacement of dysplastic epithelium in Peutz-Jeghers Polyps: the ultimate diagnostic pitfall? Am J Surg Pathol 2000;24(1): 34-39.
35. Jansen M, de Leng WW, Baas AF, Myoshi H, Mathus-Vliegen L, Taketo MM, et al. Mucosal prolapse in the pathogenesis of Peutz-Jeghers polyposis. Gut 2006;55(1):1-5.
36. Shepherd NA, Bussey HJ, Jass JR. Epithelial misplacement in Peutz-Jeghers polyps. A diagnostic pitfall. Am J Surg Pathol 1987;11(10):743-749.
37. Hizawa K, Iida M, Matsumoto T, Kohrogi N, Yao T, Fujishima M. Neoplastic transformation arising in Peutz-Jeghers polyposis. Dis Colon Rectum 1993;36(10):953-957.
38. Dozois RR, Judd ES, Dahlin DC, Bartholomew LG. The Peutz-Jeghers syndrome. Is there a predisposition to the development of intestinal malignancy? Arch Surg 1969;98(4):509-517.
39. Perzin KH, Bridge MF. Adenomatous and carcinomatous changes in hamartomatous polyps of the small intestine (Peutz-Jeghers syndrome): report of a case and review of the literature. Cancer 1982;49(5):971-983.
40. Gruber SB, Entius MM, Petersen GM, Laken SJ, Longo PA, Boyer R, et al. Pathogenesis of adenocarcinoma in Peutz-Jeghers syndrome. Cancer Res 1998;58(23):5267-5270.
41. Hemminki A. The molecular basis and clinical aspects of Peutz-Jeghers syndrome. Cell Mol Life Sci 1999;55(5):735-750.
42. Mehenni H, Gehrig C, Nezu J, Oku A, Shimane M, Rossier C, et al. Loss of LKB1 kinase activity in Peutz-Jeghers syndrome, and evidence for allelic and locus heterogeneity. Am J Hum Genet 1998; 63(6):1641-1650.

43. Buchet-Poyau K, Mehenni H, Radhakrishna U, Antonarakis SE. Search for the second Peutz-Jeghers syndrome locus: exclusion of the STK13, PRKCG, KLK10, and PSCD2 genes on chromosome 19 and the STK11IP gene on chromosome 2. Cytogenet Genome Res 2002;97(3-4):171-178.
44. Wei C, Amos CI, Zhang N, Wang X, Rashid A, Walker CL, et al. Suppression of Peutz-Jeghers polyposis by targeting mammalian target of rapamycin signaling. Clin Cancer Res 2008;14(4):1167-1171.
45. Robinson J, Lai C, Martin A, Nye E, Tomlinson I, Silver A. Oral rapamycin reduces tumour burden and vascularization in Lkb1(+/-) mice. J Pathol 2009;219(1):35-40.
46. de Jong AE, Hendriks YM, Kleibeuker JH, de Boer SY, Cats A, Griffioen G, et al. Decrease in mortality in Lynch syndrome families because of surveillance. Gastroenterology 2006;130(3):665-671.
47. Saslow D, Boetes C, Burke W, Harms S, Leach MO, Lehman CD, et al. American Cancer Society guidelines for breast screening with MRI as an adjunct to mammography. CA Cancer J Clin 2007; 57(2):75-89.
48. Tomlinson IP, Houlston RS. Peutz-Jeghers syndrome. J Med Genet 1997;34(12):1007-1011.
49. Lindor NM, Greene MH. The concise handbook of family cancer syndromes. Mayo Familial Cancer Program. J Natl Cancer Inst 1998;90(14):1039-1071.
50. Burt RW. Colon cancer screening. Gastroenterology 2000;119(3):837-853.
51. The Johns Hopkins guide for patients and families: Peutz-Jeghers syndrome. Baltimore: Johns Hopkins University. 2001.
52. McGrath DR, Spigelman AD. Preventive measures in Peutz-Jeghers syndrome. Fam Cancer 2001; 1(2):121-125.
53. Dunlop MG. Guidance on gastrointestinal surveillance for hereditary non-polyposis colorectal cancer, familial adenomatous polypolis, juvenile polyposis, and Peutz-Jeghers syndrome. Gut 2002;51 Suppl 5:V21-V27.
54. Allen BA, Terdiman JP. Hereditary polyposis syndromes and hereditary non-polyposis colorectal cancer. Best Pract Res Clin Gastroenterol 2003;17(2):237-258.
55. Hinds R, Philp C, Hyer W, Fell JM. Complications of childhood Peutz-Jeghers syndrome: implications for pediatric screening. J Pediatr Gastroenterol Nutr 2004;39(2):219-220.
56. McGarrity TJ, Amos C. Peutz-Jeghers syndrome: clinicopathology and molecular alterations. Cell Mol Life Sci 2006;63(18):2135-2144.
57. Giardiello FM, Trimbath JD. Peutz-Jeghers Syndrome and Management Recommendations. Clinical Gastroenterology and Hepatology 2006;4(4):408-415.
58. Beggs AD, Latchford AR, Vasen HF, Moslein G, Alonso A, Aretz S, et al. Peutz-Jeghers syndrome: a systematic review and recommendations for management. Gut 2010;59(7):975-986.
59. van Wijk Th W. Over het syndroom polyposis adenomatosa gastro-intestinalis generalisata heredofamiliaris gecombineerd met huid- en slijmvliespigmentaties of ziekte van Peutz (thesis). Leiden, The Netherlands: 1950.
60. Poley JW, Kluijt I, Gouma DJ, Harinck F, Wagner A, Aalfs C, et al. The Yield of First-Time Endoscopic Ultrasonography in Screening Individuals at a High Risk of Developing Pancreatic Cancer. Am J Gastroenterol 2009;104(9):2175-2181.

4

High cumulative risk of intussusception in patients with Peutz-Jeghers syndrome: Time to update surveillance guidelines?

Am J Gastroenterol. 2010 Dec 14. Epub ahead of print (doi:10.1038/ajg.2010.473)

M.G.F. van Lier[1]
E.M.H. Mathus-Vliegen[2]
A. Wagner[3]
M.E. van Leerdam[1]
E.J. Kuipers[1,4]

Departments of Gastroenterology and Hepatology[1], Clinical Genetics[3] and Internal Medicine[4], Erasmus MC, University Medical Center, Rotterdam, the Netherlands. Department of Gastroenterology and Hepatology[2], Academic Medical Center, Amsterdam, the Netherlands.

ABSTRACT

Background: Peutz-Jeghers syndrome (PJS) is characterized by gastrointestinal hamartomas. The hamartomas are located predominantly in the small-intestine and may cause intussusceptions. We aimed to assess characteristics, risk and onset of intussusception in a large cohort of PJS patients, to determine whether enteroscopy with polypectomy should be incorporated into surveillance recommendations.

Methods: All PJS patients from 2 academic hospitals were included in this cohort study (prospective follow-up between 1995 and July 2009). We obtained clinical data by interview and chart-review. Deceased family members with PJS were included retrospectively. Cumulative intussusception risks were calculated by Kaplan-Meier analysis.

Results: We included 110 PJS patients (46% males) from 50 families. Seventy-six patients (69%) experienced at least one intussusception (range 1-6), at a median age of 16 (3-50) years at first occurrence. The intussusception risk was 50% at the age of 20 years (95%CI 17-23 yrs) and the risk was independent of sex, family history and mutation status. The intussusceptions occurred in the small-intestine in 95% of events and 80% of all intussusceptions (n=128) presented as an acute abdomen. Therapy was surgical in 92.5% of events. Based on 37 histology reports, the intussusceptions were caused by polyps with a median size of 35 mm (range 15-60 mm).

Conclusions: PJS patients carry a high cumulative intussusception risk at young age. Intussusceptions are generally caused by polyps larger than 15 mm and treatment is mostly surgical. These results support the approach of enteroscopic surveillance with removal of small-intestinal polyps larger than 10-15 mm, to prevent intussusceptions. The effect of such an approach on the incidence of intussusception remains to be established in prospective trials.

INTRODUCTION

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant inherited disorder characterized by gastrointestinal hamartomas, mucocutaneous pigmentations and an elevated cancer risk.[1,2] PJS is caused by *STK11* mutations and a pathogenic germline mutation can be detected in approximately 80% of clinically affected patients.[3] The hamartomas associated with PJS are located in the small-intestine in more than 90% of patients, predominantly in the jejunum.[2] Even at young age the hamartomas may cause complications including anaemia, bleeding, abdominal pain, and intussusception.[4-6]

In the past, small-intestinal intussusceptions have been a major cause of death in PJS patients.[7] Fortunately, over the last decade the options for surveillance and treatment of small-intestinal hamartomas have increased. New techniques such as video capsule endoscopy and balloon-assisted enteroscopy (BAE) allow the entire small-intestine to be visualized.[8] These techniques are now widely available for clinical practice. An advantage of BAE is that it enables endoscopic removal of small-intestinal polyps. This may prevent complications such as intussusception, reducing the need for laparotomies and thereby improving outcome.

BAE is however an invasive procedure carrying a risk for complications and the risk of intussusception will have to be weighed against the risk of intervention. Unfortunately, there is a lack of detailed data on the risk of intussusception in PJS patients. This lack hampers the formulation of surveillance recommendations and the clinical management of PJS patients. Therefore, the aim of this study was to assess characteristics, risk and onset of intussusceptions in a large cohort of PJS patients.

METHODS

In this cohort study we included patients diagnosed with PJS on the basis either of diagnostic criteria as defined by the World Health Organisation[9] (Table 1) or of a proven *STK11* mutation. All PJS patients from two Dutch academic hospitals (Erasmus MC University Medical Centre, Rotterdam and Academic Medical Centre, Amsterdam) were included without selection for medical history. Patients were followed prospectively between January 1995 and July 2009, and clinical data from the period before 1995 were collected retrospectively. In addition, family pedigrees were traced backwards and laterally as far as possible, and data of deceased family members fulfilling the diagnostic criteria for PJS were collected retrospectively. The study was approved by the Institutional Review Board of both hospitals.

Patient information was obtained by interview and chart-review. The following data were collected per patient: sex, date of birth, diagnosis of PJS, family history of PJS, *STK11* mutation status, diagnosis and characteristics of intussusception, and date and cause of death (either intussusception-related or other cause of death). Intussusception characteristics that were

recorded included date of diagnosis as well as data on confirmation, presentation, localisation, therapy, and size of the polyp causing the intussusception. Presentation of intussusception was defined as an acute abdomen in case of acute abdominal pain in combination with nausea and vomiting. Localisation of the intussusception was classified as small-intestinal or colonic based on the site of the leading point; intussusceptions with a leading point proximal to the ileo-cecal valve were classified as small-intestinal, and those with a leading point distal to the ileo-cecal valve were classified as colonic. The small-intestinal intussusceptions were further subdivided according to the most proximal small-intestinal segment involved (i.e. a jejunoileal intussusception was classified as a jejunal). Therapy was classified as surgical, endoscopic by BAE (BAE has been available in the Netherlands since 2004) or conservative by barium-enema. Finally, data on the size of the polyps causing intussusception were derived from original histology reports whenever available (i.e. not by re-evaluation of pathological specimen). We only used the size of en-bloc resected polyps as measured (in millimeters) by the pathologist, in order to get an objective measurement. *STK11* mutation analysis was performed by denaturing gradient-gel electrophoresis and direct sequencing to detect mononucleotide changes and small deletions and insertions. Multiplex Ligation-Dependent Probe Amplification and long-range PCR were used to screen for large-scale gene deletions.

Data were analyzed using the SPSS 15.0 statistical software for Windows, and data were reported using descriptive statistics. Patients were considered at risk for intussusception from birth onwards. The cumulative intussusception risk was calculated by Kaplan-Meier analysis. Differences in intussusception risk according to sex, *STK11* mutation status and family history were analyzed with Cox's proportional hazard regression analyses. Two-sided p-values less than 0.05 were considered statistically significant.

RESULTS

A total of 110 PJS patients from 50 families were included for analysis. These 110 patients contributed to a total of 3967 person-years at risk, with a median number of 17 person-years at risk (range 3-61 years). Another 23 patients were excluded due to incomplete data on the occurrence of intussusception (18 deceased family members with PJS and 5 patients with an incomplete medical history). All included patients fulfilled the diagnostic criteria for PJS defined by the World Health Organisation (Table 1), and baseline characteristics of these 110 patients are shown in Table 2. Ninety-three individuals were born before 1995 and 17 individuals were born since January 1995. By the end of follow-up (July 2009), 24 patients had deceased at a median age of 44 years (range 3-74 years) and 86 patients were alive at a median age of 35 years (range 4-75 years). A total of 80 patients had a family history of PJS, and the number of patients affected with PJS per family ranged from 2-17 (median of 3 per family, including the proband).

Table 1. Diagnostic criteria for PJS recommended by the World Health Organisation[9] and number of patients included per criterion.

	No. of patients
A) *Positive* family history of PJS, and	80
1. Any number of histologically confirmed PJS polyps*, or	44
2. Characteristic prominent mucocutaneous pigmentation.	36
B) *Negative* family history of PJS, and	30
1. Three or more histologically confirmed PJS polyps*, or	29
2. Any number of histologically confirmed PJS polyps* and characteristic, prominent, mucocutaneous pigmentation.	1

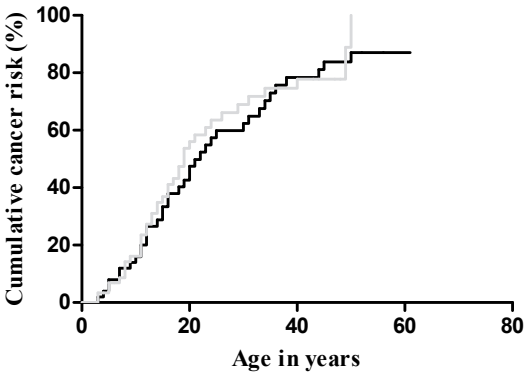
* Histology PJS polyps: A central core of smooth muscle that shows tree-like branching, covered by the mucosa native to the region which is heaped into folds producing a villous pattern.
Note: a total of 73 patients carried a proven *STK11* mutation.

Table 2. Baseline characteristics of 110 included patients.

Gender (n = 110)	**n (%)**
Male	51 (46%)
Female	59 (54%)
Age at the end of follow-up (n = 110)	**Median (range)**
Age patients alive (n = 86)	35 (4-75) years
Age deceased patients at death (n = 24)	44 (3-74) years
Cause of death (n = 24)	**n (%)**
Intussusception	8 / 24 (33%)
Other	16 / 24 (67%)
Cancer	14
Cardiovascular	2
Family history (n = 110)	**n (%)**
Positive family history of PJS (i.e. familial cases)	80 (73%)
No family history for PJS (i.e. sporadic PJS cases)	22 (20%)
Family history unknown	8 (7%)
DNA mutation analysis (n = 110)	**n (%)**
No DNA mutation analysis	34 (31%)
DNA mutation analysis performed	76 (69%)
Familial cases	51
Sporadic cases / family history unknown	25
Results DNA mutation analysis (n = 76)	**n (%)**
Pathogenic *STK11* mutation detected	73 / 76 (96%)
Familial cases	51
Sporadic cases / family history unknown	22
No *STK11* mutation detectable	3 / 76 (4%)
Intussusception occurrence (n = 110)	**n (%)**
History of intussusception	76 (69%)
No history of intussusception	34 (31%)

STK11 mutation analysis was performed in 76 patients (69%). A pathogenic germline mutation was detected in 73 patients (96% of patients tested), and in 3 patients no mutation was detectable. In all, 77 patients underwent some form of gastrointestinal surveillance, but time-intervals and diagnostic approaches could not be further specified. Ninety patients (82%)

Figure 1. Cumulative intussusception risk stratified for sex.



Gray line: Cumulative intussusception risk in females.
Black line: Cumulative intussusception risk in males.

had a history of gastrointestinal polyps (89 patients had ≥ 3 polyps, one patient had a solitary polyp). In 74 of these 90 cases the polyps were histologically classified as hamartomas as can be seen in the spectrum of PJS (i.e. polyps composed of abnormal arrangements of branching smooth muscle, covered by normal-appearing or hyperplastic glandular epithelium). In 11 patients, all younger than 20 years, no polyps had been detected yet, and in another 9 patients the presence of polyps was unknown. However, 5 of the latter 9 patients had a personal history of intussusception, so the presence of polyps is most likely in these subjects.

Seventy-six patients (69%) had experienced at least one intussusception (range 1-6 intussusceptions), and the median age at first intussusception was 16 years (range 3-50 years). Eight patients (7% of all included patients) had deceased as a result of an intussusception (all intussusception related deaths occurred before 1970), the youngest at age 3 (range 3-38 years). The Kaplan-Meier estimate for the cumulative intussusception risk was 15% (95%CI 8-22%) at age 10, 50% (95%CI 40-60%) at age 20, 65% (95%CI 55-75%) at age 30, 77% (95%CI 68-86%) at age 40, and 84% (95%CI 75-93%) at age 50. There was no significant difference in intussusception risk between males and females (hazard ratio 1.2; 95%CI 0.6 - 2.3; Figure 1), between sporadic PJS cases and patients with a family history of PJS (hazard ratio 0.7; 95%CI 0.3 - 1.3), or according to mutation status (hazard ratio 3.1; 95%CI 0.4 - 24.0).

A total of 128 intussusceptions occurred in 76 patients, presenting as an acute abdomen in 80% of all events. Forty-one intussusceptions occurred in 35 different patients during the prospective period of follow-up (1995-2009), and another 87 intussusceptions occurred in 53 patients in the retrospective period of follow-up (before 1995). The clinical characteristics of the 128 intussusceptions are shown in Table 3. On the basis of 110 intussusceptions, localisation was classified as small-intestinal in 105 events (95%) and colonic in only 5 events (5%). Localisation was unknown in 18 events. Of the small-intestinal intussusceptions, the localisation could be further subdivided in 60 events; 32 small-intestinal intussusceptions were classified as jejunal (53%) and 28 as ileal (47%). Treatment was recorded in 120 events

Table 3. Characteristics of 128 intussusceptions in 76 patients.

	Number (%)
Confirmation diagnosis	**n = 128**
Surgery report	107 / 128 (83.5%)
Imaging report (e.g. ultrasound, X-ray, CT)	11 / 128 (8.5%)
Anamnestic	10 / 128 (8%)
Presentation	**n = 112 (16 / 128 unknown *)**
Acute abdomen	90 / 112 (80%)
Abdominal pain / other complaint (e.g. blood loss)	19 / 112 (17%)
Surveillance	3 / 112 (3%)
Localisation	**n = 110 (18 / 128 unknown *)**
Small-intestinal	105 / 110 (95%)
Colonic	5 / 110 (5%)
Sub-localisation small-intestinal intussusceptions	**n = 60 (45 / 105 unknown ‡)**
Jejunal	32 / 60 (53%)
Jejuno-jejunal	31 / 32
Jejuno-ileal	1 / 32
Ileal	28 / 60 (47%)
Ileoileal	23 / 28
Ileocolic	5 / 28
Therapy	**n = 120 (8 / 128 unknown *)**
Surgery	111 / 120 (92.5%)
Enteroscopic polypectomy	6 /120 (5%)
Conservative, by barium enema	3 /120 (2.5%)

* Unknown due to missing data.

‡ Sub-localisation in 45 of 105 small-intestinal intussusceptions not further specified.

and consisted of surgery in 111 (92.5%) events, endoscopic polypectomy by BAE in 6 (5%) events (without complications), and barium enema (reduction by hydrostatic pressure) in 3 (2.5%) events. Three of the 111 surgical interventions were based on anamnestic data (i.e. no medical report was available), and one intussusception diagnosed by ultrasound had resolved spontaneously and could not be confirmed during laparotomy. Since the introduction of BAE into clinical practice in 2004, 17 intussusceptions had occurred in our cohort including 6 chronic and 11 acute events. All the chronic intussusceptions were treated by BAE with polyp removal, and the acute events by surgery (n = 10) or barium-enema (n = 1). The exact size of the intussusception-causing polyp could be determined in 37 events. These intussusceptions had been caused by hamartomas with a median size of 35 mm (range 15-60 mm). Three of these 37 hamartomas were smaller than 20 mm (15, 18 and 18 mm respectively). In addition, one hamartomatous polyp showed malignant degeneration and was histologically classified as a mucin producing adenocarcinoma invading the surrounding fat tissue, without lymphogenic or hematogenic metastasis. This patient had prior to diagnosis not been under surveillance.

DISCUSSION

This study demonstrates that PJS patients carry a high cumulative risk of intussusception already early in life (50% at the age of 20 years, 95%CI 17-23 yrs). The intussusceptions occur in the small-intestine in more than 95% of events. This is in line with the jejunum being the preferential localisation for hamartomas, which act as leading point for the intussusceptions. The intussusceptions were generally caused by hamartomas larger than or equal to 15 mm in diameter, with polyp-size probably being the most important risk factor for small-bowel intussusception. The events presented predominantly as an acute abdomen and led to surgical emergencies. The intussusception risk was independent of sex, family history and *STK11* mutation status.

Previously, only one study reported on the intussusception risk associated with PJS.[6] Our results corroborate this study on the high cumulative intussusception risks in PJS patients.[6] However, the percentage of patients experiencing one or more episodes of intussusception in our study (69%) is higher than the previously reported percentage (48%).[6] The reason for this difference is unclear. One possibility is that patients in our cohort had a more severe phenotype. However, similar inclusion criteria were used and inclusion occurred solely systematically. Conceivably, the number of at-risk person years may have been higher in our cohort than in the study of Hearle and colleagues. Unfortunately, the exact number of at-risk patient-years in the study of Hearle et al. is unknown.[6] The additional value of our study compared to the previous collaborative study[6], is that our results are based on a unique homogenous pedigree-based cohort of PJS patients with a substantial period of follow-up. Furthermore, our study is the first to report on intussusception characteristics such as intussusception localisation and size of polyps that function as leading point for the intussusceptions. These data are necessary for the development of optimal surveillance recommendations.

However, this study has some limitations, including a limited number of patients. In addition, selection bias may have resulted in an over-inflated intussusception risk, as individuals with severe phenotypes may be overrepresented in the dataset and patients with mild phenotypes may have remained undetected. To limit this form of bias, all PJS patients were included systematically regardless of a history of intussusception. Conversely, the intussusception risk may have been underestimated in this series, since some patients underwent surveillance and had small-intestinal polyps removed by BAE or intra-operative enteroscopy. One other limitation is that we do not have information on the numbers and sizes of polyps per patient, and thus also do not know what the risk is that an individual polyp will over time lead to intussusception. This information is relevant to optimize prevention strategies.

One of the intussusceptions in our cohort was caused by a hamartomatous polyp showing malignant degeneration, an interesting finding in light of surveillance. Indeed, PJS patients carry a significantly increased risk for the development of gastrointestinal and extra-gastrointestinal cancer.[10,11] Compared to the general population, high relative and cumulative risks

have been described in PJS patients for small-intestinal carcinoma (a relative risk of 520 and a cumulative risk of 13% at the age of 65 years).[10] However, it is still not known whether these and other gastrointestinal cancers evolve from PJS hamartomas, or arise in co-existing adenomas or normal mucosa.[12,13] Yet, several studies reported on a hamartoma-adenoma-carcinoma sequence, indicating endoscopic polyp-removal in order to decrease the risk for malignancy.[12,14-16]

Surveillance of PJS patients should not only be recommended for the elevated cancer risk, but also for benign polyps causing intussusceptions as well as bleeding and anaemia, leading to considerable morbidity and even mortality. Surveillance guidelines vary between institutions. Most current guidelines recommend contrast radiography of the small-intestine at intervals ranging from 2 to 5 years starting between the ages of 12 and 18 years.[17-26] Several radiographic imaging modalities can be used including small-bowel follow-through, CT enteroclysis / enterography, and MR enteroclysis / enterography, but also video capsule endoscopy. The disadvantage of small-bowel follow-through, the classical diagnostic tool for assessment of small-intestinal polyps, and CT is the associated radiation exposure. MR enteroclysis/enterography and video capsule endoscopy seem promising diagnostic techniques[27], but do not have therapeutic options.

Our findings therefore seem to justify the use of BAE in PJS patients, enabling timely endoscopic removal of small-intestinal hamartomas in order to prevent intussusception and laparotomy. However, as BAE has been available for only the past few years, the effects of such an approach on the incidence of intussusceptions as well as malignant degeneration of polyps will have to be established in prospective trials. Moreover, the potential benefit of surveillance by BAE in PJS remains to be established and has to be weighed against the burden and complication risk of the intervention.[28] Yet, in previous studies it has already been shown that BAE is a safe treatment modality for small-intestinal polyps in PJS patients[29-31], even when complete visualization of the small-intestine can be hampered by adhesions following prior laparotomies.[32]

The present data point at the potential usefulness of small-intestinal surveillance and early polyp removal in order to prevent intussusception. This can be done by MR-enterography or video capsule endoscopy, followed by BAE with polypectomy when polyps of 10-15 mm in diameter or larger are discovered. Although the smallest polyp that had led to intussusception was 15 mm in diameter in our series, we recommend to remove polyps that are 10 mm in size or larger, since the purpose of the polypectomy is prevention of intussusception. Considering the young age at onset of intussusceptions, small-intestinal surveillance for the prevention of intussusception should start at the age of 8-10 years (at this age children can undergo MRI without sedation), at 2-3 year intervals.

In conclusion, PJS patients carry a high cumulative risk of intussusception already at young age, generally caused by small-intestinal hamartomas larger than or equal to 15 mm in diameter. These findings support the approach of enteroscopic surveillance by BAE with timely

removal of small-intestinal hamartomas, in patients with known small-intestinal polyps established by radiological examination or video capsule endoscopy. However, the effect of this approach on the incidence of intussusception as well as on the malignant degeneration of polyps remains to be established and weighed against the burden and complication risk of the intervention.

REFERENCES

1. Peutz JLA. Over een zeer merkwaardige, gecombineerde familiaire polyposis van de slijmliezen van den tractus intestinalis met die van de neuskeelholte en gepaard met eigenaardige pigmentaties van huid en slijmvliezen. Ned Maandschr v Geneesk 1921;10:134-146.
2. McGarrity TJ, Kulin HE, Zaino RJ. Peutz-Jeghers syndrome. Am J Gastroenterol 2000;95(3):596-604.
3. Volikos E, Robinson J, Aittomaki K, Mecklin JP, Jarvinen H, Westerman AM, et al. LKB1 exonic and whole gene deletions are a common cause of Peutz-Jeghers syndrome. J Med Genet 2006; 43(5):e18.
4. Utsunomiya J, Gocho H, Miyanaga T, Hamaguchi E, Kashimure A. Peutz-Jeghers syndrome: its natural course and management. Johns Hopkins Med J 1975;136(2):71-82.
5. Choi HS, Park YJ, Youk EG, Yoon KA, Ku JL, Kim NK, et al. Clinical characteristics of Peutz-Jeghers syndrome in Korean polyposis patients. Int J Colorectal Dis 2000;15(1):35-38.
6. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, et al. STK11 status and intussusception risk in Peutz-Jeghers syndrome. J Med Genet 2006;43(8):e41.
7. Jeghers H MVKK. Generalized intestinal polyposis and melanin spots of the oral mucosa, lips and digits; a syndrome of diagnostic significance. N Engl J Med 1949;241:993-1005.
8. May A, Ell C. Push-and-pull enteroscopy using the double-balloon technique/double-balloon enteroscopy. Dig Liver Dis 2006;38(12):932-938.
9. Aaltonen LA, Jarvinen H, Gruber SB, Billaud M, Jass JR. World Health Organization Classification of Tumours. Pathology and Genetics. Tumours of the Digestive System. Lyon: IARC Press; 2000.
10. Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119(6):1447-1453.
11. van Lier MG, Wagner A, Mathus-Vliegen EM, Kuipers EJ, Steyerberg EW, van Leerdam ME. High Cancer Risk in Peutz-Jeghers Syndrome: A Systematic Review and Surveillance Recommendations. Am J Gastroenterol 2010;105(6):1258-1264.
12. Hizawa K, Iida M, Matsumoto T, Kohrogi N, Kinoshita H, Yao T, et al. Cancer in Peutz-Jeghers syndrome. Cancer 1993;72(9):2777-2781.
13. Jansen M, de Leng WW, Baas AF, Myoshi H, Mathus-Vliegen L, Taketo MM, et al. Mucosal prolapse in the pathogenesis of Peutz-Jeghers polyposis. Gut 2006;55(1):1-5.
14. Perzin KH, Bridge MF. Adenomatous and carcinomatous changes in hamartomatous polyps of the small intestine (Peutz-Jeghers syndrome): report of a case and review of the literature. Cancer 1982;49(5):971-983.
15. Gruber SB, Entius MM, Petersen GM, Laken SJ, Longo PA, Boyer R, et al. Pathogenesis of adenocarcinoma in Peutz-Jeghers syndrome. Cancer Res 1998;58(23):5267-5270.
16. Wang ZJ, Ellis I, Zauber P, Iwama T, Marchese C, Talbot I, et al. Allelic imbalance at the LKB1 (STK11) locus in tumours from patients with Peutz-Jeghers' syndrome provides evidence for a hamartoma-(adenoma)-carcinoma sequence. J Pathol 1999;188(1):9-13.
17. Tomlinson IP, Houlston RS. Peutz-Jeghers syndrome. J Med Genet 1997;34(12):1007-1011.
18. Lindor NM, Greene MH. The concise handbook of family cancer syndromes. Mayo Familial Cancer Program. J Natl Cancer Inst 1998;90(14):1039-1071.
19. Burt RW. Colon cancer screening. Gastroenterology 2000;119(3):837-853.
20. The Johns Hopkins guide for patients and families: Peutz-Jeghers syndrome. Baltimore: Johns Hopkins University. 2001.
21. McGrath DR, Spigelman AD. Preventive measures in Peutz-Jeghers syndrome. Fam Cancer 2001; 1(2):121-125.

22. Dunlop MG. Guidance on gastrointestinal surveillance for hereditary non-polyposis colorectal cancer, familial adenomatous polypolis, juvenile polyposis, and Peutz-Jeghers syndrome. Gut 2002;51 Suppl 5:V21-V27.
23. Allen BA, Terdiman JP. Hereditary polyposis syndromes and hereditary non-polyposis colorectal cancer. Best Pract Res Clin Gastroenterol 2003;17(2):237-258.
24. Hinds R, Philp C, Hyer W, Fell JM. Complications of childhood Peutz-Jeghers syndrome: implications for pediatric screening. J Pediatr Gastroenterol Nutr 2004;39(2):219-220.
25. McGarrity TJ, Amos C. Peutz-Jeghers syndrome: clinicopathology and molecular alterations. Cell Mol Life Sci 2006;63(18):2135-2144.
26. Giardiello FM, Trimbath JD. Peutz-Jeghers Syndrome and Management Recommendations. Clinical Gastroenterology and Hepatology 2006;4(4):408-415.
27. Caspari R, von Falkenhausen M, Krautmacher C, Schild H, Heller J, Sauerbruch T. Comparison of capsule endoscopy and magnetic resonance imaging for the detection of polyps of the small intestine in patients with familial adenomatous polyposis or with Peutz-Jeghers' syndrome. Endoscopy 2004;36(12):1054-1059.
28. Mensink PB, Haringsma J, Kucharzik T, Cellier C, Perez-Cuadrado E, Monkemuller K, et al. Complications of double balloon enteroscopy: a multicenter survey. Endoscopy 2007;39(7):613-615.
29. Ohmiya N, Taguchi A, Shirai K, Mabuchi N, Arakawa D, Kanazawa H, et al. Endoscopic resection of Peutz-Jeghers polyps throughout the small intestine at double-balloon enteroscopy without laparotomy. Gastrointest Endosc 2005;61(1):140-147.
30. Ross AS, Dye C, Prachand VN. Laparoscopic-assisted double-balloon enteroscopy for small-bowel polyp surveillance and treatment in patients with Peutz-Jeghers syndrome. Gastrointest Endosc 2006;64(6):984-988.
31. Gao H, van Lier MG, Poley JW, Kuipers EJ, van Leerdam ME, Mensink PB. Endoscopic therapy of small-bowel polyps by double-balloon enteroscopy in patients with Peutz-Jeghers syndrome. Gastrointestinal Endoscopy 2010;71(4):768-773.
32. Yamamoto H, Ell C, Binmoeller KF. Double-balloon endoscopy. Endoscopy 2008;40(9):779-783.

5

Endoscopic therapy of small-bowel polyps by double balloon enteroscopy in patients with Peutz-Jeghers syndrome

Gastrointest Endosc. 2010;71(4):768-73.

H. Gao[1,2]
M.G.F. van Lier[1]
J.W. Poley[1]
E.J. Kuipers[1,3]
M.E. van Leerdam[1]
P.B.F. Mensink[1]

Departments of Gastroenterology and Hepatology[1], and Internal Medicine[3],
Erasmus MC, University Medical Center, Rotterdam, the Netherlands
Department of Gastroenterology and Hepatology[2], Zhongshan Hospital, Fudan University,
Shanghai, China

ABSTRACT

Background: Peutz-Jeghers syndrome (PJS) is a hereditary disorder characterized by mucocutaneous pigmentations and hamartomatous polyps mainly in the small-bowel. These polyps may cause complications such as intussusception. The aim of the present study was to assess therapeutic efficacy and safety of double balloon enteroscopy (DBE) for detection and treatment of small-bowel polyps in PJS patients.

Methods: We performed a prospective cohort study in which we evaluated all PJS patients who underwent DBE at our institution. Pedunculated polyps ≥ 10 mm in diameter were considered eligible for polypectomy. DBE procedural data, location, number and size of small-bowel polyps, as well as earlier findings on gastroduodenoscopy and colonoscopy were analyzed.

Results: Thirteen PJS patients (8 males, mean age 31 years) underwent 29 DBE procedures. Ten (77%) patients had a history of partial small-bowel resections because of small-bowel polyps. Small-bowel polyps were found in all patients. The majority of polyps (94%) were located in duodenum and proximal jejunum. A total of 82 polyps ≥10 mm were detected, and 79 (96%) were endoscopically removed without complications. After the introduction of DBE, no small intestinal polyp-related complications occurred during 356 person-months of follow-up.

Conclusions: DBE is clinically useful and safe for diagnosis and therapy of small-bowel polyps in PJS patients, even in patients with a history of extensive abdominal surgery. DBE may decrease the need for laparotomy in PJS patients.

INTRODUCTION

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant hereditary disease characterized by gastrointestinal hamartomatous polyposis and mucocutaneous melanin pigmentations. In addition, PJS patients carry a significantly increased risk for the development of gastrointestinal and extra-gastrointestinal cancers, compared to the general population.[1-4] A pathogenic germline mutation in the *STK11* gene is found in approximately 70% of clinically affected patients.[5]

The predominant clinical features of PJS are the result of gastrointestinal polyposis which can lead to abdominal pain, intussusception and bleeding. Hamartomatous polyps can be detected in 88% of PJS patients and are most frequently found in the small-bowel, in order of prevalence: jejunum, ileum, duodenum, followed by the large intestine and stomach.[6] One-third of PJS patients experience symptoms because of small-bowel polyps during their first decade of life, and 50–60% of patients experience symptoms before the age of 20 years, in particular due to obstruction and intussusception.[7]

Over the last years the small-bowel follow-through (SBFT), which has been the classical diagnostic tool for the assessment of small-bowel polyps, has been partially replaced by wireless capsule endoscopy (WCE) and MR/CT-enteroclysis, or MR/CT-enterography.[8-10] Since 2001 double balloon enteroscopy (DBE) has been introduced into clinical practice, which theoretically enables endoscopic visualization of the entire small-bowel.[11,12] The main advantage of DBE is that diagnosis and therapeutic interventions can be combined in a single procedure. Until now, little is known about the clinical impact of surveillance and treatment of small-bowel polyps using DBE in PJS patients.[13,14] However in theory, timely enteroscopic removal of small-bowel polyps might prevent intussusceptions and avoid the need for laparotomy in PJS patients.[11] The aim of this prospective study was to evaluate the therapeutic efficacy, clinical impact and safety of enteroscopic therapy with DBE for intestinal polyps in PJS patients.

METHODS

All PJS patients who are under surveillance at the Erasmus Medical Center in Rotterdam were eligible for inclusion in this study. The diagnosis PJS was defined by a proven *STK11* gene mutation, or according to the diagnostic criteria developed by Giardiello and colleagues.[15] Patients were seen at the outpatient clinic and were after informed consent scheduled for DBE. After the index DBE patients were prospectively followed. Patient-characteristics including previous diagnostics (gastroduodenoscopy, ileocolonoscopy, SBFT and MR-enteroclysis), DBE findings (including number, size and location of polyps), complications, symptoms as well as need for further therapeutic intervention during follow-up, were evaluated. The study was approved by the Institutional Review Board.

DBE procedures and therapy

A polyethylene glycol solution was used for bowel preparation, and patients underwent DBE under conscious sedation using intravenous administration of midazolam and fentanyl, or general anaesthesia using propofol. The Fujinon EN-450P5 and EN-450T5 (Fujinon Inc., Saitama, Japan) enteroscopes were used. All DBE procedures were performed on an outpatient basis by the same endoscopist (PBFM), and all patients were monitored for at least 2 hours after the end of the procedure before discharge. DBE was performed via the proximal approach, unless previous small-bowel evaluation revealed suspicion of small-bowel polyps in the (distal) ileum. In that case a combined proximal and distal procedure or only a distal procedure was scheduled. Pedunculated small-bowel polyps with a diameter ≥10 mm were considered suitable for polypectomy. After injection of the stalk with diluted epinephrine-saline solution, polypectomy was performed using a polypectomy-snare and if possible the polyp was retrieved for histological evaluation.

For defining polyp-localisation, the small-bowel was divided into 4 segments for each procedure. For the proximal DBE procedures, this was done as follows: a) proximal jejunum (duodenal bulb to <150 cm beyond the ligament of Treitz), b) distal jejunum (150 – 250 cm), c) proximal ileum (250 – 350 cm) and d) distal ileum (> 350 cm beyond the ligament of Treitz). For the distal DBE procedures a similar approach was used leading to the following classification: e) distal ileum (< 150 cm), f) proximal ileum (150 – 250 cm), g) distal jejunum (250 – 350 cm), h) proximal jejunum (> 350 cm beyond the ileocecal valve). Insertion depth was measured by accumulation of net advancement of each push-and-pull maneuver as described by May et al.[16]

If the index DBE procedure had revealed small-bowel polyps, surveillance DBE was scheduled 1 – 2 years later. The DBE procedure was repeated earlier in case of incomplete removal of small-bowel polyps or when a patient developed symptoms suggestive for small-bowel obstruction. In the latter case, the treating physician was allowed to perform an MR-enteroclysis for detection of possible residual polyps or newly developed small-bowel polyps, before a repeated DBE procedure was planned. Newly diagnosed significant polyps were defined as polyps with a diameter ≥10 mm in the segment that was evaluated by DBE, but which had not been reported during the preceding DBE.

Statistical analyses

Data were analyzed using the SPSS 15.0 statistical software for Windows. Continuous variables were reported by means (and standard deviations) or medians (and range). Means were compared by the (un-)paired t-test and one-way ANOVA. A two-sided p-value < 0.05 was considered statistically significant.

Table 1. Patient characteristics (n = 13).

Number of patients	Gender	Age at PJS diagnosis	Pigmentations	*STK11* mutation	Family history of PJS	Stomach polyps	Duodenum polyps	Colon polyps	History of abdominal surgery	Number of small-bowel resections	Age at first DBE
1	M	8	*	Yes	No	Yes	Yes	Yes	Yes	2	50
2	F	16	Yes	Yes	No	Yes	No	No	Yes	1	24
3	M	26	Yes	Yes	Yes	Yes	Yes	Yes	Yes	4	45
4	F	15	Yes	Yes	*	Yes	No	Yes	Yes	1	17
5	M	*	Yes	Yes	*	Yes	Yes	Yes	Yes	2	30
6	F	19	Yes	Yes	Yes	Yes	Yes	Yes	Yes	3	35
7	F	9	Yes	Yes	No	Yes	No	Yes	Yes	1	27
8	M	6	Yes	Yes	Yes	No	No	Yes	No	0	10
9	F	*	Yes	No	No	Yes	No	*	Yes	4	47
10	M	30	Yes	No	No	Yes	Yes	Yes	Yes	0	51
11	M	5	Yes	No	No	Yes	Yes	Yes	No	0	17
12	M	11	Yes	No	Yes	Yes	Yes	*	Yes	2	30
13	M	*	Yes	No	Yes	Yes	Yes	Yes	Yes	2	33

DBE = double balloon enteroscopy, M = male, F = female, * = unknown.

RESULTS

Between October 2004 and July 2009, 13 PJS patients (8 males) were included with a median age of 31 (10–51) years (Table 1). Eleven patients (85%) had a history of abdominal surgery including 10 (77%) patients with a partial small-bowel resection due to complicated small-bowel polyps. Twelve (92%) patients had known polyps in the stomach or duodenum, and twelve patients had known colonic polyps.

In total 29 DBE procedures were performed, with a range from 1 to 6 per individual patient. These procedures included 26 enteroscopies with a proximal and 3 with a distal approach (Table 2). Thirteen of these procedures were index DBE procedures, and 16 were repeated DBE procedures. Conscious sedation was used in 15 (52%) procedures and general anaesthesia was used in the remaining 14 (48%). The mean insertion depths were 230 (140 – 320) cm and 145 (140 – 150) cm for the proximal and distal procedures, respectively. The total duration of the procedures was 70 ± 15 minutes. Complete small-bowel visualization was achieved in none of the DBE procedures. One patient was admitted for overnight observation following a piecemeal resection of a large polyp of 30 mm without signs of bleeding of perforation afterwards. No complications were reported during or after the DBE procedures and polypectomies.

Findings on the index DBE

Index DBE procedures included 13 proximal and 3 distal procedures. The indication for the index DBE included suspected small-bowel polyps based on SBFT or MR-enteroclysis (8 / 16, 50%), elective surveillance after small-bowel resection due to complicated small-bowel polyps (5 / 16, 31%) and abdominal complaints (3 / 16, 19%). Multiple small-bowel polyps were found in all patients during the index DBE procedures. In 12 patients 41 polyps with a diameter ≥10 mm were reported with a mean of three (1 - 10) per patient (Table 2). Only several minor polyps were found in one patient. All polyps ≥10 mm were pedunculated. The majority (94%) of these polyps were located in the proximal jejunum (Table 3). In total 34 (83%) polyps, with a mean diameter of 17 (10-50) mm, were completely removed. Seven polyps were not or only partially resected because they were either considered to be too large to be endoscopically removed or due to agitation of the patients. Four (57%) of these seven polyps were removed during following procedures. One patient was referred for surgical resection of 2 large polyps (over 25 mm in diameter) in the distal duodenum. Another patient with an unresected polyp died five months after the DBE procedure due to an unrelated cause (cardiac arrest). Histological examination of the retrieved polyps showed harmatomatous (76%), adenomatous (18%), and hyperplastic polypoid tissue (6%).

Table 2. DBE procedures (n = 29) and polyps (n = 82).

Number of procedures	Number of patients	Route of DBE	Insertion depth (cm)	Number of polyps (≥ 10 mm)					Max size of polyps (mm)	Number of removed polyps
				Proximal jejunum	Distal jejunum	Proximal ileum	Distal ileum	Total		
1	1	Oral	250	3	0	*	*	3	20	1
2		Oral	280	4	0	0	*	4	30	4
3	2	Oral	150	4	*	*	*	4	50	4
4		Oral	300	2	0	0	*	2	30	2
5		Oral	300	0	0	0	*	0	-	0
6		Anal	150	*	*	*	1	1	15	1
7	3	Oral	200	10	0	*	*	10	15	10
8		Oral	200	12	2	*	*	14	30	14
9		Oral	170	4	0	*	*	4	20	4
10		Oral	220	1	1	*	*	2	15	2
11		Oral	200	3	0	*	*	3	10	3
12		Oral	320	10	0	1	*	11	-	11
13	4	Oral	230	2	0	*	*	2	25	2
14		Oral	220	2	0	*	*	2	15	2
15		Anal	140	*	*	*	0	0	-	0
16	5	Oral	300	5	0	0	*	5	-	4
17	6	Oral	250	4	0	*	*	4	30	1
18		Oral	200	3	0	*	*	3	30	1
19	7	Oral	200	0	0	*	*	0	-	0
20	8	Oral	250	1	0	*	*	1	30	0
21		Oral	250	1	0	*	*	1	30	1
22	9	Oral	240	3	0	*	*	3	20	3
23	10	Oral	140	1	*	*	*	1	30	1
24		Oral	150	0	*	*	*	0	-	0
25		Anal	-	*	*	*	0	0	-	0
26	11	Oral	230	1	0	*	*	1	15	1
27	12	Oral	250	4	0	*	*	4	20	4
28	13	Oral	280	2	0	0	*	2	40	2
29		Oral	300	1	0	0	*	1	15	1

DBE = double balloon enteroscopy, * = not visualized, - = unknown / not applicable.

Findings on repeated DBE

A total of 13 repeated proximal DBE procedures were performed in 8 patients. The indications for repeated DBE were scheduled surveillance (6 / 13, 46%), retreatment of known polyps left in situ at the index procedure (4 / 13, 33%), or suspected polyps found on MR-enteroclysis during follow-up performed because of abdominal complaints (3 / 13, 23%). The insertion

Table 3. Polyps (n = 82): localization and numbers.

	Number of polyps per segment visualized	Sum of polyps	Mean number of polyps
Proximal jejunum	26	77 (94%)	3.0*
Distal jejunum	22	3 (4%)	0.1
Proximal ileum	7	1 (1%)	0.1
Distal ileum	3	1 (1%)	0.3
Total	*58*	*82 (100%)*	*1.4*

* $p < 0.001$

depth was comparable for the index and repeated DBE procedures. During the repeated DBE procedures 47 polyps ≥10 mm were found, of which 41 were defined as newly diagnosed polyps. Forty-five (96%) polyps were successfully removed. The average number of newly diagnosed polyps ≥10 mm during repeated DBE were 3.1 compared to 3.4 during index DBE procedures (not significant). The mean interval between repeated DBEs was 13 ±10 months.

Follow-up

The median follow-up time was 19 (5 – 58) months, with a total of 356 person-months. One patient, who had previously undergone repeated small-bowel resections, presented with acute small-bowel obstruction due to adhesions, which were found and treated during laparotomy. There were no reports of small-bowel obstructions or acute bleeding episodes caused by small-bowel polyps during follow-up.

DISCUSSION

PJS is characterized by hamartomatous polyposis of the gastrointestinal tract, in particular the small-bowel. These small-bowel polyps frequently give rise to symptoms, and may result in acute obstruction necessitating emergency laparotomy. Furthermore, there is an increased risk for small-bowel cancer, which may be related to the small-bowel hamartomas. For all these reasons, PJS patients are offered surveillance with SBFT or MR-/CT-enteroclysis, and more recently with WCE. These methods have the disadvantage that they do not allow any therapeutic intervention or histology sampling. PJS patients thus often undergo abdominal surgery, either scheduled for removal of small-bowel lesions, or in an emergency setting because of small-bowel obstruction. DBE is in theory very suitable to diagnose and treat small-bowel polyps in PJS patients, and may reduce the incidence of obstruction, the need for surgery, and the incidence of small-bowel cancer. However, there are so far very limited data to support this hypothesis. This study therefore presents a series of PJS patients undergoing DBE for surveillance and treatment of small-bowel polyps.

All PJS patients presented here had small-bowel polyposis, predominantly located in the proximal jejunum, and in total 79 polyps ≥10 mm in diameter were removed without complications. The majority of patients had a history of small-bowel surgery, but since the introduction of DBE with removal of the significant polyps, no intussusceptions occurred and only one laparotomy was indicated for relatively large polyps that could not be removed endoscopically. Moreover, in 7 / 8 patients undergoing repeated DBE procedures there was a clear trend (not statistically significant) for a decrease in number of newly diagnosed polyps ≥10mm in diameter.

The distribution of PJS polyps throughout the gastrointestinal tract makes surveillance and treatment challenging. Most authorities have suggested small-bowel surveillance in PJS patients by contrast radiography at 2-year intervals, usually by SBFT[6,17-22]. Recently, this surveillance has been accomplished by diagnostics such as MR-enteroclysis, CT-enteroclysis, and WCE.[9,10,23,24] It has been recommended to remove symptomatic (bleeding or obstruction) or rapidly growing small intestinal polyps, or asymptomatic polyps ≥10 – 15 mm.[6,22,25,26] Before the introduction of DBE, this was only possible by intra-operative endoscopy, surgical resection, or push enteroscopy in the case of proximal small-bowel polyps. DBE nowadays enables gastroenterologists to endoscopically remove proximal and distal small-bowel polyps, thereby preventing abdominal surgery in these patients. Our findings show that timely DBE with removal of pedunculated small-bowel polyps ≥10 mm in diameter can be safely performed. Moreover, no episodes of small-bowel obstruction or acute bleeding caused by small-bowel polyps occurred during follow-up, suggesting a trend in prevention of complicated small-bowel polyps.

It has been suggested that DBE for polyp surveillance in PJS patients may not be possible in case of a history of abdominal surgery.[27] Post-surgical intra-abdominal adhesions would prevent free motion of the small intestine within the abdominal cavity, impacting the depth of maximal insertion at DBE.[28] In the present study, DBE procedures and endoscopic therapy were performed successfully and without complications, even in patients with a history of multiple laparotomies. The fact that in none of the patients complete small-bowel visualization with DBE was achieved, did not result in complicated disease at follow-up. Therefore, previous abdominal surgery does not seem to hamper efficacy of DBE in PJS patients.

Another indication for small-bowel polyp surveillance in PJS patients is the elevated small intestinal cancer risk; a relative risk of 520 compared to the general population has been reported.[7] Although the mechanism of carcinogenesis is unknown and it is debated whether the gastrointestinal cancers originate from hamartomas, a hamartoma-adenoma-carcinoma sequence has been suggested.[29] So in theory, removal of small-bowel polyps could potentially decrease the risk for malignancies by removal of the precursor lesion. In our series, histological evaluation of resected polyps showed pre-malignant adenomatous tissue in up to 18%, but no malignancy. This underlines the importance of timely polypectomy of larger polyps and histological evaluation of all resected polyps in these patients.

This study has some limitations, in particular the limited number of patients with this relatively rare condition. However, these patients underwent repeated procedures and a large number of polypectomies, enabling some conclusions on safety and therapeutic efficacy. Furthermore, in none of the procedures the entire small-bowel was visualized, and only a small number of distal DBE procedures were performed. Most polyps were located proximal in the small-bowel, suggesting that a proximal approach might be preferable compared to a distal DBE approach in PJS patients. Only when preceding small-bowel imaging has shown distal polyps, a distal approach should be considered.

In conclusion, DBE is clinically useful as a therapeutic tool for small-bowel polyps in PJS patients, even in patients with a history of extensive abdominal surgery. Moreover, the data presented in this study suggest that a timely planned DBE might prevent future complications of small-bowel polyps and laparotomies. This clinically relevant observation has to be established in further studies.

REFERENCES

1. Utsunomiya J, Gocho H, Miyanaga T, et al. Peutz-Jeghers syndrome: its natural course and management. The Johns Hopkins Medical Journal 1975;136:71–82.
2. Spigelman AD, Murday V, Phillips RKS. Cancer and the Peutz-Jeghers syndrome. Gut 1989;30: 1588–1590.
3. Lim W, Olschwang S, Keller JJ, et al. Relative frequency and morphology of cancers in STK11 mutation carriers. Gastroenterology 2004;126:1788–1794.
4. Boardman LA, Thibodeau SN, Schaid DJ, et al. Increased risk for cancer in patients with the Peutz-Jeghers syndrome. Ann Intern Med 1998;128:896-869.
5. Aretz S, Stienen D, Uhlhaas S, et al. High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome. Hum Mutat 2005;26:513-519.
6. Giardiello FM, Trimbath JD. Peutz-Jeghers syndrome and management recommendations. Clin Gastroenterol Hepatol 2006;4:408-415.
7. Giardiello FM, Brensinger JD, Tersmette AC, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119(6):1447-1453.
8. Hartmann D, Schilling D, Bolz G et al. Capsule endoscopy, technical impact, benefits and limitations. Langenbecks Arch Surg 2004;389:225-233.
9. Caspari R, von Falkenhausen M, Krautmacher C, et al. Comparison of capsule endoscopy and magnetic resonance imaging for the detection of polyps of the small intestine in patients with familial adenomatous polyposis or with Peutz-Jeghers' syndrome. Endoscopy 2004;36:1054-1059.
10. Schulmann K, Hollerbach S, Kraus K, et al. Feasibility and diagnostic utility of video capsule endoscopy for the detection of small bowel polyps in patients with hereditary polyposis syndromes. Am J Gastroenterol 2005;100:27-37.
11. Yamamoto H, Sekine Y, Sato Y, et al. Total enteroscopy with a nonsurgical steerable double-balloon method. Gastrointest Endosc 2001;53:216-220.
12. May A, Nachbar L, Wardak A, et al. Double balloon enteroscopy: preliminary experience in patients with obscure gastrointestinal bleeding or chronic abdominal pain. Endoscopy 2003;35: 985–991.
13. Nishimura M, Yamamoto H, Kita H, et al. Gastrointestinal stromal tumor in the jejunum: diagnosis and control of bleeding with electrocoagulation by using double balloon enteroscopy. J Gastroenterol 2004;39:1001–1004.
14. Plum N, May AD, Manner H, et al. Peutz-Jeghers syndrome: endoscopic detection and treatment of small bowel polyps by double-balloon enteroscopy (in German with English abstract). Z Gastroenterol 2007;45:1049-1055.
15. Giardiello FM, Welsh SB, Hamilton SR, et al. Increased risk of cancer in the Peutz-Jeghers syndrome. N Engl J Med 1987;316:1511-1514.
16. May A, Nachbar L, Schneider M, et al. Push-and-pull enteroscopy using the double-balloon technique: method of assessing depth of insertion and training of the enteroscopy technique using the Erlangen Endo-Trainer. Endoscopy 2005;37:66-70.
17. The Johns Hopkins guide for patients and families: Peutz-Jeghers syndrome. Baltimore: Johns Hopkins University, 2001.
18. Dunlop MG. Guidance on gastrointestinal surveillance for hereditary non-polyposis colorectal cancer, familial adenomatous polyposis, juvenile polyposis, and Peutz-Jeghers syndrome. Gut 2002;51(Suppl 5):V21-7.

19. Hinds R, Philp C, Hyer W, et al. Complications of childhood Peutz-Jeghers syndrome: implications for pediatric screening. J Pediatr Gastroenterol Nutr 2004;39:219-220.
20. McGrath DR, Spigelman AD. Preventive measures in Peutz-Jeghers syndrome. Fam Cancer 2001; 1:121-125.
21. McGarrity TJ, Kulin HE, Zaino RJ. Peutz-Jeghers syndrome. Am J Gastroenterol 2000;95:596-604.
22. Tomlinson IP, Houlston RS. Peutz-Jeghers syndrome. J Med Genet 1997;34:1007-1011.
23. Soares J, Lopes L, Vilas Boas G, et al. Wireless capsule endoscopy for evaluation of phenotypic expression of small-bowel polyps in patients with Peutz-Jeghers syndrome and in symptomatic first-degree relatives. Endoscopy 2004;36:1060-1066.
24. Burke CA, Santisi J, Church J, et al. The utility of capsule endoscopy small bowel surveillance in patients with polyposis. Am J Gastroenterol 2005;100:1498-1502.
25. Heine GD, Hadithi M, Groenen MJ, et al. Double-balloon enteroscopy: indications, diagnostic yield, and complications in a series of 275 patients with suspected small-bowel disease. Endoscopy 2006;38:42-48.
26. Giardiello FM. Gastrointestinal polyposis syndromes and hereditary nonpolyposis colorectal cancer. In: Rustgi AK, et al. Gastrointestinal cancers: biology, diagnosis, and therapy. Philadelphia: Lippincott-Raven Publishers, 1995:370–371.
27. Ross A, Dye C, Prachand V. Laparoscopic-assisted double-balloon enteroscopy for small-bowel polyp surveillance and treatment in patients with Peutz-Jeghers syndrome. Gastrointestinal Endoscopy 2006;64:984-988.
28. Khashab M, Helper DJ, Johnson CS, et al. Predictors of Depth of Maximal Insertion at Double-Balloon Enteroscopy. Dig Dis Sci 2010;55(5):1391-1395.
29. Gruber SB, Entius MM, Petersen GM, et al. Pathogenesis of adenocarcinoma in Peutz-Jeghers syndrome. Cancer Res 1998;58:5267-5270.

6

Quality of life and psychological distress in patients with Peutz-Jeghers syndrome

Clin Genet. 2010;78(3):219-226

M.G.F. van Lier[1]
E.M.H. Mathus-Vliegen[2]
M.E. van Leerdam[1]
E.J. Kuipers[1,3]
C.W.N. Looman[4]
A. Wagner[5]
K. Vanheusden[5,6]

Departments of Gastroenterology and Hepatology[1], Internal Medicine[3], Public Health[4], Clinical genetics[5], and Medical Psychology and Psychotherapy[6], Erasmus University Medical Center, Rotterdam, the Netherlands.
Department of Gastroenterology and Hepatology[2], Academic Medical Center, Amsterdam, the Netherlands.

ABSTRACT

Background: Little is known about psychological distress and quality of life (QoL) in patients with Peutz-Jeghers syndrome (PJS), a rare hereditary disorder. We aimed to assess QoL and psychological distress in PJS patients compared to the general population, and to evaluate determinants of QoL and psychological distress.

Methods: In a cross-sectional study, 61 adult PJS patients from two Dutch academic hospitals were invited to complete a questionnaire on QoL, psychological distress, and illness perceptions.

Results: The questionnaire was returned by 52 patients (85% response rate, 56% females, median age 44.5 years). PJS patients reported similar anxiety (p=0.57) and depression (p=0.61) scores as the general population. They reported a lower general health perception (p=0.003), more limitations due to emotional problems (p=0.045) and a lower mental well-being (p=0.036) than the general population. Strong beliefs in negative consequences of PJS on daily life, a relapsing course of the disease, strong emotional reactions to PJS, and female gender were major determinants for a lower QoL.

Conclusions: PJS patients experience a similar level of psychological distress as the general population, but a poorer general health perception, more limitations due to emotional problems, and a poorer mental QoL. Illness perceptions and female gender were major predictors for this lower QoL. These results may help to recognize PJS patients who might benefit from psychological support.

INTRODUCTION

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant inherited disorder caused by *STK11* gene mutations.[1,2] PJS is clinically characterized by mucocutaneous pigmentations and gastrointestinal hamartomatous polyps.[3,4] The polyps can cause various complications already early in life, including abdominal pain, anaemia, bleeding, and acute intestinal obstruction caused by intussusception.[5-7] Intussusceptions may cause life-threatening surgical emergencies and considerable morbidity. In addition, PJS patients are at increased risk for the development of gastrointestinal and extra-gastrointestinal malignancies such as colorectal, gastric, breast, and gynaecological cancers.[8-10] Lifetime cumulative cancer risks as high as 93% have been described with relative risks up to 18 compared to the general population.[8,11]

Surveillance of PJS patients is recommended for both the risk of non-malignant complications of the polyps such as intussusception, as well as the elevated cancer risk. Since polyp-related complications may occur at young age and the spectrum of PJS-associated cancers is wide, surveillance usually starts at a young age and includes various intermittent invasive examinations. Surveillance generally starts between the ages of 8-12 years with physical examinations and small-bowel surveillance, later in life (between the ages of 20-30 years) extended by gastroduodenoscopy and colonoscopy at 2-3 year intervals as well as annual gynecological and breast examinations.[11-14] This can be very burdensome for patients. In addition, PJS patients may worry about their cancer and intussusception risk, and about negative effects of PJS on daily life such as work and insurance. Feelings of guilt for transmitting the disease to their offspring and the occurrence of PJS-related events in relatives may cause additional distress.

Hypothetically, PJS patients may experience more psychological distress and a lower quality of life (QoL) compared to the general population. However, data on psychological distress and QoL in PJS patients are very limited. So far, only one study concerning this topic has been published, showing that PJS patients suffer from mild depression.[15] More insight into the QoL and psychosocial distress in PJS patients is valuable for patient management. In addition, knowledge of determinants of the QoL, such as illness perceptions[16], is useful for psychological support in order to target those risk factors responsible for a lower QoL. Illness perceptions include, among others, perceptions about the likely timeline of the illness, its controllability and its consequences.[17] Other potential determinants of QoL and psychosocial distress include clinical variables (e.g. history of cancer), demographic variables (e.g. gender) and cancer worries. The first aim of this study was to compare QoL and psychological distress in PJS patients to the general population, and the second aim was to identify determinants of QoL and psychological distress.

METHODS

Patients

A total of 61 PJS patients from two Dutch academic hospitals (Erasmus MC University Medical Centre, Rotterdam, and Academic Medical Centre, Amsterdam) were invited to complete a questionnaire on QoL and psychological distress. The study was approved by the Institutional Review Boards of both participating hospitals. Patients were eligible if they were aged 18 years or older and when their diagnosis of PJS had previously been established. The diagnosis of PJS was considered to be established when patients fulfilled diagnostic criteria recommended by the World Health Organisation.[18] All eligible patients were informed about the study either by direct contact at the outpatient clinic or by telephone. Subsequently, the questionnaire, an information-folder, a consent form and a reply paid envelop were sent to all potential participants by mail. After 6 and 12 weeks a reminder was sent to potential participants. The questionnaire was returned by 52 patients (85% response rate).

Measures

The questionnaire comprised a range of demographic variables including age, gender, marital status, parenthood and educational level. Educational level was classified as high (higher secondary school, higher vocational school, university) or low (primary education only, lower or intermediate vocational school, lower secondary school). Marital status was classified as having a partner (married, living together, partner but not living together), or no partner (single, divorced, widowed). Clinical variables such as *STK11* mutation-status, history of cancer or intussusception, family history of PJS and affected relatives with a history of cancer or intussusception were derived from medical records.

Health-related QoL was measured by the 36-item Short-Form Health Survey (SF36)[19], which has been widely used in QoL studies. A validated Dutch translation is available[20] and normative data for the Netherlands have been published.[21] The SF36 comprises 36 items measuring 8 health scales; physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotional problems and mental health. Scores for all scales were transformed to a score where 0 represents worst possible health and 100 indicates best possible health. On the basis of the 8 subscales, 2 summary scores, a physical component summary score and a mental component summary score have been developed. Both summary scores were calculated based on normative data from the Dutch general population with a mean score of 50 (± 10 SD).

Psychological distress was measured by the Hospital Anxiety and Depression Scale (HADS).[22] The HADS has also been validated in the Dutch general population[23] and contains 14 items each of which is rated on a four point scale (0-3). Seven items reflect anxiety and

seven reflect depression. The sum on each subscale indicates the overall anxiety and depression score (between 0 and 21). A score of 11 or more is frequently categorized as a probable anxiety or depressive disorder.[22]

Illness perception was evaluated by the Illness Perception Questionnaire-Revised (IPQ-R).[24] We included all IPQ-R subscales: timeline acute/chronic (is PJS acute or chronic?), timeline cyclical (do symptoms come and go in cycles?), consequences (how does PJS affect my life?), personal control (what can I do about PJS?), treatment control (what can treatment do about PJS?), illness coherence (understanding of PJS), and emotional representations (emotional responses to PJS). Agreement with statements were assessed on 5-point rating scales (1 = strongly disagree, 5 = strongly agree). Higher scores on the timeline acute/chronic, timeline cyclical, and consequence subscales indicate a stronger belief in the chronicity of PJS, a relapsing course of PJS, and adverse consequences of PJS on life. High scores on the personal and treatment control, coherence, and emotional representation subscales represent positive beliefs about the controllability of PJS (either by self-care or medical care), a better understanding of PJS, and stronger emotional reactions to PJS.

We assessed concerns regarding cancer with the cancer worry scale (CWS).[25] The CWS is a four-item scale that measures worries about the risk of developing cancer and the impact of worries on daily functioning (frequency of thoughts of developing cancer, impact of thoughts about cancer on mood, impact of thoughts about cancer on daily activities and level of concern for developing cancer). Each item has 4 possible responses (1 = not at all =, 4 = almost all the time / very concerned), which are summed to create a cancer worry score between 4 and 16. A higher score indicates more concerns regarding cancer. We also investigated concerns for intussusceptions with an adapted version of the CWS, the intussusception worry scale (IWS). The IWS contains similar questions as the CWS, but the word "cancer" has been replaced by the words "acute intestinal obstruction".

Statistical analyses

Data were analyzed using the SPSS 15.0 statistical software for Windows. QoL scores and psychological distress scores of the PJS cohort were compared to normative data for the Dutch general population by the Student's t-test. A two-sided p-value < 0.05 was considered statistically significant. We also compared mean scores between patients from the two participating hospitals. Since there were no significant differences in HADS scores, SF36 scores, IPQ-R scores, and CWS scores between responders from the two participating hospitals, no adjustment for hospital was applied in further analyses.

Determinants were evaluated for QoL and psychological distress subscales (outcome measures) that showed a significant difference compared to the general population. First, univariate associations between determinants and outcome measures were examined by linear regression analysis. Second, the variables age and gender plus all variables with a p-

value < 0.1 in the univariate analyses were included in a multivariate linear regression model, using the backward selection procedure with a p-value of 0.1 for removal from the model.

RESULTS

The questionnaire was completed by 52 patients (56% females) from 34 families, with a median age of 44.5 (18-74) years. There were 19 sporadic PJS cases among the responders (i.e. no family history for PJS), and 9 patients (18%) had a history of cancer. Baseline characteristics of the 52 responders are shown in Table 1. The non-responders, 3 females and 6 males, had a similar age (p = 0.87) and gender distribution (p = 0.29) as the responders.

Table 1. Baseline characteristics of the responders (n = 52).

Female	29 (56%)
Median age (range), yrs	44.5 (18-74)
High educational level	23 (44%)
Having a partner	36 (69%)
Having one or more children	24 (46%)
Familial PJS Sporadic PJS / Family history unknown	33 (63%) 19 (37%)
Personal history of intussusception	39 (75%)
Personal history of malignancy Malignancy in family	9 (17%) 16 (31%)
DNA mutation analysis performed	37 (71%)
STK11 mutation carrier	33 / 37 (89%)

Psychological distress

There was no significant difference in mean anxiety score (p = 0.57) or depression score (p = 0.61) between our PJS cohort and the general population (in our cohort the mean anxiety score was 4.8 ± 4.0 SD, and the mean depression score 3.1 ± 3.7 SD). A potential clinical significant anxiety disorder or depression was probable in 6 PJS patients; 4 patients (8%, 3/4 females) had an anxiety score ≥ 11, and 2 other patients (4%, both female) had a depression score ≥ 11. Three of these 6 patients already received professional psychosocial support. In general, there was no correlation between the level of anxiety or depression and a personal history of cancer (anxiety; p = 0.42 and depression; p = 0.15) or intussusception (anxiety; p = 0.92 and depression; p = 0.59). Neither there was a correlation between a family history of cancer and the level of anxiety (p = 0.96) or the level of depression (p = 0.60). Furthermore, there were no significant associations between a personal history of cancer and cancer worries (p = 0.11) or between cancer in the family and cancer worries (p = 0.53).

QoL

The mean SF36 subscale scores and summary scores are depicted in Table 2. Compared to the general population, PJS patients had a significantly poorer general health perception (p = 0.003) and experienced more limitations in daily activities such as work due to emotional problems (p = 0.045). In addition, the PJS cohort scored significantly lower on the mental component summary scale (p = 0.036).

Table 2. QoL; SF36 subscale-scores in the PJS cohort compared to the general population.

SF36 subscales	**PJS cohort (n = 52) mean score (±SD)**	**General population[21] (n = 7076) mean score (±SD)**	**p**
Physical functioning	90.2 (17.5)	91.3 (16.5)	0.65
Role limitations due to physical problems	77.6 (36.9)	85.1 (30.5)	0.15
Bodily pain	83.3 (22.6)	84.8 (22.0)	0.63
General health	63.4 (24.7)	73.9 (18.2)	0.003
Vitality	66.3 (23.3)	71.1 (18.6)	0.14
Social functioning	83.7 (24.7)	89.3 (18.4)	0.11
Role limitations due to emotional problems	81.7 (35.5)	91.9 (23.8)	0.045
Mental health	76.9 (19.8)	81.5 (15.3)	0.10
Mental Component Summary (MCS)	45.8 (13.8)	50 (10)	0.036
Physical Component Summary (PCS)	48.6 (9.9)	50 (10)	0.31

p = p-value

Predictors were assessed for the two suboptimal subscales (general health perception and limitations in daily activities due to emotional problems) and the mental component summary scale. Univariate analysis showed 8 variables associated (p-value < 0.05) with a poorer general health perception (Table 3), 5 variables associated with limitations due to emotional problems (Table 4), and 6 variables associated with a lower summary component score (Table 5). These variables were included in 3 multivariate linear regression models (data not shown). Female gender as well as cancer and intussusception worries were major determinants for a lower QoL (i.e. the 3 suboptimal SF36 scores).

In contrast to what we had expected, the previous analyses showed that illness perceptions were only minor determinants of QoL. Since the limited role for illness perceptions may be explained by correlations between the determinants used in the models, we performed a correlation analysis. Indeed, there were correlations between the different illness perceptions, as well as between some illness perceptions and cancer / intussusception worries (data not shown). These correlations were more pronounced between cancer and intussusception worries on the one hand, and the perceptions concerning consequences and emotional representations on the other hand. Therefore, we repeated the multivariate analyses without the CWS and IWS scores in the models. The results of these analyses are shown in Table 3,

Table 3. QoL; determinants of general health perception (SF36).

n = 52	Univariate analysis		Multivariate linear regression analysis (excl. CWS & IWS)	
	β (95% CI)	p	β (95% CI)	p
Sex; Male/Female	22.0 (9.5 ; 34.6)	0.001	8.3 (-1.4 ; 18.0)	0.092
Age	0.2 (-0.3 ; -0.7)	0.44	-	NS
Education; High/Low	-7.6 (-21.4 ; 6.2)	0.28	NA	NA
Partner; Y/N	17.5 (3.3 ; 31.7)	0.017	17.8 (6.9 ; 28.7)	0.002
Familial; Y/N	13.8 (-1.2 ; 28.9)	0.071	NA	NA
Malignancy; Y/N	-15.5 (-33.3 ; 2.4)	0.088	NA	NA
Intussusception; Y/N	-1.6 (-20.1 ; 16.8)	0.86	NA	NA
CWS score	-6.6 (-8.9 ; -4.3)	0.000	NA*	NA*
IWS score	-9.0 (-12.2 ; -5.8)	0.000	NA*	NA*
Illness perceptions:				
Timeline acute/chronic	-0.0 (-1.9 ; 1.9)	1.00	NA	NA
Consequences	-2.4 (-3.4 ; -1.4)	0.000	-1.2 (-2.3 ; -0.2)	0.024
Personal control	0.2 (-1.3 ; 1.6)	0.84	NA	NA
Treatment control	1.8 (0.1 ; 3.4)	0.037	-	NS
Illness coherence	1.5 (-0.3 ; 3.3)	0.099	NA	NA
Timeline cyclical	-4.0 (-6.1 ; -1.9)	0.000	-2.2 (-3.9 ; -0.5)	0.013
Emotional representations	-3.3 (-4.3 ; -2.4)	0.000	-1.4 (-2.7 ; -0.4)	0.044

p = p-value, 95% CI = 95% confidence interval, NA = not analyzed, NS = not significant.
Y = yes, N = no. CWS = cancer worry scale, IWS = intussusception worry scale.
* Not in multivariate model because of correlations with illness perceptions.

Table 4, and Table 5. Not having a partner, stronger beliefs in negative effects of PJS on life, stronger beliefs about the cyclical nature of PJS and stronger emotional reactions triggered by PJS were associated with a poorer general health perception (Table 3). Female gender and stronger beliefs in negative consequences of PJS on life were risk factors for more limitations in daily life due to emotional problems (Table 4), as well as for a lower mental component summary score (Table 5).

DISCUSSION

In this study we assessed health-related QoL and psychological distress in a Dutch cohort of PJS patients. We showed that, although PJS can be associated with considerable disease burden, PJS patients experience a similar level of psychological distress as the general Dutch population. Nevertheless, PJS patients report a poorer mental QoL, they experience more limitations in daily functioning due to emotional problems, and they have a poorer general health perception. We checked whether differences in socio-demographic characteristics

Table 4. QoL; determinants of role limitations due to emotional problems (SF36).

(n = 51)	Univariate analysis		Multivariate linear regression analysis (excl. CWS & IWS)	
	β (95% CI)	p	β (95% CI)	p
Sex; Male/Female	25.4 (6.5 ; 44.3)	0.009	22.0 (3.9 ; 40.1)	0.019
Age	0.4 (-0.3 ; 1.1)	0.28	-	NS
Education; High/Low	-7.8 (-28.0 ; 12.5)	0.44	NA	NA
Partner; Y/N	15.8 (-5.4 ; 37.1)	0.14	NA	NA
Familial; Y/N	12.4 (-11.0 ; 35.9)	0.29	NA	NA
Malignancy; Y/N	-9.3 (-35.6 ; 17.1)	0.48	NA	NA
Intussusception; Y/N	-3.7 (-29.4 ; 21.9)	0.77	NA	NA
CWS score	-6.6 (-10.5 ; -2.7)	0.001	NA*	NA*
IWS score	-6.2 (-11.8 ; -0.6)	0.031	NA*	NA*
Illness perceptions:				
Timeline acute/chronic	0.5 (-2.3 ; 3.3)	0.74	NA	NA
Consequences	-2.3 (-3.9 ; -0.6)	0.007	-2.0 (-3.6 ; -0.4)	0.014
Personal control	0.4 (-1.7 ; 2.5)	0.70	NA	NA
Treatment control	2.2 (-0.2 ; 4.5)	0.077	NA	NA
Illness coherence	0.3 (-2.3 ; 3.0)	0.80	NA	NA
Timeline cyclical	-2.4 (-5.8 ; 1.0)	0.17	NA	NA
Emotional representations	-2.4 (-4.3 ; -0.6)	0.01	-	NS

p = p-value, 95% CI = 95% confidence interval, NA = not analyzed, NS = not significant.
Y = yes, N = no. CWS = cancer worry scale, IWS = intussusception worry scale.
* Not in multivariate model because of correlations with illness perceptions.

between PJS patients and the SF36 norm-population could have influenced these outcomes. The age and sex distribution were similar in both groups, and the only difference was that the PJS cohort was higher educated (p = 0.01) than the norm-population. Although educational level is a well known predictor for better mental and physical health[26], educational level was not a determinant of mental well-being, emotional problems or general health perception in our sample.

Our results corroborate an earlier report concerning a suboptimal mental well-being in PJS patients compared to healthy individuals.[15] However, there was a difference in outcome regarding depressive symptoms. Woo et al. reported that PJS patients suffered from mild depressive symptoms.[15] In contrast, our PJS cohort scored similar on the HADS depression-subscale as the general population. Differences between the results presented by Woo et al. and the present study may be explained by different measures used (CES-D versus HADS) and differences in inclusion of patients. Woo et al. recruited participants indirectly over the internet[15], whereas we systematically included patients from the out-patients clinics of the two participating hospitals.

Table 5. QoL; determinants of mental component summary (SF36).

(n = 51)	Univariate analysis		Multivariate linear regression analysis (excl. CWS & IWS)	
	β (95% CI)	p	β (95% CI)	p
Sex; Male/Female	12.4 (5.3 ; 19.4)	0.001	8.8 (1.9 ; 15.8)	0.014
Age	0.2 (-0.1 ; 0.4)	0.23	-	NS
Education; High/Low	-2.4 (-10.3 ; 5.5)	0.54	NA	NA
Partner; Y/N	7.5 (-0.6 ; 15.7)	0.07	6.8 (-0.7 ; 14.4)	0.073
Familial; Y/N	6.4 (-2.4 ; 15.3)	0.15	NA	NA
Malignancy; Y/N	-6.1 (-16.3 ; 4.0)	0.23	NA	NA
Intussusception; Y/N	-2.1 (-13.2 ; 9.1)	0.71	NA	NA
CWS score	-3.1 (-4.5 ; -1.7)	0.000	NA*	NA*
IWS score	-2.7 (-4.9 ; -0.6)	0.015	NA*	NA*
Illness perceptions:				
Timeline acute/chronic	-0.0 (-1.1 ; 1.1)	0.98	NA	NA
Consequences	-1.0 (-1.6 ; -0.4)	0.002	-1.0 (-1.5 ; -0.4)	0.002
Personal control	0.2 (-0.6 ; 1.0)	0.67	NA	NA
Treatment control	0.5 (-0.4 ; 1.5)	0.25	NA	NA
Illness coherence	0.4 (-0.6 ; 1.4)	0.40	NA	NA
Timeline cyclical	-1.5 (-2.8 ; -0.2)	0.022	-	NS
Emotional representations	-1.4 (-2.1 ; -0.7)	0.000	-	NS

p = p-value, 95% CI = 95% confidence interval, NA = not analyzed, NS = not significant.
Y = yes, N = no. CWS = cancer worry scale, IWS = intussusception worry scale.
* Not in multivariate model because of correlations with illness perceptions.

The fact that our PJS patients reported similar levels of psychological distress (and had similar scores on most SF36 health scales) as the general population, might in part be explained by the phenomenon known as response shift. This means that patients anticipate to poor health and make a QoL judgment adapted to their circumstances.[27,28] Similar levels of psychological distress as presented here have previously been described in other cancer-susceptibility syndromes such as Lynch syndrome[29] and familial adenomatous polyposis.[30]

Our PJS cohort did have lower scores on two SF36 subscales and the mental component summary. Illness perceptions were hypothesized to be important determinants of the lower SF36 scores in our PJS cohort. Yet, due to correlations between the determinants in the models of our first set of analyses, predominantly between cancer and intussusception worries and the illness perceptions, this could be confirmed only in part. After the exclusion of cancer and intussusception worries from the models, the importance of the illness perceptions became more distinct. Illness perceptions were evaluated by the IPQ-R questionnaire.[24] The IPQ-R studies the dimensions of the Leventhal's self-regulation model[31], including beliefs about the identity, cause, consequences, expected timeline and controllability of a disease. This model predicts that patients form beliefs (illness perceptions) in response to their dis-

ease. These beliefs influence coping responses, which in turn may influence QoL. Insight in illness perceptions and determinants of both health-related QoL and psychological distress are valuable in medical support. Moreover, illness perceptions are dynamic variables and can therefore be targeted by psychological support.[32,33] Our results show that stronger emotional reactions to PJS, beliefs in negative consequences of PJS on life, and beliefs in the cyclical nature of symptoms caused by PJS were predominantly associated with an impaired QoL. Indeed, a cyclical course of illness - as in PJS with complications due to the polyps that come and go - poses a psychological challenge such that patients are always on guard because symptoms of disease may strike them at any time.[34].

The variable parenthood was not used in the multivariate models to determine determinants for a lower QoL. The reason for this was that explorative statistics had remarkably shown that women with PJS less often had children than men with PJS ($p < 0.001$). This may partly be explained by the fact that the men in our cohort were on average older than the women (45 years versus 37 years, $p = 0.054$). Still, the question is raised why women with PJS less often have children than male PJS patients. There could be biological reasons for this caused by the nature of the disease (i.e. a history of hysterectomy or ovariectomy due PJS-associated tumors), but future research may also address potential psychosocial explanations for this difference.

This study is hampered by some limitations. First of all, the cross-sectional study-design makes evaluation of causal interactions impossible. Instead, we can only demonstrate statistical associations between determinants and QoL. In the future longitudinal studies are required to confirm our conclusions. Furthermore, non-responders may experience more or less distress and more or less well-being than responders, leading to underestimation or overestimation of the level of distress and well-being in our sample. In addition there may have been differences in socio-demographic characteristics between responders and non-responders, so we cannot exclude non-response bias in determinants of QoL. Yet, the response rate was over 85% and there were no significant differences in age and sex between responders and non-responders. Finally, since PJS is a rare disorder, this study is hampered by a small sample size. The power of our study might have been too low to detect statistical differences. Nevertheless, to our knowledge, this is the largest series of PJS patients assessed for health-related QoL and psychological distress.

In conclusion, this study demonstrates that PJS patients experience more emotional and mental difficulties due to their disorder than physical disabilities. More specific, PJS patients experience a similar level of psychological distress compared to the general population, but they have a poorer general health perception, a poorer mental QoL, and they experience more limitations in daily functioning due to emotional problems. Female gender and illness perceptions were major predictors for this lower QoL. These results are valuable in the medical support of PJS patients, as they help physicians to recognize patients who may benefit from psychological support. Psychosocial support such as cognitive behavioral therapy may

target illness perceptions, in order to optimalize the general health of PJS patients. Finally, our results may also apply to other hereditary colorectal cancer syndromes.

ACKNOWLEDGEMENTS

We would like to thank all participating PJS patients.

REFERENCES

1. Jenne DE, Reimann H, Nezu J, Friedel W, Loff S, Jeschke R, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat Genet 1998;18(1):38-43.
2. Hemminki A, Markie D, Tomlinson I, Avizienyte E, Roth S, Loukola A, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 1998;391(6663):184-187.
3. Peutz JLA. Over een zeer merkwaardige, gecombineerde familiaire polyposis van de slijmliezen van den tractus intestinalis met die van de neuskeelholte en gepaard met eigenaardige pigmentaties van huid en slijmvliezen. Ned Maandschr v Geneesk 1921;10:134-146.
4. McGarrity TJ, Kulin HE, Zaino RJ. Peutz-Jeghers syndrome. Am J Gastroenterol 2000;95(3):596-604.
5. Utsunomiya J, Gocho H, Miyanaga T, Hamaguchi E, Kashimure A. Peutz-Jeghers syndrome: its natural course and management. Johns Hopkins Med J 1975;136(2):71-82.
6. Choi HS, Park YJ, Youk EG, Yoon KA, Ku JL, Kim NK, et al. Clinical characteristics of Peutz-Jeghers syndrome in Korean polyposis patients. Int J Colorectal Dis 2000;15(1):35-38.
7. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, et al. STK11 status and intussusception risk in Peutz-Jeghers syndrome. J Med Genet 2006;43(8):e41.
8. Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119(6):1447-1453.
9. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 2006;12(10):3209-3215.
10. Mehenni H, Resta N, Park JG, Miyaki M, Guanti G, Costanza MC. Cancer risks in LKB1 germline mutation carriers. Gut 2006;55(7):984-990.
11. van Lier MG, Wagner A, Mathus-Vliegen EM, Kuipers EJ, Steyerberg EW, van Leerdam ME. High Cancer Risk in Peutz-Jeghers Syndrome: A Systematic Review and Surveillance Recommendations. Am J Gastroenterol 2010;105(6):1258-1264.
12. The Johns Hopkins guide for patients and families: Peutz-Jeghers syndrome. Baltimore: Johns Hopkins University. 2001.
13. Hinds R, Philp C, Hyer W, Fell JM. Complications of childhood Peutz-Jeghers syndrome: implications for pediatric screening. J Pediatr Gastroenterol Nutr 2004;39(2):219-220.
14. Giardiello FM, Trimbath JD. Peutz-Jeghers syndrome and management recommendations. Clin Gastroenterol Hepatol 2006;4(4):408-415.
15. Woo A, Sadana A, Mauger DT, Baker MJ, Berk T, McGarrity TJ. Psychosocial impact of Peutz-Jeghers Syndrome. Fam Cancer 2009;8(1):59-65.
16. Testa MA, Simonson DC. Assesment of quality-of-life outcomes. N Engl J Med 1996;334(13):835-840.
17. Leventhal H., Diefenbach M. The active side of illness cognition. In: Skelton J.A., Croyle R.T., editors. Mental representation in health and illness.New York: Springer-Verlag; 1991.
18. Aaltonen LA, Jarvinen H, Gruber SB, Billaud M, Jass JR. World Health Organization Classification of Tumours. Pathology and Genetics. Tumours of the Digestive System. Lyon: IARC Press; 2000.
19. Ware JE, Jr., Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30(6):473-483.
20. Aaronson NK, Muller M, Cohen PD, Essink-Bot ML, Fekkes M, Sanderman R, et al. Translation, validation, and norming of the Dutch language version of the SF-36 Health Survey in community and chronic disease populations. J Clin Epidemiol 1998;51(11):1055-1068.

21. Kruijshaar ME, Hoeymans N, Bijl RV, Spijker J, Essink-Bot ML. Levels of disability in major depression: findings from the Netherlands Mental Health Survey and Incidence Study (NEMESIS). J Affect Disord 2003;77(1):53-64.
22. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand 1983; 67(6):361-370.
23. Spinhoven P, Ormel J, Sloekers PP, Kempen GI, Speckens AE, Van Hemert AM. A validation study of the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. Psychol Med 1997;27(2):363-370.
24. Moss-Morris R, Weinman J, Petrie KJ, Horne R, Cameron LD, Buick D. The revised illness perception questionnaire (IPQ-R). Psychology and Health 2002;17(1):1-16.
25. Lerman C, Trock B, Rimer BK, Jepson C, Brody D, Boyce A. Psychological side effects of breast cancer screening. Health Psychol 1991;10(4):259-267.
26. Regidor E, Barrio G, de la FL, Domingo A, Rodriguez C, Alonso J. Association between educational level and health related quality of life in Spanish adults. J Epidemiol Community Health 1999; 53(2):75-82.
27. Jansen SJ, Stiggelbout AM, Nooij MA, Noordijk EM, Kievit J. Response shift in quality of life measurement in early-stage breast cancer patients undergoing radiotherapy. Qual Life Res 2000;9(6): 603-615.
28. Schwartz CE, Sprangers MA. Methodological approaches for assessing response shift in longitudinal health-related quality-of-life research. Soc Sci Med 1999;48(11):1531-1548.
29. Geirdal AO, Reichelt JG, Dahl AA, Heimdal K, Maehle L, Stormorken A, et al. Psychological distress in women at risk of hereditary breast/ovarian or HNPCC cancers in the absence of demonstrated mutations. Fam Cancer 2005;4(2):121-126.
30. Douma KF, Aaronson NK, Vasen HF, Gerritsma MA, Gundy CM, Janssen EP, et al. Psychological distress and use of psychosocial support in familial adenomatous polyposis. Psychooncology 2010;19(3):289-298.
31. Leventhal H, Safer MA, Panagis DM. The impact of communications on the self-regulation of health beliefs, decisions, and behavior. Health Educ Q 1983;10(1):3-29.
32. Kreuter MW, Strecher VJ. Changing inaccurate perceptions of health risk: results from a randomized trial. Health Psychol 1995;14(1):56-63.
33. Edwards A, Hood K, Matthews E, Russell D, Russell I, Barker J, et al. The effectiveness of one-to-one risk communication interventions in health care: a systematic review. Med Decis Making 2000; 20(3):290-297.
34. Rolland JS. Cancer and the family: an integrative model. Cancer 2005;104(11 Suppl):2584-2595.

Peutz-Jeghers syndrome and family planning: The attitude towards prenatal diagnosis and preimplantation genetic diagnosis

Submitted

M.G.F. van Lier[1]
S.E. Korsse[1]
E.M.H. Mathus-Vliegen[2]
E.J. Kuipers[1,3]
A.M.W. van den Ouweland[4]
K. Vanheusden[4,5]
M.E. van Leerdam[1]
A. Wagner[4]

Departments of Gastroenterology and Hepatology[1], Internal Medicine[3], Clinical Genetics[4] and Psychology[5], Erasmus University Medical Centre, Rotterdam, the Netherlands.
Department of Gastroenterology and Hepatology[2], Academic Medical Centre, Amsterdam, the Netherlands.

ABSTRACT

Background: Peutz-Jeghers syndrome (PJS) is a hereditary disorder caused by *STK11* gene mutations, and is associated with considerable morbidity and decreased life expectancy. This study was conducted to assess the genetic test uptake among PJS patients, and their attitude towards family planning, prenatal diagnosis (PND) and pregnancy termination, and preimplantation genetic diagnosis (PGD).

Methods: In a cross-sectional study, 61 adult PJS patients from two Dutch academic hospitals were asked to complete a questionnaire concerning genetic testing, family planning, PND and PGD.

Results: The questionnaire was completed by 52 patients (85% response rate, 56% females) with a median age of 45 (range 18-74) years. Thirty-seven (71%) respondents had undergone genetic testing. Female gender and parenthood were positive predictors for genetic test uptake. Twenty-four respondents (46%, 18/24 males) had children. Fifteen (29%) respondents reported that their diagnosis of PJS had influenced their decisions regarding family planning, including 10 patients (19%, 9/10 females) who did not want to have children because of their PJS. Termination of pregnancy after PND in the case of a fetus with PJS in a personal situation was considered 'acceptable' for 15% of the respondents, whereas 52% considered PGD acceptable.

Conclusions: The diagnosis of PJS influences the decisions regarding family planning in one third of PJS patients, especially in women. Most patients have a negative attitude towards pregnancy termination after PND, while PGD in case of PJS is judged more acceptable. These results emphasize the importance of discussing aspects regarding family planning with PJS patients, including PND and PGD.

INTRODUCTION

Peutz-Jeghers syndrome (PJS) is a rare autosomal dominant inherited disorder caused by germline mutations in the *STK11* gene, also known as the *LKB1* gene.[1,2] The syndrome is clinically characterized by gastrointestinal hamartomas and mucocutaneous pigmentations.[3,4] Hamartomatous polyps can develop already early in life and may cause various complications, including anaemia, bleeding and acute intestinal obstruction caused by intussusception.[5,6] Furthermore, PJS is associated with an increased cancer risk in adult life. Lifetime cumulative cancer risks as high as 93% have been described, with a relative cancer risk between 10 and 18 compared to the general population.[7,8]

All these aspects lead to considerable morbidity and decreased life expectancy, which can affect the psychological condition and quality of life of PJS patients. PJS patients suffer from mild depression and experience a poorer mental quality of life, more limitations in daily functioning due to emotional problems, and a poorer general health perception compared to the general population.[9,10] Interestingly, the latter study showed that women with PJS less often had children than male PJS patients, raising questions regarding family planning, reproductive decision making and attitude towards genetic testing.

Family planning is one of the main motives to undergo genetic testing for a hereditary cancer predisposition.[11] On that account, accurate counseling is important for patients suffering from PJS, not only with regard to genetic testing, but also with respect to decisions regarding family planning. Given the autosomal dominant heredity of the syndrome, there is a 50% chance for the offspring of PJS patients to inherit the mutated gene. Genetic testing before birth is available through prenatal diagnosis (PND) (i.e. chorionic villus sampling and amniocentesis), which may result in termination of pregnancy in the case of an affected fetus. In addition, preimplantation genetic diagnosis (PGD) has become available. PGD involves in vitro fertilization (IVF). One or two cells of a 3-day old embryo created *in vitro* are analyzed for the genetic defect and only embryos with an unaffected genotype are selected for transfer to the uterus.[12] Although PND and PGD are available for hereditary cancer syndromes in most European countries, the application of these techniques remains controversial in the social, ethical and political domain.

In PJS patients, data concerning genetic test uptake, reproductive decision making, attitude towards PND with the implication of pregnancy termination, and PGD are lacking. Therefore, the aim of this study was to investigate predictors for genetic testing in PJS patients, their desire to have children and their attitudes towards PND and PGD. In addition, we tried to identify sociodemographic and clinical determinants associated with these issues.

METHODS

Patients

A total of 61 PJS patients from 39 families from two Dutch academic hospitals (Erasmus University Medical Centre, Rotterdam, and Academic Medical Centre, Amsterdam) were invited to complete a questionnaire on genetic testing, family planning, PND, and PGD. The study was approved by the Institutional Review Boards of both participating hospitals. Patients were eligible if they were aged 18 years or older and when their diagnosis of PJS had previously been established according to the diagnostic criteria recommended by the World Health Organisation.[13] All eligible patients were informed about the study either by direct contact at the outpatient clinic or by telephone. Subsequently, the questionnaire, an information-folder, a consent form and a reply paid envelop were sent to all potential participants by mail. After 6 and 12 weeks a reminder was sent to non-respondents.

Measures

The questionnaire comprised a range of demographic variables including age, gender, marital status, parenthood and educational level. Educational level was classified as high (higher secondary school, higher vocational school, university) or low (primary education only, lower or intermediate vocational school, lower secondary school). Marital status was classified as having a partner (married, living together, partner but not living together), or no partner (single, divorced, widowed). Clinical variables such as history of cancer, family history of PJS and affected relatives with a history of cancer were derived from medical records. As a psychological determinant, concerns regarding cancer were assessed with the cancer worry scale (CWS).[14] The CWS measures worries about the risk of developing cancer and the impact of these worries on daily functioning. A higher score indicates more concerns regarding cancer.

In addition, respondents were asked whether or not they had undergone genetic testing and, if they had, what the result had been. Self-reported data regarding genetic testing were confirmed by medical records if possible. Questions were posed about the current desire to have (more) children, and if the diagnosis of PJS had influenced the desire to have (more) children. Furthermore, after a short introductory text about PND, respondents were asked whether or not they considered termination of pregnancy after PND acceptable in four different scenarios; (1) in general, (2) if the fetus has Down syndrome, (3) if the fetus is a carrier of PJS (not in a personal situation), and (4) if in their personal situation the fetus is a carrier of PJS. Subsequently, a short explanation was given about PGD and respondents were similarly asked about their attitude towards the use of PGD in four different scenarios; (1) in general, (2) if the fetus was at increased risk for Down syndrome, (3) if the fetus was at increased

risk for PJS (not in a personal situation), and (4) if in their personal situation the fetus was at increased risk for PJS. For all questions, response categories were 'yes', 'no', or 'unsure'.[15]

Statistical analyses

Data were analyzed using the SPSS 15.0 statistical software for Windows. Descriptive statistics were used to characterize the study sample. Continuous variables were reported by means (and standard deviation) and medians (and range). Univariate analyses (χ^2, Fisher's exact test, independent *t*-test and Mann-Whitney U test) were used to investigate which sociodemographic, clinical and psychological variables were related to attitudes towards genetic testing and PND and PGD. A two-sided p-value < 0.05 was considered statistically significant. Logistic regression analyses were carried out to determine univariate associations between possible determinants (gender, age, parenthood, clinical history of cancer and familial history of PJS or cancer worries) and three outcome measures; genetic testing ('yes' or 'no'), termination of pregnancy after PND acceptable in case of PJS in a personal situation ('yes' or 'no/unsure') and PGD acceptable in case of PJS in a personal situation ('yes' or 'no/unsure'). Subsequently, the variables age and gender plus all variables showing at least a marginally significant association (p < 0.10) were included in a multivariate logistic regression model, using the backward selection procedure with a *P*-value of 0.1 for removal from the model.

RESULTS

Response

The questionnaire was completed by 52 PJS patients (response rate 85%) from 34 families with a median age of 44.5 (18-74) years. There were no statistically significant differences in age (p = 0.86) and gender distribution (p = 0.29) between respondents and non-respondents. Baseline characteristics of the respondents and non-respondents are shown in Table 1.

Genetic testing

Of the 52 patients who completed the questionnaire, 37 patients had undergone genetic testing, and 33 patients (89%) were actually carrier of a pathogenic *STK11* mutation. Univariate analysis showed that a higher age and parenthood were positively associated with a tendency to perform genetic testing in patients with PJS (Table 2). The multivariate model showed female gender (p = 0.035) and parenthood (p = 0.016) as independent predictors for genetic test uptake, whereas age, family history of PJS and a history of cancer were not.

Table 1. Baseline characteristics of respondents and non-respondents.

	52 Respondents n (%)	**9 Non-respondents** n (%)
Median age (range)	44.5 (18-74)	34 (18-67)
≤ 45 yrs (childbearing age)	29 (55.8)	5 (55.6)
> 45 yrs	23 (44.2)	4 (44.4)
Gender		
Male	23 (44.2)	6 (66.7)
Female	29 (55.8)	3 (33.3)
Partner		
Yes	36 (69.2)	Unknown
No	16 (30.8)	Unknown
Children		
Yes	24 (46.2)	5 (55.6)
No	28 (53.8)	4 (44.4)
Educational level		
Low	29 (55.8)	Unknown
High	23 (44.2)	Unknown
Genetic testing performed		
Yes	37 (71.2)	9 (100)
No	15 (28.8)	0 (0)
Family history		
Familial PJS	33 (63)	5 (55.6)
Sporadic PJS / Family history unknown	19 (37)	4 (44.4)

Table 2. Determinants of genetic testing (N=52).

	Univariate regression analysis		**Multivariate regression analysis**	
	OR (95% CI)	p-value	OR (95% CI)	p-value
Gender; male/female	0.597 (0.178;1.997)	0.402	0.088 (0.009;0.846)	0.035
Age	1.042 (0.995;1.092)	0.080	-	>0.1
Children; yes/no	3.235 (0.869;12.043)	0.080	17.664 (1.726;180.818)	0.016
PJS familial; yes/no	1.333 (0.357;4.985)	0.669	NA	NA
Malignancy; yes/no	1.517 (0.277;8.310)	0.631	NA	NA

OR = odds ratio, 95% CI = 95% confidence interval, NA = not analyzed.

Parenthood and influence of PJS on family planning

Twenty-four respondents (46%; median age 50 years) had children. Female PJS patients less often had children than male patients (25% versus 75%, $p < 0.001$). Fifteen of the 52 respondents (29%, median age 44 years) reported that their diagnosis of PJS had influenced their desire to have children (i.e. less or no children), due to the hereditary nature of PJS and/or the morbidity associated with the syndrome. Ten of these 15 respondents (19% of all respondents; median age 45 years) stated that they had decided to have no children because of PJS, including 9 females and one male, the latter whom had adopted a child. Cancer inci-

dence was higher in these 10 patients (56% vs. 44%, p = 0.011) and they scored higher on the cancer worry scale (8 vs. 5.23, p = 0.039) compared to the other respondents. No significant difference was found in age (p = 0.77). Twenty-three of the respondents (44%, median age 45 years) indicated that PJS had not influenced their desire to have children.

Attitude towards termination of pregnancy after PND

In general, 17% of respondents considered termination of pregnancy after PND as 'unacceptable', whereas 62% felt this was 'acceptable' and 19% were unsure (Table 3). More specifically, termination of pregnancy in the case of a fetus with Down syndrome was 'acceptable' for 29% of respondents. In the case of a fetus with PJS (not in a personal situation), termination of pregnancy was considered 'acceptable' by 15% of respondents and 'unacceptable' by 62%. In the case of a fetus with PJS in a personal situation, also 15% of the respondents considered termination of pregnancy 'acceptable', whereas 73% reported this as 'unacceptable'. Univariate logistic regression analyses for the variables gender, age, children, CWS and a personal history of cancer showed that only a higher age (p = 0.042) predicted a negative attitude

Table 3. Attitude of PJS patients towards termination of pregnancy after PND (n = 52).

	n (%)
Termination of a pregnancy is **unacceptable** in every situation	
Yes	9 (17)
No	32 (62)
Don't know	10 (19)
Missing	*1 (2)*
Termination of a pregnancy is **acceptable** if the fetus has Down syndrome	
Yes	15 (29)
No	13 (25)
Don't know	23 (44)
Missing	*1 (2)*
Termination of a pregnancy is **acceptable** if the fetus is carrier of the genetic mutation causing PJS	
Yes	8 (15)
No	32 (62)
Don't know	11 (21)
Missing	*1 (2)*
Termination of **my** pregnancy is **acceptable** if the fetus is carrier of the genetic mutation causing PJS	
Yes	8 (15)
No	38 (73)
Don't know	5 (10)
Missing	*1 (2)*

PND = prenatal diagnosis.

towards pregnancy termination after PND in the case of a fetus with PJS in a personal situation. Multivariate logistic regression analyses did not show any significant associations (data not shown).

Attitude towards PGD

Regardless of the situation, 61% of respondents indicated PGD as 'acceptable', 6% as 'unacceptable', and 29% were unsure (Table 4). In case of a high risk of a fetus with Down syndrome, PGD was 'acceptable' for 54% and 'unacceptable' for 11%, whereas 31% was unsure. In case of a high risk of a fetus with PJS (not in a personal situation), PGD was considered 'acceptable' by 62% of respondents. For 15% this was 'unacceptable'. Fifty-two percent reported this as 'acceptable' for themselves in the case of a fetus at increased risk for PJS, whereas for 17% this was 'unacceptable'. With regard to PJS in a personal situation, both univariate as well as multivariate logistic regression analyses for the variables gender, age, children, CWS and a personal history of cancer did not show any significant associations with the attitude towards PGD (data not shown).

Table 4. Attitude of PJS patients towards pre-implantation genetic diagnosis (n = 52).

	n (%)
PGD is **unacceptable** in every situation	
Yes	3 (6)
No	32 (61)
Don't know	15 (29)
Missing	*2 (4)*
PGD is **acceptable** if the fetus has Down syndrome	
Yes	28 (54)
No	6 (11)
Don't know	16 (31)
Missing	*2 (4)*
PGD is **acceptable** if the fetus is carrier of the genetic mutation causing PJS	
Yes	32 (62)
No	8 (15)
Don't know	10 (19)
Missing	*2 (4)*
PGD is **acceptable for me** if the fetus is carrier of the genetic mutation causing PJS	
Yes	27 (52)
No	9 (17)
Don't know	11 (21)
Missing	*5 (10)*

PGD = preimplantation genetic diagnosis.

DISCUSSION

This is the first survey among PJS patients that evaluated predictors for genetic test uptake, their decisions regarding family planning, and their attitude towards PND and pregnancy termination, and PGD. Seventy-one percent of patients had undergone genetic testing, a requisite for PND and PGD. This proportion is similar to the genetic test uptake (62-97%) in patients with familial adenomatous polyposis (FAP), another autosomal dominant inherited disorder characterized by gastrointestinal polyps and an elevated cancer risk.[16,17] Our multivariate analysis furthermore showed an association between uptake of genetic testing and the determinants female gender and parenthood, which are well known predictors for genetic testing for other hereditary cancer syndromes.[17-19]

Nearly a third of our study population (29%) indicated that they opted for less children or no children at all because of their PJS diagnosis, a similar proportion as previously reported by FAP patients (37% and 35%).[15,20] Interestingly, 90% of our patients (9 / 10) who explicitly indicated that they did not want to have children because of PJS were female. Moreover, female patients less often had children than men with PJS ($p < 0.001$), as mentioned in our previous report.[10] In contrast, in the general Dutch population, men are more often childless than women (www.cbs.nl). The reason for this difference in our study population is not clear. As PJS is associated with an increased risk for the development of gynaecological tumors[8,21], physical inabilities (e.g. hysterectomy or oophorectomy) might have prevented female patients from having children. However, this was the case in only 2 females from our cohort (at the age of 36 and 39 years). In addition, there was no significant difference in cancer incidence between male and female respondents ($p = 0.144$). Therefore, one could postulate that psychosocial explanations for this difference exist. Women might, for example, have a higher sense of responsibility towards their offspring.[22]

All respondents, irrespective of parenthood or not, were asked about their attitude towards termination of pregnancy after PND. Although the majority (62%) did not reject termination of pregnancy after PND in general, the majority of individuals (62%) did not consider pregnancy termination as an acceptable option for PJS. Even more patients (73%) reported that pregnancy termination was unacceptable for themselves in the case of a fetus with PJS, whereas only 15% indicated this as acceptable. Similarly, a previous study showed that the majority of FAP patients did not consider pregnancy termination as a viable option for FAP; only 15% did.[15] In our PJS cohort, pregnancy termination for Down syndrome was reported to be more acceptable than pregnancy termination for PJS. Approximately one third of our patients accepted termination of pregnancy for Down syndrome, corresponding with the general Dutch population.[23]

In contrast, attitude towards PGD was more positive in our cohort. More patients accepted the use of PGD in the case of PJS than pregnancy termination after PND, suggesting a preference for PGD. This preference has also been observed for late-onset neurodegenerative dis-

eases (e.g. Huntington's disease)[24] and cancer susceptibility syndromes including hereditary breast and ovarian cancer[25] and FAP.[20] The preference for PGD above PND might be explained by the fact that PGD is considered a morally and psychologically more acceptable option for genetic testing before birth[24], since it offers patients the possibility to have an unaffected genetically related child while termination of a pregnancy can be avoided. Though, many individuals with a hereditary condition for which PGD has been permitted, are unfamiliar with the technique[26], which is physically and psychologically burdensome.[27] Our questionnaire did not explore the knowledge of respondents about PND and PGD. Although both techniques were shortly described, the information might have been too limited. Furthermore, positive attitudes towards PND and PGD do not necessarily translate into actual use. One study showed that following an informational intake session and/or the provision of written materials about PGD, 44% of couples who were referred for PGD declined its use.[28]

This study is hampered by some limitations. First of all, the cross-sectional study design makes evaluation of causal interactions impossible. Instead, we can only demonstrate statistical associations between determinants and the attitude towards genetic testing and reproductive decision making. Second, only affected individuals were asked to fill in the questionnaire, not their partners, yet it is likely that partners of PJS patients play an important role in the reproductive decision making and family planning. Third, the actual use of PND and subsequent pregnancy termination and PGD amongst PJS patients is not known and questions regarding religion were not included in our questionnaire, while religion can be of influence on the attitude towards both PND as well as PGD. Finally, in spite of the response rate of over 85%, our conclusions are drawn from a small sample size. Since PJS is a rare disorder it is difficult to assess a larger group. However, we managed to approach nearly all known Dutch PJS patients, thereby creating a heterogeneous cohort of patients enrolled in similar surveillance programs and with similar access to medical care. To our knowledge this is the first report concerning reproductive decision making and the attitude towards antenatal diagnostics amongst PJS patients.

In conclusion, this study demonstrates that the diagnosis of PJS influences decisions regarding family planning in approximately one third of PJS patients, especially in women. The majority of patients undergo genetic testing, and many PJS patients have a positive attitude towards PGD as an option to prevent transmission of PJS to their offspring. In contrast, the attitude of respondents was predominantly negative towards pregnancy termination after PND in case of a fetus affected with the syndrome. Our results emphasize not only the importance of accurate genetic counseling for these patients; they also indicate that medical specialists dealing with patients suffering from hereditary cancer syndromes, including PJS, should inform their patients about the possibilities of prenatal testing such as PGD.

ACKNOWLEDGEMENTS

We would like to thank all participating PJS patients.

REFERENCES

1. Hemminki A, Markie D, Tomlinson I, et al. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. Nature 1998;391:184-187.
2. Jenne DE, Reimann H, Nezu J, et al. Peutz-Jeghers syndrome is caused by mutations in a novel serine threonine kinase. Nat Genet 1998;18:38-43.
3. Peutz JLA. Over een zeer merkwaardige, gecombineerde familiaire polyposis van de slijmliezen van den tractus intestinalis met die van de neuskeelholte en gepaard met eigenaardige pigmentaties van huid en slijmvliezen. Ned Maandschr v Geneesk 1921;10:134-146.
4. McGarrity TJ, Amos C. Peutz-Jeghers syndrome: clinicopathology and molecular alterations. Cell Mol Life Sci 2006;63:2135-2144.
5. Utsunomiya J, Gocho H, Miyanaga T, et al. Peutz-Jeghers syndrome: its natural course and management. Johns Hopkins Med J 1975;136:71-82.
6. Hearle N, Schumacher V, Menko FH, et al. STK11 status and intussusception risk in Peutz-Jeghers syndrome. J Med Genet 2006;43:e41.
7. Giardiello FM, Brensinger JD, Tersmette AC, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119:1447-1453.
8. van Lier MG, Wagner A, Mathus-Vliegen EM, et al. High Cancer Risk in Peutz-Jeghers Syndrome: A Systematic Review and Surveillance Recommendations. Am J Gastroenterol 2010;105:1258-1264.
9. Woo A, Sadana A, Mauger DT, et al. Psychosocial impact of Peutz-Jeghers Syndrome. Fam.Cancer 2009;8:59-65.
10. van Lier MG, Mathus-Vliegen EM, van Leerdam ME, et al. Quality of life and psychological distress in patients with Peutz-Jeghers syndrome. Clin Genet 2010;78:219-226.
11. Pasacreta JV. Psychosocial issues associated with genetic testing for breast and ovarian cancer risk: an integrative review. Cancer Invest 2003;21:588-623.
12. Sermon K, Van Steirteghem A, Liebaers I. Preimplantation genetic diagnosis. Lancet 2004;363: 1633-1641.
13. Hamilton SR, Aaltonen LA. World Health Organization Classification of Tumours. Pathology and Genetics. Tumours of the Digestive System. Lyon, IARC Press, 2001.
14. Lerman C, Trock B, Rimer BK, et al. Psychological side effects of breast cancer screening. Health Psychol 1991;10:259-267.
15. Douma KF, Aaronson NK, Vasen HF, et al. Attitudes toward genetic testing in childhood and reproductive decision-making for familial adenomatous polyposis. Eur J Hum Genet 2010;18:186-193.
16. Douma KF, Aaronson NK, Vasen HF, et al. Psychosocial issues in genetic testing for familial adenomatous polyposis: a review of the literature. Psychooncology 2008;17:737-745.
17. Ramsoekh D, van Leerdam ME, Tops CM, et al. The use of genetic testing in hereditary colorectal cancer syndromes: genetic testing in HNPCC, (A)FAP and MAP. Clin Genet 2007;72:562-567.
18. Meijers-Heijboer EJ, Verhoog LC, Brekelmans CT, et al. Presymptomatic DNA testing and prophylactic surgery in families with a BRCA1 or BRCA2 mutation. Lancet 2000;355:2015-2020.
19. Evans DG, Maher ER, Macleod R, et al. Uptake of genetic testing for cancer predisposition. J Med Genet 1997;34:746-748.
20. Kastrinos F, Stoffel EM, Balmana J, et al. Attitudes toward prenatal genetic testing in patients with familial adenomatous polyposis. Am J Gastroenterol 2007;102:1284-1290.
21. Hearle N, Schumacher V, Menko FH, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 2006;12:3209-3215.

22. d'Agincourt-Canning L, Baird P. Genetic testing for hereditary cancers: the impact of gender on interest, uptake and ethical considerations. Crit Rev Oncol Hematol 2006;58:114-123.
23. Schielen PCJI, Elvers LH, Loeber JG. Downsyndroom-kansbepaling met de eerstetrimester-combinatietest 2004-2006 (deels 2007). RIVM 2008; rapport 230024002.
24. Niermeijer MF, de Die-Smulders CE, Page-Christiaens GC, et al. Genetic cancer syndromes and reproductive choice: dialogue between parents and politicians on preimplantation genetic diagnosis. Ned Tijdschr Geneeskd 2008;152:1503-1506.
25. Menon U, Harper J, Sharma A, et al. Views of BRCA gene mutation carriers on preimplantation genetic diagnosis as a reproductive option for hereditary breast and ovarian cancer. Hum Reprod 2007;22:1573-1577.
26. Musters MA, Repping S, Twisk M, et al. Doelgroep is onwetend: onbekendheid met pre-implantatie genetische diagnostiek is groot. Medisch Contact (Bussum) 2008;63:980-983.
27. Van Voorhis BJ. Clinical practice. In vitro fertilization. N Engl J Med 2007;356:379-386.
28. de Die-Smulders CE, Land JA, Dreesen JC, et al. Results from 10 years of preimplantation-genetic diagnostics in The Netherlands. Ned Tijdschr Geneeskd 2004;148:2491-2496.

A review on the molecular diagnostics of Lynch syndrome: A central role for the pathology laboratory

8

J Cell Mol Med. 2010;14(1-2):181-197

M.G.F. van Lier[1]
A. Wagner[2]
M.E. van Leerdam[1]
K. Biermann[3]
E.J. Kuipers[1,4]
E.W. Steyerberg[5]
H.J. Dubbink[3]
W.N.M. Dinjens[3]

Departments of Gastroenterology and Hepatology[1], Clinical Genetics[2], Pathology[3], Internal Medicine[4], and Public Health[5], Erasmus MC, University Medical Center Rotterdam, The Netherlands

ABSTRACT

Lynch syndrome (LS) is caused by mutations in mismatch repair (MMR) genes and is characterized by a high cumulative risk for the development of mainly colorectal carcinoma and endometrial carcinoma. Early detection of LS is important since surveillance can reduce morbidity and mortality. However, the diagnosis of LS is complicated by the absence of a pre-morbid phenotype and germline mutation analysis is expensive and time-consuming. Therefore it is standard practice to precede germline mutation analysis by a molecular diagnostic work-up of tumors, guided by clinical and pathological criteria, to select patients for germline mutation analysis. In this review we address these molecular analyses, the central role for the pathologist in the selection of patients for germline diagnostics of LS, as well as the molecular basis of LS.

LYNCH SYNDROME

Colorectal cancer (CRC) is the most common malignancy within the European Union and ranks second to lung cancer as a cause of cancer-related mortality.[1] CRC results from both genetic and environmental factors. The most common genetic susceptibility for CRC is Lynch syndrome (LS), formerly known as hereditary non-polyposis colorectal cancer (HNPCC). LS accounts for approximately 3% of all CRCs[2,3], and also for 2% of all endometrial cancers.[4] The burden of LS is considerably greater than these percentages imply, as the cancers are diagnosed at a young age and synchronous or metachronous malignancies occur in 30% of the patients.[5,6]

LS is characterized by a high lifetime risk for the development of CRC (20-70%), endometrial cancer (15-70%), and other extra-colonic cancers (< 15%).[7-14] These extra-colonic malignancies include carcinomas of the small intestine, stomach, pancreas and biliary tract, ovaries, brain, upper urinary tract and skin. LS is caused by germline mutations in mismatch repair (MMR) genes[15], and the definitive diagnosis is currently made by identification of an inactivating germline mutation in one of the MMR genes *MLH1*, *MSH2*, *MSH6* or *PMS2*.[16] Early detection of LS is of great importance, particularly in pre-symptomatic mutation carriers, since colonoscopic surveillance has proven to reduce CRC morbidity and mortality by 65-70%[17-19] and prophylactic surgery may prevent endometrial and ovarian carcinoma effectively.[20] Individuals with a predisposing mutation are candidates for participation in surveillance programs.

The diagnosis of LS is hampered by the absence of specific diagnostic features and the first manifestation in many patients is the presence of an advanced cancer. Furthermore, DNA mutation analysis is time-consuming and expensive. For these reasons, DNA analysis is generally preceded by a molecular diagnostic work-up to select patients as candidates for genetic tests. This molecular diagnostic work-up may be guided by several clinical and pathological criteria such as the presence of LS associated malignancies, number of malignancies and age at cancer diagnosis, family history, as well as histological tumor features such as mucinous or signet-ring differentiation. In this review, we address the central role for the pathologist in the selection of patients for germline diagnostics of LS, the molecular analyses to identify LS, as well as the molecular basis of LS.

IDENTIFICATION OF PATIENTS AT RISK FOR LYNCH SYNDROME

Different models and strategies have been developed to identify patients with LS. In 1990 the Amsterdam Criteria I were developed to provide a basis for uniformity in collaborative studies to find the disease-causing gene (Table 1).[21] These criteria were designed to be highly specific at the expense of the sensitivity.[3,22] They were criticized because extra-colonic tumors were not taken into account, thereby excluding classical LS families. Therefore, the Amsterdam

Table 1. Amsterdam criteria I.[21]

Families must fulfill all criteria:
1. There should be at least three relatives with a CRC.
2. One should be a first-degree relative of the other two.
3. At least two successive generations should be affected.
4. At least one should be diagnosed before the age of 50 years.
5. Familial adenomatous polyposis (FAP) should be excluded.
6. Tumors should be verified by pathological examination.

CRC = colorectal cancer.

Table 2. Amsterdam criteria II.[23]

Families must fulfill all criteria:
1. There should be at least three relatives with a LS-associated cancer*.
2. One should be a first-degree relative of the other two.
3. At least two successive generations should be affected.
4. At least one should be diagnosed before the age of 50 years.
5. Familial adenomatous polyposis (FAP) should be excluded in the CRC case(s), if any.
6. Tumors should be verified by pathological examination.

CRC = colorectal cancer.
* Colorectal cancer, cancer of the endometrium, small-bowel, ureter, or renal pelvis.

Criteria II were established in 1999 (Table 2).[23] However, many families with the syndrome (i.e. mutation carriers) do not meet these criteria[24], usually because these families are too small or there is a late onset of the disease. In addition, obtaining a thorough family history is difficult in clinical practice[25] and patients may have limited knowledge of their family history.[26,27]

In 1997 the Bethesda Guidelines were published to select patients whose tumors should be analyzed for molecular features associated with LS, i.e. microsatellite instability (MSI), to identify potential mutation carriers (Table 3).[28] The Bethesda Guidelines have been revised in 2004 to make them more suitable for use in clinical practice, and are not only based on family history, but also on age at cancer diagnosis, number of LS-associated carcinomas and certain histological tumor features (Table 4).[29] These histological tumor features, associated with LS, include the presence of tumor-infiltrating lymphocytes, a Crohn's-like lymphocytic reaction, mucinous or signet-ring cell differentiation, and a medullary or undifferentiated and solid growth pattern. The additional value of these pathology characteristics in the selection of tumors for further testing for LS has been described previously.[30,31] However, these histological features are related to both microsatellite unstable sporadic tumors as well as LS tumors. Therefore the ability to identify LS patients alone on the basis of these tumor features is limited.[32] In addition, the assessment of these histological tumor features indicating MSI is poorly implemented in daily clinical practice.[32]

At present, the most widely accepted recommendation for the identification of patients with LS is based on the combination of these revised Bethesda Guidelines and MSI-testing.

Table 3. Original Bethesda Guidelines.[28]

Individuals meeting any one of the following should undergo MSI-testing:
1. Individuals with cancer in families that meet the Amsterdam criteria.
2. Individuals with two LS-related cancers, including synchronous and metachronous CRCs or associated extra-colonic cancers*.
3. Individuals with CRC and a first-degree relative with CRC and/or LS-related extracolonic cancer and/or a colorectal adenoma; one of the cancers diagnosed at age < 45 years, and the adenoma diagnosed at age < 40 years.
4. Individuals with CRC or endometrial cancer diagnosed at age < 45 years.
5. Individuals with right-sided CRC with an undifferentiated pattern (solid/cribriform) on histopathology diagnosed at age < 45 years ‡.
6. Individuals with signet-ring-cell-type CRC (more than 50% signet ring cells) diagnosed at age < 45 years.
7. Individuals with adenomas diagnosed at age < 40 years.

LS = Lynch syndrome, CRC = colorectal cancer.
* Endometrial, ovarian, gastric, hepatobiliary, or small-bowel cancer or transitional cell carcinoma of the renal pelvis or ureter.
‡ Solid/cribriform defined as poorly differentiated or undifferentiated carcinoma composed of irregular, solid sheets of large eosinophilic cells and containing small gland-like spaces.

Table 4. Revised Bethesda Guidelines.[29]

Individuals meeting any one of the following should undergo MSI-testing:
1. CRC diagnosed in an individual under age 50 years.
2. Presence of synchronous, metachronous colorectal, or other LS-associated tumors*, regardless of age.
3. CRC with the MSI-H histology‡, in a patient < 60 years of age.
4. CRC in 1 or more first-degree relatives with a LS-related tumor*, with 1 of the cancers being diagnosed under age 50 years.
5. CRC diagnosed in 2 or more first- or second-degree relatives with LS-related tumors*, regardless of age.

LS = Lynch syndrome, CRC = colorectal cancer.
* Endometrial, ovarian, gastric, small-bowel, pancreas, hepatobiliary tract, renal pelvis or ureter, and brain tumors, sebaceous gland adenomas and keratoacanthomas.
‡ Presence of tumor-infiltrating lymphocytes, Crohn's like lymphocytic reaction, mucinous or signet-ring differentiation, or medullary growth pattern.

This combination has proven to be an effective and efficient strategy for LS identification, with a sensitivity for detection of mutation carriers reported from 72%[3] up to 100%[33-36], and a specificity ranging from 77-98%.[33,35,36] However, these criteria have been criticized because of the use of broad and complex variables, and families with *MSH6* and possibly also *PMS2* mutations remain undetected.[37] It has also been shown in several studies that these criteria are poorly implemented in clinical practice.[32,38-40]

In 2005, a Dutch group therefore developed a new strategy for the detection of LS.[41] In this strategy the pathologist selects newly diagnosed patients fulfilling one of the following criteria for MSI analysis; 1) CRC before the age of 50 years, 2) two LS-associated tumors, including synchronous or metachronous CRCs or LS-associated tumors, or 3) adenoma before the age of 40 years. These criteria, known as MIPA criteria, simplify the Bethesda guidelines in such a way that pathologists, without knowledge of family history, can easily apply them. These criteria were found to be effective, efficient and feasible in daily practice.[41,42]

Table 5. Dutch guideline for MSI-testing (*www.oncoline.nl*).

The pathologist is advised to requests MSI-testing (and immunohistochemistry of the MMR proteins) in the following patients:
1. CRC or endometrial carcinoma before the age of 50 years.
2. A second CRC before the age of 70 years.
3. CRC before the age of 70 years AND another synchronous or previous LS-associated tumor*.

LS = Lynch syndrome, CRC = colorectal cancer.
* Colorectal, endometrial, ovarian, gastric, small-bowel, pancreas, hepatobiliary tract, renal pelvis or ureter cancer, and brain tumors, sebaceous gland adenomas and keratoacanthomas.

In the Netherlands, the diagnosis of LS is currently based on a nationwide guideline for MSI analysis (Table 5), that was introduced in January 2008 (*www.oncoline.nl*). This guideline resembles the MIPA criteria. MSI analysis (and immunohistochemistry of the MMR proteins) is requested by the pathologist in patients newly diagnosed with CRC or endometrial carcinoma before the age of 50 years, or patients with 2 LS-associated tumors (including synchronous and metachronous CRCs or LS-associated tumors) before the age of 70 years. Presence of multiple LS-associated cancers is registered in PALGA, the nationwide network and registry of histopathology and cytopathology in the Netherlands (*www.palga.nl*). For MSI analysis based on a positive family history, referral to a clinical geneticist is indicated. In those cases MSI analysis will generally be performed when the (revised) Bethesda or Amsterdam Criteria are met and if archival paraffin-embedded tumor tissue can be obtained.

Since clinical criteria do not quantify the likelihood of being a mutation carrier, refined algorithms and multivariable models have been developed to make a quantitative estimation of the risk of carrying a germline MMR gene mutation, without the requirement of tissue.[36] Several models that combine personal and familial data have been developed, such as the Leiden model, the Edinburgh Model, Premm1,2 and the MMR-pro model.[43-46] One of the advantages of the quantitative models is that the threshold for sensitivity or specificity of the model can be adjusted based upon the clinical situation. However, the role for these models in daily clinical practice remains to be determined.

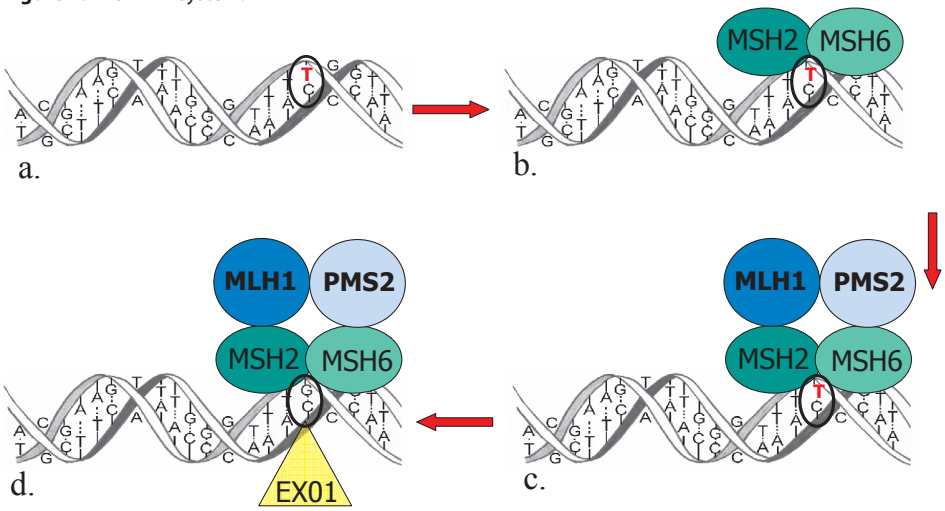
At present a study (called LIMO and coordinated by the Erasmus MC, Rotterdam, The Netherlands) is performed to determine whether further improvement of LS diagnostics can be obtained by the performance of MSI analysis in CRC patients up to the age of 70 years. MSI analysis is performed in a prospective consecutive series of 1,000 newly diagnosed CRC patients ≤ 70 years, and the results are expected in 2010.

MOLECULAR BASIS OF LYNCH SYNDROME AND SPORADIC MMR-DEFICIENT TUMORS

LS is caused by a germline mutation in one of the MMR genes; most commonly *MLH1* and *MSH2* (± 90%)[47,48], but also *MSH6* and *PMS2*.[37,49,50] LS patients are born with a germline mutation in one of these MMR genes, and acquire inactivation of the second wild-type allele in their tumors, fulfilling Knudson's two hit hypothesis for inactivation of tumor suppressor genes.[51] Because of the high chance of inactivation of the homologous wild-type allele during life, LS transmits phenotypically in an autosomal dominant fashion. The somatic inactivation of the corresponding wild-type allele occurs almost exclusively by small mutations or (partial) gene loss, and bi-allelic inactivation then leads to complete abolition of the protein function. This results in a defective DNA MMR system, since the protein products of the MMR genes are involved in correction of nucleotide base mismatches and small insertions or deletions that arise during DNA replication.[52-54]

The mechanism of MMR has been largely elucidated (Figure 1). MSH2 (mutS homolog 2) forms a heterodimer with MSH6 (mutS homolog 6), sliding along the DNA as a clamp to identify single nucleotide mispairs and small insertions and deletions.[55,56] MLH1 (mutL homolog 1) dimerizes with PMS2 (post-meiotic segregation 2) and binds to the MSH2-MSH6 complex.

Figure 1. The MMR system.

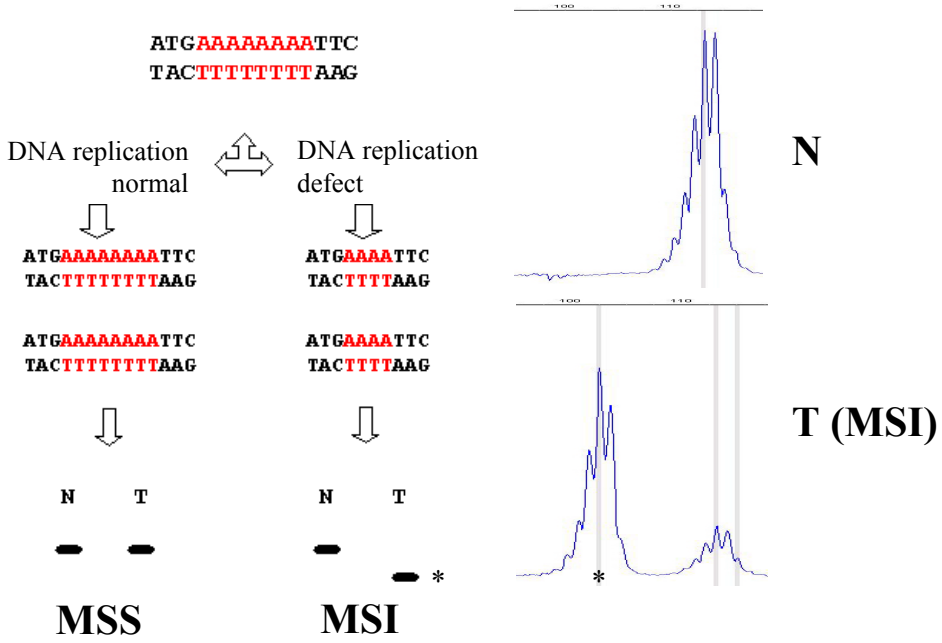


During DNA-replication, insertions or deletions of one or more nucleotides and single nucleotide mismatches may occur. For example;
a) A single nucleotide mismatch occurs (G>T).
b) MSH2 and MSH6 form a heterodimer and recognize the mismatch.
c) MLH1 and PMS2 dimerize and bind to the *MSH2-MSH6* complex.
d) The complex of four proteins activates an exonuclease to perform the DNA-repair.

Together this group of four proteins recruits an exonuclease to perform the DNA-repair.[57,58] If any of the four major proteins (MSH2, MLH1, MSH6, or PMS2) is functionally inactive, mismatches are not repaired. A defective DNA MMR system increases the mutation rate and makes the cell vulnerable to mutations in genes controlling cell growth (including tumor suppressor genes and oncogenes), resulting in an elevated cancer risk.

In case of a defective MMR system, mutations occur frequently in small (usually mononucleotide or dinucleotide) repetitive DNA sequences, known as microsatellites.[59,60] In MMR deficient tumor cells the number of nucleotide repeat units of microsatellites can deviate from the corresponding normal DNA; the number of repeats is usually decreased, but occasionally increased (Figure 2). This variation in repeat units and thus length or size of microsatellites is called microsatellite instability (MSI). MSI (formerly referred to as MIN, another abbreviation for microsatellite instability, or replication error abbreviated as RER) is the molecular hallmark of LS since approximately 95% of all LS-associated cancers show MSI.[61-63] MSI thereby serves as a reliable phenotypic marker of MMR deficiency which is easy to evaluate in order to preselect patients for germline mutation analysis of the MMR genes.

Figure 2. Microsatellite instability (MSI).



A schematic microsatellite is indicated (poly A track). When the tumor cells have an intact MMR system the size of the microsatellite will be the same in DNA isolated from normal (N) and from tumor (T) cells: microsatellite stable (MSS) tumor. In case of a defect in MMR the size of the microsatellite (number of repeat units) can change (in most cases becomes shorter) when comparing N with T DNA: microsatellite unstable (MSI) tumor. Asterisks indicate the microsatellite unstable tumor DNA fragment.

Despite the fact that tumor MSI is a reliable marker for MMR deficiency, it is a marker for LS with limited specificity since 15% of sporadic CRCs also demonstrate a MSI phenotype. This is mainly caused by somatic hypermethylation of the *MLH1* gene promoter.[64,65] DNA methylation is an epigenetic DNA modification that specifically targets cytosine residues at CpG dinucleotides. Genomic regions that contain a high frequency of CpG dinucleotides are called CpG islands, present in the promoters of about 40% of all human genes, including the *MLH1* gene.[66] Hypermethylation of CpG islands in the *MLH1* promoter causes severe inhibition of gene transcription thereby functionally mimicking an inactivating gene mutation. If both copies of the gene are inactivated (mainly by bi-allelic hypermethylation), the DNA MMR function of *MLH1* is lost. This leads to microsatellite unstable cancers, especially in older patients.[65] MLH1 deficient microsatellite unstable tumors can be assessed for *MLH1* hypermethylation to distinguish sporadic CRCs from LS-related cancers. Theoretically, sporadic hypermethylation of the other MMR genes is possible but has not yet been demonstrated.

Specific activating mutations in the *BRAF* oncogene, usually V600E missense mutations (formerly reported as V599E), can be detected in 40-87% of all sporadic microsatellite unstable tumors. An oncogenic *BRAF* mutation has been described only once[67] in numerous investigated LS tumors.[68-76] These results indicate that *BRAF* mutations are closely correlated with *MLH1* methylation in sporadic CRCs.[69-72,76,77] Therefore, *BRAF* mutation status can be used to identify sporadic microsatellite unstable tumors, although it has been demonstrated that determination of hypermethylation of the *MLH1* gene promoter is more sensitive to detect sporadic MSI tumors.[69]

In addition to sporadic forms of *MLH1* promoter hypermethylation, germline epimutations of *MLH1* (soma-wide mono-allelic hypermethylation of the gene promoter) have also been reported.[78-85] Germline *MLH1* hypermethylation, often showing some degree of mosaicism, is functionally equivalent to an inactivating mutation and produces a clinical phenotype that resembles LS. Inheritance of epimutations is weak as the methylation can be cleared on passage through the germline (germline *MLH1* promoter epimutations are reversible during meiosis) and so can display non-Mendelian inheritance. Heritability of epimutations might also be explained by the inheritance of an unknown predisposition to epimutations, rather than the inheritance of the epimutation itself.[86] Although very rare, germline *MLH1* promoter methylation should be considered in younger individuals or individuals with multiple LS-associated tumors without a family history who present with an MSI tumor showing loss of MLH1 expression.[81,82]

Besides germline *MLH1* hypermethylation, a new mechanism of germline *MSH2* hypermethylation has recently been discovered.[87] Ligtenberg et al showed that a germline deletion of the last two exons of *TACSTD1*, the gene just upstream of *MSH2* encoding epithelial cell adhesion molecule (EpCAM), leads to inactivation of the *MSH2* gene by promoter hypermethylation exclusively in tissues expressing EpCAM (mosaic pattern). This mechanism may cause LS in patients with MSH2 deficient microsatellite unstable tumors with an undetectable

MSH2 germline mutation. Identification of these cases is possible by the determination of the methylation status of the *MSH2* gene promoter in the tumor and in EpCAM expressing normal tissues (e.g. normal colorectal mucosa). In addition, evidence for the presence of *MSH2* methylation can be obtained by detection of deletions in the 3'end of the *TACSTD1* gene.

MOLECULAR DIAGNOSTICS OF LYNCH SYNDROME

The molecular diagnostics of LS usually starts with MSI analysis. MSI analysis is traditionally performed with a panel of 5 microsatellite markers proposed by a NCI (National Cancer Institute) sponsored consensus conference, also known as the Bethesda panel.[29] With these markers, microsatellites in tumor DNA are compared to microsatellites in corresponding DNA from normal tissue. Tumors with more than one unstable marker (or ≥ 40% of markers) are categorized as having a high degree of microsatellite instability (MSI-H), which is suspect for LS or epigenetic *MLH1* silencing.[88-91] Those with one unstable marker (20-40% of markers) are categorized as having a low degree of microsatellite instability (MSI-L) and tumors with no instability (≤20%) are categorized as being microsatellite stable (MSS), seen in sporadic carcinomas.[92] Although there are no clear differences in clinical or pathological features between MSI-L and MSS tumors, it has been speculated that MSI-L tumors comprise an independent phenotype.[93] However, there is nowadays no role for separating MSI-L from MSS tumors in the diagnostic work-up. Furthermore, MSI-testing seems not only important for recognition of LS, but may in the future also improve the clinical management of CRC patients. This is because patients with microsatellite unstable CRCs appear to have a better prognosis than patients with MSS tumors[94-97] and they do not seem to benefit from adjuvant chemotherapy with 5-fluorouracil.[98-100]

The Bethesda panel, comprising 2 mononucleotide repeats (BAT-25 and BAT-26) and 3 dinucleotide repeats (D2S123, D5S346 and D17S250)[62], does have some limitations, mainly caused by the dinucleotide repeats. These repeats are highly polymorphic and less sensitive and specific in the identification of MSI-H tumors than mononucleotide repeats. Their use in MSI-screening requires analysis of corresponding germline DNA[101] and the interpretation of size-alterations in dinucleotide repeats is more difficult due to stutter, a PCR artefact. Their use can result in misclassification of MSI-L tumors as MSI-H.[102,103] Furthermore, *MSH6* mutation carriers may develop tumors (predominantly endometrial cancer) without alteration in these dinucleotide repeats leading to false MSI-L or MSS results.[104,105] The limitations of the Bethesda panel have lead to the development of a pentaplex panel, which comprises 5 quasi-monomorphic mononucleotide repeats (see below). This panel shows less variation in size among different ethnic populations and has been shown to be superior to the Bethesda panel for the detection of MSI-H tumors.[102,106] Because the pentaplex analysis is carried out in a single multiplex PCR, this method is simple to use and is free of errors due to mixing samples.

To gain insight into what gene might be affected in patients with MSI-H tumors, MLH1, MSH2, MSH6 and PMS2 protein expression can be assessed by immunohistochemistry. The combination of MSI analysis and MMR protein immunostaining is generally considered as the superior strategy for the identification of suspected LS patients.[107] Absence of MMR protein nuclear staining within the tumor cells can be compared to nuclear staining in the normal cells within the same tumor specimen (and same histological section). The latter then serve as internal positive control.

Due to their heterodimeric nature, different immunohistochemical staining patterns of the MMR proteins can be observed (Table 6). Loss of MLH1 protein due to *MLH1* gene mutation or promoter hypermethylation is usually accompanied with absence of PMS2 in the tumor (Figure 3). Similarly, absence of MSH2 due to *MSH2* mutations results in absence of MSH6 (Figure 3), since MSH6 and PMS2 will disintegrate without their obligatory partners MSH2 and MLH1, respectively. A mutation in either *PMS2* or *MSH6* does not lead to loss of MLH1 and MSH2 protein, respectively (Figure 3) because of the formation of other heterodimers than MLH1-PMS2 and MSH2-MSH6. MLH1 can for instance dimerize with either MLH3 or PMS1[108,109] and MSH2 can also bind to MSH3.[110] Due to the binding of MLH1 and MSH2 to other MMR proteins in the absence of PMS2 or MSH6, there is no concurrent loss of MLH1 and MSH2.[111] To date no bona fide involvement of *PMS1*, *MLH3* or *MSH3* (inactivating mutations) has been demonstrated in LS.

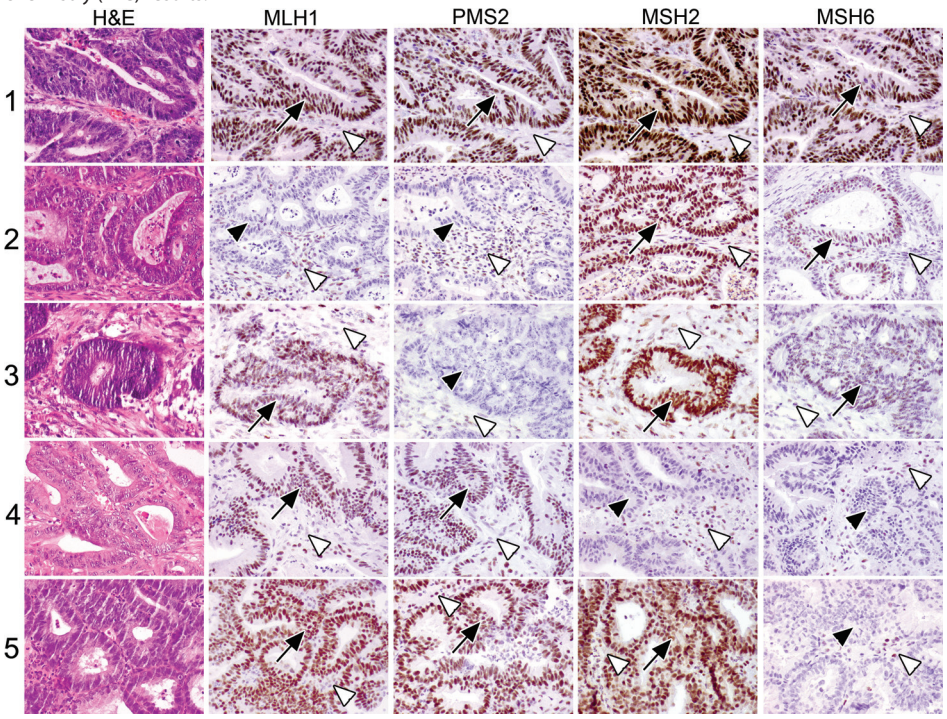
Table 6. Immunohistochemical expression patterns associated with MMR gene mutations.

		MMR gene mutation			
		MLH1	*MSH2*	*MSH6*	*PMS2*
Protein expression	MLH1	– *	+	+	+
	MSH2	+	–	+	+
	MSH6	+	–	–	+
	PMS2	–	+	+	–

* Absent MLH1 protein expression can be associated with either *MLH1* germline mutations as well as epigenetic *MLH1* silencing by promoter methylation.
+ = present nuclear protein expression in tumor cells (as well as in normal cells).
– = absent nuclear protein expression in tumor cells (and present staining in normal cells, thus serving as internal positive control).

In general, absent MSH2, MSH6 or PMS2 expression in tumor cells with present staining in normal cells is suspect for underlying LS and calls for germline testing. Absent MLH1 (and PMS2) expression, can indicate either LS or a sporadic tumor with epigenetically silenced *MLH1*.[111] If epigenetic *MLH1* silencing has been excluded by the analysis of *MLH1* hypermethylation and / or *BRAF* mutation analysis, *MLH1* germline mutation testing is indicated. Furthermore, it might theoretically be possible that immunohistochemical absence of PMS2 or MSH6 without concomitant absence of MLH1 or MSH2, respectively, is due to mutations in

Figure 3. Five colorectal cancer cases; H&E staining and MLH1, PMS2, MSH2 and MSH6 immunohistochemistry (IHC) results.

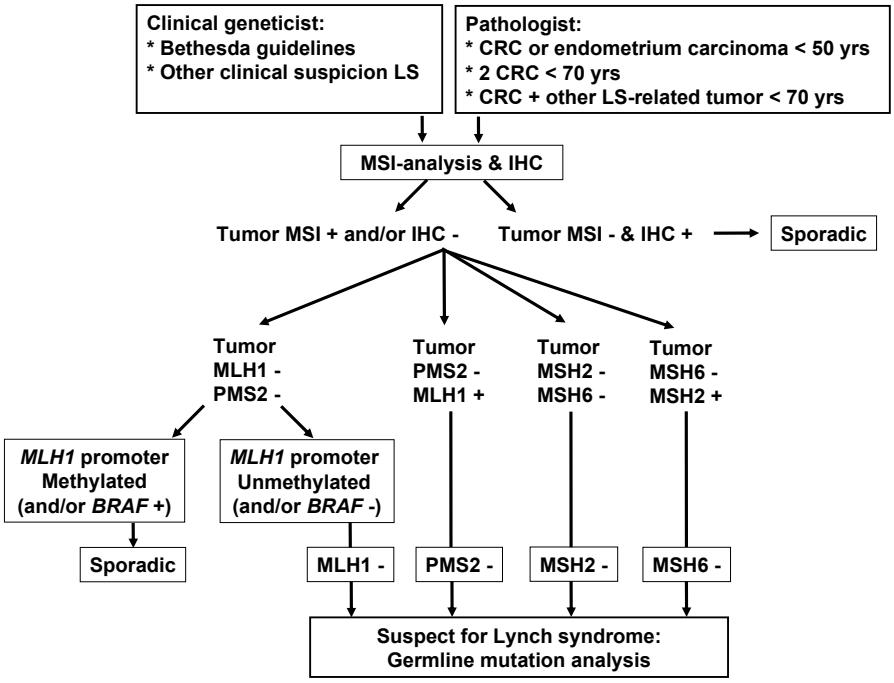


Case 1: normal IHC in the tumor cells.
Case 2: absence of MLH1 and PMS2 in the tumor cells.
Case 3: Absence of PMS2 in the tumor cells.
Case 4: Absence of MSH2 and MSH6 in the tumor cells.
Case 5: Absence of MSH6 in the tumor cells.
Arrows point to IHC-positive tumor cells, filled arrow heads point to IHC-negative tumor cells and open arrow heads point to IHC-positive stromal cells.

MLH1 or *MSH2*. These mutations do not lead to decreased MLH1 and MSH2 immunostaining, while binding to and expression of PMS2 and MSH6 respectively, is abrogated. Therefore, absent PMS2 or MSH6 immunostaining without detectable mutations in the *PMS2* or *MSH2* gene, asks for mutation analysis of *MLH1* or *MSH2* respectively.

At our institution, MSI analysis and immunohistochemistry are requested either by the pathologist when patients fulfil the criteria as depicted in Table 3, or by the clinical geneticist (or clinician) when individuals meet the Bethesda Guidelines. The flowchart of the molecular diagnostics of LS in the Netherlands is depicted in Figure 4. All these different molecular diagnostic procedures will be described in more detail in the next paragraphs.

Figure 4. Flowchart for molecular diagnostics of LS in the Netherlands.

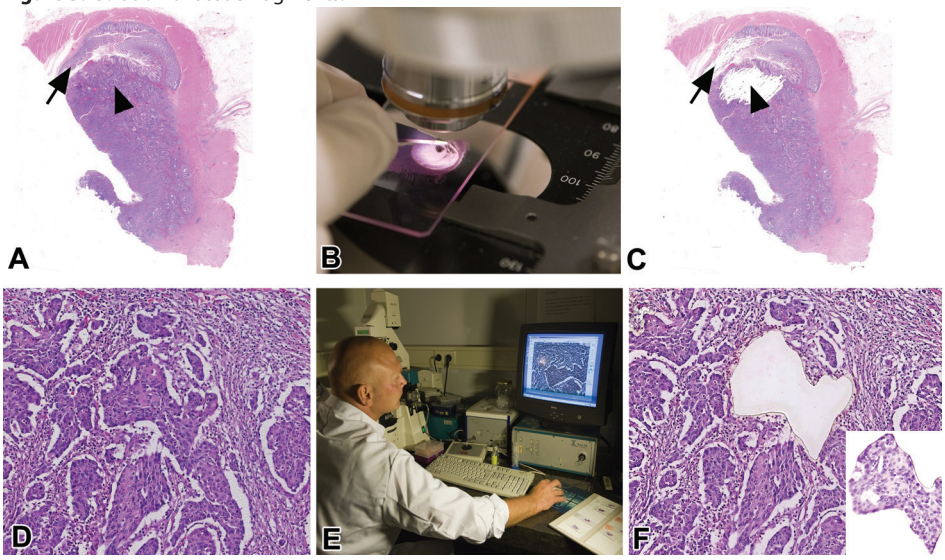


DESCRIPTION OF MOLECULAR ANALYSES

MSI analysis

From routine formalin fixed and paraffin embedded (FFPE) tumor tissue specimens 10 to 20 consecutive sections of 4µm are cut and routinely glued on microscope glass slides. The number of sections is determined by the size of the tissue fragments that need to be isolated for DNA analysis. All sections are deparaffinized and the first and the last section of the series are routine Mayer hematoxylin and eosin (H&E) stained. These sections are used as reference for the isolated tissue parts. The intermediate sections are stained in hematoxylin and rinsed in distilled water. The indicated tumor and normal tissue fragments are then manually scraped in distilled water from the glass slide and transferred to Eppendorf vials. From the remaining tissue fragments on the glass slides, routine microscopic preparations are made after additional staining with eosin. With these preparations the isolated tissue fragments can be verified (Figure 5). Occasionally, when large and easy recognizable tissue fragments can be isolated, scraping is performed from paraffin sections on glass slides without deparaffinization. Furthermore, when the tissue fragments to be isolated are too small for manual isolation, laser microdissection is used on H&E stained sections glued on membrane containing glass slides (PALM Membrane Slides, P.A.L.M. Microlaser Technologies AG, Bernried, Germany)(Figure 5).

Figure 5. Isolation of tissue fragments.



A, B, C. Manual macrodissection of a normal tissue fragment (arrow) and a region of the tumor composed of a high percentage of tumor cells (arrow head), before, during and after macrodissection, respectively. **D, E, F.** Laser capture microdissection (LCM) of a small tumor tissue fragment surrounded by abundant stromal cells, before, during and after LCM, respectively. Insert in F shows the microdissected fragment.

Although MSI can be reliably detected even when DNA is isolated from a tissue fragment composed of only 10% neoplastic cells (unpublished data), tumor DNA is isolated preferably from a tissue fragment with a high percentage (>70%) of tumor cells. DNA isolated from tissue with a high percentage of tumor cells can also be used for reliable additional investigations (*BRAF* mutation and *MLH1* hypermethylation). In the case of an adenoma the fragment with the highest grade of dysplasia should be used for DNA isolation. For isolation of normal DNA a tissue fragment composed of normal cells, preferably from the normal epithelial counterpart of the tumor (e.g. normal colorectal or normal endometrial mucosa), is used to circumvent heterogeneity problems that can be caused by mosaicism (e.g. mosaic *MLH1* promoter germline hypermethylation or *MSH2* promoter hypermethylation only in Epcam expressing cells). However, since these mosaic phenomena are very rare, other normal tissue fragments (e.g. a tumor-negative lymph node) can be used for normal DNA isolation in cases where there is no or not easy to isolate normal mucosa available.

From the microdissected FFPE tissue fragments DNA is extracted by addition of 100 to 200µl (when very small tissue fragments are used digestion is performed in a volume down to 25µl) lysis buffer (10mM Tris/HCL pH 8.0, 1mM EDTA pH 8.0, 0.01% Tween 20) containing 2mg/ml proteinase K and 5% Chelex 100 resin. Following overnight incubation at 56°C, proteinase K is inactivated at 100°C for 10 minutes. Next, dissolved DNA is separated from cell debris by centrifugation at maximum speed in a microcentrifuge for 5 minutes. The DNA-containing supernatant is carefully pipetted from the Chelex resin-containing pellet (Chelex resin inhibits

polymerase activity) and transferred to another Eppendorf vial. In case un-deparaffinized sections were used for DNA isolation, the DNA-containing supernatant is collected by carefully poking the pipette tip through the solidified paraffin layer on top of the supernatant

Different methods for MSI analysis are currently available. In our laboratory we use the MSI analysis system of Promega (Promega, Madison, WI, USA)[103], a fluorescent multiplex PCR-based assay in which the PCR products are separated by capillary electrophoresis using an ABI PRISM 3130*xl* genetic analyzer (Applied Biosystems, Foster City, CA, USA). PCR is performed according to the kit instructions in a total volume of 10µl including 2µl of an 80 fold dilution of the isolated DNA solution. The output data are analyzed with GeneMarker software (SoftGenetics, State College, PA, USA) with pathologist review, to determine MSI-status of tumor samples. This system includes fluorescently labelled primers for co-amplification of five quasi-monomorphic mononucleotide repeat markers BAT-25, BAT-26, NR-21, NR-24 and MONO-27. In addition, 2 pentanucleotide markers (Penta C and Penta D) characterized by a high level of polymorphism have been added to provide information on possible sample mix-up or contamination. Because of the low size variation in the population of the selected mononucleotide markers, this analysis allows in most cases that only tumor DNA is investigated for MSI. DNA from a microsatellite stable cell line suffices as normal DNA reference. If inconclusive results are obtained, for example due to the infrequent occurrence of bi-allelic variation or borderline shifts of the marker peaks, the assay is repeated with both tumor and patient matched normal DNA. Furthermore, additional mononucleotide MSI-markers such as BAT-40 can be used in the case of a MSS tumor with a strong clinical suspicion for underlying LS. Results of microsatellite stable and microsatellite unstable tumors are shown in Figure 6.

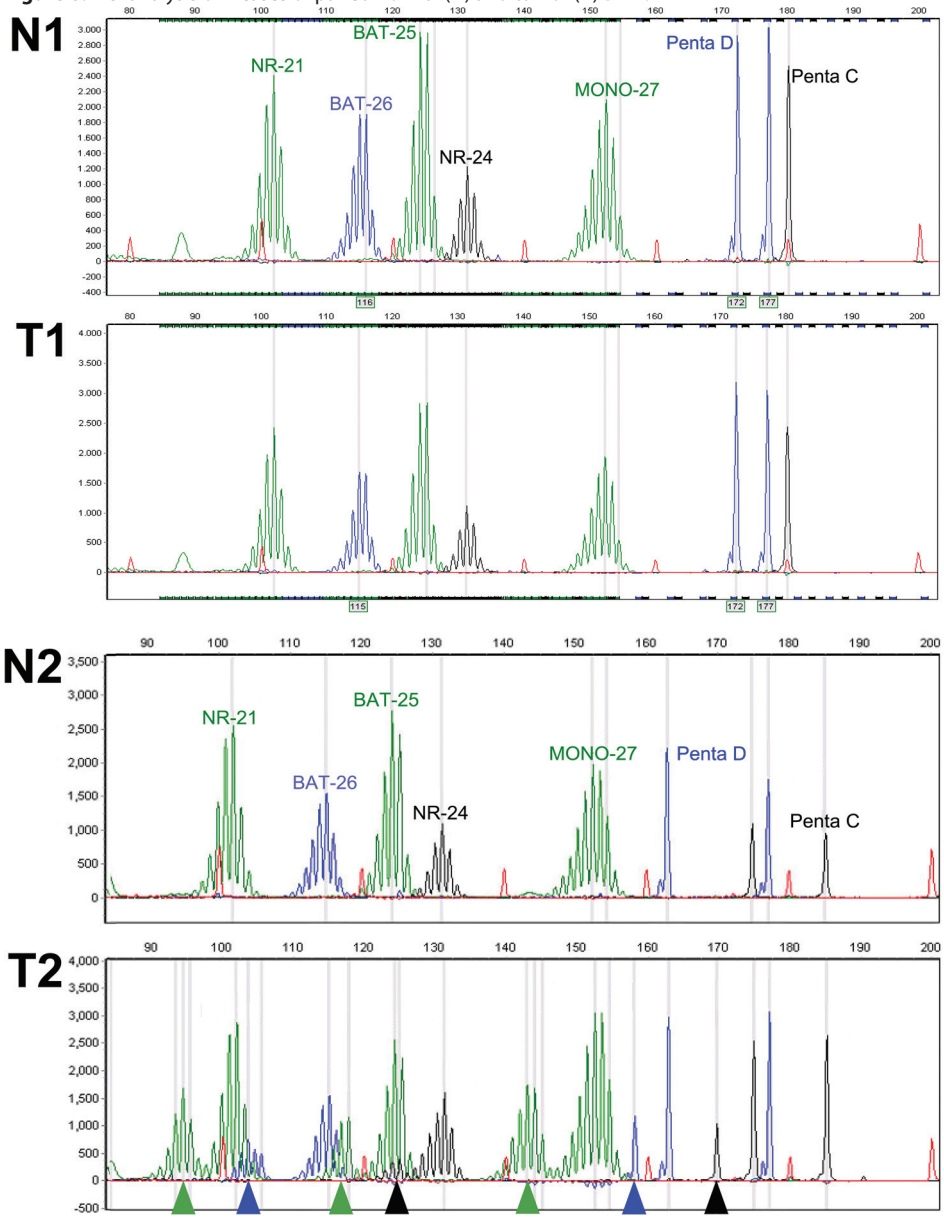
Immunohistochemistry

Our method of immunohistochemistry was described in detail previously.[112] Briefly, FFPE tissue sections (4µm) are dewaxed, and antigen retrieval is performed in 10mM Tris-EDTA buffer, (pH 9.0) in a microwave oven for 45 minutes at 100°C. Primary antibodies anti-MLH1 (Pharmingen BD, Alphen aan den Rijn, the Netherlands; clone G168-728; dilution, 1:20), anti-MSH2 (Pharmingen BD; clone G219-1129; dilution, 1:300), anti- MSH6 (Pharmingen BD; clone 44; dilution, 1:100), and anti-PMS2 (Pharmingen BD; clone A16-4; dilution, 1:50) are applied for 1 hour at room temperature. After washing, immunoreactivity is visualized with the Envision kit (Dako, Glostrup, Denmark). Subsequently, the sections are counterstained with Mayer hematoxylin and evaluated under a light microscope (Figure 3).

MLH1 promoter hypermethylation assay

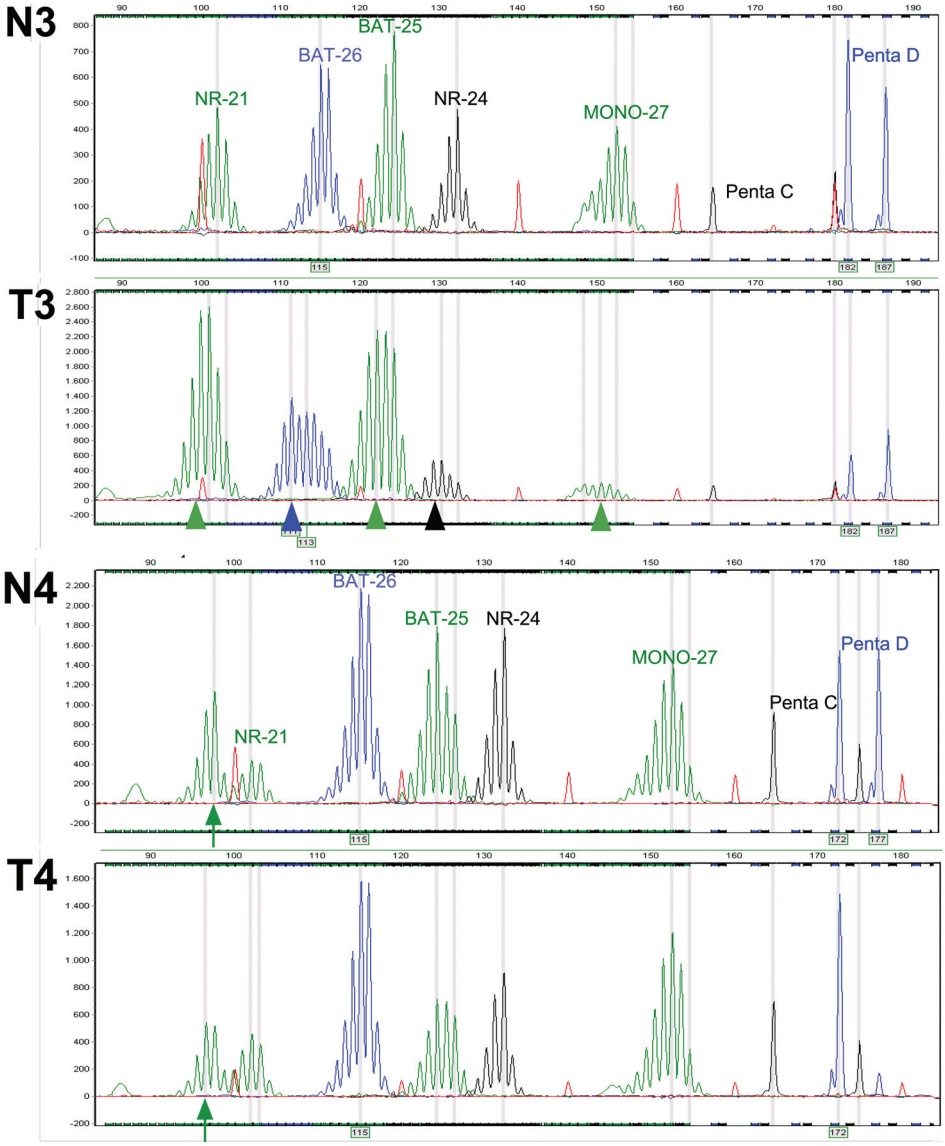
In case of absent MLH1 expression in tumor cells, the methylation status of the *MLH1* promoter can be determined by different methods such as Methylation-Specific PCR[113] and

Figure 6. MSI analysis of 4 cases of paired normal (N) and tumor (T) DNA.



A:
Case 1: N1 and T1, colorectal cancer without MSI, i.e. MSS.
Case 2: N2 and T2, colorectal cancer with clear MSI.

Methylation-Specific Multiplex Ligation-Dependent Probe Amplification (MS-MLPA).[114] MS-MLPA is performed using the SALSA MS-MLPA Kit ME011-A1 for MMR genes (MRC-Holland, Amsterdam, The Netherlands). The analysis is performed according to the kit instructions with



B:

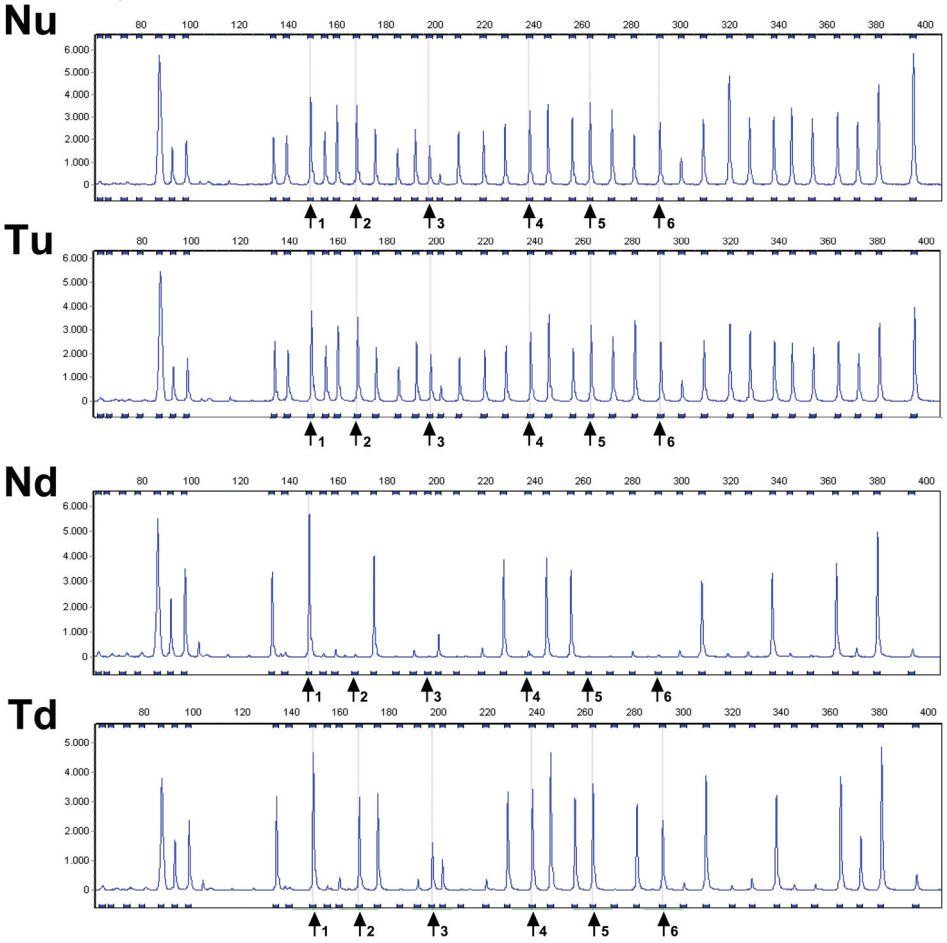
Case 3: N3 and T3, endometrial carcinoma with MSI (subtle microsatellite shifts).
Case 4: N4 and T4, colorectl cancer without MSI but with heterozygous NR-21 microsatellite alleles (present in normal and tumor DNA).
Arrow heads indicate the MSI shifts, arrows indicate the variant NR-21 allele.

3µl undiluted DNA solution as input. The assay takes advantage of methylation-sensitive endonuclease *HhaI*, which only cleaves unmethylated DNA fragments. The MS-MLPA kit contains 8 control probe sequences and 21 methylation-sensitive probes of which 5 recognize CpG dinucleotides within the *MLH1* promoter. The methylation-sensitive probes contain a restriction

site for *Hha*I. Comparison of a *HhaI*-digested DNA sample (yielding only signal of methylated DNA) to its undigested counterpart (yielding signal of both methylated and unmethylated DNA) provides insight into the degree of methylation. Details of the MS-MLPA protocol are freely available on the website of the manufacturer (http://www.mrc-holland.com).

Basically, tumor DNA is hybridized to the probe mix. After hybridization, half of the sample is subjected to a ligation step joining both adjacently hybridized fragments of a probe set, whereas the other half of the sample is subjected to both ligation and *HhaI* digestion, leaving

Figure 7. *MLH1* promoter hypermethylation assay; MS-MLPA analysis of a MSI CRC with absence of MLH1 and PMS2 expression.



Results are shown of paired undigested normal (Nu) and undigested tumor (Tu) DNA and of the methylation-sensitive endonuclease *HhaI* digested normal (Nd) and tumor (Td) DNA. Arrows indicate six *MLH1* promoter probes, nr 1 representative for a fragment without and nrs 2-6 representative for fragments with a *HhaI* restriction site. In the *HhaI* digested DNA, probes 2-6 are clearly present in the tumor DNA (Td) and not in the paired normal DNA (Nd) indicating tumor-specific methylation of the *MLH1* promoter fragments 2-6.

only methylated sequences intact. Subsequent PCR amplification exponentially amplifies all ligated, but undigested, probes. The signal generated with the part of the sample that has undergone both ligation and digestion represents the amount of methylated DNA present in the tumor. For fragment analysis, PCR products are separated by capillary gel electrophoresis using an ABI PRISM 3130*xl* genetic analyzer (Applied Biosystems) and quantified with GeneMarker software version 1.7 (SoftGenetics). The MS-MLPA results are normalized by dividing the peak height of each *MLH1* probe signal by the mean peak height of the eight control fragments obtained with the same sample (Figure 7). The degree of methylation for individual *MLH1* probes can be assessed by dividing normalized values of each *MLH1* probe within digested DNA samples by normalized values of the probe in corresponding undigested samples. The MS-MLPA assay is performed with both tumor and normal mucosal DNA to detect possible germline *MLH1* promoter hypermethylation.

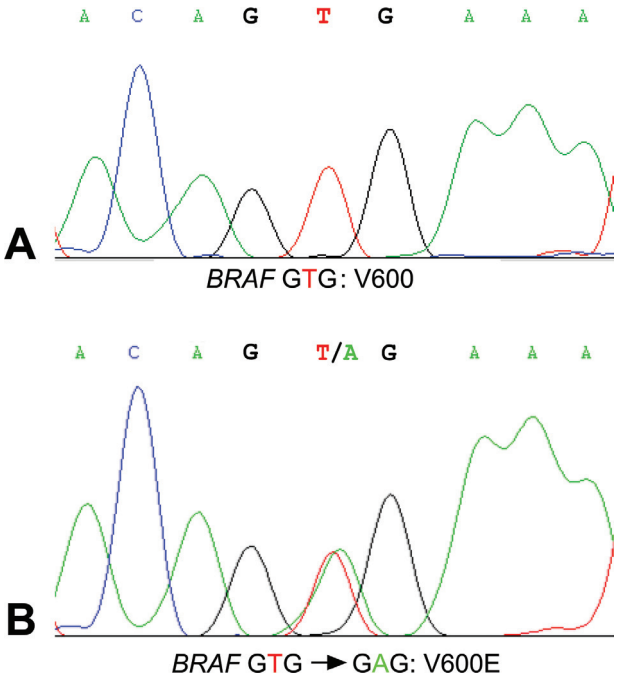
BRAF mutation analysis

BRAF alterations of mutational hotspot codon V600 are determined by bi-directional cycle sequencing of PCR-amplified fragments. PCR amplification is performed by M13-tailed forward primer 5'-TGT AAA ACG ACG GCC AGT AAA CTC TTC ATA ATG CTT GCT CTG -3' and M13-tailed reverse primer 5'-CAG GAA ACA GCT ATG ACC GGC CAA AAA TTT AAT CAG TGG AA-3'. PCR products are generated in a 15µl reaction mixture including 1.0µl undiluted DNA solution, 10µmol of each primer, 25mM $MgCl_2$, 10mM dNTP's and 1U Taq polymerase (Promega, Madison, WI, USA). The PCR reaction is performed using a thermocycler (Biometra, Göttingen, Germany) with an initial denaturating step (95°C) for 3 minutes, followed by 35 cycles consisting of denaturation (95°C) for 30 seconds, annealing (60°C) for 45 seconds and extension (72°C) for 45 seconds. After the final cycle, an extension period of 10 minutes at 72°C is performed. The PCR products are sequenced with M13 forward primer 5'-TGT AAA ACG ACG GCC AGT-3'and M13 reverse primer 5'-CAG GAA ACA GCT ATG ACC-3'using the ABI PRISM *BigDye* Terminator v3.1 kit (Applied Biosystems). Sequence analyses are performed on an ABI PRISM 3130*xl* genetic analyzer (Applied Biosystems). Samples are analyzed using Mutation Surveyor software (SoftGenetics) with pathologist review, and are compared with the public sequence of GenBank (NT-007914). Examples of *BRAF* mutation analysis results are shown in Figure 8.

LIMITATIONS OF MOLECULAR ANALYSES

Over the last decade, the diagnostics of LS have improved considerably. Nevertheless, there still remain some limitations that need to be addressed. It has to be taken into account that the described procedures provide information on the chance that a certain tumor arose in

Figure 8. Mutation analysis of codon 600 of the *BRAF* gene.



A: Tumor DNA without *BRAF* codon 600 mutation.
B: Tumor DNA with a heterozygous oncogenic mutation GTG-GAG, leading to a V600E amino acid substitution.

the context of LS and are not diagnostic for LS in an absolute sense. The false negative rate of MSI analysis is very low (< 5%) but cannot be completely ruled out. MSI can be very subtle or escape detection particularly in low grade lesions as adenomas, in endometrial carcinomas (Figure 6B, panel N3/T3) and in samples with a low percentage of neoplastic cells.[115] These false-negative results may lead to the exclusion of LS patients (and affected family members) from necessary surveillance programs and subsequent failure to detect (secondary) cancers in an early stage. In addition, although rare, sporadic MSS tumors can occur in LS patients and MSI analysis then fails to indicate LS. To exclude false-negative MSI results as much as possible it is necessary to isolate DNA from a tissue fragment with a high percentage of tumor cells. For this, laser microdissection might be preferable instead of manual microdissection. However, laser microdissection is a time-consuming and labour-intensive procedure to obtain sufficient tissue fragments for DNA isolation.[116] In general, it is recommended to refer patients with a high clinical or familial suspicion of LS to a clinical genetics department, irrespective of the MSI-status. In addition, other hereditary CRC syndromes such as attenuated familial adenomatous polyposis (aFAP), MYH-associated polyposis (MAP), Cowden syndrome, or Peutz-Jeghers syndrome might need to be excluded.

Although the assessment of MMR protein expression by immunohistochemistry is a fast and simple procedure, the interpretation of the results can be difficult. Interpretation may be impeded by absence or low intensity of the nuclear staining in tumor and normal tissue due to fixation artifacts, especially in old archival specimens.[117] In case of missense mutations, the inactive protein may be (partly) expressed and detectable by immunohistochemistry. The interpretation is also hampered by some degree of observer-variation and the value of immunohistochemistry partially depends on the experience of the pathologist.[118,119] For these reasons immunohistochemistry cannot replace MSI-testing to detect LS, and this underlines the importance of the combined application of MSI analysis and MMR protein immunostaining to detect LS.

In the evaluation of *MLH1* promoter methylation, it is important to study the correct promoter regions since MLH1 expression only correlates with methylation of the proximal promoter regions (mainly region C, but also region D).[113,120,121] Nevertheless, there are still studies published in which the distal *MLH1* promoter regions were analyzed, which are not or only poorly associated with gene silencing. Moreover, epigenetic inactivation of the second normal *MLH1* allele by promoter methylation (second hit), may also play a role in individuals with LS[122,123], and it should be realized that the detection of *MLH1* promoter methylation can not completely rule out LS. In the case of a strong clinical suspicion, referral to a clinical geneticist is indicated. The exact frequencies of *MLH1* promoter methylation in LS patients (either as a second inactivating event, or as a heritable germline epimutation), are unknown. It has been reported that in tumors from *MLH1* mutation carriers, the wild-type allele is hypermethylated in 0-46% of the tumors.[65,76,80,122,124-129] However, only one study evaluated the proximal promoter region (region D) associated with gene silencing in 55 CRCs and endometrial cancers of *MLH1* germline mutation carriers.[129] Hypermethylation was seen in 7.3% of all tumors (16% of CRCs). In the other studies, promoter regions not associated with *MLH1* silencing were investigated (i.e. the distal promoter regions).[65,76,80,122,124-128]

There are some other points of concern in the molecular diagnostics of LS. First, the value of MSI-testing and immunohistochemistry in other LS-related tumors than CRC is largely unknown.[130] In endometrial tumors, the second most common malignancy in LS, MSI can escape detection by the occurrence of only subtle shifts in the size of the markers.[131] Therefore, MSI analysis in endometrial cancers is performed with patient matched normal DNA as the reference, and molecular pre-screening has been found feasible (Figure 6B, panel N3/T3).[4,132,133] Furthermore, the quality of DNA extracted from FFPE tumors can occasionally be poor and therefore not suitable for MSI analysis.[130] And last but not least, some individuals might have ethical objections against MSI-testing or immunohistochemistry, since the diagnosis of LS can be very likely after the described molecular examinations, which might have negative social consequences and raise concerns e.g. about insurance risks. Therefore, we believe that the clinician should inform the patient about the fact that the pathological examinations may

not only give information about the nature of the tumor, but may also indicate an elevated risk of an underlying hereditary disorder.

CONCLUSIONS

Different diagnostic strategies have been developed for LS as discussed in this review and the optimal method for the identification of LS patients is still debated and in flux. In the previous paragraphs the molecular diagnostic approach of LS in The Netherlands (Erasmus MC, University Medical Center, Rotterdam) has been described (Figure 4). This approach combines MSI analysis and MMR protein immunostaining and is in our opinion a productive way of pre-selecting patients for germline mutation analysis, with a central role for the pathologist. Nevertheless, if the clinical suspicion for LS is very high, e.g. because of a positive family history for LS-associated cancers or a LS-associated malignancy diagnosed at a very young age, referral to a clinical geneticist is strongly recommended, even in the case of tumors without MMR deficiency (i.e. MSS).

ACKNOWLEDGEMENTS

We thank Frank van der Panne for photographic work.

REFERENCES

1. Boyle P, Ferlay J. Cancer incidence and mortality in Europe, 2004. Ann Oncol 2005;16(3):481-488.
2. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338(21):1481-1487.
3. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Feasibility of Screening for Lynch Syndrome Among Patients With Colorectal Cancer. J Clin Oncol 2008;26(35):5783-5788.
4. Hampel H, Frankel W, Panescu J, Lockman J, Sotamaa K, Fix D, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res 2006;66(15):7810-7817.
5. Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM, et al. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. Gastroenterology 1993;104(5):1535-1549.
6. Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. Cancer 1993;71(3):677-685.
7. Quehenberger F, Vasen HF, van Houwelingen HC. Risk of colorectal and endometrial cancer for carriers of mutations of the hMLH1 and hMSH2 gene: correction for ascertainment. J Med Genet 2005;42(6):491-496.
8. Vasen HF, Wijnen JT, Menko FH, Kleibeuker JH, Taal BG, Griffioen G, et al. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. Gastroenterology 1996;110(4):1020-1027.
9. Plaschke J, Engel C, Kruger S, Holinski-Feder E, Pagenstecher C, Mangold E, et al. Lower incidence of colorectal cancer and later age of disease onset in 27 families with pathogenic MSH6 germline mutations compared with families with MLH1 or MSH2 mutations: the German Hereditary Nonpolyposis Colorectal Cancer Consortium. J Clin Oncol 2004;22(22):4486-4494.
10. Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la Chapelle A, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999;81(2):214-218.
11. Vasen HF, Stormorken A, Menko FH, Nagengast FM, Kleibeuker JH, Griffioen G, et al. MSH2 mutation carriers are at higher risk of cancer than MLH1 mutation carriers: a study of hereditary nonpolyposis colorectal cancer families. J Clin Oncol 2001;19(20):4074-4080.
12. Hendriks YM, Wagner A, Morreau H, Menko F, Stormorken A, Quehenberger F, et al. Cancer risk in hereditary nonpolyposis colorectal cancer due to MSH6 mutations: impact on counseling and surveillance. Gastroenterology 2004;127(1):17-25.
13. Hampel H, Stephens JA, Pukkala E, Sankila R, Aaltonen LA, Mecklin JP, et al. Cancer risk in hereditary nonpolyposis colorectal cancer syndrome: later age of onset. Gastroenterology 2005;129(2):415-421.
14. Senter L, Clendenning M, Sotamaa K, Hampel H, Green J, Potter JD, et al. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology 2008;135(2):419-428.
15. Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. J Med Genet 1999;36(11):801-818.
16. Boland CR. Evolution of the nomenclature for the hereditary colorectal cancer syndromes. Fam Cancer 2005;4(3):211-218.
17. Jarvinen HJ, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomaki P, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000;118(5):829-834.

18. de Jong AE, Hendriks YM, Kleibeuker JH, de Boer SY, Cats A, Griffioen G, et al. Decrease in mortality in Lynch syndrome families because of surveillance. Gastroenterology 2006;130(3):665-671.
19. Stupart DA, Goldberg PA, Algar U, Ramesar R. Surveillance colonoscopy improves survival in a cohort of subjects with a single mismatch repair gene mutation. Colorectal Dis 2009;11(2):126-130.
20. Schmeler KM, Lynch HT, Chen LM, Munsell MF, Soliman PT, Clark MB, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. N Engl J Med 2006;354(3):261-269.
21. Vasen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991;34(5):424-425.
22. Kievit W, de Bruin JH, Adang EM, Ligtenberg MJ, Nagengast FM, van Krieken JH, et al. Current clinical selection strategies for identification of hereditary non-polyposis colorectal cancer families are inadequate: a meta-analysis. Clin Genet 2004;65(4):308-316.
23. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 1999;116(6):1453-1456.
24. Lindor NM, Petersen GM, Hadley DW, Kinney AY, Miesfeldt S, Lu KH, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006;296(12):1507-1517.
25. Church J, McGannon E. Family history of colorectal cancer: how often and how accurately is it recorded? Dis Colon Rectum 2000;43(11):1540-1544.
26. Katballe N, Juul S, Christensen M, Orntoft TF, Wikman FP, Laurberg S. Patient accuracy of reporting on hereditary non-polyposis colorectal cancer-related malignancy in family members. Br J Surg 2001;88(9):1228-1233.
27. Sijmons RH, Boonstra AE, Reefhuis J, Hordijk-Hos JM, de Walle HE, Oosterwijk JC, et al. Accuracy of family history of cancer: clinical genetic implications. Eur J Hum Genet 2000;8(3):181-186.
28. Rodriguez-Bigas MA, Boland CR, Hamilton SR, Henson DE, Jass JR, Khan PM, et al. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. J Natl Cancer Inst 1997;89(23):1758-1762.
29. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96(4):261-268.
30. Jenkins MA, Hayashi S, O'Shea AM, Burgart LJ, Smyrk TC, Shimizu D, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology 2007;133(1):48-56.
31. Truta B, Chen YY, Blanco AM, Deng G, Conrad PG, Kim YH, et al. Tumor histology helps to identify Lynch syndrome among colorectal cancer patients. Fam Cancer 2008;7(3):267-274.
32. Sanchez JA, Vogel JD, Kalady MF, Bronner MP, Skacel M, Church JM. Identifying Lynch syndrome: we are all responsible. Dis Colon Rectum 2008;51(12):1750-1756.
33. Wolf B, Gruber S, Henglmueller S, Kappel S, Bergmann M, Wrba F, et al. Efficiency of the revised Bethesda guidelines (2003) for the detection of mutations in mismatch repair genes in Austrian HNPCC patients. Int J Cancer 2006;118(6):1465-1470.
34. Pinol V, Castells A, Andreu M, Castellvi-Bel S, Alenda C, Llor X, et al. Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. JAMA 2005;293(16):1986-1994.
35. Rodriguez-Moranta F, Castells A, Andreu M, Pinol V, Castellvi-Bel S, Alenda C, et al. Clinical performance of original and revised Bethesda guidelines for the identification of MSH2/MLH1 gene

carriers in patients with newly diagnosed colorectal cancer: proposal of a new and simpler set of recommendations. Am J Gastroenterol 2006;101(5):1104-1111.
36. Balmana J, Balaguer F, Castellvi-Bel S, Steyerberg EW, Andreu M, Llor X, et al. Comparison of predictive models, clinical criteria and molecular tumour screening for the identification of patients with Lynch syndrome in a population-based cohort of colorectal cancer patients. J Med Genet 2008;45(9):557-563.
37. Ramsoekh D, Wagner A, van Leerdam ME, Dinjens WN, Steyerberg EW, Halley DJ, et al. A high incidence of MSH6 mutations in Amsterdam criteria II-negative families tested in a diagnostic setting. Gut 2008;57(11):1539-1544.
38. van Dijk DA, Oostindier MJ, Kloosterman-Boele WM, Krijnen P, Vasen HF. Family history is neglected in the work-up of patients with colorectal cancer: a quality assessment using cancer registry data. Fam Cancer 2007;6(1):131-134.
39. Overbeek LI, Hoogerbrugge N, van Krieken JH, Nagengast FM, Ruers TJ, Ligtenberg MJ, et al. Most patients with colorectal tumors at young age do not visit a cancer genetics clinic. Dis Colon Rectum 2008;51(8):1249-1254.
40. van Lier MG, De Wilt JH, Wagemakers JJ, Dinjens WN, Damhuis RA, Wagner A, et al. Underutilization of microsatellite instability analysis in colorectal cancer patients at high risk for Lynch syndrome. Scand J Gastroenterol 2009;44(5):600-604.
41. Kievit W, de Bruin JH, Adang EM, Severens JL, Kleibeuker JH, Sijmons RH, et al. Cost effectiveness of a new strategy to identify HNPCC patients. Gut 2005;54(1):97-102.
42. de Bruin JH, Ligtenberg MJ, Nagengast FM, Adang EM, van Krieken JH, Hoogerbrugge N. Optimizing the detection of hereditary non-polyposis colorectal cancer: an update. Scand J Gastroenterol Suppl 2006;(243):146-152.
43. Wijnen JT, Vasen HF, Khan PM, Zwinderman AH, van der KH, Mulder A, et al. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. N Engl J Med 1998; 339(8):511-518.
44. Barnetson RA, Tenesa A, Farrington SM, Nicholl ID, Cetnarskyj R, Porteous ME, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. N Engl J Med 2006;354(26):2751-2763.
45. Balmana J, Stockwell DH, Steyerberg EW, Stoffel EM, Deffenbaugh AM, Reid JE, et al. Prediction of MLH1 and MSH2 mutations in Lynch syndrome. JAMA 2006;296(12):1469-1478.
46. Chen S, Wang W, Lee S, Nafa K, Lee J, Romans K, et al. Prediction of germline mutations and cancer risk in the Lynch syndrome. JAMA 2006;296(12):1479-1487.
47. Peltomaki P, Vasen H. Mutations associated with HNPCC predisposition - Update of ICG-HNPCC/INSiGHT mutation database. Dis Markers 2004;20(4-5):269-276.
48. Vasen HF, Boland CR. Progress in genetic testing, classification, and identification of Lynch syndrome. JAMA 2005;293(16):2028-2030.
49. Miyaki M, Konishi M, Tanaka K, Kikuchi-Yanoshita R, Muraoka M, Yasuno M, et al. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 1997;17(3): 271-272.
50. Hendriks YM, Jagmohan-Changur S, van der Klift HM, Morreau H, Van PM, Tops C, et al. Heterozygous mutations in PMS2 cause hereditary nonpolyposis colorectal carcinoma (Lynch syndrome). Gastroenterology 2006;130(2):312-322.
51. Knudson AG. Two genetic hits (more or less) to cancer. Nat Rev Cancer 2001;1(2):157-162.
52. Chung DC, Rustgi AK. DNA mismatch repair and cancer. Gastroenterology 1995;109(5):1685-1699.

53. Papadopoulos N, Nicolaides NC, Liu B, Parsons R, Lengauer C, Palombo F, et al. Mutations of GTBP in genetically unstable cells. Science 1995;268(5219):1915-1917.
54. Baker SM, Bronner CE, Zhang L, Plug AW, Robatzek M, Warren G, et al. Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. Cell 1995; 82(2):309-319.
55. Fishel R, Ewel A, Lescoe MK. Purified human MSH2 protein binds to DNA containing mismatched nucleotides. Cancer Res 1994;54(21):5539-5542.
56. Yang Q, Zhang R, Wang XW, Linke SP, Sengupta S, Hickson ID, et al. The mismatch DNA repair heterodimer, hMSH2/6, regulates BLM helicase. Oncogene 2004;23(21):3749-3756.
57. Genschel J, Bazemore LR, Modrich P. Human exonuclease I is required for 5′ and 3′ mismatch repair. J Biol Chem 2002;277(15):13302-13311.
58. Gruber SB. New developments in Lynch syndrome (hereditary nonpolyposis colorectal cancer) and mismatch repair gene testing. Gastroenterology 2006;130(2):577-587.
59. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 1993; 363(6429):558-561.
60. Umar A, Boyer JC, Thomas DC, Nguyen DC, Risinger JI, Boyd J, et al. Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. J Biol Chem 1994;269(20):14367-14370.
61. Aaltonen LA, Peltomaki P, Mecklin JP, Jarvinen H, Jass JR, Green JS, et al. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. Cancer Res 1994;54(7):1645-1648.
62. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58(22):5248-5257.
63. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003;348(10):919-932.
64. Kane MF, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, et al. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. Cancer Res 1997;57(5):808-811.
65. Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci U S A 1998;95(12):6870-6875.
66. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003;349(21):2042-2054.
67. Wang L, Cunningham JM, Winters JL, Guenther JC, French AJ, Boardman LA, et al. BRAF mutations in colon cancer are not likely attributable to defective DNA mismatch repair. Cancer Res 2003; 63(17):5209-5212.
68. Miyaki M, Iijima T, Yamaguchi T, Kadofuku T, Funata N, Mori T. Both BRAF and KRAS mutations are rare in colorectal carcinomas from patients with hereditary nonpolyposis colorectal cancer. Cancer Lett 2004;211(1):105-109.
69. McGivern A, Wynter CV, Whitehall VL, Kambara T, Spring KJ, Walsh MD, et al. Promoter hypermethylation frequency and BRAF mutations distinguish hereditary non-polyposis colon cancer from sporadic MSI-H colon cancer. Fam Cancer 2004;3(2):101-107.

70. Kambara T, Simms LA, Whitehall VL, Spring KJ, Wynter CV, Walsh MD, et al. BRAF mutation is associated with DNA methylation in serrated polyps and cancers of the colorectum. Gut 2004;53(8): 1137-1144.
71. Domingo E, Laiho P, Ollikainen M, Pinto M, Wang L, French AJ, et al. BRAF screening as a low-cost effective strategy for simplifying HNPCC genetic testing. J Med Genet 2004;41(9):664-668.
72. Deng G, Bell I, Crawley S, Gum J, Terdiman JP, Allen BA, et al. BRAF mutation is frequently present in sporadic colorectal cancer with methylated hMLH1, but not in hereditary nonpolyposis colorectal cancer. Clin Cancer Res 2004;10(1 Pt 1):191-195.
73. Young J, Barker MA, Simms LA, Walsh MD, Biden KG, Buchanan D, et al. Evidence for BRAF mutation and variable levels of microsatellite instability in a syndrome of familial colorectal cancer. Clin Gastroenterol Hepatol 2005;3(3):254-263.
74. Loughrey MB, Waring PM, Tan A, Trivett M, Kovalenko S, Beshay V, et al. Incorporation of somatic BRAF mutation testing into an algorithm for the investigation of hereditary non-polyposis colorectal cancer. Fam Cancer 2007;6(3):301-310.
75. Bessa X, Balleste B, Andreu M, Castells A, Bellosillo B, Balaguer F, et al. A prospective, multicenter, population-based study of BRAF mutational analysis for Lynch syndrome screening. Clin Gastroenterol Hepatol 2008;6(2):206-214.
76. Nagasaka T, Koi M, Kloor M, Gebert J, Vilkin A, Nishida N, et al. Mutations in both KRAS and BRAF may contribute to the methylator phenotype in colon cancer. Gastroenterology 2008;134(7): 1950-1960.
77. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology 2008;135(4):1079-1099.
78. Gazzoli I, Loda M, Garber J, Syngal S, Kolodner RD. A hereditary nonpolyposis colorectal carcinoma case associated with hypermethylation of the MLH1 gene in normal tissue and loss of heterozygosity of the unmethylated allele in the resulting microsatellite instability-high tumor. Cancer Res 2002;62(14):3925-3928.
79. Suter CM, Martin DI, Ward RL. Germline epimutation of MLH1 in individuals with multiple cancers. Nat Genet 2004;36(5):497-501.
80. Miyakura Y, Sugano K, Akasu T, Yoshida T, Maekawa M, Saitoh S, et al. Extensive but hemiallelic methylation of the hMLH1 promoter region in early-onset sporadic colon cancers with microsatellite instability. Clin Gastroenterol Hepatol 2004;2(2):147-156.
81. Hitchins M, Williams R, Cheong K, Halani N, Lin VA, Packham D, et al. MLH1 germline epimutations as a factor in hereditary nonpolyposis colorectal cancer. Gastroenterology 2005;129(5):1392-1399.
82. Hitchins MP, Wong JJ, Suthers G, Suter CM, Martin DI, Hawkins NJ, et al. Inheritance of a cancer-associated MLH1 germ-line epimutation. N Engl J Med 2007;356(7):697-705.
83. Valle L, Carbonell P, Fernandez V, Dotor AM, Sanz M, Benitez J, et al. MLH1 germline epimutations in selected patients with early-onset non-polyposis colorectal cancer. Clin Genet 2007;71(3):232-237.
84. Morak M, Schackert HK, Rahner N, Betz B, Ebert M, Walldorf C, et al. Further evidence for heritability of an epimutation in one of 12 cases with MLH1 promoter methylation in blood cells clinically displaying HNPCC. Eur J Hum Genet 2008;16(7):804-811.
85. Hitchins MP, Ward RL. Constitutional (germline) MLH1 epimutation as an aetiological mechanism for hereditary non-polyposis colorectal cancer. J Med Genet 2009;46(12):793-802.
86. Dobrovic A, Kristensen LS. DNA methylation, epimutations and cancer predisposition. Int J Biochem Cell Biol 2009;41(1):34-39.

87. Ligtenberg MJ, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. Nat Genet 2009;41(1):112-117.
88. Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschoff J. Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. Cancer Res 1997; 57(21):4749-4756.
89. Thibodeau SN, French AJ, Cunningham JM, Tester D, Burgart LJ, Roche PC, et al. Microsatellite instability in colorectal cancer: different mutator phenotypes and the principal involvement of hMLH1. Cancer Res 1998;58(8):1713-1718.
90. Veigl ML, Kasturi L, Olechnowicz J, Ma AH, Lutterbaugh JD, Periyasamy S, et al. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. Proc Natl Acad Sci U S A 1998;95(15):8698-8702.
91. Jass JR, Walsh MD, Barker M, Simms LA, Young J, Leggett BA. Distinction between familial and sporadic forms of colorectal cancer showing DNA microsatellite instability. Eur J Cancer 2002; 38(7):858-866.
92. Laiho P, Launonen V, Lahermo P, Esteller M, Guo M, Herman JG, et al. Low-level microsatellite instability in most colorectal carcinomas. Cancer Res 2002;62(4):1166-1170.
93. Pawlik TM, Raut CP, Rodriguez-Bigas MA. Colorectal carcinogenesis: MSI-H versus MSI-L. Dis Markers 2004;20(4-5):199-206.
94. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med 2000;342(2):69-77.
95. Wright CM, Dent OF, Barker M, Newland RC, Chapuis PH, Bokey EL, et al. Prognostic significance of extensive microsatellite instability in sporadic clinicopathological stage C colorectal cancer. Br J Surg 2000;87(9):1197-1202.
96. Chang EY, Dorsey PB, Frankhouse J, Lee RG, Walts D, Johnson W, et al. Combination of microsatellite instability and lymphocytic infiltrate as a prognostic indicator in colon cancer. Arch Surg 2009; 144(6):511-515.
97. Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. Science 1993;260(5109):816-819.
98. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349(3):247-257.
99. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 2005;23(3):609-618.
100. Jover R, Zapater P, Castells A, Llor X, Andreu M, Cubiella J, et al. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. Gut 2006; 55(6):848-855.
101. Chialina SG, Fornes C, Landi C, de la Vega Elena CD, Nicolorich MV, Dourisboure RJ, et al. Microsatellite instability analysis in hereditary non-polyposis colon cancer using the Bethesda consensus panel of microsatellite markers in the absence of proband normal tissue. BMC Med Genet 2006;7: 5.
102. Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, et al. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. Gastroenterology 2002;123(6):1804-1811.
103. Bacher JW, Flanagan LA, Smalley RL, Nassif NA, Burgart LJ, Halberg RB, et al. Development of a fluorescent multiplex assay for detection of MSI-High tumors. Dis Markers 2004;20(4-5):237-250.

104. Akiyama Y, Sato H, Yamada T, Nagasaki H, Tsuchiya A, Abe R, et al. Germ-line mutation of the hMSH6/GTBP gene in an atypical hereditary nonpolyposis colorectal cancer kindred. Cancer Res 1997;57(18):3920-3923.
105. Berends MJ, Wu Y, Sijmons RH, Mensink RG, van der ST, Hordijk-Hos JM, et al. Molecular and clinical characteristics of MSH6 variants: an analysis of 25 index carriers of a germline variant. Am J Hum Genet 2002;70(1):26-37.
106. Xicola RM, Llor X, Pons E, Castells A, Alenda C, Pinol V, et al. Performance of different microsatellite marker panels for detection of mismatch repair-deficient colorectal tumors. J Natl Cancer Inst 2007;99(3):244-252.
107. Halvarsson B, Lindblom A, Rambech E, Lagerstedt K, Nilbert M. Microsatellite instability analysis and/or immunostaining for the diagnosis of hereditary nonpolyposis colorectal cancer? Virchows Arch 2004;444(2):135-141.
108. Chen PC, Dudley S, Hagen W, Dizon D, Paxton L, Reichow D, et al. Contributions by MutL homologues Mlh3 and Pms2 to DNA mismatch repair and tumor suppression in the mouse. Cancer Res 2005;65(19):8662-8670.
109. Cannavo E, Marra G, Sabates-Bellver J, Menigatti M, Lipkin SM, Fischer F, et al. Expression of the MutL homologue hMLH3 in human cells and its role in DNA mismatch repair. Cancer Res 2005; 65(23):10759-10766.
110. Acharya S, Wilson T, Gradia S, Kane MF, Guerrette S, Marsischky GT, et al. hMSH2 forms specific mispair-binding complexes with hMSH3 and hMSH6. Proc Natl Acad Sci U S A 1996;93(24):13629-13634.
111. Shia J. Immunohistochemistry versus microsatellite instability testing for screening colorectal cancer patients at risk for hereditary nonpolyposis colorectal cancer syndrome. Part I. The utility of immunohistochemistry. J Mol Diagn 2008;10(4):293-300.
112. Poley JW, Wagner A, Hoogmans MM, Menko FH, Tops C, Kros JM, et al. Biallelic germline mutations of mismatch-repair genes: a possible cause for multiple pediatric malignancies. Cancer 2007; 109(11):2349-2356.
113. Capel E, Flejou JF, Hamelin R. Assessment of MLH1 promoter methylation in relation to gene expression requires specific analysis. Oncogene 2007;26(54):7596-7600.
114. Schouten JP, McElgunn CJ, Waaijer R, Zwijnenburg D, Diepvens F, Pals G. Relative quantification of 40 nucleic acid sequences by multiplex ligation-dependent probe amplification. Nucleic Acids Res 2002;30(12):e57.
115. Heinmoller E, Renke B, Beyser K, Dietmaier W, Langner C, Ruschoff J. Piffalls in diagnostic molecular pathology - significance of sampling error. Virchows Arch 2001;439(4):504-511.
116. Muller A, Giuffre G, Edmonston TB, Mathiak M, Roggendorf B, Heinmoller E, et al. Challenges and pitfalls in HNPCC screening by microsatellite analysis and immunohistochemistry. J Mol Diagn 2004;6(4):308-315.
117. Mangold E, Pagenstecher C, Friedl W, Fischer HP, Merkelbach-Bruse S, Ohlendorf M, et al. Tumours from MSH2 mutation carriers show loss of MSH2 expression but many tumours from MLH1 mutation carriers exhibit weak positive MLH1 staining. J Pathol 2005;207(4):385-395.
118. Muller W, Burgart LJ, Krause-Paulus R, Thibodeau SN, Almeida M, Edmonston TB, et al. The reliability of immunohistochemistry as a prescreening method for the diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC) - results of an international collaborative study. Fam Cancer 2001;1(2):87-92.

119. Overbeek LI, Ligtenberg MJ, Willems RW, Hermens RP, Blokx WA, Dubois SV, et al. Interpretation of immunohistochemistry for mismatch repair proteins is only reliable in a specialized setting. Am J Surg Pathol 2008;32(8):1246-1251.
120. Matsubara N. Diagnostic application of hMLH1 methylation in hereditary non-polyposis colorectal cancer. Dis Markers 2004;20(4-5):277-282.
121. Deng G, Chen A, Hong J, Chae HS, Kim YS. Methylation of CpG in a small region of the hMLH1 promoter invariably correlates with the absence of gene expression. Cancer Res 1999;59(9):2029-2033.
122. Esteller M, Fraga MF, Guo M, Garcia-Foncillas J, Hedenfalk I, Godwin AK, et al. DNA methylation patterns in hereditary human cancers mimic sporadic tumorigenesis. Hum Mol Genet 2001; 10(26):3001-3007.
123. Rahner N, Friedrichs N, Steinke V, Aretz S, Friedl W, Buettner R, et al. Coexisting somatic promoter hypermethylation and pathogenic MLH1 germline mutation in Lynch syndrome. J Pathol 2008; 214(1):10-16.
124. Cunningham JM, Christensen ER, Tester DJ, Kim CY, Roche PC, Burgart LJ, et al. Hypermethylation of the hMLH1 promoter in colon cancer with microsatellite instability. Cancer Res 1998;58(15): 3455-3460.
125. Kuismanen SA, Holmberg MT, Salovaara R, Schweizer P, Aaltonen LA, de la Chapelle A, et al. Epigenetic phenotypes distinguish microsatellite-stable and -unstable colorectal cancers. Proc Natl Acad Sci U S A 1999;96(22):12661-12666.
126. Kuismanen SA, Holmberg MT, Salovaara R, de la Chapelle A, Peltomaki P. Genetic and epigenetic modification of MLH1 accounts for a major share of microsatellite-unstable colorectal cancers. Am J Pathol 2000;156(5):1773-1779.
127. Wheeler JM, Loukola A, Aaltonen LA, Mortensen NJ, Bodmer WF. The role of hypermethylation of the hMLH1 promoter region in HNPCC versus MSI+ sporadic colorectal cancers. J Med Genet 2000;37(8):588-592.
128. Potocnik U, Glavac D, Golouh R, Ravnik-Glavac M. Causes of microsatellite instability in colorectal tumors: implications for hereditary non-polyposis colorectal cancer screening. Cancer Genet Cytogenet 2001;126(2):85-96.
129. Ollikainen M, Hannelius U, Lindgren CM, Abdel-Rahman WM, Kere J, Peltomaki P. Mechanisms of inactivation of MLH1 in hereditary nonpolyposis colorectal carcinoma: a novel approach. Oncogene 2007;26(31):4541-4549.
130. Ramsoekh D, van Leerdam ME, Wagner A, Kuipers EJ. Review article: Detection and management of hereditary non-polyposis colorectal cancer (Lynch syndrome). Aliment Pharmacol Ther 2007; 26 Suppl 2:101-111.
131. Ferreira AM, Westers H, Wu Y, Niessen RC, Olderode-Berends M, van der ST, et al. Do microsatellite instability profiles really differ between colorectal and endometrial tumors? Genes Chromosomes Cancer 2009;48(7):552-557.
132. de Leeuw WJ, Dierssen J, Vasen HF, Wijnen JT, Kenter GG, Meijers-Heijboer H, et al. Prediction of a mismatch repair gene defect by microsatellite instability and immunohistochemical analysis in endometrial tumours from HNPCC patients. J Pathol 2000;192(3):328-335.
133. Hewitt MJ, Wood N, Quinton ND, Charlton R, Taylor G, Sheridan E, et al. The detection of microsatellite instability in blind endometrial samples--a potential novel screening tool for endometrial cancer in women from hereditary nonpolyposis colorectal cancer families? Int J Gynecol Cancer 2006;16(3):1393-1400.

9

Underutilization of microsatellite instability analysis in colorectal cancer patients at high risk for Lynch syndrome

Scand J Gastroenterol. 2009;44(5):600-604

M.G.F. van Lier[1]
J.H.W. de Wilt[2]
J.J.M.F. Wagemakers[3]
W.N.M. Dinjens[4]
R.A.M. Damhuis[3]
A. Wagner[5]
E.J. Kuipers[1,6]
M.E. van Leerdam[1]

Departments of Gastroenterology and Hepatology[1], Surgery[2], Pathology[4], Clinical Genetics[5] and Internal Medicine[6], Erasmus MC, University Medical Center, Rotterdam, the Netherlands. Comprehensive Cancer Center Rotterdam, the Netherlands[3]

ABSTRACT

Background: The revised Bethesda Guidelines were published to improve the efficiency of recognizing Lynch syndrome by identifying Lynch syndrome-related malignancies that should be analyzed for microsatellite instability (MSI). The aim of this study was to evaluate whether MSI analysis was performed in colorectal cancer patients at risk for Lynch syndrome according to the revised Bethesda Guidelines.

Methods: Patients diagnosed with colorectal cancer in 11 Dutch hospitals in 2005 and 2006 were selected from a regional database. Patients were included if they met any of the following; 1) diagnosed with colorectal cancer < 50 years, 2) a second Lynch syndrome-associated tumor prior to the diagnosis of colorectal cancer in 2005/2006, and 3) colorectal cancer < 60 years with a tumor displaying mucinous or signet-ring differentiation or medullary growth pattern. The use of MSI analysis in these patients was evaluated.

Results: Of 1905 colorectal cancer patients, 169 met at least one of the inclusion criteria. MSI analysis had been performed in 23 (14%) of the 169 tumors. MSI-status had been determined in 18 of 80 included patients < 50 years of age, in 4 of 70 patients with a second Lynch syndrome-related tumor, and in 3 of 41 patients < 60 years with high-risk pathology features.

Conclusions: There is marked underutilization of MSI analysis in patients at risk for Lynch syndrome. As a result Lynch syndrome might be underdiagnosed in patients with colorectal cancer and their relatives.

INTRODUCTION

Within the European community 400,000 people are annually diagnosed with colorectal carcinoma (CRC), resulting in approximately 220,000 deaths every year. As such, CRC is the most common malignancy within the European Union, and ranks second to lung cancer as a cause of cancer-related mortality.[1] Approximately 3% of these colorectal cancers occur on the basis of Lynch syndrome (LS), previously designated Hereditary Non-Polyposis Colorectal Cancer (HNPCC). LS is an autosomal dominant inherited disorder caused by a germline mutation in one of the mismatch repair (MMR) genes, *MLH1*, *MSH2*, *MSH6*, and *PMS2*. Mutation carriers are at high risk for developing CRC (60-90%), endometrial cancer (20-50%) and other extra-colonic cancers (< 15%).[2,3] Many of these malignancies occur at a relatively young age, and carriers also suffer from a high risk for synchronous and metachronous CRC.

Early detection of LS carriers is important to decrease the incidence of CRC by colonoscopic surveillance. A Finnish study reported that colonoscopic surveillance at 3-year intervals reduces the occurrence of CRC and decreases overall mortality by approximately 65% in LS families.[4] In a similar Dutch study, colonoscopic surveillance at 1-2 year intervals led to a 70% reduction of CRC-related mortality.[5]

The clinical criteria for LS, the Amsterdam Criteria, were primarily established to provide a basis for uniformity in collaborative studies.[6] These criteria rely on patients' recall of family history and family size, and unfortunately have a limited sensitivity.[7] In about half of the families meeting the Amsterdam Criteria a MMR gene mutation can be detected, whereas many families with the syndrome (i.e. mutation carriers) do not meet these criteria.[8] Therefore the less stringent Bethesda Guidelines were published to improve the efficiency of recognizing LS by the identification of LS-related malignancies on which microsatellite instability analysis should be performed.[9] Microsatellite instability (MSI) is the molecular hallmark of LS. More than 90% of LS-associated colorectal carcinomas display MSI. The Bethesda Guidelines have been revised in 2004[10] and the application of these revised Bethesda Guidelines in combination with MSI analysis is nowadays considered a good strategy for the detection of patients at high risk for LS.

However, the revised Bethesda Guidelines have also been criticized for being too complex to use in clinical practice[11], and a considerable proportion of patients with LS are probably not yet identified.[7,12] Hence, we hypothesize there is only a moderate implementation of the revised Bethesda Guidelines in clinical practice. The aim of this study was to evaluate retrospectively whether MSI analysis is performed in CRC patients at high risk for Lynch syndrome according to the revised Bethesda Guidelines in the South-western part of the Netherlands.

METHODS

All patients diagnosed with invasive CRC in the South-western part of the Netherlands between January 2005 and January 2007 were selected from the database of the regional Comprehensive Cancer Center Rotterdam (CCCR). This Rotterdam Cancer Registry covers a region with approximately 2.3 million inhabitants. The following data were anonymously collected; age, age at CRC diagnosis, gender, previous LS-associated tumors (see below) and CRC characteristics including stage, localization and pathological characteristics: mucinous or signet-ring differentiation or medullary growth pattern. For tumor localization and morphology the ICD-O classification system (third edition) was used. Tumors were defined to be right-sided when located at or proximal to the splenic flexure, and left-sided when distal to the splenic flexure. The study was approved by the institutional Ethics Committee.

Patients from 10 regional hospitals and one academic center were included if they met any of the following criteria, derived from the revised Bethesda Guidelines (Table 1); 1) patients diagnosed with CRC < 50 years, 2) patients diagnosed with a second LS-associated tumor prior to the diagnosis of CRC, and 3) patients < 60 years with CRC displaying mucinous or signet-ring differentiation or medullary growth pattern. LS-associated tumors include colorectal, endometrial, ovarian, gastric, small-bowel, and pancreatic cancer, as well as tumors in the hepatobiliary tract, renal pelvis or ureter, sebaceous glands, and brain. Family history and presence of tumor-infiltrating lymphocytes or Crohn's-like lymphocytic reaction in the tumor could not be taken into account since these data were not recorded in the database. Patients with carcinoid tumors and lymphomas and patients already identified as MMR gene mutation carrier were excluded.

The 10 general hospitals refer their patients to the Erasmus Medical Centre for MSI analysis and genetic counseling. Data from the department of molecular pathology concerning all MSI analyses performed in the complete CCCR region between January 2005 and August 2007 were available for matching. For MSI analyses the following markers were used; BAT25, BAT26, BAT40, D2S123 and D5S346. Tumors with more than one unstable marker were

Table 1. Revised Bethesda Guidelines.[10]

Individuals meeting any one of the following should undergo MSI-testing:
1. CRC diagnosed in an individual under age 50 years.
2. Presence of synchronous, metachronous colorectal, or other LS-associated tumors*, regardless of age.
3. CRC with the MSI-H histology (presence of tumor-infiltrating lymphocytes, Crohn's like lymphocytic reaction, mucinous /signet-ring differentiation, or medullary growth pattern), in a patient < 60 years of age.
4. CRC in 1 or more first-degree relatives with a LS-related tumor*, with 1 of the cancers being diagnosed under age 50 years.
5. CRC diagnosed in 2 or more first- or second-degree relatives with LS-related tumors*, regardless of age.

CRC = colorectal cancer, LS = Lynch syndrome, MSI-H = microsatellite instable.
* Colorectal, endometrial, ovarian, gastric, small-bowel, pancreas, hepatobiliary tract, renal pelvis or ureter cancer, and brain tumors, sebaceous gland adenomas and keratoacanthomas.

categorized as having a high degree of microsatellite instability (MSI-H). Those with one unstable marker were categorized as having a low degree of microsatellite instability (MSI-L) and tumors with no instability were categorized as being microsatellite stable (MSS).[10] In addition, tumors of all cases referred for MSI analysis were investigated for MLH1, MSH2, MSH6 and PMS2 expression by immunohistochemistry.

Statistical analyses

Data were analyzed using the SPSS 12.1 statistical software for Windows. Descriptive statistics were used to analyze and report the data. MSI performance rates were analyzed using chi square statistics. For comparisons, patients were divided in three different risk groups; 1) patients < 50 years, 2) patients with multiple tumors and 3) patients < 60 years with tumors showing MSI-H histology. Those fulfilling more than one of these inclusion-criteria were allocated to the "< 50 years" group, and if older than 50 years to the "multiple tumors" group.

RESULTS

An invasive colorectal adenocarcinoma was diagnosed in 1905 patients in the 11 hospitals in the South-western part of the Netherlands in 2005 and 2006. A total of 169 (8%) patients (56% male, 44% female) met at least one of the 3 inclusion criteria derived from the revised Bethesda Guidelines. There were no known LS mutation carriers. The median age at CRC diagnosis was 53 years (range 24-93).

MSI analysis had been performed on 23 (14%) of the 169 colorectal tumors. Patient and tumor characteristics including the number of MSI analyses are shown in Table 2. Of the 169 patients, 80 met the first inclusion criterion, being younger than 50 years at CRC diagnosis. MSI-testing had been performed in 18 (22%) of them. Seventy patients had already been diagnosed with another LS-associated tumor prior to the diagnosis of CRC: 63 colorectal, 3 endometrial, 1 ovarian and 3 gastric carcinomas. The mean age in this group was 72 years and only 4 patients (6%) were referred for MSI analysis. Forty-one patients fulfilled the third criterion, showing one of the specific pathology features at age < 60 years. In three (7%) of them MSI analysis had been requested. In only 2 (11%) of the 19 patients fulfilling more than one of the inclusion criteria MSI-status was determined.

Six of the 70 patients with multiple LS-related malignancies were diagnosed with 3 LS-associated tumors. These 6 patients were all > 50 years, and in none MSI-status of their tumor had been determined. Six patients had a prior LS-associated tumor with high-risk pathology features, however MSI analysis had not been requested in any of them. Thirty-two patients were diagnosed with synchronous LS-associated cancers. Compared to patients with only

Table 2. Patient characteristics and MSI performance.

	n (%)	MSI performed (%)
Gender		
Male	95 (56)	13 (14)
Female	74 (44)	10 (14)
Age at CRC diagnosis		
< 50	80 (47)	18 (22) ‡
> 50	89 (53)	5 (6)
LS-associated tumors		
> 1 tumors	70 (41)	4 (6) ‡
1 tumor	99 (59)	19 (19)
High risk pathology features		
Mucinous	33 (30)	2 (6)
Signet-ring	8 (5)	1 (12)
Medullary	0	0
Total	41 (24)	3 (7) ‡
CRC localization		
Left	94 (56)	15 (16)
Right	75 (44)	8 (11)
Total	169 (100)	23 (14)

CRC = colorectal cancer, MSI = microsatellite instability, LS = Lynch syndrome.
‡ MSI analysis was performed significantly more often in patients < 50 years than in patients diagnosed with another LS-associated tumor or patients with a tumor displaying high risk pathology features (p = 0.001).

one LS-associated tumor, MSI analysis was significantly more often performed in patients with synchronous malignancies (p = 0.026).

In patients with CRC < 50 years MSI analysis had significantly more often been performed than in patients fulfilling one of the other two inclusion criteria (p = 0.001). Gender and tumor localization did not influence MSI performance in our selected cases. Institutional variability (e.g. variability between academic and non-academic hospitals) could not be evaluated since these data were blinded.

In the 23 cases in which MSI analysis was performed, five (22%) showed either a MSI-H pattern or loss of immunohistochemical expression of one or more of the MMR proteins, suspect for underlying LS. Another 17 tumors were MSS and showed normal immunohistochemical expression of the MMR proteins and in one case the tumor displayed a MSI-L phenotype with normal MMR-protein expression. Ultimately, there was one mutation-carrier detected with a germline defect in the *MSH2* gene and one patient with an MSI-H CRC with loss of MSH6 protein expression was not analyzed for a germline mutation. In 3 patients with an absent MLH1 protein expression, either hypermethylation of the *MLH1* promoter or a *BRAF* mutation was demonstrated, which is seen in sporadic CRC.

DISCUSSION

It is of great importance to recognize potential LS mutation carriers in the general CRC patient population, since regular surveillance colonoscopies have proven to decrease CRC morbidity and mortality.[4,5] In addition, patients with LS are at considerable risk for developing a second LS-associated malignancy and by the identification of germline mutations genetic counseling can be offered to at risk family members and mutation carriers can enter surveillance programs. According to the revised Bethesda Guidelines, all colorectal carcinomas of the patients included in this study should have been assessed for microsatellite instability. Our results show that MSI analysis was nevertheless performed in only 14% of these patients, despite the increasing attention for hereditary disorders and excellent access for MSI-testing. In these 23 analyzed CRC's, five (22%) showed abnormal results (i.e. MSI-H and / or absence of MMR-protein expression) with at least one, and possibly 2 mutation carriers (9%) among them. Assuming absence of selection bias, another 32 aberrant molecular results and approximately 11 mutation carriers may have been missed in the remaining 146 patients not referred for MSI analysis.

Despite optimization of selection criteria and enhancements in molecular techniques for identifying families with LS, many cases are not recognized.[7,13] Our results corroborate earlier reports concerning the moderate implementation of MSI analysis in clinical practice. Van Dijk et al investigated patients with multiple LS-associated tumors and patients with CRC < 50 years of age, and concluded that MSI analysis was performed in only 9% of these patients.[13] Underutilisation of MSI analysis is likely to considerably contribute to the underdiagnosis of LS.

Nevertheless, the current most widely accepted recommendation for the identification of patients with LS, is based on the combination of the revised Bethesda Guidelines and MSI-testing. This combination has proven to be an effective and efficient strategy for Lynch syndrome identification, with a sensitivity for identifying mutation carriers ranging from 72%[14] to 100% in several publications.[15-18] However, these clinical criteria have been criticized because of the use of broad and complex variables and a specificity ranging from 77 to 98% in recent publications.[15-18]

In our study MSI analysis was significantly more often performed in patients with CRC < 50 years of age than in patients fulfilling one of the other 2 inclusion criteria ($p < 0.01$). In only 7% of patients with high-risk pathology features, and 6% of patients with 2 or more LS-associated tumors MSI-status was determined.

However, Jenkins et al confirmed the importance of pathology features in the identification of LS by the investigation of 1098 CRC's in patients < 60 years.[19] Mucinous (n = 115), signet ring (n =10) or medullary (n = 1) histology was seen in 11% of the tumors with a sensitivity of 28% and specificity of 91% for MSI-H status. Twenty-six percent showed tumor infiltrating lymphocytes with a sensitivity of 72% and specificity of 82%. Crohn's like lymphocytic

reaction was seen in 28% of all tumors with a sensitivity for MSI-H of 56% and a specificity of 77%. It is imperative that pathologists and clinicians should pay more attention to these tumor characteristics. Another group compared the clinical performance of the original and revised Bethesda Guidelines for the detection of LS in CRC patients, and concluded that two LS-related cancers achieved the highest sensitivity.[16]

Family history is the other hallmark of LS, besides young age, pathology features and multiple LS-associated tumors. However, obtaining a thorough family history is difficult in clinical practice. In an American study, a family history of CRC was present in only 54% of the medical records of surgical CRC patients. A family history of extra-colonic tumors was not even considered.[20] Furthermore, several other studies showed that CRC patients reported the diagnosis of CRC in first-degree relatives in only 68-91% accurately.[21,22]

Other reasons for missing LS include inadequate general knowledge of LS among health care professionals, leading to suboptimal compliance with recommended guidelines and notification of at-risk relatives.[23,24] Alberto et al studied adequacy of family history taking in 101 CRC patients, and found that in none of the 3 families fulfilling the Amsterdam Criteria further action was taken.[25]

We believe that clinical physicians should be more aware of the possibility of LS in CRC patients and the significance of the Bethesda Guidelines should be more appreciated. Therefore attention for hereditary CRC and the Bethesda Guidelines, as well as continuous education is necessary. Besides the clinical geneticist and the clinician, also the pathologists may play an important role in selecting tumors for MSI-testing.[11]

The strength of this study lies in the fact that a complete population-based database was used covering a large region. From all included patients, MSI data and data considering known LS-mutations were available up to August 2007. Limitation of this study is the fact that not all Bethesda Guidelines were considered. Family history and presence of tumor-infiltrating lymphocytes or Crohn's-like lymphocytic reaction in the tumor were not taken into account since these data were not recorded in the population-based database. Therefore, the performance of MSI-testing in 14% of cases fulfilling the revised Bethesda Guidelines might be an overestimation as well as an underestimation.

In conclusion, despite a multidisciplinary approach of oncology and easy access to a tertiary referral center and clinical genetics facilities, there is marked underutilization of MSI analysis in CRC patients at high risk for Lynch syndrome. As a result Lynch syndrome is still underdiagnosed in patients with CRC and their relatives. The clinical implications of this underdiagnosis of LS are devastating due to the high probability of (preventable) malignancies with a potential fatal outcome.

REFERENCES

1. Boyle P, Ferlay J. Cancer incidence and mortality in Europe, 2004. Ann Oncol 2005;16(3):481-488.
2. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003;348(10):919-932.
3. Vasen HF, Wijnen JT, Menko FH, Kleibeuker JH, Taal BG, Griffioen G, et al. Cancer risk in families with hereditary nonpolyposis colorectal cancer diagnosed by mutation analysis. Gastroenterology 1996;110(4):1020-1027.
4. Jarvinen HJ, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomaki P, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000;118(5):829-834.
5. de Jong AE, Hendriks YM, Kleibeuker JH, de Boer SY, Cats A, Griffioen G, et al. Decrease in mortality in Lynch syndrome families because of surveillance. Gastroenterology 2006;130(3):665-671.
6. Vasen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991;34(5):424-425.
7. Kievit W, de Bruin JH, Adang EM, Ligtenberg MJ, Nagengast FM, van Krieken JH, et al. Current clinical selection strategies for identification of hereditary non-polyposis colorectal cancer families are inadequate: a meta-analysis. Clin Genet 2004;65(4):308-316.
8. Lindor NM, Petersen GM, Hadley DW, Kinney AY, Miesfeldt S, Lu KH, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006;296(12):1507-1517.
9. Rodriguez-Bigas MA, Boland CR, Hamilton SR, Henson DE, Jass JR, Khan PM, et al. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. J Natl Cancer Inst 1997;89(23):1758-1762.
10. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96(4):261-268.
11. Kievit W, de Bruin JH, Adang EM, Severens JL, Kleibeuker JH, Sijmons RH, et al. Cost effectiveness of a new strategy to identify HNPCC patients. Gut 2005;54(1):97-102.
12. de Bruin JH, Ligtenberg MJ, Nagengast FM, Adang EM, van Krieken JH, Hoogerbrugge N. Optimizing the detection of hereditary non-polyposis colorectal cancer: an update. Scand J Gastroenterol Suppl 2006;(243):146-152.
13. van Dijk DA, Oostindier MJ, Kloosterman-Boele WM, Krijnen P, Vasen HF. Family history is neglected in the work-up of patients with colorectal cancer: a quality assessment using cancer registry data. Fam Cancer 2007;6(1):131-134.
14. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Feasibility of Screening for Lynch Syndrome Among Patients With Colorectal Cancer. J Clin Oncol 2008;26(35):5783-5788.
15. Pinol V, Castells A, Andreu M, Castellvi-Bel S, Alenda C, Llor X, et al. Accuracy of revised Bethesda guidelines, microsatellite instability, and immunohistochemistry for the identification of patients with hereditary nonpolyposis colorectal cancer. JAMA 2005;293(16):1986-1994.
16. Rodriguez-Moranta F, Castells A, Andreu M, Pinol V, Castellvi-Bel S, Alenda C, et al. Clinical performance of original and revised Bethesda guidelines for the identification of MSH2/MLH1 gene carriers in patients with newly diagnosed colorectal cancer: proposal of a new and simpler set of recommendations. Am J Gastroenterol 2006;101(5):1104-1111.
17. Balmana J, Balaguer F, Castellvi-Bel S, Steyerberg EW, Andreu M, Llor X, et al. Comparison of predictive models, clinical criteria and molecular tumour screening for the identification of patients

with Lynch syndrome in a population-based cohort of colorectal cancer patients. J Med Genet 2008;45(9):557-563.

18. Wolf B, Gruber S, Henglmueller S, Kappel S, Bergmann M, Wrba F, et al. Efficiency of the revised Bethesda guidelines (2003) for the detection of mutations in mismatch repair genes in Austrian HNPCC patients. Int J Cancer 2006;118(6):1465-1470.
19. Jenkins MA, Hayashi S, O'Shea AM, Burgart LJ, Smyrk TC, Shimizu D, et al. Pathology features in Bethesda guidelines predict colorectal cancer microsatellite instability: a population-based study. Gastroenterology 2007;133(1):48-56.
20. Church J, McGannon E. Family history of colorectal cancer: how often and how accurately is it recorded? Dis Colon Rectum 2000;43(11):1540-1544.
21. Katballe N, Juul S, Christensen M, Orntoft TF, Wikman FP, Laurberg S. Patient accuracy of reporting on hereditary non-polyposis colorectal cancer-related malignancy in family members. Br J Surg 2001;88(9):1228-1233.
22. Sijmons RH, Boonstra AE, Reefhuis J, Hordijk-Hos JM, de Walle HE, Oosterwijk JC, et al. Accuracy of family history of cancer: clinical genetic implications. Eur J Hum Genet 2000;8(3):181-186.
23. de la Chapelle A. The incidence of Lynch syndrome. Fam Cancer 2005;4(3):233-237.
24. Schroy PC, III, Barrison AF, Ling BS, Wilson S, Geller AC. Family history and colorectal cancer screening: a survey of physician knowledge and practice patterns. Am J Gastroenterol 2002;97(4): 1031-1036.
25. Alberto VO, Harocopos CJ, Patel AA, Clark SK. Family and personal history in colorectal cancer patients: what are we missing? Colorectal Dis 2006;8(7):612-614.

10

Yield of routine molecular analyses in colorectal cancer patients ≤ 70 years to detect underlying Lynch syndrome

Manuscript in preparation.

M.G.F. van Lier[1]
C.H.M. Leenen[1]
A. Wagner[2]
D. Ramsoekh[1]
H.J. Dubbink[3]
P.J. Westenend[4]
E.J.R. de Graaf[5]
L.M.M. Wolters[6]
W.W. Vrijland[7]
E.J. Kuipers[1,8]
M.E. van Leerdam[1]
E.W. Steyerberg[9*]
W.N.M. Dinjens[3*]

[*] Equal contribution

Departments of Gastroenterology and Hepatology[1], Clinical Genetics[2], Pathology[3], Internal Medicine[8], and Public Health[9], Erasmus MC, University Medical Center, Rotterdam, the Netherlands. Departments of Pathology[4] and Gastroenterology and Hepatology[6], Albert Schweitzer Ziekenhuis, Dordrecht, the Netherlands. Department of Surgery[5], IJsselland Ziekenhuis, Capelle a/d IJssel, the Netherlands. Department of Surgery[7], Sint Franciscus Gasthuis, Rotterdam, the Netherlands.

On behalf of the LIMO study group:
A.M.W. van den Ouweland, dept of clinical genetics, Erasmus MC, Rotterdam; G. van Tilburg, dept of pathology, Erasmus MC, Rotterdam, S. Hulspas, dept of pathology, Erasmus MC, Rotterdam, Prof. dr. J.F. Lange, dept of surgery, Erasmus MC, Rotterdam; Dr R.F. Hoedemaeker, dept of Pathology, Pathan, Rotterdam; Dr. W.A. Bode, dept of gastroenterology and hepatology, IJsselland Ziekenhuis, Capelle a/d IJssel; Dr. J.H. van Dam, dept of surgery, Havenziekenhuis, Rotterdam; Dr. P.J. Wismans, dept of internal medicine, Havenziekenhuis, Rotterdam; Dr. R. den Toom, dept of surgery, Ruwaard van Putten Ziekenhuis, Spijkenisse; Drs. E.J.H.M. van de Weijgert, dept of internal medicine, Ruwaard van Putten Ziekenhuis, Spijkenisse; Dr. A.J.P. van Tilburg, dept of gastroenterology and hepatology, Sint Franciscus Gasthuis, Rotterdam; Drs. H.E. Lont, dept of surgery, Vlietland Ziekenhuis, Schiedam; Drs. B.W.M. van 't Hoff, dept of gastroenterology and hepatology, Vlietland Ziekenhuis, Schiedam; Dr. R.J. Oostenbroek, dept of surgery, Albert Schweitzer Ziekenhuis, Dordrecht; Dr. F. Logeman, dept of surgery, Beatrixziekenhuis, Gorinchem; Dr. O. van Baalen, dept of gastroenterology and hepatology, Beatrixziekenhuis, Gorinchem; dr. H.F.G.M. van den Ingh, dept of pathology, Maasstad ziekenhuis Rotterdam; Dr. E. van der Harst, dept of surgery, Maasstad ziekenhuis Rotterdam; Dr. F.J.G.M. Kubben, dept of gastroenterology and hepatology, Maasstad ziekenhuis Rotterdam; Dr. W.F. Weidema, dept of surgery, Ikazia Ziekenhuis, Rotterdam; Dr. R.J.Th. Ouwendijk, dept of gastroenterology and hepatology, Ikazia Ziekenhuis, Rotterdam; Drs. R.H. van Rijssel, dept of pathology, Deventer Ziekenhuis, Deventer; Dr. E.H. Eddes, dept of surgery, Deventer Ziekenhuis, Deventer; Dr. F. ter Borg, dept of gastroenterology and hepatology, Deventer Ziekenhuis, Deventer.

ABSTRACT

Background: Although early detection of Lynch Syndrome (LS) is important, a considerable proportion of patients at risk for LS is not recognized. We aimed to study the yield of routine molecular analyses in colorectal cancer (CRC) and advanced adenoma patients for early LS detection.

Methods: We prospectively included consecutive CRC patients ≤ 70 years and patients with advanced colorectal adenomas ≤ 45 years. Tumor specimens were analyzed for microsatellite instability (MSI), immunohistochemical mismatch repair protein expression (IHC), and *MLH1* promoter methylation. Tumors were classified as either 1) suspect for LS, 2) sporadic MSI-H, or 3) microsatellite stable (MSS). Predictive factors for LS were determined by multivariable logistic regression analyses.

Results: A total of 1117 CRC patients (57% males, median age 61 years, IQR 55-66) were included. Fifty patients (4.5%; 95% CI 3.4-5.9) were suspect for LS, and 71 had a sporadic MSI-H tumor (6.4%; 95% CI 5.1-8.0). Thirty-five patients suspect for LS (70%) were > 50 years. A molecular profile suspect for LS was detected in 10% (15/144) of patients ≤ 50 years, in 4% (15/377) of those aged 51-60, and in 3% (20/596) of patients older than 61. Compared to MSS cases, patients suspect for LS were significantly younger (OR 3.9; 95% CI 1.7-8.7), had more often right-sided CRCs (OR 14; 95% CI 6.0-34) and had cancers with a lower TNM-stage at diagnosis (OR 0.44; 95% CI 0.28-0.69). Among 125 advanced adenoma patients (58% males, median age 41 years, IQR 37-44), three were suspect for LS (2.4%; 95% CI 0.5-7.1%).

Conclusions: Molecular screening for LS in CRC patients ≤ 70 years leads to identification of a profile pathognomic for LS in 4.5% of patients, with most of them not fulfilling the age-criterion (≤ 50 years) routinely used for LS-assessment. This supports routine use of MSI-testing in these patients, although cost-effectiveness of this strategy has to be determined.

INTRODUCTION

Lynch Syndrome (LS) is the most common form of hereditary colorectal cancer (CRC), responsible for approximately 3% of all CRCs.[1,2] LS is caused by a germline mutation in one of the mismatch repair (MMR) genes; *MLH1*, *MSH2*, *MSH6* and *PMS2*. The burden of LS is considerable, as the cancers are generally diagnosed at a young age and synchronous or metachronous malignancies occur in 30% of the patients. Furthermore, extra-colonic LS-associated malignancies frequently occur.[3-5]

Early detection of LS is important, since colonoscopic surveillance has proven to reduce CRC morbidity and mortality by 65-70%.[6-8] However, the diagnosis of LS is complicated by the absence of a pre-morbid phenotype and DNA mutation analysis to confirm the diagnosis is time-consuming and expensive. MMR gene mutations lead to microsatellite instability (MSI) in tumor DNA, the molecular hallmark of LS. As MSI can be detected in approximately 95% of all LS-associated cancers, MSI analysis can be used in the diagnostic approach of LS.[5,9,10] The revised Bethesda Guidelines have been developed to select patients for MSI analysis, in order to identify patients at high risk for LS (Table 1).[11]

The combination of the revised Bethesda Guidelines and MSI-testing is currently the most widely accepted approach for the identification of LS patients. However, the Bethesda guidelines have been criticized for being too complex for readily use[12], and it has been shown that these criteria are poorly implemented in clinical practice.[13-15] In addition, several prediction models have been developed to quantitatively estimate the risk of LS on the basis of personal and familial data[16-21], but the implementation of these models into clinical practice is limited. Together, this leads to a suboptimal detection of LS and the concern that many if not most mutation-carriers are not being identified.[22,23]

Therefore clinicians and researchers are searching for new simple strategies to improve the detection of LS. The aim of the present prospective population-based study therefore was to evaluate the yield of routine molecular analyses, including MSI analysis, in consecutive CRC patients ≤ 70 years and patients with advanced colorectal adenomas ≤ 45 years.

Table 1. Revised Bethesda Guidelines.[11]

Individuals meeting any one of the following should undergo MSI-testing:
1. CRC diagnosed in an individual under age 50 years.
2. Presence of synchronous, metachronous colorectal, or other LS-associated tumors*, regardless of age.
3. CRC with the MSI-H histology (presence of tumor-infiltrating lymphocytes, Crohn's like lymphocytic reaction, mucinous /signet-ring differentiation, or medullary growth pattern), in a patient < 60 years of age.
4. CRC in 1 or more first-degree relatives with a LS-associated tumor*, with 1 of the cancers being diagnosed under age 50 years.
5. CRC diagnosed in 2 or more first- or second-degree relatives with LS-associated tumors*, regardless of age.

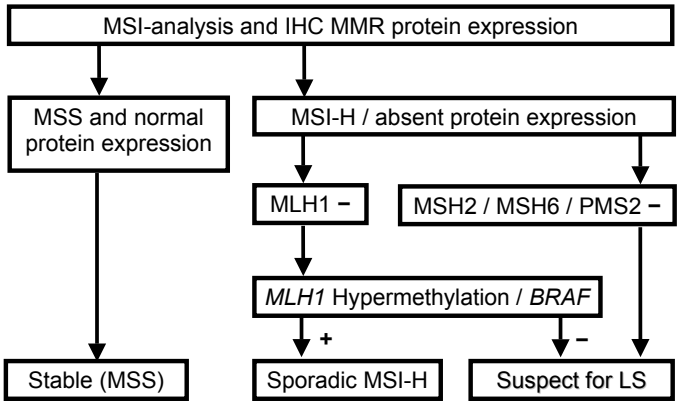
CRC = colorectal cancer, MSI = microsatellite instability, LS = Lynch syndrome.
* Colorectal, endometrial, ovarian, gastric, small-bowel, pancreas, hepatobiliary tract, renal pelvis or ureter cancer, and brain tumors, sebaceous gland adenomas and keratoacanthomas.

METHODS

In this prospective multicenter population-based study, we included all consecutive patients newly diagnosed with either an invasive colorectal adenocarcinoma ≤ 70 years, or an advanced colorectal adenoma ≤ 45 years. Adenomas were considered advanced when they were either ≥ 10 mm in diameter, showed a villous component or high-grade dysplasia, or when at least 3 synchronous adenomas (regardless of size and histology) were found. Patients were included between May 2007 and September 2009 in 11 Dutch hospitals including one academic medical center and 10 general hospitals (5 pathology laboratories). Patients were identified by monthly electronic searches in the institutional pathology databases. The following data were anonymously collected from the original pathology reports (i.e. not by re-evaluation of pathological specimen): gender, age at diagnosis, tumor-characteristics including MSI-related histology features (see Table 1), TNM-stage (5th edition) and localization. Tumors were defined as right-sided if located at or proximal to the splenic flexure, and left-sided when distal to the splenic flexure. Patients previously diagnosed with (attenuated) FAP, MAP, or a known MMR gene defect were excluded.

Routine formalin-fixed and paraffin-embedded (FFPE) tissue blocks, either from a surgical resection or, if not available, a diagnostic biopsy specimen, were collected from all included patients. Whenever possible, a biopsy specimen as well as a resection specimen were collected from rectal cancer patients treated with neoadjuvant radiotherapy or chemoradiation (i.e. TNM ≥ T2), to evaluate possible therapy-effect on MSI-status. The collected tissue-samples were analyzed as shown in Figure 1. First, MSI analysis and evaluation of immunohistochemical MMR protein expression (IHC) were performed in all patients. In the case of a microsatellite instable tumor (MSI-H, see below) with absent MLH1 protein expression, we also studied hypermethylation of the *MLH1* promoter and somatic *BRAF* mutations. This was done as MSI

Figure 1. Flow chart of molecular analyses.



MSI = microsatellite instability, IHC = immunohistochemical, MMR = Mismatch Repair, MSS = microsatellite stable, MSI-H = high degree of microsatellite instability, LS = Lynch Syndrome.

can be seen in approximately 15% of sporadic CRCs due to *MLH1* promoter methylation.[24] *MLH1* promoter methylation is in its turn associated with somatic *BRAF* mutations.[25]

The study was approved by the Institutional Review Boards of the participating hospitals, and patients received written information. This also allowed them to lodge an objection to the molecular analyses. If they did object, their archival tissue-blocks were anonymously collected informing neither the patient nor their doctor about the results of the additional analyses. Otherwise the results were discussed with the patient by their doctor. Patients with a tumor showing a molecular profile suspect for LS who did want to be informed about the results, were referred to the department of clinical genetics for counseling and germline mutation analysis.

MSI analysis

MSI analyses were performed on DNA derived from FFPE tumor tissue, using a panel of pentaplex markers as previously described.[26] Tumors with more than one unstable marker were categorized as having a high degree of microsatellite instability (MSI-H), suspect for LS. Those with one or no unstable marker were categorized microsatellite stable (MSS), not associated with LS.

Immunohistochemistry, MLH1 promoter hypermethylation assay, & BRAF mutation analysis

Immunohistochemistry, *MLH1* promoter hypermethylation assay, and *BRAF* mutation analysis were performed as previously described.[26] IHC analyses were performed for the mismatch repair proteins MLH1, MSH2, MSH6 and PMS2. If there was no MLH1 expression in tumor cells, the methylation status of the *MLH1* promoter was determined by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA). In tumor specimens that did not express MLH1 protein, *BRAF* alterations of mutational hotspot codon V600 were additionally determined by bi-directional cycle- sequencing of PCR-amplified fragments.

Analyzed tumors were classified as either 1) suspect for LS if MSI-H and simultaneously showing absent MMR protein expression, with exclusion of *MLH1* promoter hypermethylation and/or *BRAF* mutation in the case of absent MLH1 expression, 2) sporadic MSI-H tumors displaying absent MLH1 expression and established *MLH1* promoter hypermethylation and/or *BRAF* mutation, or 3) sporadic, microsatellite stable (MSS) tumors. If difficulties occurred in the interpretation of the MSI or IHC results, the analyses were repeated on biopsy tissue if available.

Statistical analyses

Data were analyzed using the SPSS 15.0 statistical software for Windows, and were reported using descriptive statistics. The incidence of a profile suspect for LS and a sporadic MSI-H phenotype were analyzed, and predictive factors for LS and sporadic microsatellite instability were determined by multivariable logistic regression analyses. The correlation between MSI and corresponding IHC results were evaluated, as well as the correlation between *MLH1* hypermethylation and *BRAF* mutations. Finally, we assessed differences in MSI-status before and after neo-adjuvant therapy in cases with advanced rectal cancers. Two-sided p-values less than 0.05 were considered significant.

RESULTS

Colorectal cancer cases

A total of 1136 CRC patients were eligible for inclusion in this study. Nineteen patients had to be excluded as there was either no vital tumor tissue left for the analyses (n = 9), or the tumor specimen could not be collected (n = 10). The 1117 included cases (57% males) had a median age of 61 years (interquartile range 55-66) and the youngest patient was 27 years old. Most CRC patients were older than 50 years and 28% of all CRCs were located in the right colon (Table 2). Only 4 patients (0.4%) lodged an objection to the molecular analyses.

The molecular analyses revealed a profile suspect for LS in 50 of the 1117 CRCs (4.5%; 95% CI 3.4-5.9) and 71 sporadic MSI-H tumors (6.4%; 95% CI 5.1-8.0). Thirty-five of the 50 patients suspect for LS (70%) were older than 50 years at CRC diagnosis. On the basis of immunohistochemical protein expression, 19 patients were suspect for a *MLH1* gene defect, 12 for *MSH2*, 12 for *MSH6* and 6 for a *PMS2* defect. In one patient with an MSI-H tumor, all proteins were expressed in the tumor, but germline mutation analysis revealed a pathogenic *MSH2* mutation. A pathogenic MMR mutation has thus far been found in 16 of them (3 *MLH1*, 5 *MSH2*, 6 *MSH6*, and 2 *PMS2* mutations), and results of germline analyses of the remaining 34 cases are awaited. Multivariable analyses demonstrated that CRC patients suspect for LS were significantly younger at cancer diagnosis (p = 0.001; OR 3.9; 95% CI 1.7-8.7), had more often right-sided CRCs (p < 0.001; OR 14; 95% CI 6.0-34) and had cancers with a lower TNM-stage at diagnosis (p < 0.001; OR 0.44; 95% CI 0.28-0.69) than patients with MSS tumors.

A profile suspect for LS was detected predominantly in younger patients: in 15 of the 144 patients (10%) ≤ 50 years, in 15 of 377 patients (4%) aged 51-60 years, and in 20 of 596 patients (3%) aged 61 years or older. Conversely, a sporadic MSI-H status was more often detected in older patients; in 1/144 (1%) of those aged ≤ 50 years, in 15/377 (4%) of those aged 51-60 years, and in 55/596 (9%) of the patients aged 61 or older (Figure 2).

Table 2. Characteristics of 1117 CRC patients and results of molecular analyses.

	MSS	Suspect for LS	Sporadic MSI-H	Total
	n = 996 (89.2%)	n = 50 (4.5%)	n = 71 (6.4%)	n = 1117 (100%)
Age in years				
Median (IQR)	61 (55-66)	57 (49-65)	64 (61-68)	61 (55-66)
Age ≤ 50 years	128 (13%)	15 (30%)	1 (1.5%)	144 (13%)
Gender				
Males	579 (58%[a])	35 (70%)	19 (27%)	633 (57%)
Localisation	n = 947 (49 NS)	n = 48 (2 NS)	n = 69 (2 NS)	n = 1064 (53 NS)
Right-sided [b]	204 (21.5%)	34 (71%)	62 (90%)	300 (28%)
MSI-H histology [c]	n = 107 (11%)	n = 13 (26%)	n = 22 (31%)	n 142 (13%)
Mucinous	99	12	20	131
Signet ring cells	14	3	2	19
Medullary	-	-	-	-
TIL's [d]	-	-	1	1
Crohn's reaction [e]	-	-	-	-
TNM-stage	n = 660 (336 NS)	n = 38 (12 NS)	n = 58 (13 NS)	n = 756 (361 NS)
I	159	11	8	178
IIA	158	12	23	193
IIB	33	4	6	43
IIIA	53	-	-	52
IIIB	113	6	7	126
IIIC	106	5	11	122
IV	38	-	3	41

CRC = colorectal carcinoma, MSS = microsatellite stable, LS = Lynch syndrome, MSI-H = high degree of microsatellite instability, NS = not further specified, IQR = interquartile range.

[a] i.e. 58% of all patients with an MSS tumor were male.

[b] CRCs were defined to be right-sided if located at or proximal to the splenic flexure, and left-sided when distal to the splenic flexure.

[c] Several tumors (n = 18) displayed both a mucinous differentiation as well as signet ring cells.

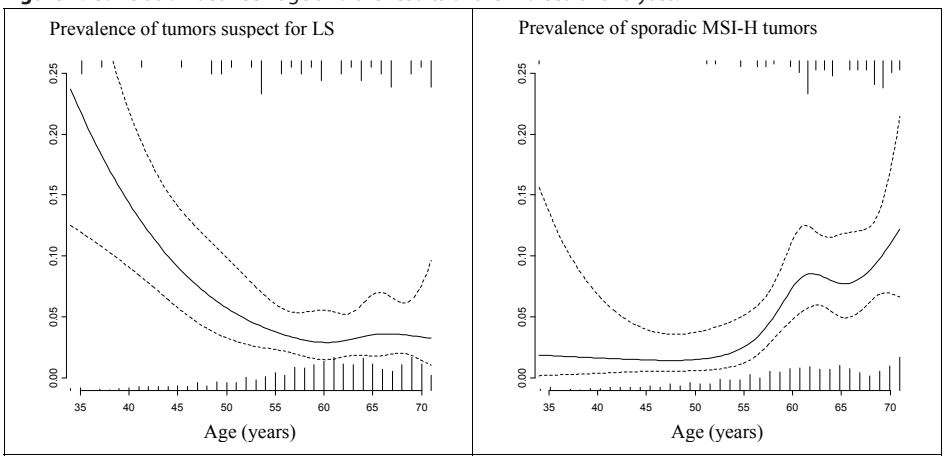
[d] Tumor-infiltrating lymphocytes.

[e] Crohn's-like lymphocytic reaction.

Furthermore, compared to MSS tumors, sporadic MSI-H tumors were significantly more often located right-sided ($p < 0.001$; OR 43; 95% CI 15-125), more often displayed MSI-H histology features ($p = 0.015$; OR 2.4; 95% CI 1.2-4.8) and had a lower TNM-stage at time of diagnosis ($p = 0.031$; OR 0.65; 95% CI 0.44-0.96). In addition, patients with sporadic MSI-H tumors were more often female ($p = 0.001$; OR 0.34; 95% CI 0.17-0.66) and older than 50 years ($p = 0.041$; OR 0.12; 95% CI 0.02-0.92) compared to patients with a MSS tumor. In 46 of the 71 sporadic MSI-H tumors a V600E *BRAF* mutation was detected.

There was a good correlation between MSI and corresponding IHC results. In 5 MSS CRCs IHC results were questionable (e.g. due to absent MMR protein expression in normal tissue), all other MSS tumors (n = 991) showed normal protein expression for the MMR proteins. IHC showed absent protein expression for one or more of the MMR proteins in 119 of 121 MSI-H tumors. In one MSI-H tumor all proteins were normally expressed although germline muta-

Figure 2. Correlation between age and the results of the molecular analyses.



LS = Lynch syndrome. MSI-H = high degree of microsatellite instability.
Left panel: Prevalence of tumors suspect for LS according to age; the chance to detect a patient suspect for LS by molecular analyses clearly decreases with age.
Right panel: Prevalence of sporadic MSI-H tumors according to age; the chance to detect a patient with a sporadic MSI-H tumor by molecular analyses clearly increases with age.

tion analysis revealed an *MSH2* mutation, and in another MSI-H tumor the MMR proteins were absent in both tumor and normal tissue (i.e. not conclusive).

Of 122 included patients with advanced rectal cancer who were treated by neo-adjuvant chemoradiation prior to resection, both a biopsy specimen as well as a resection specimen were analyzed. MSI-status did not differ between biopsy and corresponding resection specimen, suggesting that neo-adjuvant therapy has no effect on MSI-status.

Advanced adenoma cases

A total of 130 patients with an advanced adenoma were eligible for inclusion in this study. In 125 (96%) of these cases, adenomatous tissue was available for molecular analyses. These 125 subjects (58% males) had a median age of 41 years (interquartile range 37-44, Table 3). Three male adenoma patients (2.4%; 95% CI 0.5-7.1%) aged 34, 41, and 44 years, were suspect for underlying LS. Only one of these 3 patients fulfilled the Amsterdam II criteria, and none of them fulfilled the revised Bethesda guidelines. IHC showed lack of MLH1 expression in 2 of these 3 patients, and lack of MSH6 expression in 1 patient. So far, 2 pathogenic MMR gene germline mutations have been found by DNA analysis (one *MLH1* and one *MSH6* mutation). Mutation analysis is still pending in the third patient.

Table 3. Characteristics of patients with advanced adenoma and results of molecular analyses

	MSS n = 121 (96.8%)	Suspect for LS n = 3 (2.4%)	Sporadic MSI-H n = 1 (0.8%)	Total n = 125
Age in years				
Median (IQR)	41 (37-44)	41 (34-44)	44	41 (37-44)
Gender				
Males	70	3	0	73 (58%)
Localisation				n = 113 (12 NS)
Right-sided #	31	0	0	31
Advanced				
Villous component	77	2	1	80
High grade dysplasia	30	0	0	30
Size ≥ 10 mm	47	1	0	48
≥ 3 synchronous adenomas	14	0	0	14

MSS = microsatellite stable, LS = Lynch syndrome, MSI-H = high degree of microsatellite instability, NS = not further specified, IQR = interquartile range.

Adenomas were defined to be right-sided if located at or proximal to the splenic flexure, and left-sided when distal to the splenic flexure.

DISCUSSION

This prospective study shows that routine molecular screening for LS in CRC patients ≤ 70 years leads to the identification of a profile pathognomic for LS in 4.5%. Seventy percent of these patients are older than 50 years at the time of CRC diagnosis and do not meet the age-criterion routinely used for LS assessment. In patients with an advanced adenoma ≤ 45 years a molecular profile pathognomic for LS was detected in 2.4%. These adenoma patients would not have been detected by the current screening guidelines such as the revised Bethesda guidelines. The detection of CRC and adenoma patients suspect for LS is of great importance since these patients and their family members at risk (carriers) can enter surveillance programs which have been proven to reduce CRC morbidity and mortality by 65-70%.[6-8] Our analyses furthermore revealed a sporadic MSI-H status in 6.4% of all analyzed CRCs. The establishment of a sporadic MSI-H status by *MLH1* promoter hypermethylation assay considerably reduced the number of patients referred for counseling and germline genetic testing at the clinical genetics department. Furthermore, the establishment of a sporadic MSI-H status reduced the number of patients worrying about being a MMR gene mutation carrier.

Immunohistochemistry showed absent MSH6 protein expression in 24% of all CRC patients suspect for LS, and absent PMS2 expression in 12%. These percentages are higher than expected[27-29], but in line with a previous report on LS detection in the Netherlands, describing a high incidence of *MSH6* mutations.[30] The high percentage of absent *MSH6* and *PMS2* expression may be explained by the fact that *MSH6* and *PMS2* mutations have for a long time been underestimated due to the more atypical presentation of disease in *MSH6* and *PMS2* families.[31,32] Another explanation can be the presence of founder mutations.

Since we do not have data on family history of all included patients, we can not compare the yield of our strategy in terms of LS detection to other strategies including the revised Bethesda guidelines[11], the Amsterdam II Criteria[33] and several prediction models.[16-21] However, the Bethesda guidelines have been criticized for being too complex to use and have been proven to be poorly implemented in clinical practice.[13-15,34] The Amsterdam II criteria are predominantly hampered by a low sensitivity[35], and although some of the prediction models to estimate the risk of LS have been validated in a population-based cohort of CRC patients[36-38], the implementation of these models in clinical practice is still in its infancy. Therefore the MIPA criteria (MSI-testing-Indicated-by-a-Pathologist) have been developed.[12] The MIPA criteria simplify the Bethesda guidelines in such a way that pathologists, without knowledge of family history, can select patients for MSI analysis. They resemble our strategy, yet the MIPA criteria recommend MSI analysis in patients newly diagnosed with CRC before age 50, or before age 70 in patients diagnosed with two LS-associated cancers. As we demonstrate that most CRC patients suspect for LS are > 50 years, our strategy may thus help to detect more LS patients.

As neoadjuvant chemotherapy may alter MMR protein expression in cancer cells[39] and exposure to ionising radiation promotes the development of MSI in mouse tumors[40], one might hypothesize that neo-adjuvant (chemo)radiation as advocated for advanced rectal cancers[41], may influence MSI-status. This is an interesting hypothesis, as MSI analyses are usually performed on surgical resection specimens. Therefore we assessed differences in MSI-status in 122 rectal cancers before and after neo-adjuvant therapy. No differences in MSI-status were found, suggesting that neo-adjuvant (chemo)radiation has no effect on MSI-status and that surgical resection specimens can be used for MSI analysis.

In contrast to the revised Bethesda guidelines, the original Bethesda guidelines recommended MSI analysis on adenomas of patients < 40 years.[42] Yet, this was found to be ineffective to identify new LS cases.[43,44] Nevertheless, it has been demonstrated that most adenomas of LS patients show MSI.[45,46] Furthermore, LS-associated adenomas have been demonstrated to be larger and to have a higher proportion of villous component and / or high grade dysplasia than patients without LS[47], and MSI analysis is more reliable in these high risk adenomas.[48] Therefore we also analyzed advanced adenomas of patients younger than 45 years, revealing 3 patients with LS that would not have been detected by current screening guidelines. It remains to be established whether or not it is cost-effective to screen advanced adenomas of young patients for LS.

The strength of this study lies in the fact that we performed a prospective population-based study in which we evaluated routine molecular screening for LS in CRC patients using a high age cut-off. The high age cut-off allowed us to gain insight in the correlation between age and diagnostic yield (i.e. LS detection, Figure 2). We chose for an age cut-off of 70 in order to compromise between the feasibility of MSI analysis in a large number of patients on the one hand, and a maximum detection of patients suspect for LS on the other hand. In the

future, it might be considered to subject all newly diagnosed CRC patients to MSI analysis, as MSI analysis is not only valuable for detection of LS, but also has prognostic and therapeutic implications. Regardless of stage at diagnosis, microsatellite unstable CRCs (including sporadic MSI-H cancers) are associated with a better prognosis than MSS tumors[49,50] and patients with MSI-H tumors do not seem to benefit from adjuvant chemotherapy with 5-fluorouracil.[50-52] However, as the optimal strategy for adjuvant therapy of MSI-H cancers still needs to be established, we do not believe that it is justified to screen all CRCs for MSI at this stage.

This study also has some limitations. As mentioned before, data on family history are lacking. Therefore we cannot compare the yield of our strategy in terms of LS detection to other strategies in which family history is one of the cornerstones. However, obtaining a thorough family history is difficult in clinical practice[53], CRC patients frequently report their family history inaccurately[54-56], and it may become more difficult to identify LS patients on the basis of family history as family sizes are decreasing. Second, the molecular analyses used in this study also have some drawbacks as previously described[26], and because we could not perform DNA mutation analysis of the MMR genes in all included subjects, some LS cases may have been missed. Another limitation is that LS has not been confirmed by germline mutation analysis in all cases suspect for LS yet. Germline analyses are still pending in most cases and some patients deceased before they could be referred to the clinical geneticist. Furthermore, in a small proportion of patients suspect for LS, no germline MMR gene mutation can be detected by DNA mutation analysis. Yet, these patients might still suffer from an inherited MMR defect, for example caused by germline *MSH2* hypermethylation[57] or other mechanisms that still need to be discovered. Finally, some clinicians may have ethical objections against molecular screening for LS prior to genetic counseling, since the results of the molecular analyses, especially the IHC results, can be very suggestive for LS. However the molecular analyses do not establish the diagnosis of LS, but can make underlying LS more likely. Therefore, we believe that the benefits of accurate diagnosis outweigh potential negative effects, and the clinician should inform the patient that the pathological examinations may not only give information about the nature of the tumor, but may also indicate an elevated risk of an underlying hereditary disorder.

In conclusion, this study demonstrates that routine molecular screening for LS in CRC patients ≤ 70 years previously not diagnosed with LS, leads to the identification of a profile pathognomic for LS in 4.5%. For advanced adenoma patients ≤ 45 years a molecular profile suspect for LS was detected in 2.5%. Identification of LS is of major relevance for these patients as well as their affected family members, as CRC-related morbidity and mortality can be reduced by colonoscopic surveillance. As most CRC patients suspect for LS were older than 50 years and do not meet the age-criterion routinely used for LS assessment, and because routine molecular screening is easy to implement in clinical practice, our strategy may help to increase the detection of LS. However, the cost-effectiveness of this approach as well as the optimal age cut-off for molecular screening remain to be established.

REFERENCES

1. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338(21):1481-1487.
2. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Feasibility of Screening for Lynch Syndrome Among Patients With Colorectal Cancer. J Clin Oncol 2008;26(35):5783-5788.
3. Lynch HT, Smyrk TC, Watson P, Lanspa SJ, Lynch JF, Lynch PM, et al. Genetics, natural history, tumor spectrum, and pathology of hereditary nonpolyposis colorectal cancer: an updated review. Gastroenterology 1993;104(5):1535-1549.
4. Watson P, Lynch HT. Extracolonic cancer in hereditary nonpolyposis colorectal cancer. Cancer 1993;71(3):677-685.
5. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003;348(10):919-932.
6. Jarvinen HJ, Aarnio M, Mustonen H, Aktan-Collan K, Aaltonen LA, Peltomaki P, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000;118(5):829-834.
7. de Jong AE, Hendriks YM, Kleibeuker JH, de Boer SY, Cats A, Griffioen G, et al. Decrease in mortality in Lynch syndrome families because of surveillance. Gastroenterology 2006;130(3):665-671.
8. Stupart DA, Goldberg PA, Algar U, Ramesar R. Surveillance colonoscopy improves survival in a cohort of subjects with a single mismatch repair gene mutation. Colorectal Dis 2009;11(2):126-130.
9. Aaltonen LA, Peltomaki P, Mecklin JP, Jarvinen H, Jass JR, Green JS, et al. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. Cancer Res 1994;54(7):1645-1648.
10. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58(22):5248-5257.
11. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96(4):261-268.
12. Kievit W, de Bruin JH, Adang EM, Severens JL, Kleibeuker JH, Sijmons RH, et al. Cost effectiveness of a new strategy to identify HNPCC patients. Gut 2005;54(1):97-102.
13. Overbeek LI, Hoogerbrugge N, van Krieken JH, Nagengast FM, Ruers TJ, Ligtenberg MJ, et al. Most patients with colorectal tumors at young age do not visit a cancer genetics clinic. Dis Colon Rectum 2008;51(8):1249-1254.
14. Van Lier MG, De Wilt JH, Wagemakers JJ, Dinjens WN, Damhuis RA, Wagner A, et al. Underutilization of microsatellite instability analysis in colorectal cancer patients at high risk for Lynch syndrome. Scand J Gastroenterol 2009;44(5):600-604.
15. Singh H, Schiesser R, Anand G, Richardson PA, El-Serag HB. Underdiagnosis of Lynch syndrome involves more than family history criteria. Clin Gastroenterol Hepatol 2010;8(6):523-529.
16. Wijnen JT, Vasen HF, Khan PM, Zwinderman AH, van der KH, Mulder A, et al. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. N Engl J Med 1998; 339(8):511-518.

17. Lipton LR, Johnson V, Cummings C, Fisher S, Risby P, Eftekhar Sadat AT, et al. Refining the Amsterdam Criteria and Bethesda Guidelines: testing algorithms for the prediction of mismatch repair mutation status in the familial cancer clinic. J Clin Oncol 2004;22(24):4934-4943.
18. Barnetson RA, Tenesa A, Farrington SM, Nicholl ID, Cetnarskyj R, Porteous ME, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. N Engl J Med 2006;354(26):2751-2763.
19. Balmana J, Stockwell DH, Steyerberg EW, Stoffel EM, Deffenbaugh AM, Reid JE, et al. Prediction of MLH1 and MSH2 mutations in Lynch syndrome. JAMA 2006;296(12):1469-1478.
20. Chen S, Wang W, Lee S, Nafa K, Lee J, Romans K, et al. Prediction of germline mutations and cancer risk in the Lynch syndrome. JAMA 2006;296(12):1479-1487.
21. Kastrinos F, Steyerberg EW, Mercado R, Balmana J, Holter S, Gallinger S, et al. The PREMM(1,2,6) Model Predicts Risk of MLH1, MSH2, and MSH6 Germline Mutations Based on Cancer History. Gastroenterology 2011;140(1):73-81.
22. Lynch HT, Riley BD, Weissman SM, Coronel SM, Kinarsky Y, Lynch JF, et al. Hereditary nonpolyposis colorectal carcinoma (HNPCC) and HNPCC-like families: Problems in diagnosis, surveillance, and management. Cancer 2004;100(1):53-64.
23. Terdiman JP. It is time to get serious about diagnosing Lynch syndrome (hereditary nonpolyposis colorectal cancer with defective DNA mismatch repair) in the general population. Gastroenterology 2005;129(2):741-744.
24. Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP, et al. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci U S A 1998;95(12):6870-6875.
25. Grady WM, Carethers JM. Genomic and epigenetic instability in colorectal cancer pathogenesis. Gastroenterology 2008;135(4):1079-1099.
26. Van Lier MG, Wagner A, van Leerdam ME, Biermann K, Kuipers EJ, Steyerberg EW, et al. A review on the molecular diagnostics of Lynch syndrome: a central role for the pathology laboratory. J Cell Mol Med 2010;14(1-2):181-197.
27. Peterlongo P, Nafa K, Lerman GS, Glogowski E, Shia J, Ye TZ, et al. MSH6 germline mutations are rare in colorectal cancer families. Int J Cancer 2003;107(4):571-579.
28. Peltomaki P, Vasen H. Mutations associated with HNPCC predisposition - Update of ICG-HNPCC/INSiGHT mutation database. Dis Markers 2004;20(4-5):269-276.
29. Talseth-Palmer BA, McPhillips M, Groombridge C, Spigelman A, Scott RJ. MSH6 and PMS2 mutation positive Australian Lynch syndrome families: novel mutations, cancer risk and age of diagnosis of colorectal cancer. Hered Cancer Clin Pract 2010;8(1):5.
30. Ramsoekh D, Wagner A, van Leerdam ME, Dinjens WN, Steyerberg EW, Halley DJ, et al. A high incidence of MSH6 mutations in Amsterdam criteria II-negative families tested in a diagnostic setting. Gut 2008;57(11):1539-1544.
31. Wagner A, Hendriks Y, Meijers-Heijboer EJ, de Leeuw WJ, Morreau H, Hofstra R, et al. Atypical HNPCC owing to MSH6 germline mutations: analysis of a large Dutch pedigree. J Med Genet 2001;38(5):318-322.
32. Boland CR, Koi M, Chang DK, Carethers JM. The biochemical basis of microsatellite instability and abnormal immunohistochemistry and clinical behavior in Lynch syndrome: from bench to bedside. Fam Cancer 2008;7(1):41-52.
33. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 1999;116(6):1453-1456.

34. van Dijk DA, Oostindier MJ, Kloosterman-Boele WM, Krijnen P, Vasen HF. Family history is neglected in the work-up of patients with colorectal cancer: a quality assessment using cancer registry data. Fam Cancer 2007;6(1):131-134.
35. Lindor NM, Petersen GM, Hadley DW, Kinney AY, Miesfeldt S, Lu KH, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006;296(12):1507-1517.
36. Balmana J, Balaguer F, Castellvi-Bel S, Steyerberg EW, Andreu M, Llor X, et al. Comparison of predictive models, clinical criteria and molecular tumour screening for the identification of patients with Lynch syndrome in a population-based cohort of colorectal cancer patients. J Med Genet 2008;45(9):557-563.
37. Balaguer F, Balmana J, Castellvi-Bel S, Steyerberg EW, Andreu M, Llor X, et al. Validation and extension of the PREMM1,2 model in a population-based cohort of colorectal cancer patients. Gastroenterology 2008;134(1):39-46.
38. Green RC, Parfrey PS, Woods MO, Younghusband HB. Prediction of Lynch syndrome in consecutive patients with colorectal cancer. J Natl Cancer Inst 2009;101(5):331-340.
39. Samimi G, Fink D, Varki NM, Husain A, Hoskins WJ, Alberts DS, et al. Analysis of MLH1 and MSH2 expression in ovarian cancer before and after platinum drug-based chemotherapy. Clin Cancer Res 2000;6(4):1415-1421.
40. Haines J, Bacher J, Coster M, Huiskamp R, Meijne E, Mancuso M, et al. Microsatellite instability in radiation-induced murine tumours; influence of tumour type and radiation quality. Int J Radiat Biol 2010;86(7):555-568.
41. Peeters KC, Marijnen CA, Nagtegaal ID, Kranenbarg EK, Putter H, Wiggers T, et al. The TME trial after a median follow-up of 6 years: increased local control but no survival benefit in irradiated patients with resectable rectal carcinoma. Ann Surg 2007;246(5):693-701.
42. Rodriguez-Bigas MA, Boland CR, Hamilton SR, Henson DE, Jass JR, Khan PM, et al. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. J Natl Cancer Inst 1997;89(23):1758-1762.
43. Velayos FS, Allen BA, Conrad PG, Gum J, Jr., Kakar S, Chung DC, et al. Low rate of microsatellite instability in young patients with adenomas: reassessing the Bethesda guidelines. Am J Gastroenterol 2005;100(5):1143-1149.
44. Ferreira S, Claro I, Lage P, Filipe B, Fonseca R, Sousa R, et al. Colorectal adenomas in young patients: microsatellite instability is not a useful marker to detect new cases of Lynch syndrome. Dis Colon Rectum 2008;51(6):909-915.
45. Iino H, Simms L, Young J, Arnold J, Winship IM, Webb SI, et al. DNA microsatellite instability and mismatch repair protein loss in adenomas presenting in hereditary non-polyposis colorectal cancer. Gut 2000;47(1):37-42.
46. Muller A, Beckmann C, Westphal G, Bocker ET, Friedrichs N, Dietmaier W, et al. Prevalence of the mismatch-repair-deficient phenotype in colonic adenomas arising in HNPCC patients: results of a 5-year follow-up study. Int J Colorectal Dis 2006;21(7):632-641.
47. de Jong AE, Morreau H, Van PM, Eilers PH, Wijnen J, Nagengast FM, et al. The role of mismatch repair gene defects in the development of adenomas in patients with HNPCC. Gastroenterology 2004;126(1):42-48.
48. Loukola A, Salovaara R, Kristo P, Moisio AL, Kaariainen H, Ahtola H, et al. Microsatellite instability in adenomas as a marker for hereditary nonpolyposis colorectal cancer. Am J Pathol 1999;155(6):1849-1853.

49. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med 2000;342(2):69-77.
50. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 2005;23(3):609-618.
51. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349(3):247-257.
52. Jover R, Zapater P, Castells A, Llor X, Andreu M, Cubiella J, et al. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. Gut 2006; 55(6):848-855.
53. Church J, McGannon E. Family history of colorectal cancer: how often and how accurately is it recorded? Dis Colon Rectum 2000;43(11):1540-1544.
54. Katballe N, Juul S, Christensen M, Orntoft TF, Wikman FP, Laurberg S. Patient accuracy of reporting on hereditary non-polyposis colorectal cancer-related malignancy in family members. Br J Surg 2001;88(9):1228-1233.
55. Sijmons RH, Boonstra AE, Reefhuis J, Hordijk-Hos JM, de Walle HE, Oosterwijk JC, et al. Accuracy of family history of cancer: clinical genetic implications. Eur J Hum Genet 2000;8(3):181-186.
56. Mitchell RJ, Brewster D, Campbell H, Porteous ME, Wyllie AH, Bird CC, et al. Accuracy of reporting of family history of colorectal cancer. Gut 2004;53(2):291-295.
57. Ligtenberg MJ, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, et al. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3′ exons of TACSTD1. Nat Genet 2009;41(1):112-117.

11

Prospective evaluation of molecular screening for Lynch syndrome in patients with endometrial cancer ≤ 70 years

Manuscript in preparation.

C.H.M. Leenen[1]
M.G.F. van Lier[1]
H.C. van Doorn[2]
M.E. van Leerdam[1]
G.S. Kooi[3]
J. de Waard[4]
R.F. Hoedemaeker[5]
A.M.W. van den Ouweland[6]
H.J. Dubbink[7]
E.J. Kuipers[1,8]
A. Wagner[6]
W.N.M. Dinjens[7*]
E.W. Steyerberg[9*]

[*] Equal contribution
Departments of Gastroenterology and Hepatology[1], Gynaecology[2], Clinical Genetics[6], Pathology[7], Internal Medicine[8], and Public Health[9], Erasmus MC, University Medical Center, Rotterdam, the Netherlands. Department of Gynaecology[3], Albert Schweitzer Ziekenhuis, Dordrecht, the Netherlands. Department of Gynaecology[4], Sint Franciscus Gasthuis, Rotterdam, the Netherlands. Department of Pathology[5], Pathan, Rotterdam, the Netherlands.

On behalf of the LIMO study group:
P.A.A van Hagen, MD, department of Gynaecology, IJsselland Ziekenhuis, Capelle a/d IJssel; A. Bouman, MD, PhD, department of Gynaecology, Deventer Ziekenhuis, Deventer; F.J.M. Huikeshoven, department of Gynaecology, MD, PhD, Ruwaard van Putten Ziekenhuis, Spijkenisse; A.K. Westendorp, MD, PhD, department of Gynaecology, Vlietland Ziekenhuis, Schiedam; L. Pijpers, MD, PhD, department of Gynaecology, Havenziekenhuis, Rotterdam; P.J. Westenend, MD, PhD, department of Pathology, Albert Schweitzer Ziekenhuis, Dordrecht; R.H. van Rijssel, MD, department of Pathology, Deventer Ziekenhuis, Deventer; G. van Tilburg, department of Pathology, Erasmus MC, Rotterdam; S. Hulspas, department of Pathology, Erasmus MC, Rotterdam.

ABSTRACT

Background: Lynch syndrome (LS) is a hereditary syndrome that predisposes to multiple malignancies including endometrial cancer (EC). Although identification of LS is important, detection is far from optimal. Current screening strategies for LS include microsatellite instability (MSI) analysis and immunohistochemical (IHC) staining for mismatch repair (MMR) proteins in tumor tissue. We aimed to evaluate a new diagnostic strategy for LS based on routine molecular analyses of EC in all newly diagnosed patients ≤ 70 years in the Netherlands.
Methods: Consecutive EC patients ≤ 70 years were included in this prospective multicenter study. EC specimens were analyzed for MSI, IHC of four MMR proteins, and *MLH1* promoter hypermethylation. Tumors were classified as; 1) suspect for LS, 2) sporadic MSI-H, or 3) microsatellite stable (MSS). We advised referral to a clinical geneticist for germline mutation analysis in patients suspect for LS.
Results: Tumor specimens of 172 patients (median age 61 years, IQR 56-67) were analyzed. Ten patients, all > 50 years (range 53-69), were suspect for LS (6%; 95% CI 3-10%) including 1 patient suspect for an *MLH1*, 2 for an *MSH2*, 5 for an *MSH6* and 2 for a *PMS2* gene defect. Eight of these ten patients suspect for LS were referred to a clinical geneticist. So far, DNA mutation analysis confirmed an *MSH6* mutation in 3 of them (1.7%). In addition, 28 sporadic MSI-H tumors (16%; 95% CI 12-23%) with *MLH1* promoter hypermethylation were identified.
Conclusions: Molecular screening for LS in patients with EC diagnosed ≤ 70 years, leads to identification of a profile suspect for LS in 6% of cases. New screening guidelines for LS are needed, including recommendations for EC patients older than 50 years of age.

INTRODUCTION

Lynch syndrome (LS) is an autosomal dominant inherited syndrome that predisposes to multiple malignancies including endometrial cancer (EC). The lifetime risk of women with LS to develop EC is 40 to 60%. In addition, patients with LS carry a lifetime risk of 50 to 85% to develop colorectal cancer (CRC) and also an increased risk of up to 15% to develop other malignancies including gastric, ovarian, small-bowel and urinary tract cancers.[1-3]

LS is caused by a germline mutation in one of the mismatch repair (MMR) genes; *MLH1*, *MSH2*, *MSH6* and *PMS2*. Mutations in these MMR genes lead to microsatellite instability (MSI) in tumor DNA. MSI is the molecular hallmark of LS, and can be detected in more than 90% of all CRCs and ECs in LS mutation carriers.[4] Therefore, MSI analysis can be used in the diagnostic approach of LS.[5] However, MSI can also be detected in 17 to 23% of sporadic ECs[6,7], which is mostly caused by transcriptional silencing of the *MLH1* gene by promoter hypermethylation.[8,9] Yet, in addition to MSI analysis, immunohistochemical (IHC) analyses can be performed to evaluate the expression of the four MMR proteins, and tumors showing absent MLH1 expression can be selected for a *MLH1* promoter methylation assay. In case of microsatellite instability and loss of MMR protein expression, with exclusion of *MLH1* promoter hypermethylation in case of absent MLH1 expression, further germline DNA testing is available for all four LS associated MMR genes.

Early detection of LS in EC patients is of great importance, since LS carriers are at risk of other cancers, especially CRC. Surveillance by regular colonoscopy reduces CRC morbidity and mortality by 65-70%.[10-12] Moreover, the diagnosis of LS in EC patients is of great importance for at risk relatives. After genetic testing relatives with an MMR gene defect can enter surveillance programs in order to reduce cancer morbidity and mortality, and relatives without an MMR germline mutation can be reassured and dismissed from surveillance.

Selection of patients for molecular testing for LS is currently based on clinical criteria, including the Amsterdam II criteria and the revised Bethesda guidelines.[13,14] In the Amsterdam II criteria EC is included as a diagnostic criterion. However, the Amsterdam II criteria lack sensitivity, particularly in cases of small families or when extensive family history information is not available.[15,16] The revised Bethesda criteria focus primarily on patients with CRC and not with EC. This contributes to the concern that EC patients with LS remain undetected. Therefore the aim of the present prospective multicenter study was to evaluate the feasibility and the yield of large scale molecular analyses in patients newly diagnosed with EC under the age of 70 years.

METHODS

Endometrial cancer patient population

All consecutive patients ≤ 70 years newly diagnosed with an invasive EC of epithelial origin were included at eight Dutch hospitals, including one academic medical center, between May 2007 and September 2009. Patients were identified by monthly electronic searches in these institutions' pathology databases. Data were collected on age at diagnosis and tumor-characteristics including histology and stage. Patients with endometrial sarcomas were excluded.

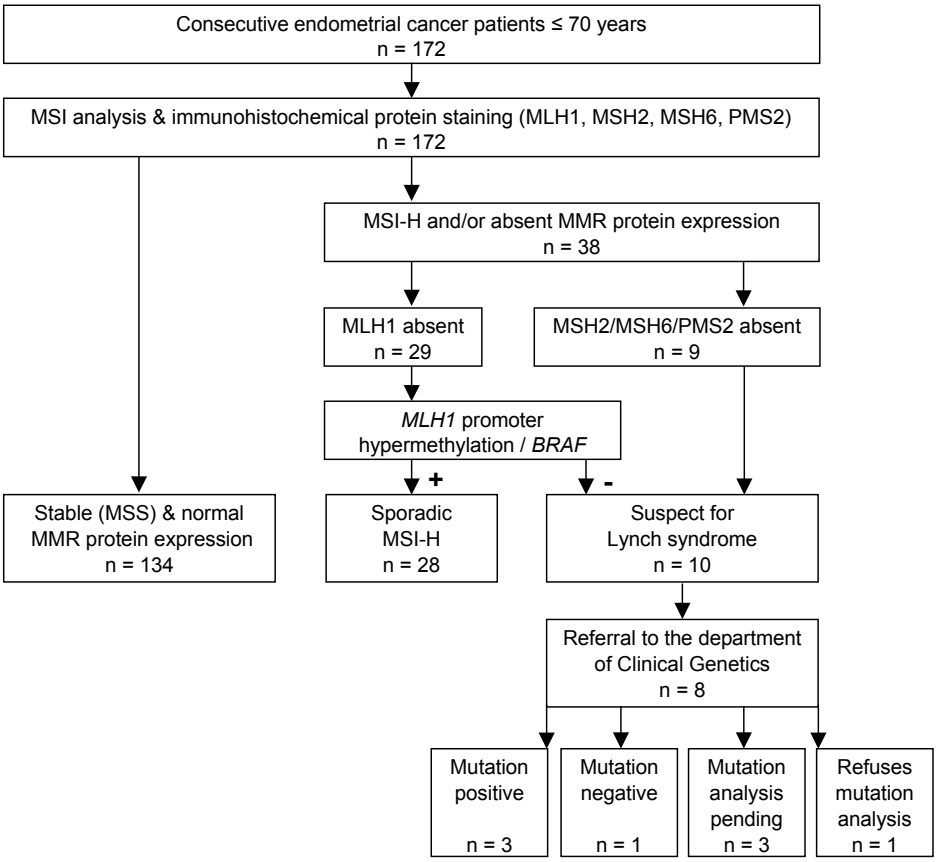
The study was approved by the Ethical Committees of the participating hospitals, and patients were informed about the study by a folder which they received from their gynaecologist. This folder also enabled patients to lodge an objection to the molecular analyses by a reply card. If they objected, their archival tissue-blocks were collected anonymously without informing the patient or their doctor about the additional analyses. Otherwise the results were discussed with the patient by their physician. Patients who wanted to be informed and were diagnosed with a tumor with a molecular profile suspect for LS, were referred to the department of clinical genetics for counseling and germline mutation analysis.

Routine formalin-fixed, paraffin-embedded (FFPE) tissue blocks were collected from all included EC patients. The collected tissue-samples were analyzed as shown in Figure 1. First, MSI analysis and immunohistochemical evaluation of MMR protein expression (IHC) were performed. In patients with a microsatellite instable tumor (i.e. MSI-H, see below) and absent MLH1 protein expression, also hypermethylation of the *MLH1* promoter was investigated and the presence of somatic *BRAF* mutations was analyzed. Tumors were classified as either 1) sporadic, microsatellite stable (MSS) tumors, 2) suspect for LS if MSI-H and simultaneously showing absent MMR protein expression, with exclusion of *MLH1* promoter hypermethylation and/or *BRAF* mutation in the case of absent MLH1 expression, or 3) sporadic MSI-H tumors with absent MLH1 expression and established *MLH1* promoter hypermethylation and/or *BRAF* mutation.

MSI analysis

Analysis for MSI was performed on FFPE tumor and normal tissue, using a panel of pentaplex markers as previously described.[17] Tumors with more than one unstable marker were categorized as having a high degree of microsatellite instability (MSI-H), suspect for LS. Tumors with one or no unstable markers were categorized as being microsatellite stable (MSS) and not suspect for LS.

Figure 1. Flow diagram of the diagnostic strategy and results of molecular analyses.



MSI = microsatellite instability, MSI-H = high degree of microsatellite instability, IHC = immunohistochemistry.

Immunohistochemistry, MLH1 promoter hypermethylation assay, & BRAF mutation analysis

IHC analysis was performed for four mismatch repair proteins: MLH1, MSH2, MSH6 and PMS2, according to the standard procedure.[17] If there was no MLH1 expression in tumor cells, the methylation status of the *MLH1* promoter was determined by methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA). MS-MLPA was performed with the SALSA MSMLPA Kit ME011-A1 for MMR genes (MRC-Holland, Amsterdam, the Netherlands), as previously described.[17] In tumor specimens with loss of MLH1 expression additional *BRAF* sequence analysis of the mutation hotspot codon V600 was performed by bi-directional cycle- sequencing of PCR-amplified fragments, using a previously described method.[17]

Germline mutation analysis

To all patients suspect for LS and referred to the department of clinical genetics, germline mutation analysis was offered. In DNA isolated from peripheral blood samples of these patients all coding regions and intron-exon boundaries of the *MLH1*, *MSH2* and *MSH6* genes were completely and systematically analyzed using direct sequence analysis. Reaction products were analyzed using a capillary automated sequencer (details of method and primer sequences available on request). In addition, all three genes were analyzed for genomic rearrangements using MLPA analysis (MRC Holland). Mutation analysis of *PMS2* was performed as described previously.[18]

Statistical analyses

Data were analyzed using SPSS 17.0 statistical software for Windows, and reported using descriptive statistics. The prevalence of LS and a sporadic MSI-H phenotype were analyzed. The Mann-Whitney U test was used to compare the different groups. Two-sided p-values less than 0.05 were considered statistically significant.

RESULTS

Endometrial cancer patient population

A total of 172 EC patients were included (Figure 1). The median age at EC diagnosis was 61 years (interquartile range: 56-67 years). Fourteen patients were ≤ 50 years (8%) at time of diagnosis. Most ECs (81%) showed an endometrioid type histology. Tumorspecimens were found to be grade 1 in 66 patients, grade 2 in 40 patients, and grade 3 in 24 patients (Table 1).

Molecular analyses

Overall, 134 tumors were found to be MSS and 38 tumors displayed an MSI-H phenotype including 28 sporadic MSI-H tumors (16%; 95% CI 11-22%). Ten tumors were classified as suspect for LS (6%; 95% CI 3-10%). On the basis of immunohistochemical protein expression, one of these patients was suspect for an *MLH1* gene defect, two for an *MSH2*, five for an *MSH6*, and two for a *PMS2* defect (Table 2). The correlation between age and the results of the molecular analyses is shown in Figure 2. There was a 100% concordance between IHC results and MSI-status in analyzed EC tumor tissues (Table 2). *BRAF* mutation analysis was performed in all MSI-H tumors, but no *BRAF* mutations were detected.

Table 1. Patient characteristics and results of molecular analyses.

	MSS n = 134 (78%)	**Sporadic MSI-H** n = 28 (16%)	**Suspect for LS** n = 10 (6%)	**Total** n = 172
Median Age (IQR)	61 (56-67)	61 (57-69)	61 (53-67)	61 (56-67)
Histology				
Endometroid adenocarcinoma	104 (78%)	27 (96%)	9 (90%)	140 (81%)
Adenosquamous carcinoma	1 (1%)	0	0	1 (1%)
Serous adenocarcinoma (papill)	6 (5%)	0	0	6 (4%)
Mixed adenocarcinoma	7 (5%)	0	1 (10%)	8 (5%)
Clear cell carcinoma	2 (2%)	0	0	2 (1%)
Serous adenocarcinoma	1 (1%)	0	0	1 (1%)
Squamous cell carcinoma	1 (1%)	0	0	1 (1%)
Adenocarcinoma not further specified	12 (9%)	1 (4%)	0	13 (8%)
Tumor grade				
1	54 (40%)	10 (35%)	2 (20%)	66 (38%)
2	27 (20%)	9 (32%)	4 (40%)	40 (23%)
3	16 (12%)	5 (18%)	3 (30%)	24 (14%)
Grade unknown	37 (28%)	4 (14%)	1 (10%)	42 (24%)
Endometrial cancer stage				
I	47 (35%)	10 (36%)	5 (50%)	62 (36%)
II	4 (3%)	1 (4%)	0	5 (3%)
III	8 (6%)	1 (4%)	0	9 (5%)
IV	0	0	0	0
Stage unknown	75 (56%)	16 (57%)	5 (50%)	96 (56%)

MSS = microsatellite stable, MSI-H = high degree of microsatellite instability, LS = Lynch syndrome, IQR = interquartile range.

Table 2. Concordance between MSI and IHC results.

MMR protein expression (n = 172)	**MSI-H**	**MSS**
Normal expression	0	134
Absent protein expression	38	0
MLH1 staining absent	29	0
MSH2 staining absent	2	0
MSH6 staining absent	5	0
PMS2 staining absent	2	0

MMR = mismatch repair, MSI-H = high degree of microsatellite instability,
MSS = microsatellite stable.

Patients suspect for Lynch syndrome

Ten patients were suspect for LS on the basis of the molecular and IHC analyses (Table 3). The median age of these patients was 61 years (interquartile range: 53-67 years), and they were all older than 50 years at time of EC diagnosis (Figure 2). There was no difference in age at EC diagnosis between patients suspect for LS and patients with either sporadic MSI-H tumors (p = 0.41) or patients with MSS tumors (p = 0.99). Comparing tumor grade, histology and stage there were also no differences between patients suspect for LS and patients with

sporadic MSI-H tumors (p=0.32). The gynaecologists of the ten patients whose EC displayed a phenotype suspect for LS, were advised to refer their patients to the department of clinical genetics for counseling and germline mutation analysis. So far, eight patients have been referred and counseled. Five of these patients did not have a family history suspect for LS. One patient declined germline DNA mutation analysis and in the other seven patients three *MSH6* mutations have been found so far. Mutation analysis is still pending in three patients and in another patient whose tumor showed absent MSH2 and MSH6 expression, DNA mutation analysis of *MSH2* and *MSH6* was negative.

Table 3. Characteristics of the 10 endometrial cancer patients suspect for Lynch syndrome.

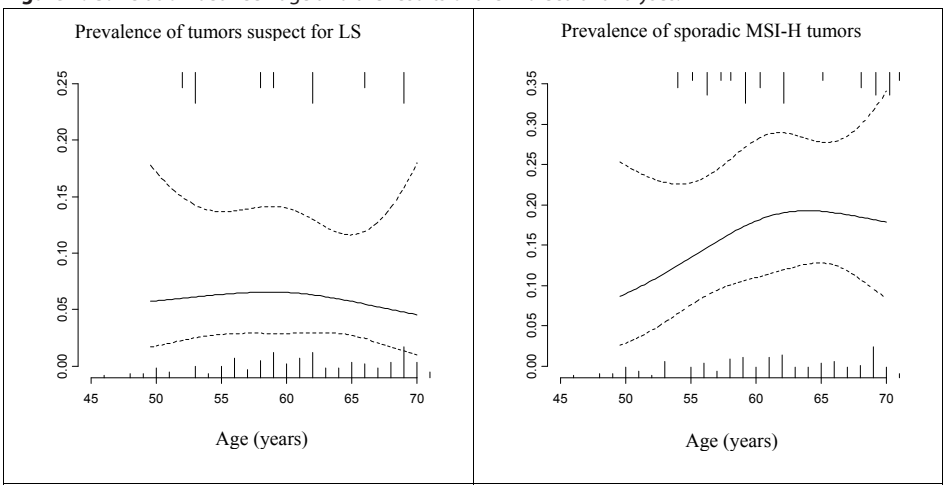
Patient no.	Age at EC diagnosis	Histology	Grade	AC	rBC	No. of pos. MSI markers	Absent MMR protein (IHC)	*MLH1* promoter methylation	Germline analysis	Mutation
110	52	Endometroid adenoca	1	No	No	3/5	MSH6	ND	Yes	*MSH6*
153	66	Endometroid adenoca	1	No	No	3/5	MLH1/PMS2	No	Declines DNA analysis	
93	69	Mixed adenoca	3	No	No	3/5	PMS2	ND	Pending	
112	53	Endometroid adenoca	2	NA	Yes	4/5	MSH6	ND	Not referred	
37	58	Endometroid adenoca	2	No	No	5/5	MSH2/MSH6	ND	Yes	No *MSH2* or *MSH6* mutation detectable
43	53	Endometroid adenoca	3	NA	NA	5/5	MSH2/MSH6	ND	Pending	
92	62	Endometroid adenoca	3	Yes	No	5/5	MSH6	ND	Yes	*MSH6*
64	59	Endometroid adenoca	NA	No	Yes	5/5	MSH6	ND	Yes	*MSH6*
161	69	Endometroid adenoca	2	NA	NA	5/5	PMS2	ND	Not referred	
57	62	Endometroid adenoca	2	NA	NA	5/5	MSH6	ND	Pending	

EC = endometrial cancer, adenoca = adenocarcinoma, LS = Lynch syndrome, rBG = revised Bethesda Guidelines, AC = Amsterdam Criteria II, IHC = Immunohistochemistry, NA = not available, ND = not done.

DISCUSSION

In this prospective multicenter population-based study, we showed that routine molecular analyses lead to the identification of a profile suspect for LS in 6% (95% CI 3-11%) of all newly diagnosed EC patients younger than 70 years. So far, three germline mutations in an MMR gene (*MSH6*) have been confirmed (1.7%). All patients suspect for LS were older than 50 years at EC diagnosis. The detection of EC patients suspect for LS is of great importance since these

Figure 2. Correlation between age and the results of the molecular analyses.



LS = Lynch syndrome. MSI-H = high degree of microsatellite instability.
Left panel: Prevalence of tumors suspect for LS according to age; the chance to detect a patient suspect for LS by molecular analyses is not related to age.
Right panel: Prevalence of sporadic MSI-H tumors according to age; the chance to detect a patient with a sporadic MSI-H tumor by molecular analyses clearly increases to the age of 63 years.

patients are at high risk for synchronous carcinomas, especially CRC. Moreover, the diagnosis of LS is of great importance to at risk relatives. EC patients with LS and their family members harbouring an MMR gene mutation can enter surveillance programs which have been proven to reduce CRC morbidity and mortality by 65-70%.[10-12] For female LS carriers gynaecologic surveillance programmes, including endometrial biopsy and transvaginal ultrasound, are available. However, gynaecological surveillance is currently based on expert opinion since no controlled trials have been published on the effectiveness of surveillance.[19] Instead of surveillance, prophylactic surgery can be considered after childbearing has been completed, as this may prevent endometrial and ovarian carcinoma effectively.[20]

Other prospective studies on the prevalence of LS among EC patients have been conducted, using different strategies of molecular analyses and different age cut-offs.[21-23] We chose for an age cut-off of 70 years to compromise between the feasibility of the study and an optimal detection of patients suspect for LS. Previous studies have been conducted to evaluate the yield of molecular screening for LS. In the largest unselected cohort of endometrial cancer patients (n = 543), 1.8% of newly diagnosed EC patients were diagnosed with an MMR germline mutation.[24] However this study may have been biased by the fact that almost half of the eligible patients declined molecular screening of their tumor tissue.[24] Our strategy appears more effective in detecting patients at high risk of LS (6%), but one can discuss whether patients are sufficiently aware of the possible consequences of molecular tumor screening for LS, being informed by their gynaecologist and a folder. Backes et al. noted a low acceptance of genetic consultation in 15 EC patients at high risk of LS.[21] In our experience, it took a lot

of explanation and time to clarify the relevance of referral to a clinical genetics department to gynaecologists and their patients, leading to a delay in genetic counseling for LS. So far, two of ten patients at high risk for LS are not referred. Of the eight referred patients, one patient declined further DNA analysis due to religious considerations. Another known reason to decline genetic testing is fear of social consequences e.g. obtaining insurance coverage.[25] In our strategy we experienced that logistic and communication problems were the main causes for delay in informing patients about the molecular test results and planning of an appointment at the genetics department. This indicates that better implementation of genetic service in all hospitals may contribute to a better uptake for genetic counseling and testing.

In our study we performed both MSI and IHC for four MMR proteins. The concordance rate in our study between MSI analysis and immunohistochemical analysis was 100%. From previous research it is known that effectiveness of MSI compared to IHC in EC is similar with a concordance rate between MSI and IHC of 92%.[26-28] In the literature therefore strategies are proposed using exclusively immunohistochemical staining in patients with EC to detect patients at high risk for LS[21,23,29-31], since immunohitochemistry is relatively cheap, easy to perform and points directly towards the gene most likely to be affected. However, immunohistochemical analyses can miss carriers of deleterious missense mutations. Furthermore not yet defined genes which may play a role in the development of LS are not detected by the current MMR proteins stained for. Therefore, the combination of MSI and IHC analyses to select patients at high risk for LS remains superior in our opinion.

In addition to MSI and IHC analyses, we performed *MLH1* promoter methylation assay in MSI-H tumors with absent MLH1 expression. These analyses revealed a sporadic MSI-H status in 16% of all analyzed ECs. This result is in line with findings from previous studies.[8,9] The establishment of a sporadic MSI-H status considerably reduced the number of patients referred for counseling and germline genetic testing at the clinical genetics department. In all MSI-H tumors we also performed *BRAF* mutation analysis. In concordance with a recent report by Kawguchi et al.[32], we did not detect any *BRAF* V600E mutation in the MSI-H tumor cases. Therefore, *MLH1* promoter hypermethylation analysis seems more suitable to identify sporadic MSI-H EC cases than *BRAF* mutation analysis.

Our study, as well as previous studies[21-24,26,30,33], demonstrate the urge to implement EC in diagnostic criteria for LS. The Dutch MIPA criteria and the international SGO guidelines recognise the importance of selecting EC patients for molecular analyses for LS.[34,35] However, these guidelines include only EC patients diagnosed before the age of 50 years, unless another LS associated tumor is also present. In our study all ten EC patients suspect for LS were over 50 years of age at EC diagnosis. On the basis of these data and data from the literature[22,24], MSI and immunohistochemical analysis for LS should not be limited to EC patients under the age of 50 years. More studies on the optimal age criterion for molecular testing and cost-benefit analysis data are desirable.

A limitation of our study is the lack of data on family history of included patients. Therefore we can not compare our strategy to the Amsterdam II criteria, the Bethesda guidelines or predictive models for LS.[13,36] Five of eight families of the referred patients suspect for LS did not fulfil the Amsterdam II criteria nor the revised Bethesda guidelines. In the study by Hampel et al., the families of 7/10 EC patients who appeared to have LS did not fulfil Amsterdam II criteria or the Bethesda guidelines.[24] This indicates that these guidelines may not be suitable to detect LS in EC patients. Recently, Backes et al. evaluated current LS predictive models for patients with EC, and concluded that these models worked reasonably well to identify EC patients at high risk for LS.[21] Further research is needed to develop specific LS predictive models for EC patients.[36]

Another limitation is that we did not perform germline testing on all patients in this study, nor in all MSI-H cases. In case of an MSI-H tumor tissue with absent MLH1 protein expression and hypermethylation of the *MLH1* promoter we concluded this tumor to be sporadic MSI-H. This could have lead to underestimation of LS in our study population. However, previous studies indicate that *MLH1* promoter hypermethylation is a sufficient tool to detect sporadic tumors.[8,9,37]

In conclusion, routine molecular screening for LS by MSI analysis, IHC analysis of MMR protein expression and *MLH1* promoter hypermethylation assay in EC patients under the age of 70 years, contributes to the detection of more patients at high risk for LS. New screening guidelines for LS are needed, including recommendations for EC patients older than 50 years of age. Physicians and patients have to be educated about the importance of early detection of LS in EC patients and the importance of surveillance programmes, leading to better implementation of genetic counseling and testing in clinical practice.

REFERENCES

1. Aarnio M, Sankila R, Pukkala E, Salovaara R, Aaltonen LA, de la Chapelle A, et al. Cancer risk in mutation carriers of DNA-mismatch-repair genes. Int J Cancer 1999;81(2):214-218.
2. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 2005;352(18):1851-1860.
3. Koornstra JJ, Mourits MJ, Sijmons RH, Leliveld AM, Hollema H, Kleibeuker JH. Management of extracolonic tumours in patients with Lynch syndrome. Lancet Oncol 2009;10(4):400-408.
4. Loukola A, Eklin K, Laiho P, Salovaara R, Kristo P, Jarvinen H, et al. Microsatellite marker analysis in screening for hereditary nonpolyposis colorectal cancer (HNPCC). Cancer Res 2001;61(11):4545-4549.
5. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003;348(10):919-932.
6. Burks RT, Kessis TD, Cho KR, Hedrick L. Microsatellite instability in endometrial carcinoma. Oncogene 1994;9(4):1163-1166.
7. Risinger JI, Berchuck A, Kohler MF, Watson P, Lynch HT, Boyd J. Genetic instability of microsatellites in endometrial carcinoma. Cancer Res 1993;53(21):5100-5103.
8. Buttin BM, Powell MA, Mutch DG, Rader JS, Herzog TJ, Gibb RK, et al. Increased risk for hereditary nonpolyposis colorectal cancer-associated synchronous and metachronous malignancies in patients with microsatellite instability-positive endometrial carcinoma lacking MLH1 promoter methylation. Clin Cancer Res 2004;10(2):481-490.
9. Simpkins SB, Bocker T, Swisher EM, Mutch DG, Gersell DJ, Kovatich AJ, et al. MLH1 promoter methylation and gene silencing is the primary cause of microsatellite instability in sporadic endometrial cancers. Hum Mol Genet 1999;8(4):661-666.
10. de Jong AE, Hendriks YM, Kleibeuker JH, de Boer SY, Cats A, Griffioen G, et al. Decrease in mortality in Lynch syndrome families because of surveillance. Gastroenterology 2006;130(3):665-671.
11. Jarvinen HJ, Aarnio M. Surveillance on mutation carriers of DNA mismatch repair genes. Ann Chir Gynaecol 2000;89(3):207-210.
12. Stupart DA, Goldberg PA, Algar U, Ramesar R. Surveillance colonoscopy improves survival in a cohort of subjects with a single mismatch repair gene mutation. Colorectal Dis 2009;11(2):126-130.
13. Umar A, Risinger JI, Hawk ET, Barrett JC. Testing guidelines for hereditary non-polyposis colorectal cancer. Nat Rev Cancer 2004;4(2):153-158.
14. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 1999;116(6):1453-1456.
15. Ramsoekh D, Wagner A, van Leerdam ME, Dinjens WN, Steyerberg EW, Halley DJ, et al. A high incidence of MSH6 mutations in Amsterdam criteria II-negative families tested in a diagnostic setting. Gut 2008;57(11):1539-1544.
16. Syngal S, Fox EA, Eng C, Kolodner RD, Garber JE. Sensitivity and specificity of clinical criteria for hereditary non-polyposis colorectal cancer associated mutations in MSH2 and MLH1. J Med Genet 2000;37(9):641-645.
17. van Lier MG, Wagner A, van Leerdam ME, Biermann K, Kuipers EJ, Steyerberg EW, et al. A review on the molecular diagnostics of Lynch syndrome: a central role for the pathology laboratory. J Cell Mol Med 2010;14(1-2):181-197.

18. van der Klift HM, Tops CM, Bik EC, Boogaard MW, Borgstein AM, Hansson KB, et al. Quantification of sequence exchange events between PMS2 and PMS2CL provides a basis for improved mutation scanning of Lynch syndrome patients. Hum Mutat 2010;31(5):578-587.
19. Burke W, Petersen G, Lynch P, Botkin J, Daly M, Garber J, et al. Recommendations for follow-up care of individuals with an inherited predisposition to cancer. I. Hereditary nonpolyposis colon cancer. Cancer Genetics Studies Consortium. JAMA 1997;277(11):915-919.
20. Schmeler KM, Lynch HT, Chen LM, Munsell MF, Soliman PT, Clark MB, et al. Prophylactic surgery to reduce the risk of gynecologic cancers in the Lynch syndrome. N Engl J Med 2006;354(3):261-269.
21. Backes FJ, Leon ME, Ivanov I, Suarez A, Frankel WL, Hampel H, et al. Prospective evaluation of DNA mismatch repair protein expression in primary endometrial cancer. Gynecol Oncol 2009;114(3): 486-490.
22. Goodfellow PJ, Buttin BM, Herzog TJ, Rader JS, Gibb RK, Swisher E, et al. Prevalence of defective DNA mismatch repair and MSH6 mutation in an unselected series of endometrial cancers. Proc Natl Acad Sci U S A 2003;100(10):5908-5913.
23. Lu KH, Schorge JO, Rodabaugh KJ, Daniels MS, Sun CC, Soliman PT, et al. Prospective determination of prevalence of lynch syndrome in young women with endometrial cancer. J Clin Oncol 2007;25(33):5158-5164.
24. Hampel H, Frankel W, Panescu J, Lockman J, Sotamaa K, Fix D, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res 2006;66(15):7810-7817.
25. Hadley DW, Jenkins J, Dimond E, Nakahara K, Grogan L, Liewehr DJ, et al. Genetic counseling and testing in families with hereditary nonpolyposis colorectal cancer. Arch Intern Med 2003;163(5): 573-582.
26. Modica I, Soslow RA, Black D, Tornos C, Kauff N, Shia J. Utility of immunohistochemistry in predicting microsatellite instability in endometrial carcinoma. Am J Surg Pathol 2007;31(5):744-751.
27. Mueller J, Gazzoli I, Bandipalliam P, Garber JE, Syngal S, Kolodner RD. Comprehensive molecular analysis of mismatch repair gene defects in suspected Lynch syndrome (hereditary nonpolyposis colorectal cancer) cases. Cancer Res 2009;69(17):7053-7061.
28. Shia J, Black D, Hummer AJ, Boyd J, Soslow RA. Routinely assessed morphological features correlate with microsatellite instability status in endometrial cancer. Hum Pathol 2008;39(1):116-125.
29. Matthews KS, Estes JM, Conner MG, Manne U, Whitworth JM, Huh WK, et al. Lynch syndrome in women less than 50 years of age with endometrial cancer. Obstet Gynecol 2008;111(5):1161-1166.
30. Ollikainen M, Abdel-Rahman WM, Moisio AL, Lindroos A, Kariola R, Jarvela I, et al. Molecular analysis of familial endometrial carcinoma: a manifestation of hereditary nonpolyposis colorectal cancer or a separate syndrome? J Clin Oncol 2005;23(21):4609-4616.
31. Walsh MD, Young J, Spurdle A, Obermair A. Lynch syndrome in women less than 50 years of age with endometrial cancer. Obstet Gynecol 2008;112(4):943.
32. Kawaguchi M, Yanokura M, Banno K, Kobayashi Y, Kuwabara Y, Kobayashi M, et al. Analysis of a correlation between the BRAF V600E mutation and abnormal DNA mismatch repair in patients with sporadic endometrial cancer. Int J Oncol 2009;34(6):1541-1547.
33. Black D, Soslow RA, Levine DA, Tornos C, Chen SC, Hummer AJ, et al. Clinicopathologic significance of defective DNA mismatch repair in endometrial carcinoma. J Clin Oncol 2006;24(11):1745-1753.
34. Richtlijn Erfelijke Darmkanker 2008. Vereniging Klinische Genetica Nederland; Kwaliteitsinstituut voor de gezondheidszorg CBO, 2008.

35. Lancaster JM, Powell CB, Kauff ND, Cass I, Chen LM, Lu KH, et al. Society of Gynecologic Oncologists Education Committee statement on risk assessment for inherited gynecologic cancer predispositions. Gynecol Oncol 2007;107(2):159-162.
36. Backes FJ, Hampel H, Backes KA, Vaccarello L, Lewandowski G, Bell JA, et al. Are prediction models for Lynch syndrome valid for probands with endometrial cancer? Fam Cancer 2009;8(4):483-487.
37. Walsh CS, Blum A, Walts A, Alsabeh R, Tran H, Koeffler HP, et al. Lynch syndrome among gynecologic oncology patients meeting Bethesda guidelines for screening. Gynecol Oncol 2010;116(3): 516-521.

12

General discussion and conclusions

PART I: PEUTZ-JEGHERS SYNDROME (PJS)

PJS is difficult to assess due to its rarity, leading to a lack of detailed clinical and epidemiological data on the syndrome. This lack of data hampers patient management including the development of optimal surveillance strategies. In this thesis we therefore studied risk for bowel intussusception, cancer risks, and mortality associated with PJS. In addition, we assessed the effect of PJS on the quality of life and on family planning, in order to improve clinical management of PJS patients.

Gastrointestinal (GI) and extra-GI malignancies associated with PJS have been reported since the late 1950s.[1-3] A systematic review (Chapter 2) showed high cumulative and relative cancer risks, with a mean age at cancer diagnosis of 42 years. The young age at cancer diagnosis is consistent with the identification of the *STK11* gene as a tumor suppressor gene.[4] The most common malignancy was colorectal cancer (CRC), followed by breast, small-bowel, gastric and pancreatic cancer.[5] The review comprised large collaborative studies evaluating heterogeneous groups of patients and small cohort studies. Consequently, the review revealed rather wide ranges in reported cancer risks: life time cumulative cancer risks ranged from 37 to 93% and relative cancer risks between 9.9 and 18 had been reported.

We therefore performed a large pedigree-based cohort study among 133 Dutch PJS patients and showed a life time cumulative cancer risk of more than 75%. The relative cancer risk adjusted for age, sex, and calendar period was nearly 10 times as high as in the general population (Chapter 3). These elevated cancer risks are in line with previous reports on the increased cancer risk associated with PJS. However, the cumulative and relative cancer risks in our study are slightly lower than previously reported.[6-11] We furthermore found that cancer risks are higher in females than in males with PJS, which can be explained by the additional risk of breast cancer and gynaecological cancers. In addition to the elevated cancer risks, we also showed an increased mortality in PJS patients compared to the general population (hazard ratio 3.5). The increased mortality can largely be explained by the elevated cancer risk. Acute bowel intussusception was, at least before 1970, another important cause of death.

The cumulative intussusception risk in the Dutch PJS cohort was 50% at the age of 20 years, increasing to 75% at the age of 36 years (Chapter 4). The risk was independent of sex. The intussusceptions occurred in the small-intestine in more than 95% of events and were generally caused by hamartomas larger than or equal to 15 mm in diameter. The increased intussusception risk corroborates a previous collaborative study on the intussusception risk in PJS patients.[12] However, our study is the first to report on intussusception characteristics such as intussusception localisation and size of polyps that function as leading point for the intussusceptions. These data are relevant for the improvement of surveillance recommendations, especially since new techniques including balloon-assisted enteroscopy (BAE) have been introduced into clinical practice.[13,14] BAE may play a role in small intestinal surveillance of PJS patients as BAE enables endoscopic visualization of the entire small intestine and allows the

diagnosis of small intestinal polyps as well as therapeutic interventions to be combined in a single procedure. In this thesis we demonstrated that BAE can actually play an important role in small intestinal surveillance of PJS patients, as BAE is not only clinically useful and safe for diagnosis and therapy of small-intestinal polyps, but BAE may also prevent complications of small-intestinal polyps including intussusception and laparotomies (Chapter 5).

Data on psychological distress and quality of life in PJS patients are very limited as only one study on this topic has previously been performed.[15] Moreover, data concerning genetic test uptake, reproductive decision making, attitude towards prenatal diagnosis with the implication of pregnancy termination, and preimplantation genetic diagnosis are lacking. The adult PJS patients from our cohort were therefore invited to complete a questionnaire on these issues. PJS patients experienced a similar level of psychological distress as the general population, but a poorer general health perception, more limitations due to emotional problems, and a poorer mental quality of life (Chapter 6). Illness perceptions and female gender were major predictors for this lower quality of life. These results may help to recognize PJS patients who might benefit from psychological support. Psychological support can target illness perceptions among other things, as these perceptions are dynamic variables.[16,17] Furthermore, 71% percent of the responders had undergone DNA mutation analysis, a requisite for prenatal diagnosis and preimplantation genetic diagnosis. Female gender and parenthood were, as expected on the basis of literature, positive predictors for DNA mutation analysis.[18-20] The diagnosis of PJS influenced decisions regarding family planning (i.e. less or no children) in one third of PJS patients, especially in women. This could be explained by the fact that women may have a higher sense of responsibility towards their offspring than men.[21] More patients accepted the use of preimplantation genetic diagnosis in case of PJS (52%) than pregnancy termination after prenatal diagnosis (15%). This could be due to the fact that preimplantation genetic diagnosis is considered a morally and psychologically more acceptable option for genetic testing before birth[22], since it offers patients the possibility to have an unaffected genetically related child while termination of a pregnancy can be avoided. (Chapter 7). These results emphasize the importance of discussing aspects regarding family planning with PJS patients, including prenatal diagnosis and pregnancy termination and preimplantation genetic diagnosis.

Conclusions and directions for future research on Peutz-Jeghers syndrome

PJS is associated with high cancer and intussusception risks, leading to considerable morbidity and mortality. The intussusceptions predominate the first three decades of life, whereas malignancies become more common thereafter. PJS patients experience a poorer mental quality of life, more limitations in daily functioning due to emotional problems, and a poorer general health perception compared to the general population. Furthermore, the diagnosis

of PJS influences the desire to have children (i.e. less or no children). These findings can help in the clinical management and counseling of PJS patients.

The high cancer and intussusception risks justify surveillance, predominantly of the GI tract. We have formulated a surveillance recommendation on the basis of the results presented in this thesis. Surveillance has two aims in PJS patients. The first aim is to reduce the polyp burden and the intussusception risk, predominantly in young PJS patients. The second aim is to detect cancer in an early phase in order to improve outcome and potentially reduce the cancer risk in adult PJS patients. The main difference between our surveillance recommendation and previously published recommendations[23-32], is the incorporation of new surveillance and treatment techniques such as BAE. However, all surveillance recommendations published so far are based on consensus and expert opinion, since no controlled trials have been published on the effectiveness of surveillance. Therefore, future research, including decision analytic modelling, is required to establish the effect of surveillance on the cancer and intussusception incidence and to weigh a potential beneficial effect against the burden and complication risk of the interventions.

Another unresolved question, important in light of surveillance, is whether the malignancies in the GI tract originate from the hamartomas, from coexisting adenomas, or from otherwise normal appearing mucosa.[33,34] Several studies have reported a hamartoma-adenoma-carcinoma sequence[35-37], suggesting that endoscopic polyp-removal could potentially decrease the risk for malignancies. Yet, other facts contradict this hypothesis. For example, the location of GI cancers does not always correlate with the location of the hamartomas.[34] To answer the question whether or not hamartomas are pre-malignant and to gain more insight into PJS-related carcinogenesis, further basal research is required. Although a second gene locus responsible for PJS has been suggested[38,39], future research should primarily focus on the *STK11* gene function, since *STK11* mutations can already be detected in more than 90% of patients with new available techniques.[40,41] It is relevant to gain more insight in genotype-phenotype correlations and to investigate whether differences in *STK11* mutation types are related to cancer and intussusception risks. The development of hamartomas and malignancies might be independent stromal and epithelial processes[4], which complicates the elucidation of the molecular mechanisms underlying *STK11*-associated carcinogenesis. The exact role of *STK11* in the carcinogenic pathway is still unclear, but up-regulation of mTOR signalling seems to be an important step as mTOR inhibitors have been shown to reduce tumor burden in mouse models[42,43] and a recent case report.[44] Elucidating the molecular background of cancer susceptibility in PJS patients might reveal therapeutic options and may help in the improvement of surveillance recommendations.

PART II: LYNCH SYNDROME (LS)

LS is a hereditary cancer susceptibility syndrome, responsible for 3% of all CRCs[45,46] and 2% of all endometrial cancers.[47] Early detection of LS is a major challenge as the syndrome lacks a pre-morbid phenotype. Yet, early detection of LS is of great importance because colonoscopic surveillance can significantly reduce CRC morbidity and mortality.[48-50] Furthermore, prophylactic surgery may prevent endometrial and ovarian carcinoma.[51] Accordingly, part II of this thesis focuses on LS detection and the improvement of LS detection.

Over the last 2 decades several strategies have been developed to identify patients with LS (Chapter 8). In 1990 the Amsterdam Criteria were developed to identify families eligible for the identification of the LS-causing gene in the period when these genes were not known yet.[52] These criteria were designed to be highly specific at the expense of the sensitivity.[46,53] They were criticized because extra-colonic tumors were not taken into account, leading to the publication of the Amsterdam Criteria II.[54] However, also the Amsterdam Criteria II are still hampered by a low sensitivity.[55]

Between 1993 and 1995 the mismatch repair (MMR) genes were discovered to cause LS.[56-61] MMR gene mutations lead to microsatellite instability (MSI) in tumor DNA. As MSI can be detected in approximately 95% of all LS-associated cancers, MSI analysis can be used in the diagnostic approach of LS.[62-64] In 1997 the Bethesda Guidelines were published to select patients for MSI analysis in order to identify patients at high risk for LS[65], and these guidelines have been revised in 2004 to make them more suitable for use in clinical practice.[66]

The revised Bethesda Guidelines are based on family history, age at cancer diagnosis, number of LS-associated carcinomas and certain histological tumor features. The combination of the revised Bethesda Guidelines and MSI-testing is currently the most widely accepted approach for the identification of LS patients. However, these guidelines have been criticized for being too complex to use.[67] Moreover, we demonstrated (Chapter 9) that MSI analysis was performed in only 14% of patients fulfilling the revised Bethesda Guidelines. As there is marked underutilization of MSI analysis in CRC patients at high risk for LS, LS is still under-diagnosed in CRC patients and their relatives. The clinical implications of this underdiagnosis of LS are devastating due to the high probability of (preventable) malignancies with a potential fatal outcome.

Since clinical criteria such as the Bethesda Guidelines do not quantify the likelihood of being a mutation carrier, several prediction models have been developed. These models make a quantitative estimation of the risk of carrying a germline MMR gene mutation on the basis of personal and familial data, without the requirement of tissue.[68-72] However, the implementation of these models into clinical practice is still in its infancy. Family history is one of the cornerstones of the prediction models as well as of the clinical criteria including the revised Bethesda Guidelines. However, obtaining a thorough family history is difficult in clinical practice[73], CRC patients frequently report their family history inaccurately[74-76], and it

may become more difficult to identify LS patients on the basis of family history as family sizes are decreasing.

As a considerable proportion of patients at high risk for LS is not recognized and the most optimal strategy for the detection of LS is still debatable, we performed a large population-based prospective study. The aim of this study was to determine whether further improvement of LS detection can be obtained by routine molecular analyses in CRC and endometrial cancer (EC) patients ≤ 70 years, and patients with advanced colorectal adenomas ≤ 45 years (Chapters 10 and 11). The molecular analyses including MSI analysis, immunohistochemical analysis of MMR protein expression, and *MLH1* promoter methylation assay, revealed a profile pathognomic for LS in 4.5% of 1117 CRC patients, in 2.4% of 125 advanced adenoma patients and in 5.8% of 172 endometrial cancer patients. Remarkably, 70% of the CRC patients (n=50) and all EC patients (n=10) suspect for LS were older than 50 years at cancer diagnosis, and do not meet the age-criterion routinely used for LS assessment. This it indicates that the age-criterion used in the Bethesda Guidelines (CRC < 50 years) may be suboptimal. Moreover, the MIPA criteria have recently been introduced in the Netherlands.[67] The MIPA criteria resemble our strategy, yet the MIPA criteria recommend MSI analysis in patients newly diagnosed with CRC or endometrial carcinoma before age 50, or before age 70 in patients diagnosed with two LS-associated cancers. As we demonstrate that most CRC and EC patients suspect for LS are older than 50 years at cancer diagnosis, our strategy may help to detect more LS patients.

Conclusions and directions for future research on Lynch syndrome

LS is still under-diagnosed in CRC patients and their relatives. Therefore further improvements in LS detection are necessary. We propose a strategy of routine molecular screening of all newly diagnosed CRC and EC patients younger than 70 years. The optimal age cut-off (at least higher than 50 years), as well as the cost-effectiveness of such an approach need to be determined. In the future, it may likely be desirable to subject all newly diagnosed CRC patients to MSI analysis[77], as MSI analysis is not only valuable for detection of LS, but also has prognostic and therapeutic implications. Regardless of stage at diagnosis, microsatellite unstable CRCs (including sporadic MSI-H cancers) are associated with a better prognosis than MSS tumors[78,79] and patients with MSI-H tumors may not benefit from adjuvant chemotherapy with 5-fluorouracil.[79-81] However, as the optimal strategy for adjuvant therapy of MSI-H cancers remains debatable, we do not believe that it is justified to screen all CRCs for MSI at this stage. More research, including cost-effectiveness modelling and prospective trials, should be performed to determine the optimal treatment of microsatellite instable CRCs.

REFERENCES

1. Bailey D. Polyposis of gastro-intestinal tract: the Peutz syndrome. Br Med J 1957;2(5042):433-439.
2. Bartholomew LG, Dahlin DC, Waugh JM. Intestinal polyposis associated with mucocutaneous melanin pigmentation (Peutz-Jeghers syndrome). Proc Staff Meet Mayo Clin 1957;32(24):675-680.
3. Christian CD, McLoughlin TG, Cathcart ER, Eisenberg MM. Peutz-Jeghers Syndrome Associated with Functioning Ovarian Tumor. JAMA 1964;190:935-938.
4. Hezel AF, Bardeesy N. LKB1; linking cell structure and tumor suppression. Oncogene 2008;27(55): 6908-6919.
5. van Lier MG, Wagner A, Mathus-Vliegen EM, Kuipers EJ, Steyerberg EW, van Leerdam ME. High cancer risk in Peutz-Jeghers syndrome: a systematic review and surveillance recommendations. Am J Gastroenterol 2010;105(6):1258-1264.
6. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, et al. Frequency and spectrum of cancers in the Peutz-Jeghers syndrome. Clin Cancer Res 2006;12(10):3209-3215.
7. Mehenni H, Resta N, Park JG, Miyaki M, Guanti G, Costanza MC. Cancer risks in LKB1 germline mutation carriers. Gut 2006;55(7):984-990.
8. Lim W, Olschwang S, Keller JJ, Westerman AM, Menko FH, Boardman LA, et al. Relative frequency and morphology of cancers in STK11 mutation carriers. Gastroenterology 2004;126(7):1788-1794.
9. Giardiello FM, Brensinger JD, Tersmette AC, Goodman SN, Petersen GM, Booker SV, et al. Very high risk of cancer in familial Peutz-Jeghers syndrome. Gastroenterology 2000;119(6):1447-1453.
10. Choi HS, Park YJ, Youk EG, Yoon KA, Ku JL, Kim NK, et al. Clinical characteristics of Peutz-Jeghers syndrome in Korean polyposis patients. Int J Colorectal Dis 2000;15(1):35-38.
11. Giardiello FM, Welsh SB, Hamilton SR, Offerhaus GJ, Gittelsohn AM, Booker SV, et al. Increased risk of cancer in the Peutz-Jeghers syndrome. N Engl J Med 1987;316(24):1511-1514.
12. Hearle N, Schumacher V, Menko FH, Olschwang S, Boardman LA, Gille JJ, et al. STK11 status and intussusception risk in Peutz-Jeghers syndrome. J Med Genet 2006;43(8):e41.
13. Yamamoto H, Sekine Y, Sato Y, Higashizawa T, Miyata T, Iino S, et al. Total enteroscopy with a nonsurgical steerable double-balloon method. Gastrointest Endosc 2001;53(2):216-220.
14. May A, Nachbar L, Wardak A, Yamamoto H, Ell C. Double-balloon enteroscopy: preliminary experience in patients with obscure gastrointestinal bleeding or chronic abdominal pain. Endoscopy 2003;35(12):985-991.
15. Woo A, Sadana A, Mauger DT, Baker MJ, Berk T, McGarrity TJ. Psychosocial impact of Peutz-Jeghers Syndrome. Fam Cancer 2009;8(1):59-65.
16. Kreuter MW, Strecher VJ. Changing inaccurate perceptions of health risk: results from a randomized trial. Health Psychol 1995;14(1):56-63.
17. Edwards A, Hood K, Matthews E, Russell D, Russell I, Barker J, et al. The effectiveness of one-to-one risk communication interventions in health care: a systematic review. Med Decis Making 2000; 20(3):290-297.
18. Ramsoekh D, van Leerdam ME, Tops CM, Dooijes D, Steyerberg EW, Kuipers EJ, et al. The use of genetic testing in hereditary colorectal cancer syndromes: genetic testing in HNPCC, (A)FAP and MAP. Clin Genet 2007;72(6):562-567.
19. Meijers-Heijboer EJ, Verhoog LC, Brekelmans CT, Seynaeve C, Tilanus-Linthorst MM, Wagner A, et al. Presymptomatic DNA testing and prophylactic surgery in families with a BRCA1 or BRCA2 mutation. Lancet 2000;355(9220):2015-2020.
20. Evans DG, Maher ER, Macleod R, Davies DR, Craufurd D. Uptake of genetic testing for cancer predisposition. J Med Genet 1997;34(9):746-748.

21. d'Agincourt-Canning L, Baird P. Genetic testing for hereditary cancers: the impact of gender on interest, uptake and ethical considerations. Crit Rev Oncol Hematol 2006;58(2):114-123.
22. Niermeijer MF, de Die-Smulders CE, Page-Christiaens GC, de Wert GM. [Genetic cancer syndromes and reproductive choice: dialogue between parents and politicians on preimplantation genetic diagnosis]. Ned Tijdschr Geneeskd 2008;152(27):1503-1506.
23. Allen BA, Terdiman JP. Hereditary polyposis syndromes and hereditary non-polyposis colorectal cancer. Best Pract Res Clin Gastroenterol 2003;17(2):237-258.
24. Lindor NM, McMaster ML, Lindor CJ, Greene MH. Concise handbook of familial cancer susceptibility syndromes - second edition. J Natl Cancer Inst Monogr 2008;(38):1-93.
25. Tomlinson IP, Houlston RS. Peutz-Jeghers syndrome. J Med Genet 1997;34(12):1007-1011.
26. The Johns Hopkins guide for patients and families: Peutz-Jeghers syndrome. Baltimore: Johns Hopkins University. 2001.
27. Burt RW. Colon cancer screening. Gastroenterology 2000;119(3):837-853.
28. McGrath DR, Spigelman AD. Preventive measures in Peutz-Jeghers syndrome. Fam Cancer 2001; 1(2):121-125.
29. Dunlop MG. Guidance on gastrointestinal surveillance for hereditary non-polyposis colorectal cancer, familial adenomatous polypolis, juvenile polyposis, and Peutz-Jeghers syndrome. Gut 2002;51 Suppl 5:V21-V27.
30. Hinds R, Philp C, Hyer W, Fell JM. Complications of childhood Peutz-Jeghers syndrome: implications for pediatric screening. J Pediatr Gastroenterol Nutr 2004;39(2):219-220.
31. McGarrity TJ, Amos C. Peutz-Jeghers syndrome: clinicopathology and molecular alterations. Cell Mol Life Sci 2006;63(18):2135-2144.
32. Giardiello FM, Trimbath JD. Peutz-Jeghers syndrome and management recommendations. Clin Gastroenterol Hepatol 2006;4(4):408-415.
33. Hizawa K, Iida M, Matsumoto T, Kohrogi N, Yao T, Fujishima M. Neoplastic transformation arising in Peutz-Jeghers polyposis. Dis Colon Rectum 1993;36(10):953-957.
34. Dozois RR, Judd ES, Dahlin DC, Bartholomew LG. The Peutz-Jeghers syndrome. Is there a predisposition to the development of intestinal malignancy? Arch Surg 1969;98(4):509-517.
35. Perzin KH, Bridge MF. Adenomatous and carcinomatous changes in hamartomatous polyps of the small intestine (Peutz-Jeghers syndrome): report of a case and review of the literature. Cancer 1982;49(5):971-983.
36. Hizawa K, Iida M, Matsumoto T, Kohrogi N, Kinoshita H, Yao T, et al. Cancer in Peutz-Jeghers syndrome. Cancer 1993;72(9):2777-2781.
37. Gruber SB, Entius MM, Petersen GM, Laken SJ, Longo PA, Boyer R, et al. Pathogenesis of adenocarcinoma in Peutz-Jeghers syndrome. Cancer Res 1998;58(23):5267-5270.
38. Mehenni H, Gehrig C, Nezu J, Oku A, Shimane M, Rossier C, et al. Loss of LKB1 kinase activity in Peutz-Jeghers syndrome, and evidence for allelic and locus heterogeneity. Am J Hum Genet 1998; 63(6):1641-1650.
39. Buchet-Poyau K, Mehenni H, Radhakrishna U, Antonarakis SE. Search for the second Peutz-Jeghers syndrome locus: exclusion of the STK13, PRKCG, KLK10, and PSCD2 genes on chromosome 19 and the STK11IP gene on chromosome 2. Cytogenet Genome Res 2002;97(3-4):171-178.
40. Volikos E, Robinson J, Aittomaki K, Mecklin JP, Jarvinen H, Westerman AM, et al. LKB1 exonic and whole gene deletions are a common cause of Peutz-Jeghers syndrome. J Med Genet 2006; 43(5):e18.
41. Aretz S, Stienen D, Uhlhaas S, Loff S, Back W, Pagenstecher C, et al. High proportion of large genomic STK11 deletions in Peutz-Jeghers syndrome. Hum Mutat 2005;26(6):513-519.

42. Wei C, Amos CI, Zhang N, Wang X, Rashid A, Walker CL, et al. Suppression of Peutz-Jeghers polyposis by targeting mammalian target of rapamycin signaling. Clin Cancer Res 2008;14(4):1167-1171.
43. Robinson J, Lai C, Martin A, Nye E, Tomlinson I, Silver A. Oral rapamycin reduces tumour burden and vascularization in Lkb1(+/-) mice. J Pathol 2009;219(1):35-40.
44. Klumpen HJ, Queiroz KC, Spek CA, van Noesel CJ, Brink HC, de Leng WW, et al. mTOR Inhibitor Treatment of Pancreatic Cancer in a Patient With Peutz-Jeghers Syndrome. J Clin Oncol 2010. in press
45. Aaltonen LA, Salovaara R, Kristo P, Canzian F, Hemminki A, Peltomaki P, et al. Incidence of hereditary nonpolyposis colorectal cancer and the feasibility of molecular screening for the disease. N Engl J Med 1998;338(21):1481-1487.
46. Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, et al. Feasibility of screening for Lynch syndrome among patients with colorectal cancer. J Clin Oncol 2008;26(35):5783-5788.
47. Hampel H, Frankel W, Panescu J, Lockman J, Sotamaa K, Fix D, et al. Screening for Lynch syndrome (hereditary nonpolyposis colorectal cancer) among endometrial cancer patients. Cancer Res 2006;66(15):7810-7817.
48. Jarvinen HJ, Aarnio M, Mustonen H, ktan-Collan K, Aaltonen LA, Peltomaki P, et al. Controlled 15-year trial on screening for colorectal cancer in families with hereditary nonpolyposis colorectal cancer. Gastroenterology 2000;118(5):829-834.
49. de Jong AE, Hendriks YM, Kleibeuker JH, de Boer SY, Cats A, Griffioen G, et al. Decrease in mortality in Lynch syndrome families because of surveillance. Gastroenterology 2006;130(3):665-671.
50. Stupart DA, Goldberg PA, Algar U, Ramesar R. Surveillance colonoscopy improves survival in a cohort of subjects with a single mismatch repair gene mutation. Colorectal Dis 2009;11(2):126-130.
51. Schmeler KM, Sun CC, Bodurka DC, White KG, Soliman PT, Uyei AR, et al. Prophylactic bilateral salpingo-oophorectomy compared with surveillance in women with BRCA mutations. Obstet Gynecol 2006;108(3 Pt 1):515-520.
52. Vasen HF, Mecklin JP, Khan PM, Lynch HT. The International Collaborative Group on Hereditary Non-Polyposis Colorectal Cancer (ICG-HNPCC). Dis Colon Rectum 1991;34(5):424-425.
53. Kievit W, de Bruin JH, Adang EM, Ligtenberg MJ, Nagengast FM, van Krieken JH, et al. Current clinical selection strategies for identification of hereditary non-polyposis colorectal cancer families are inadequate: a meta-analysis. Clin Genet 2004;65(4):308-316.
54. Vasen HF, Watson P, Mecklin JP, Lynch HT. New clinical criteria for hereditary nonpolyposis colorectal cancer (HNPCC, Lynch syndrome) proposed by the International Collaborative group on HNPCC. Gastroenterology 1999;116(6):1453-1456.
55. Lindor NM, Petersen GM, Hadley DW, Kinney AY, Miesfeldt S, Lu KH, et al. Recommendations for the care of individuals with an inherited predisposition to Lynch syndrome: a systematic review. JAMA 2006;296(12):1507-1517.
56. Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, et al. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 1993;75(5):1027-1038.
57. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, et al. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 1994;368(6468):258-261.
58. Papadopoulos N, Nicolaides NC, Wei YF, Ruben SM, Carter KC, Rosen CA, et al. Mutation of a mutL homolog in hereditary colon cancer. Science 1994;263(5153):1625-1629.

59. Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, et al. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 1994;371(6492):75-80.
60. Drummond JT, Li GM, Longley MJ, Modrich P. Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. Science 1995;268(5219):1909-1912.
61. Miyaki M, Konishi M, Tanaka K, Kikuchi-Yanoshita R, Muraoka M, Yasuno M, et al. Germline mutation of MSH6 as the cause of hereditary nonpolyposis colorectal cancer. Nat Genet 1997;17(3): 271-272.
62. Aaltonen LA, Peltomaki P, Mecklin JP, Jarvinen H, Jass JR, Green JS, et al. Replication errors in benign and malignant tumors from hereditary nonpolyposis colorectal cancer patients. Cancer Res 1994;54(7):1645-1648.
63. Boland CR, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, et al. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998;58(22):5248-5257.
64. Lynch HT, de la Chapelle A. Hereditary colorectal cancer. N Engl J Med 2003;348(10):919-932.
65. Rodriguez-Bigas MA, Boland CR, Hamilton SR, Henson DE, Jass JR, Khan PM, et al. A National Cancer Institute Workshop on Hereditary Nonpolyposis Colorectal Cancer Syndrome: meeting highlights and Bethesda guidelines. J Natl Cancer Inst 1997;89(23):1758-1762.
66. Umar A, Boland CR, Terdiman JP, Syngal S, de la Chapelle A, Ruschoff J, et al. Revised Bethesda Guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. J Natl Cancer Inst 2004;96(4):261-268.
67. Kievit W, de Bruin JH, Adang EM, Severens JL, Kleibeuker JH, Sijmons RH, et al. Cost effectiveness of a new strategy to identify HNPCC patients. Gut 2005;54(1):97-102.
68. Wijnen JT, Vasen HF, Khan PM, Zwinderman AH, van der KH, Mulder A, et al. Clinical findings with implications for genetic testing in families with clustering of colorectal cancer. N Engl J Med 1998; 339(8):511-518.
69. Barnetson RA, Tenesa A, Farrington SM, Nicholl ID, Cetnarskyj R, Porteous ME, et al. Identification and survival of carriers of mutations in DNA mismatch-repair genes in colon cancer. N Engl J Med 2006;354(26):2751-2763.
70. Balmana J, Stockwell DH, Steyerberg EW, Stoffel EM, Deffenbaugh AM, Reid JE, et al. Prediction of MLH1 and MSH2 mutations in Lynch syndrome. JAMA 2006;296(12):1469-1478.
71. Chen S, Wang W, Lee S, Nafa K, Lee J, Romans K, et al. Prediction of germline mutations and cancer risk in the Lynch syndrome. JAMA 2006;296(12):1479-1487.
72. Kastrinos F, Steyerberg EW, Mercado R, Balmana J, Holter S, Gallinger S, et al. The PREMM(1,2,6) Model Predicts Risk of MLH1, MSH2, and MSH6 Germline Mutations Based on Cancer History. Gastroenterology 2011;140(1):73-81.
73. Church J, McGannon E. Family history of colorectal cancer: how often and how accurately is it recorded? Dis Colon Rectum 2000;43(11):1540-1544.
74. Katballe N, Juul S, Christensen M, Orntoft TF, Wikman FP, Laurberg S. Patient accuracy of reporting on hereditary non-polyposis colorectal cancer-related malignancy in family members. Br J Surg 2001;88(9):1228-1233.
75. Sijmons RH, Boonstra AE, Reefhuis J, Hordijk-Hos JM, de Walle HE, Oosterwijk JC, et al. Accuracy of family history of cancer: clinical genetic implications. Eur J Hum Genet 2000;8(3):181-186.
76. Mitchell RJ, Brewster D, Campbell H, Porteous ME, Wyllie AH, Bird CC, et al. Accuracy of reporting of family history of colorectal cancer. Gut 2004;53(2):291-295.

77. Hampel H. Point: justification for Lynch syndrome screening among all patients with newly diagnosed colorectal cancer. J Natl Compr Canc Netw 2010;8(5):597-601.
78. Gryfe R, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, et al. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N Engl J Med 2000;342(2):69-77.
79. Popat S, Hubner R, Houlston RS. Systematic review of microsatellite instability and colorectal cancer prognosis. J Clin Oncol 2005;23(3):609-618.
80. Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, et al. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 2003;349(3):247-257.
81. Jover R, Zapater P, Castells A, Llor X, Andreu M, Cubiella J, et al. Mismatch repair status in the prediction of benefit from adjuvant fluorouracil chemotherapy in colorectal cancer. Gut 2006; 55(6):848-855.

Summary
Samenvatting

SUMMARY

Chapter 1 of this thesis contains a general introduction and outline of the thesis, including an overview of the hereditary colorectal cancer (CRC) syndromes. This thesis focuses on two hereditary CRC syndromes associated with an elevated gastrointestinal cancer risk, as well as an elevated risk for extra-gastrointestinal malignancies; Peutz-Jeghers syndrome (Part I) and Lynch syndrome (Part II).

Part I

The first part of this thesis focuses on Peutz-Jeghers syndrome (PJS). PJS is a rare autosomal dominant inherited disorder caused by *STK11* gene mutations. PJS is characterized by mucocutaneous pigmentations, gastrointestinal hamartomas, and an elevated cancer risk. As PJS is a rare disorder, few extensive studies on PJS have been conducted, leading to a lack of detailed data on the syndrome. This lack of data hampers patient management including the development of optimal surveillance strategies. Therefore we reviewed the literature and studied a large pedigree-based cohort of Dutch PJS patients in order to gain more insight into PJS.

Chapter 2 provides a systematic review of literature evaluating PJS-associated cancer risks. This review includes one meta-analysis and 20 cohort studies, the latter contributing to a total of 1644 patients. Of these 1644 patients, 349 patients developed 384 malignancies at a mean age of 42 years. The most common malignancy was colorectal cancer, followed by breast, small-bowel, gastric and pancreatic cancer. The reported life time risk for any cancer varied between 37 and 93% with relative risks ranging from 9.9 to 18 in comparison with the general population.

Our review revealed a wide range in reported cancer risk estimates. In **Chapter 3** cancer and mortality risks were therefore studied in133 Dutch PJS patients from 54 families (48% males), contributing to 5004 person-years of follow-up. Forty-nine cancers (including 25 gastrointestinal cancers, 6 gynaecological cancers, and 6 breast cancers) were diagnosed in 42 patients at a median age of 45 years at first cancer diagnosis. The overall cumulative cancer risk was 20% at age 40, increasing to 76% at age 70. The gastrointestinal cancer risk was 51% at age 70. PJS patients carry a substantially higher cancer risk than the general population (Hazard ratio (HR) 9.0), with higher relative risks in female patients (HR 20.4) than in males with PJS (HR 4.8). At the end of follow-up 42 patients had deceased at a median age of 45 years, including 28 cancer-related deaths. The mortality among PJS patients was significantly increased compared to the general population (HR 3.5). As these results justify surveillance to detect malignancies and their precursors in an early phase in order to improve outcome, we developed a surveillance recommendation.

Surveillance of PJS patients should not only be recommended for the elevated cancer risk, but also for benign polyps causing intussusceptions, leading to considerable morbidity. In order to improve surveillance recommendations and clinical management of PJS patients, we assessed characteristics, risk and onset of intussusception in the Dutch cohort of PJS patients in **Chapter 4**. In all, 110 patients from 50 families were analyzed (23 patients from the cohort had to be excluded due to incomplete data on the occurrence of intussusception). Seventy-six patients (69%) experienced at least one intussusception (range 1-6), at a median age of 16 years at the first event. The intussusception risk was 50% at the age of 20 years, increasing to 75% at the age of 36 years. The risk was independent of sex, family history and mutation status. The intussusceptions (n=128) occurred in the small-intestine in 95% of events and 80% of all intussusceptions presented as an acute abdomen. Therapy was surgical in 92.5% of events, and intussusceptions were caused by polyps with a median size of 35 mm (range 15-60 mm). These data support the approach of enteroscopic surveillance with removal of small-intestinal polyps larger than 10-15 mm, in order to prevent intussusceptions.

As balloon-assisted enteroscopy (BAE) may play a role in the surveillance of PJS patients, we evaluated its therapeutic efficacy and safety for detection and treatment of small-intestinal polyps in **Chapter 5**. Between October 2004 and July 2009, 13 PJS patients (62% males) with a median age of 31 (range 10–51) years underwent 29 BAE procedures. Small-bowel polyps were found in all patients, located predominantly in the duodenum and proximal jejunum (94%). A total of 82 polyps larger than or equal to 10 mm were detected, and 79 (96%) were endoscopically removed without complications. After the introduction of BAE, no small-intestinal polyp-related complications occurred during a follow-up period of 356 person-months. Hence, BAE is not only clinically useful and safe for diagnosis and therapy of small-intestinal polyps, but may also prevent complications of small-intestinal polyps and laparotomies in PJS patients.

Sixty-one adult PJS patients from the cohort were furthermore invited to complete a questionnaire on quality of life and psychological distress (**Chapter 6**), as well as genetic test uptake, and attitude towards family planning, prenatal diagnosis and pregnancy termination, and preimplantation genetic diagnosis (**Chapter 7**). The questionnaire was returned by 51 patients (84% response rate). The survey revealed that PJS patients experience a similar level of psychological distress, but a poorer mental quality of life, more limitations in daily functioning due to emotional problems, and a poorer general health perception compared to the general population. Illness perceptions and female gender were major determinants for the decline in quality of life. Moreover, the diagnosis of PJS influences the desire to have children (i.e. less or no children) in nearly one third of PJS patients, especially in women. Accordingly, females in our cohort less often had children than males. Most PJS patients have a positive attitude towards preimplantation genetic diagnosis as technique for antenatal diagnosis of PJS. In contrast, attitude was predominantly negative towards pregnancy termination in case of a fetus with PJS after prenatal diagnosis. These data indicate that medical specialists dealing

with patients suffering from hereditary cancer syndromes including PJS, should inform their patients about the possibilities of prenatal testing such as preimplantation genetic diagnosis.

Part II

The second part of this thesis focuses on Lynch syndrome (LS), another autosomal dominant inherited disorder caused by germline mutations in the mismatch repair genes. LS is the commonest form of hereditary CRC and is responsible for approximately 3% of all CRC cases. Additionally, LS is associated with extra-colonic cancers, mostly endometrial carcinoma. Early detection of LS is very important, especially for healthy at risk relatives, since colonoscopic surveillance can reduce morbidity and mortality. However, the diagnosis of LS is complicated by the absence of a pre-morbid phenotype and germline mutation analysis of the mismatch-repair genes to confirm the diagnosis is expensive and time-consuming. Therefore it is standard practice to precede germline mutation analysis by a molecular diagnostic work-up of tumors, guided by clinical and pathological criteria, to select patients for germline mutation analysis. In **Chapter 8** these molecular analyses, the clinical and pathological criteria including the revised Bethesda Guidelines, as well as the molecular basis of LS are addressed.

Mismatch repair gene mutations lead to microsatellite instability (MSI) in LS-associated tumors. MSI is the molecular hallmark of LS. The revised Bethesda Guidelines were developed to improve the efficiency of recognizing LS by identifying LS-related malignancies that should be analyzed for MSI. This is currently the most widely accepted approach to identify LS patients. In **Chapter 9** we evaluated the implementation of the revised Bethesda Guidelines into clinical practice. We included 169 patients diagnosed with CRC in 2005 and 2006 in the South-western part of the Netherlands, fulfilling the Bethesda guidelines on the basis of; 1) a CRC diagnosed < 50 years, 2) a second LS-associated tumor, and 3) patients < 60 years with CRC displaying mucinous or signet-ring differentiation or medullary growth pattern. MSI analysis had been performed in only 23 (14%) of the 169 CRC patients. These results show that there is marked underutilization of MSI analysis in CRC patients at high risk for LS. As a result LS is still underdiagnosed in patients with CRC and their relatives.

As a considerable proportion of patients at high risk for LS is not recognized, we prospectively studied the yield of routine molecular analyses in 1117 CRC patients ≤ 70 years and 125 patients with advanced colorectal adenomas ≤ 45 years in **Chapter 10**. Tumor specimens were analyzed for MSI, immunohistochemical MMR protein expression (IHC), and *MLH1* promoter methylation. Tumors were classified as either 1) suspect for LS, 2) sporadic MSI-H (*MLH1* promoter methylation), or 3) microsatellite stable (MSS). Analyses of the 1117 CRCs (57% males, median age 61) revealed 50 patients (4.5%) suspect for LS, 71 sporadic MSI-H tumors (6.4%), and 966 MSS tumors. Thirtyfive patients suspect for LS (70%) were > 50 years at CRC diagnosis. A profile suspect for LS was detected in 10% of patients ≤ 50 years, in 4% of those aged 51-60 years, and in 3% of patients older than 61 years. Among the 125 advanced

adenoma patients (58% males, median age 41 years), 3 were suspect for underlying LS (2.4%). Similarly, in **Chapter 11** we studied the yield of the molecular analyses in 172 consecutive endometrial cancer (EC) patients ≤ 70 years. These women had a median age of 61 years at EC diagnosis. The molecular analyses revealed 10 patients suspect for LS (5.8%), all older than 50 years at EC diagnosis (median age 61, range 52-69 years). In addition, 28 sporadic MSI-H tumors were detected (16.3%), and the remaining 134 ECs were MSS. There was no significant difference in age at EC diagnosis between patients suspect for LS and patients with either a sporadic MSI-H tumor ($p = 0.41$) or patients with a MSS tumor ($p = 0.99$). These results indicate that young age does not seem to be a useful criterion to select EC patients for MSI analysis. Moreover, the results presented in chapters 10 and 11 show that routine molecular screening may help to identify more LS patients. The cost-effectiveness of such an approach and the optimal age cut-off for molecular screening have to be determined.

The main findings of this thesis and directions for future research are discussed in **Chapter 12**.

SAMENVATTING

In **Hoofdstuk 1** wordt een inleiding op dit proefschrift gegeven, en wordt de opbouw van het proefschrift beschreven. In deze inleiding wordt onder andere een overzicht gegeven van de bestaande erfelijke colorectaal kanker syndromen. In dit proefschrift zal vervolgens op twee van deze syndromen, beide geassocieerd met een verhoogd risico op zowel gastrointestinale als extra-gastrointestinale maligniteiten, dieper worden ingegaan: het Peutz-Jeghers syndroom (Deel I) en het Lynch syndroom (Deel II)

Deel I

Het eerste deel van dit proefschrift gaat over het Peutz-Jeghers syndroom (PJS). PJS is een zeldzame autosomaal dominant overervende aandoening veroorzaakt door mutaties in het *STK11* gen. PJS wordt gekenmerkt door pigmentaties op de huid en slijmvliezen, poliepen (hamartomen) in het maag-darmkanaal, en een verhoogd risico op kanker. Omdat PJS een zeldzame aandoening is, zijn er weinig studies naar verricht waardoor relevante informatie over dit syndroom ontbreekt. Daarom hebben we een literatuur studie verricht en een groot cohort van Nederlandse PJS patiënten bestudeerd om meer inzicht in PJS te krijgen.

Hoofdstuk 2 omvat een systematische literatuurstudie, waarin het kanker risico wordt beschreven geassocieerd met PJS. In deze literatuurstudie werden 1 meta-analyse en 20 cohort studies geïncludeerd. In de cohort studies werden 1644 patiënten beschreven waarvan 349 patiënten in totaal 384 maligniteiten ontwikkelden op een gemiddelde leeftijd van 42 jaar. De meest voorkomende maligniteit was dikke darm kanker, gevolgd door borst, dunne darm, maag en alvleesklier kanker. De gerapporteerde cumulatieve kanker risico's varieerden van 37 tot 93%, en de relatieve kanker risico's van 9.9 tot 18 in vergelijking met de algemene bevolking.

De literatuurstudie toonde een vrij grote spreiding in gerapporteerde kanker risico's. Daarom hebben we in **Hoofdstuk 3** het kanker risico en de sterfte onderzocht in een cohort van 133 Nederlandse PJS patiënten uit 54 verschillende families (48% mannen, 5004 persoonsjaren follow-up). In 42 van deze 133 patiënten werden in totaal 49 maligniteiten gediagnosticeerd (25 tumoren in het maag-darmkanaal, 6 gynaecologische en 6 borst kankers) op een mediane leeftijd van 42 jaar bij de eerste tumor. Het cumulatieve kanker risico was 20% op de leeftijd van 40 jaar, oplopend naar 76% het 70^{e} jaar. Het risico op kanker in het maag-darmkanaal was 51% op de leeftijd van 70 jaar. Het kankerrisico in PJS patiënten bleek duidelijk verhoogd ten opzichte van de algemene bevolking (hazard ratio (HR) 9.0), met een hoger relatief kanker risico voor vrouwelijke PJS patiënten (HR 20.4) dan voor mannen met het syndroom (HR 4.8). Aan het einde van de follow-up waren 42 patiënten overleden op een mediane leeftijd van 45 jaar, waarvan 28 aan de gevolgen van kanker. De mortaliteit bleek duidelijk verhoogd ten opzichte van de algemene bevolking (HR 3.5). Deze resultaten

rechtvaardigen surveillance om kanker en de voorlopers daarvan in een vroeg stadium op te sporen om zo de overleving te verbeteren.

Surveillance van PJS patiënten dient niet alleen gericht zijn op de vroege detectie van kanker, maar moet ook gericht zij op de goedaardige poliepen die onder andere tot darmafsluitingen, invaginaties, kunnen leiden. Om surveillance aanbevelingen te kunnen verbeteren, hebben we in **Hoofdstuk 4** de karakteristieken en het risico op invaginaties onderzocht in het Nederlandse cohort van PJS patiënten. In totaal werden hiervoor 110 patiënten uit 50 families geanalyseerd (23 patiënten werden geëxcludeerd omdat de gegevens over het al dan niet optreden van een invaginatie incompleet waren). Zesenzeventig patiënten maakten 1 of meerdere invaginaties door (maximaal 6), op een mediane leeftijd van 16 jaar bij de eerste invaginatie. Het invaginatie-risico was 50% op de leeftijd van 20 jaar, oplopend tot 75% op het 36^{e} jaar. Het risico was onafhankelijk van het geslacht, de familieanamnese en de mutatiestatus. De invaginaties (in totaal 128) waren in 95% van de gevallen in de dunne darm gelokaliseerd, en 80% presenteerde zich als een acute buik (hevige buikpijn en braken waarbij snel ingrijpen noodzakelijk is). Therapie was chirurgisch in 92.5% van de gevallen, en de invaginaties werden veroorzaakt door poliepen met een mediane grootte van 35 mm (15-60 mm). Deze resultaten onderstrepen het belang van dunne darm surveillance met het verwijderen van poliepen groter dan 10-15 mm, om invaginaties te voorkomen.

Ballon-enteroscopie zou een rol zou kunnen spelen in de dunne darm surveillance van PJS patiënten. Daarom hebben we in **Hoofdstuk 5** de therapeutische effectiviteit en de veiligheid hiervan bestudeerd voor de detectie en behandeling van dunne darm poliepen. Tussen oktober 2004 en juli 2009 ondergingen 13 PJS patiënten met een mediane leeftijd van 31 jaar in totaal 29 enteroscopiëen. Alle patiënten bleken dunne darm poliepen te hebben, welke met name (94%) in het duodenum en proximale jejunum waren gelokaliseerd. Er werden 82 poliepen gedetecteerd met een diameter groter of gelijk aan 10 mm, waarvan er 79 endoscopisch werden verwijderd zonder complicaties. Na de introductie van ballon-enteroscopie traden gedurende een periode van 356 persoonsmaanden follow-up geen complicaties op veroorzaakt door dunne darm poliepen. Daarom is ballon-enteroscopie niet alleen veilig te gebruiken voor de detectie en verwijdering van dunne darm poliepen, maar ballon-enteroscopie kan ook invaginaties en operaties voorkomen.

Vervolgens werden 61 volwassen patiënten uit het cohort uitgenodigd om een vragenlijst in te vullen over enerzijds kwaliteit van leven, angst en depressie (**Hoofdstuk 6**) en anderzijds over opvattingen over DNA-onderzoek, kinderwens, prenatale diagnostiek met zwangerschapsafbreking, en pre-implantatie genetische diagnostiek (**Hoofdstuk 7**). De vragenlijst werd geretourneerd door 51 personen (84% respons). Het onderzoek toonde dat PJS patiënten net zo veel angst en depressieve klachten ervaren als de algemene bevolking, maar ze ervaren een slechtere mentale gezondheid, meer beperkingen in het dagelijks leven door emotionele problemen en een slechtere algemene gezondheidsbeleving. Bovendien beïnvloed de diagnose PJS de kinderwens in bijna een derde van de patiënten (geen of

minder kinderen), met name onder vrouwen. Vrouwen in het cohort bleken hierbij minder vaak kinderen te hebben dan de mannen in het cohort. De meeste PJS patiënten hebben een positieve mening over pre-implantatie genetische diagnostiek, en een negatieve mening over prenatale diagnostiek met zwangerschapsafbreking in het geval van een foetus met PJS. Deze data laten zien dat medisch specialisten die patiënten begeleiden met erfelijke kanker syndromen zoals PJS, hun patiënten zouden moeten informeren over de mogelijkheden van prenatale diagnostiek waaronder pre-implantatie genetische diagnostiek.

Deel II

Het tweede deel van dit proefschrift gaat over het Lynch syndroom (LS), een andere autosomaal dominant overervende aandoening, veroorzaakt door mutaties in de DNA herstel genen. LS is de meest voorkomende vorm van erfelijke darmkanker, verantwoordelijk voor ongeveer 3% van alle dikke darmkankers. Daarnaast komen ook carcinomen buiten de darm, met name in de baarmoeder, vaker voor. Vroege detectie van LS is van groot belang, met name voor gezonde familieleden, aangezien colonoscopische surveillance de morbiditeit en mortaliteit aanzienlijk kan reduceren. Echter, de diagnose LS wordt gecompliceerd door het ontbreken van fenotypische kenmerken. Daarnaast is kiembaanmutatie-analyse van de DNA herstel genen duur en tijdrovend. Daarom wordt mutatie-analyse voorafgegaan door moleculaire analyses van tumoren aan de hand van klinische en pathologische criteria, om patiënten te selecteren voor mutatie-analyse. In **Hoofdstuk 8** worden de moleculaire basis van LS, deze moleculaire analyses, en de klinische en pathologische criteria waaronder de Bethesda criteria en uitvoerig beschreven.

Mutaties in de DNA herstel genen leiden tot microsatelliet instabiliteit (MSI) in LS-geassocieerde tumoren. De gereviseerde Bethesda criteria zijn ontwikkeld om de detectie van LS te verbeteren door tumoren te selecteren voor MSI analyse. In **Hoofdstuk 9** hebben we de implementatie van deze criteria in de praktijk onderzocht. Er werden 169 patiënten geïncludeerd die in 2005 en 2006 waren gediagnosticeerd met dikke darmkanker in het Zuidwesten van Nederland, en aan de Bethesda criteria voldeden op basis van; 1) darmkanker voor het 50^{e} jaar, 2) een 2^{e} LS-geassocieerde tumor, of 3) patiënten jonger dan 60 jaar met darmkanker met een mucineuze of zegelringcel differentiatie of een medullair groeipatroon. MSI analyse was slechts verricht in 23 (14%) van de 169 patiënten. Op basis van dit resultaat kan geconcludeerd worden dat MSI analyse onvoldoende wordt toegepast in darmkanker patiënten met een verhoogd risico op LS, en dat er aanzienlijke onderdiagnostiek is van LS.

Omdat een aanzienlijk deel van alle LS patiënten niet als zodanig wordt herkend, hebben we in **Hoofdstuk 10** prospectief de opbrengst bestudeerd van routinematige moleculaire analyses in 1117 darmkanker patiënten ≤ 70 jaar en 125 patiënten met een advanced adenoom (darmpoliep) < 45 jaar. Tumorweefsel werd geanalyseerd op MSI, immunohistochemische DNA herstel eiwit expressie en *MLH1* promoter methylatie. Tumoren werden geclassificeerd

als; 1) verdacht voor LS, 2) sporadisch MSI-H (*MLH1* promoter methylatie), of 3) microsatelliet stabiel (MSS). De analyses van de 1117 darmkanker patiënten (57% mannen, mediane leeftijd 61) toonden 50 patiënten (4.5%) verdacht voor LS, 71 sporadische MSI-H tumoren (6.4%), en 966 MSS tumoren. Vijfendertig patiënten verdacht voor LS (70%) waren > 50 jaar bij de darmkanker diagnose. En moleculair profiel verdacht voor LS werd gevonden in 10% van alle geanalyseerde patiënten ≤ 50 jaar, in 4% van alle patiënten tussen de 51 en de 60 jaar, en in 3% van de patiënten ouder dan 61 jaar. Van de 125 patiënten met een advanced adenoom (58% mannen, mediane leeftijd 41 jaar) waren er 3 verdacht voor LS (2.4%). In **Hoofdstuk 11** hebben we op een soortgelijke manier de opbrengst bestudeerd van de moleculaire analyses in 172 patiënten met baarmoeder kanker ≤ 70 jaar. Deze vrouwen hadden een mediane leeftijd van 61 jaar bij de baarmoeder kanker diagnose, en de moleculaire analyses toonden 10 patiënten (5.8%) verdacht voor LS, allen ouder dan 50 jaar (52-69 jaar, mediaan 61 jaar). Daarnaast werden er 28 sporadische MSI-H tumoren gevonden (16.3%). Er was geen significant verschil in leeftijd bij de baarmoeder kanker diagnose tussen patiënten verdacht voor LS en patiënten met een sporadische MSI-H tumor ($p = 0.41$) of een MSS tumor ($p = 0.99$). Deze resultaten laten zien dat jonge leeftijd geen bruikbaar criterium is om patiënten met baarmoeder kanker te selecteren voor MSI analyse. Bovendien tonen deze resultaten dat routinematige moleculaire analyses er toe bij kunnen dragen dat meer LS patiënten worden opgespoord. De kosteneffectiviteit van deze aanpak en de optimale leeftijdsgrens moeten in de toekomst bepaald worden.

Tot slot worden de belangrijkste bevindingen uit dit proefschrift en aanbevelingen voor toekomstig onderzoek besproken in **Hoofdstuk 12**.

Publications
PhD portfolio
Dankwoord
Curriculum Vitae

A

PUBLICATIONS

1. van Lier M.G.F., Westerman A.M., Wagner A., Looman C.W.N., Wilson J.H.P., de Rooij F.W.M., Lemmens V.E.P.P., Kuipers E.J., Mathus-Vliegen E.M.H., van Leerdam M.E. High cancer risk and increased mortality in patients with Peutz-Jeghers syndrome. Gut 2011;60(2):141-147.

2. van Lier M.G.F., Mathus-Vliegen E.M.H., Wagner A., van Leerdam M.E., Kuipers E.J. High Cumulative Risk of Intussusception in Patients With Peutz-Jeghers Syndrome: Time to Update Surveillance Guidelines? Am J Gastroenterol. 2010 Dec 14. Epub ahead of print

3. van Lier M.G.F., Mathus-Vliegen E.M.H., van Leerdam M.E., Kuipers E.J., Looman C.W.N., Wagner A., Vanheusden K. Quality of life and psychological distress in patients with Peutz-Jeghers syndrome. Clin Genet. 2010;78(3):219-226.

4. Gao H., van Lier M.G.F., Poley J.W., Kuipers E.J., van Leerdam M.E., Mensink P.B.F. Endoscopic therapy of small-bowel polyps by double-balloon enteroscopy in patients with Peutz-Jeghers syndrome. Gastrointest Endosc. 2010;71(4):768-773.

5. van Lier M.G.F., Wagner A., Mathus-Vliegen E.M.H., Kuipers E.J., Steyerberg E.W., van Leerdam M.E. High cancer risk in Peutz-Jeghers syndrome: A systematic review and surveillance recommendations. Am J Gastroenterol. 2010;105(6):1258-1264.

6. van Lier M.G.F., Wagner A., van Leerdam M.E., Biermann K., Kuipers E.J., Steyerberg E.W., Dubbink H.J., Dinjens W.N.M. A review on the molecular diagnostics of Lynch syndrome: A central role for the pathology laboratory. J Cell Mol Med. 2010;14(1-2):181-197.

7. van Lier M.G.F., De Wilt J.H.W., Wagemakers J.J.M.F., Dinjens W.N.M., Damhuis R.A.M., Wagner A., Kuipers E.J., van Leerdam M.E. Underutilization of microsatellite instability analysis in colorectal cancer patients at high risk for Lynch syndrome. Scand J Gastroenterol. 2009;44(5):600-604.

8. Van Lier M.G.F., Bomhof F.J., Leendertse I., Flens M., Balk A.T., Loffeld R.J.L.F. Cytokeratin phenotyping does not help in distinguishing oesophageal adenocarcinoma from cancer of the gastric cardia. J Clin Pathol. 2005;58(7):722-724.

PHD PORTFOLIO

Oral presentations

High intussusception risk at young age in patients with Peutz-Jeghers syndrome: Time to update surveillance guidelines?
Digestive Disease Week 2010, New Orleans, USA.
Dutch Society of Gastroenterology 2010, Veldhoven, the Netherlands.

High cumulative and relative cancer risk and increased mortality in patients with Peutz-Jeghers syndrome.
Dutch Society of Gastroenterology 2010, Veldhoven, the Netherlands.

Towards improved detection of Lynch syndrome.
Regional symposium Comprehensive Cancer Center Rotterdam 2010, Ridderkerk, the Netherlands.

Routine MSI analysis in colorectal cancer patients ≤ 70 years leads to the identification of more patients at high risk for Lynch syndrome.
Dutch Society of Gastroenterology 2009, Veldhoven, the Netherlands.

High cumulative risk of intussusceptions in patients with Peutz-Jeghers syndrome.
International Society for Gastrointestinal Hereditary Tumors (InSight) 2009, Dusseldorf, Germany.
Dutch Society of Gastroenterology 2009, Veldhoven, the Netherlands.

Poor compliance with MSI analysis in patients with colorectal cancer at high risk for Lynch syndrome.
Dutch Society of Gastroenterology 2008, Veldhoven, the Netherlands.
The Netherlands Foundation for the Detection of Hereditary Tumors (STOET) symposium 2008, Utrecht, the Netherlands.

Poster presentations

High cumulative and relative cancer risk and increased mortality in patients with Peutz-Jeghers syndrome.
Digestive Disease Week 2010, New Orleans, USA.

Quality of life and psychological distress in patients with Peutz-Jeghers syndrome.
Digestive Disease Week 2010, New Orleans, USA.

Routine MSI analysis in colorectal cancer patients ≤ 70 years leads to the identification of more patients at high risk for Lynch syndrome.
Digestive Disease Week 2009, Chicago, USA.
International Society for Gastrointestinal Hereditary Tumors (InSight) 2009, Dusseldorf, Germany.

High cumulative risk of intussusceptions in patients with Peutz-Jeghers syndrome.
Digestive Disease Week 2009, Chicago, USA.

Surveillance and endoscopic treatment of large small-bowel polyps by double balloon enteroscopy in patients with Peutz-Jeghers syndrome.
Digestive Disease Week 2009, Chicago, USA.

Poor compliance with MSI analysis in patients with colorectal cancer at high risk for Lynch syndrome.
Digestive Disease Week 2008, San Diego, USA.
Dutch Society of Gastroenterology 2008, Veldhoven, the Netherlands.
The Netherlands Foundation for the Detection of Hereditary Tumors (STOET) symposium 2008, Utrecht, the Netherlands.

Memberships

2007: Dutch Society of Gastroenterology
2008: Dutch Peutz-Jeghers Working Group

Courses

March 2009: English Biomedical Writing and Communication.
January 2008: Introduction to Clinical Research, Nihes Rotterdam.
January 2008: Biostatistics for Clinicians, Nihes Rotterdam.
August 2007: Principles of Research in Medicine and Epidemiology, Nihes Rotterdam.

DANKWOORD

De totstandkoming van dit proefschrift heeft heel wat voeten in de aarde gehad. Hierbij wil ik iedereen bedanken die er aan heeft bijgedragen dat ik mijn promotie met succes heb kunnen afronden.

Allereerst mijn beide promotoren: Professor Dr. E.J. Kuipers en Professor Dr. E.W. Steyerberg.
Beste Ernst, ik durf niets meer te schrijven over een roze bril, maar je aanstekelijke optimisme en enthousiasme zijn altijd een enorme stimulans geweest. Ik was elke keer weer verbluft hoe je manuscripten en revision letters wist aan te scherpen, vaak met acceptatie als resultaat. Dank voor de kans om onder jouw begeleiding te mogen promoveren!
Beste Ewout, voordat ik aan mijn promotietraject begon, was ik bang dat mijn statistische en epidemiologische kennis tekort zouden schieten. Jij had vertrouwen in me en hebt me veel geleerd. Het was me daarnaast een waar genoegen om tijdens de Insight in Düsseldorf samen de hardloopschoenen onder te binden ondanks je recent geopereerde heup. Dank voor alles!

Heel graag wil ik ook mijn copromotoren ontzettend bedanken.
Beste Monique, bedankt dat je me wegwijs hebt willen maken in de wondere wereld van het "onderzoek". Ik heb bewondering voor de manier waarop je de begeleiding van al je promovendi weet te combineren met het moederschap en je baan als MDL-arts. Dank voor al je steun van de afgelopen jaren en ik kijk er naar uit om na mijn interne vooropleiding mijn verdere opleiding tot MDL-arts mede onder jouw begeleiding te vervolgen!
Lieve Anja, dank voor je onafgelaten steun van de afgelopen jaren! Ik bewonder de manier waarop jij altijd belangeloos voor iedereen klaar staat, als dokter, maar bovenal als mens. Ik heb veel gehad aan je relativerende opmerkingen zoals "je moet jezelf niet te serieus nemen" tijdens onze werkbesprekingen, waar ook altijd veel gelachen werd. Ook al zit mijn promotietraject er nu op, ik hoop contact met je te houden.

Mijn dank gaat tevens uit naar alle leden die zitting hebben willen nemen in de promotiecommissie.

Een manuscript schrijven doe je niet alleen. Verschillende auteurs hebben bijgedragen aan de hoofdstukken in dit proefschrift, waarvoor ik hen allen hartelijk wil bedanken. Een aantal mensen wil hierbij in het bijzonder noemen, om te beginnen Winand Dinjens en Erik Jan Dubbink. Winand en Erik Jan, de LIMO studie bleek iets meer werk dat we van tevoren hadden gedacht, maar mede dankzij jullie inspanningen hebben we de studie tot een goed einde weten te brengen. Ontzettend bedankt voor de fijne en gezellige samenwerking! De analisten (Monique, Carry, Gabriëlle en Sanne) wil ik graag danken voor hun inzet, evenals

alle betrokken artsen in de participerende ziekenhuizen. Caspar Looman, dank voor je hulp bij de statistiek die mijn pet zo nu en dan te boven dreigde te gaan. Kathleen Vanheusden, het was leuk om met je samen te mogen werken aan onze QoL studies.

Dan de afdeling MDL van het Erasmus MC:
Wendy Holleman en Bernadette Lourens, dank voor al jullie hulp en ondersteuning. Peter Mensink, het was een voorrecht om tijdens de afwezigheid van Monique bij je op de kamer te mogen zitten, en ik wil jou en Hong nogmaals danken voor de prettige samenwerking welke heeft geleid tot een mooie publicatie.
Natuurlijk wil ik de (arts-)onderzoekers en assistenten bedanken, die er zowel op de werkplek als daarbuiten (o.a. op de piste), een feestje van wisten te maken. Juud, Nicoline, Dees en Vera; ik heb genoten van de weekjes vakantie die we aan de congressen in Amerika hebben geplakt en de gezelligheid op o.a. de flex en Ca-425! Ook dank aan alle andere collega's van de "flex" en het "dak" die er aan bijdroegen dat de meeste werkdagen, congressen en borrels een dolle boel werden; Jildou, Aafke, Leonie, Femme, Edmee, Lieke, Celine, Susanne, Paul, Jerome, kleine en grote Vincent, Daphne, Robert, Ad, Roeland, Milan, Edith, Edith, Jur, Erik, Caroline, Renate, Lauran, Lisette, Marjolein, Aria, Dew, Suzanne en Sanna. Celine en Susanne; top dat jullie de LIMO studie en het Peutz onderzoek zo enthousiast doorzetten; ik kijk nu al uit naar jullie boekje!

Sinds juni 2010 heb ik mijn plek gevonden binnen de afdeling interne geneeskunde van het Maasstad ziekenhuis. Ik wil de maatschap interne en de leuke club aan arts-assistenten hartelijk bedanken voor de goede sfeer en de fijne samenwerking. Het zijn deze collega's die ervoor zorgen dat ik elke dag met plezier aan het werk ga en snel heb kunnen inburgeren in een nieuw ziekenhuis in een nieuwe stad.

Graag zou ik nog twee mensen willen noemen die mij enthousiast hebben gemaakt voor de Maag-Darm-Leverziekten en het onderzoek: Allereerst Ruud Loffeld. Beste Ruud, mijn eerste publicatie heb ik aan jou te danken, net als mijn enthousiasme voor de MDL. Al tijdens het 2e jaar van mijn studie heb ik bij jou een kijkje op de endoscopieafdeling mogen nemen, een ervaring die mij direct een baan als MDL-arts deed ambiëren. Deze ambitie werd versterkt door Prof. Mathus-Vliegen. Beste Lisbeth, al tijdens de colleges in het AMC was het geweldig om je bevlogenheid te aanschouwen. Het was een eer dat ik tijdens mijn promotie-onderzoek met je heb mogen samenwerken.

Een proefschrift schrijven lukt wat mij betreft niet zonder vrienden en familie om je heen. Lieve Mic en Marjon, al heel wat jaren mag ik ondertussen van jullie gezelligheid genieten; in Wormer, op de piste in Evolène, en in de Pyreneeën. Dank voor al deze fijne momenten, en voor al jullie interesse en enthousiasme!

Ik had mijn boekje niet af kunnen maken zonder de oppeppende weekendjes weg, eetdates en borrels met mijn lieve vriendinnen: Klaar, Sabien, Anouk, Jon, Bella, Kiki, Anouk, Nienke en Daniëlle. Ik heb jullie de laatste maanden lang niet zo veel heb gezien als ik zou willen, maar dat gaat vanaf nu beter worden!
Lieve Jon en Anouk, al sinds de basisschool hebben we ontzettend veel lol samen. Van voetballen op het schoolplein in Koog aan de Zaan tot theetjes, taartjes (Anouk dan), borrels en etentjes in Amsterdam; ik hoop dat er nog heel veel meer gaan komen. Thanks for being my friends!

Mijn paranimfen:
Lieve Sabien, samen naar middelbare school, samen naar het AMC, samen lopen, samen in de kroeg, een heleboel samen en altijd eindeloze gesprekken. Ondanks het feit dat ik nu in Rotterdam woon en jij samen met jouw Isa en Wouter in Amsterdam, hoop ik dat er nog heel veel samen komt. Dank dat je me vandaag net als altijd bij wil staan!
Lieve Tul, 1989... Do I need to say more? Of jij nou in Edinburgh woonde of ik in Melbourne, het maakte allemaal niet uit: altijd was je er. In al die jaren hebben we ontzettend veel mooie momenten beleefd en buitengewoon veel lol gehad. Ik hoop dat er nog heel veel van die mooie momenten komen gaan, ik heb er in ieder geval alle vertouwen in. You rock!

Dit dankwoord is niet compleet zonder mijn lieve vader, moeder en broer, die ik in de aanloop naar 25 maart veel te weinig heb gezien, een warm hart toe te dragen. Lieve pap en mam, jullie hebben mij gestimuleerd om het onderste uit de kan te halen en jullie trots is altijd een enorme prikkel geweest om beter te willen. Jullie hebben alles voor ons mogelijk gemaakt waarvoor duidend maal dank! Zelf hebben jullie altijd hard gewerkt, en ik hoop dat jullie de komende jaren in goede gezondheid van het goede leven mogen genieten. Stan, ik vind het helemaal geweldig dat jij, mijn broer, de omslag van dit boekje hebt ontworpen; van Lier en van Lier. Ik ben er ontzettend blij mee; jij komt er wel als kunstenaar!

Tot slot kom ik bij jou, lieve Bart. In 2002 hebben we elkaar leren kennen in het door ons zo geliefde Amsterdam. Nu, 9 jaar later, bouwen we samen een toekomst op in Rotterdam. Ik geniet elke dag van je. Altijd weet je met jouw humor en rust de storm in mijn hoofd te doen gaan liggen. Lieverd, we gaan er samen iets moois van maken (en sparen voor die botter)!

CURRICULUM VITAE

Margot van Lier werd op 22 februari 1981 geboren te Nijmegen. Na het behalen van het diploma Voortgezet Wetenschappelijk Onderwijs aan het Sint Michaël College te Zaandam, ging zij in 1999 Geneeskunde studeren aan de Universiteit van Amsterdam. In 2003 heeft zij onderzoek gedaan naar complicaties van diabetes mellitus aan de Universiteit van Melbourne. Na deze onderzoeksstage heeft zij haar studie een aantal maanden onderbroken om onder begeleiding van Dr. R.J.L.F. Loffeld onderzoek te doen naar slokdarmkanker (Zaans Medisch Centrum). Op 28 juli 2006 werd het artsexamen behaald, waarna ze gedurende 8 maanden als arts-assistent interne geneeskunde heeft gewerkt in het Spaarne ziekenhuis te Hoofddorp. Van juni 2007 tot juni 2010 was zij werkzaam als arts-onderzoeker aan de afdeling MDL van het Erasmus MC onder begeleiding van promotoren Prof. Dr. E.J. Kuipers en Prof. Dr. E.W. Steyerberg, en copromotoren Dr. M.E. van Leerdam en Dr. A. Wagner. Sinds 1 juni 2010 is zij in opleiding tot MDL-arts (opleider Dr. R.A. de Man). Op dit moment doet zij haar vooropleiding interne geneeskunde in het Maasstad ziekenhuis te Rotterdam (opleider Dr. M.A. van den Dorpel).