KCE REPORT 242

Federaal Kenniscentrum voor de Gezondheidszorg
Centre Fédéral d'Expertise des Soins de Santé
Belgian Health Care Knowledge Center

# ONCOGENETIC TESTING FOR PERSONS WITH HEREDITARY ENDOCRINE CANCER SYNDROMES

KCE REPORT 242
GOOD CLINICAL PRACTICE

Federaal Kenniscentrum voor de Gezondheidszorg
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# ONCOGENETIC TESTING FOR PERSONS WITH HEREDITARY ENDOCRINE CANCER SYNDROMES

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# COLOPHON


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# LIST OF ABBREVIATIONS

| ABBREVIATION | DEFINITION |
|---|---|
| 95%CI | 95% confidence interval |
| AACE | American Association of Clinical Endocrinologists |
| ATA | American Thyroid Association |
| CEBAM | Belgian Centre for Evidence-Based Medicine |
| DNA | Deoxyribonucleic acid |
| FMTC | Familial medullary thyroid carcinoma |
| GDG | Guideline development group |
| GRADE | Grading of Recommendations Assessment, Development and Evaluation |
| HNPGL | Head and neck paraganglioma |
| HTA | Health technology assessment |
| KCE | Belgian Health Care Knowledge Centre |
| MEN | Multiple Endocrine Neoplasia |
| MSAC | Medical Services Advisory Committee |
| MTC | Medullary thyroid carcinoma |
| NIHDI | National Institute for Health and Disability Insurance |
| NPV | Negative predictive value |
| OR | Odds ratio |
| PCR | Polymerase Chain Reaction |
| PGL | Paraganglioma |
| PHEO | Phaeochromocytoma |
| PICO | Population – Intervention – Comparator – Outcomes |
| PPV | Positive predictive value |
| RCT | Randomized controlled trial |
| RET | REarranged during Transfection proto-oncogene |
| RR | Relative risk |
| SDH | Succinate Dehydrogenase |
| STOET | Stichting opsporing erfelijke tumoren |
| VHL | von Hippel-Lindau |

# GLOSSARY

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|---|---|
| **Family History** | A family history of disease in an individual is the occurrence of the disease in a blood relative of that individual. |
| **Gene** | A gene is a molecular unit of heredity of a living organism. |
| **Genetic Counselling** | A service delivered by a qualified health professional that provides a comprehensive evaluation of familial risk for inherited disorders using kindred analysis and other methods, patient education, discussion of the benefits and harms of genetic testing, interpretation of results after testing (consequences and nature of the disorder, probability of developing or transmitting it), and discussion of management options. |
| **Genetic Counsellor** | A healthcare professional providing individuals and families with information on the nature, inheritance, and implications of genetic disorders to help them make informed medical and personal decisions. If it is appropriate, they will discuss genetic testing, coordinate any testing, interpret test results, and review all additional testing, surveillance, surgical, or research options that are available to members of the family. |
| **Genetic testing** | Genetic testing is a type of medical test that identifies changes in chromosomes, genes, or proteins. The results of a genetic test can confirm or rule out a suspected genetic condition or help determine a person's probability of developing or passing on a genetic disorder. |
| **Germline** | The cells from which eggs or sperm (i.e., gametes) are derived. |
| **Penetrance** | A characteristic of a genotype; it refers to the likelihood that a clinical condition will occur when a particular genotype is present. |
| **Proband** | The individual through whom a family with a genetic disorder is identified. |
| **Relatives – First-degree relatives** | These are the closest blood relatives (relatives by marriage do not count). These include father, mother, son, daughter, brother, sister. |
| **Relatives – Second-degree relatives** | These are blood related grandparents, grandchildren, uncles, aunts, nephews and nieces, half-brothers and half-sisters, on mother's or father's side of the family. |
| **Relatives – Third-degree relatives** | These are blood related great grandparents, great grandchildren, great uncles, great aunts, first cousins, grand-nephews and grand-nieces, on mother's or father's side of the family. |

# SCIENTIFIC REPORT

## 1 INTRODUCTION

This clinical practice guideline is based on a joint effort of the Belgian Health Care Knowledge Centre (KCE), the College of Human Genetics and the College of Oncology. This guideline is the third report in a short series of oncogenetic testing guidelines.

### 1.1 Background

Oncogenetic tests are tests that assist in the diagnosis of specific cancers that have an important hereditary component. Such tests may also assist to identify family members at risk of developing specific forms of cancer. This guideline will address the indications for genetic testing in the following selection of endocrine tumours / syndromes: Multiple Endocrine Neoplasia type 1 (MEN1), Multiple Endocrine Neoplasia type 2 (MEN2), von Hippel-Lindau (VHL) syndrome, phaeochromcytoma and paraganglioma.

MEN2 is a group of disorders associated with endocrine tumours (typically of the thyroid, parathyroid and adrenals).[1] Nearly all patients develop a medullary thyroid carcinoma (MTC). In general, three major phenotypes are distinguished. MEN2A (60% of all MEN2 cases) combines MTC with phaeochromocytoma (10-50% of MEN2A cases) and/or primary hyperparathyroidism (5-20% of MEN2A cases). MEN2B (5% of all MEN2 cases) combines MTC with phaeochromocytoma (50% of MEN2B cases) and typical phenotypic features such as a Marfan-type dysmorphism, ganglioneuromatosis and/or skeletal abnormalities. Finally, in familial MTC (35% of all MEN2 cases), the other components of the disease are absent. MEN2 is typically associated with mutations of the proto-oncogene RET. Epidemiological data are not available for Belgium, but the prevalence is estimated to be 2.5 per 100 000 in the general population.[1]

MEN1 is a polyglandular genetic syndrome characterized by tumours of the parathyroid glands, pancreatic islet cells and/or anterior pituitary gland. Parathyroid tumours with primary hyperparathyroidism is the most common presentation (95% of all MEN1 cases). In addition to these three ‘major’ locations, tumours can also occur in ‘minor’ locations, such as the adrenal cortex. MEN1 is usually inherited (as an autosomal dominant disorder), but *de novo* mutations of the MEN1 gene (menin) are found in about 10% of patients. Epidemiological data are not available for Belgium, but the incidence has been estimated to be 0.25% from postmortem studies.[2]

The VHL syndrome is associated with a variety of benign and malignant tumours, in particular haemangioblastomas of the retina and central nervous system, endolymphatic sac tumours, phaeochromocytomas, renal cell carcinomas and cysts in various organs including the kidney, pancreas and liver.[3] The VHL syndrome is inherited, and caused by germline mutations in the VHL tumour suppressor gene. Epidemiological data are not available for Belgium, but the prevalence is estimated to be 1 in 90 000 people.[3]

Phaeochromocytomas are tumours arising from adrenomedullary chromaffin cells that commonly produce catecholamines.[4] Paragangliomas are tumours derived from extra-adrenal chromaffin cells of the sympathetic paravertebral ganglia of thorax, abdomen, and pelvis, or from parasympathetic ganglia located along the glossopharyngeal and vagal nerves in the neck and at the base of the skull. In addition to the three syndromes described above, phaeochromocytomas and paragangliomas can also occur sporadically, i.e. without syndromic features. Several susceptibility genes have been described, with SDH mutations occurring most frequently. In 2011, 16 phaeochromocytomas and 9 paragangliomas were registered at the Belgian Cancer Registry (personal communication), with a European Standardized Rate of 0.14 and 0.06 per 100 000 person years, respectively. However, these incidences are probably underestimated because of underregistration.

## 1.2 The need for a guideline

At present, eight genetic centres are recognized in Belgium. However, no national guideline exists on the indications for genetic testing, and most centres follow their own protocols. Therefore, uniform criteria are needed for the identification and referral of patients to genetic centres for counselling, possibly followed by germline mutation analysis. It is important to provide such guidance to all clinicians active in the field. This guideline is timely because the new nomenclature, introduced on 1/1/2013, for genetic tests (article 33) and the agreement on genetic testing consultation led to redistribute the NIHDI budget between genetic counselling (€4,288 millions) and laboratory procedures (€37,795 millions). There is a need to standardise the use of these tests and base their use on available evidence. Early identification of persons at risk makes the initiation strategies possible that may reduce morbidity or be lifesaving, including enhanced surveillance and prophylactic surgery. It may also help the patient in making decisions concerning preconception and antenatal screening and reproduction in general.

## 1.3 Scope

**This guideline will cover following populations:**

- Multiple Endocrine Neoplasia type 1
- Multiple Endocrine Neoplasia type 2
- Von-Hippel Lindau syndrome
- Phaeochromocytoma
- Paraganglioma
- Relatives of patients with one of the syndromes / tumours above
- Patients with a suspicion of one of the syndromes / tumours above

**The guideline will cover following issues:**

- Genetic testing for Succinate Dehydrogenase B, C and D (SDHB, SDHC, SDHD), menin, VHL and RET mutations

**The guideline will not cover following issues:**

- Genetic testing for mutations other than SDHB, SDHC, SDHD, menin, VHL and RET mutations
- Clinical, biochemical and imaging follow-up of persons testing positive for a mutation
- Prenatal screening for a mutation

## 1.4 Remit of the guideline

### *1.4.1 Overall objectives*

This guideline provides recommendations based on current scientific evidence for the genetic testing of patients with the endocrine syndromes described above and their relatives. Clinicians are encouraged to interpret these recommendations in the context of the individual patient situation, values and preferences.

The guidelines are based on clinical evidence and may not always be in line with the current criteria for NIHDI (RIZIV/INAMI) reimbursement of diagnostic and therapeutic interventions. The NIHDI may consider adaptation of reimbursement/funding criteria based on these guidelines.

### *1.4.2 Target users of the guideline*

This guideline is intended to be used by care providers involved in genetic counseling, testing and follow-up of patients with the endocrine syndromes described above. It also contains recommendations for persons that must decide when to refer for genetic counselling and testing such as general practitioners, endocrinologists, oncologists, surgeons, and pathologists. It can also be of interest for patients and their families, hospital managers and policy makers.

## 1.5 Statement of intent

Clinical guidelines are designed to improve the quality of health care and decrease the use of unnecessary or harmful interventions. This guideline has been developed by clinicians and researchers for use within the Belgian healthcare context. It provides advice regarding the care and management of patients presenting with MEN1, MEN2, VHL or phaeochoromocytoma/paraganglioma and their relatives by care providers involved in genetic counseling, testing and follow-up of patients with the endocrine syndromes described above.

The recommendations are not intended to indicate an exclusive course of action or to serve as a standard of care. Standards of care are determined on the basis of all the available clinical data for an individual case and are subject to change as scientific knowledge and technology advance and patterns of care evolve. Variations, which take into account individual circumstances, clinical judgement and patient choice, may also be appropriate. The information in this guideline is not a substitute for proper diagnosis, treatment or the provision of advice by an appropriate health professional. It is advised, however, that significant deviations from the national guideline are fully documented in the patient's file at the time the relevant decision is taken.

## 1.6 Funding and declaration of interest

KCE is a federal institution funded for the largest part by INAMI/RIZIV, but also by the Federal Public Service of Health, Food chain Safety and Environment, and the Federal Public Service of Social Security. The development of clinical practice guidelines is part of the legal mission of the KCE. Although the development of guidelines is paid by KCE's budget, the sole mission of the KCE is providing scientifically valid information. KCE has no interest in companies (commercial or non-commercial i.e. hospitals and universities), associations (e.g. professional associations, unions), individuals or organisations (e.g. lobby groups) that could be positively or negatively affected (financially or in any other way) by the implementation of these guidelines. All clinicians involved in the Guideline Development Group (GDG) or the peer-review process completed a declaration of interest form. Information on potential conflicts of interest is published in the colophon of this report. All members of the KCE Expert Team make yearly declarations of interest and further details of these are available upon request.

# 2 METHODOLOGY

## 2.1 Introduction

This guideline was developed using a standard methodology based on a systematic review of the evidence. Further details about KCE and the guideline development methodology are available at https://kce.fgov.be/content/kce-processes.

Several steps were followed to elaborate this guideline. Firstly, clinical questions were developed and the inclusion and exclusion criteria were defined in collaboration with members of the Guideline Development Group (see Appendix 1). Secondly a literature review was conducted (including a search for recent, high-quality guidelines). Thirdly, on the basis of the results of the literature review, recommendations were formulated and graded according to the GRADE approach.

## 2.2 The Guideline Development Group

This guideline was developed as a result of a collaboration between multidisciplinary groups of practising clinicians and KCE experts. The composition of the GDG is documented in Appendix 1.

Guideline development and literature review expertise, support, and facilitation were provided by the KCE Expert Team.

The roles assigned to the GDG were:

- To define the clinical questions, in close collaboration with the KCE Expert Team and stakeholders;
- To identify critical and important outcomes;
- To provide feedback on the selection of studies and identify further relevant manuscripts which may have been missed;
- To provide feedback on the content of the guideline;
- To provide judgement about indirectness of evidence;
- To provide feedback on the draft recommendations;
- To address additional concerns to be reported under a section on 'other considerations'.

## 2.3 Clinical research questions and definitions

The CPG addresses the following clinical questions:

- What is the clinical effectiveness of genetic testing in patients presenting with MEN1 (MEN1 mutations), MEN2 (RET mutations), VHL (VHL mutations) or phaeochromocytoma / paraganglioma (SDH, VHL and RET mutations)?
- What is the diagnostic accuracy of clinical features for the triage for genetic testing of patients presenting with MEN1, MEN2, VHL or phaeochromocytoma / paraganglioma?
- What is the clinical effectiveness of genetic testing of relatives of MEN1, RET, VHL or SDH mutation carriers?

## 2.4 General approach

To verify if high-quality, recent guidelines are available that address the clinical research questions, a GCP project always starts with a search for published guidelines. If such guidelines are available, the ADAPTE methodology is followed (http://www.g-i-n.net/working-groups/adaptation). However, we assess and summarize the underlying evidence where the recommendations of the guideline are based on. We only adopt the recommendation if the GDG agrees with the interpretation and considers the guideline applicable to the Belgian context.

If no high-quality, recent guidelines are available, the general approach begins with the search for systematic reviews.

For each research question, a search for systematic reviews was conducted in MEDLINE, Embase and The Cochrane Library (Cochrane Database of Systematic Reviews, DARE and HTA database). If no systematic review was available, a search for primary studies was performed in the same databases, without time restriction. Members of the GDG were also consulted to identify additional relevant evidence that might have been missed by the search.

## 2.5 Literature search and study selection

### 2.5.1 Study design

- Inclusion criteria for the study design: guidelines, systematic reviews, meta-analyses, RCTs, observational studies.
- Exclusion criteria for study design:
  - Narrative review
  - Cadaver/animal studies
  - Case reports
- Articles in Dutch, English and French were included.

To be included, a systematic review had to:

- address at least one of the research questions;
- evaluate at least one of the selected outcomes;
- search MEDLINE and at least one other electronic database;

If more than one systematic review was identified for a particular research question, the focus was on the most complete systematic review.

To be included, a primary study had to:

- be an RCT or an observational study;
- address at least one of the research questions;
- evaluate at least one of the selected outcomes.

### 2.5.2 Databases and date limits

The following databases were included in the literature search:

- The Cochrane Library (http://www.cochrane.org)
- Medline (http://www.ncbi.nlm.nih.gov/pubmed)
- Embase (http://www.embase.com/)

Guidelines were identified through the search for systematic reviews and primary studies, and through a search of the websites of the following organisations: STOET (www.stoet.nl), American Thyroid Association (ATA, www.thyroid.org), American Association of Clinical Endocrinologists (AACE, www.aace.com), Endocrine Society (www.endocrine.org), and the European Thyroid Association (ETA, www.eurothyroid.com). Members of the GDG were also consulted to identify relevant evidence that might have been missed during the search process.

The search for systematic reviews was limited to studies published since 2008. The search for primary studies was not limited in time.

### 2.5.3 Search strategy

The search strategy and number of articles per database are detailed in Appendix 2.

Studies were screened on **title and abstract** by one researcher (JV). In case of doubt, the content experts were consulted. In a second step, the remaining papers were screened by reading their **full-text**. If no full-text was available, the study was excluded for the final recommendations. Reference lists of the selected studies were hand searched for additional relevant manuscripts. Due to limited resources only articles available through the Vesalius Documentation and Information centre or Interlibrary Loan were retained.

## 2.6 Quality appraisal

Detailed results of the quality appraisal can be found in Appendix 3.

### 2.6.1 Clinical practice guidelines

The AGREE II instrument was used to critically appraise guidelines retrieved (JV).

### 2.6.2 Systematic reviews

Selected (systematic) reviews were critically appraised by a single KCE expert (JV) using the AMSTAR checklist (http://amstar.ca/Amstar_Checklist.php). In case of doubt, a second KCE expert was consulted.

### 2.6.3 Primary articles

Critical appraisal of each study was performed by a single KCE expert (JV). In case of doubt, a second KCE expert was consulted.

## 2.7 Data extraction

For each included CPG the relevant recommendations were extracted.

For each systematic review, the search date, publication year, included studies and main results were extracted. For RCTs and observational studies, the following data were extracted: publication year, study population, study intervention and outcomes.

Data extraction was performed by one researcher (JV) and entered in evidence tables using standard KCE templates. All evidence tables are reported in Appendix 4.

## 2.8 Statistical analyses

Diagnostic meta-analyses were performed using the metandi or gllamm commands of STATA version 12.1 in case at least 4 studies were available. In case no meta-analysis was possible, median sensitivities and specificities were calculated. Medians were also calculated for the positive predictive value (PPV) and negative predictive value (NPV).

## 2.9 Grading evidence

Due to current methodological limitations of the GRADE system for diagnostic tests, GRADE was not applied to the recommendations. However, the GRADE tools available on www.guidelinedevelopment.org were used to inform the GDG about the number of false positives and negatives associated with clinical features when used to predict the presence of a mutation. Furthermore, the general philosophy of the GRADE system for interventions (see KCE process book, http://processbook.kce.fgov.be/node/51) was also used for this report (more specifically for the grading of the recommendations).

For the conclusions regarding the diagnostic accuracy, pragmatic categories were defined: a sensitivity or specificity of at least 90% was considered high, 80-90% was considered moderate and below 80% was considered low. The following considerations were made regarding diagnostic accuracy outcomes when evaluating the clinical features that are predictive of mutations:

- True positives: would undergo genetic testing, would test positive and would receive screening for new manifestations;
- False positives: would undergo genetic testing, but would test negative (i.e. inappropriate genetic testing);
- True negatives: would not undergo genetic testing, would not undergo screening for new manifestations, and would probably never develop new manifestations;
- False negatives: would not undergo genetic testing, would not undergo screening for new manifestations, but some would develop new manifestations.

The guideline development group considered a high number of true negatives to be more important for the patients and their relatives than a high number of true positives. Furthermore, a high number of false positives was not considered having a high budgetary impact (because of the low prevalences), and avoiding a high number of false negatives was considered to be more important.

## 2.10 Formulation of recommendations

Based on the retrieved evidence, a first draft of recommendations was prepared. This first draft was, together with the evidence tables, circulated to the GDG two weeks prior to the face-to-face meetings (September 11, 2014; December 5, 2014). Based on the discussion meetings a second draft of recommendations was prepared and once more circulated to the GDG for final approval (January 16, 2015).

## 2.11 Final validation and external review

As part of the standard KCE procedures, an external scientific evaluation (i.e. validation) of the report was conducted prior to its publication (February 12, 2015). The current guideline was reviewed by 3 independent assessors (cf. names in the colophon). The validation of the report results from a consensus or a voting process between the validators.

After the validation a final discussion with the GDG and stakeholders was organised on February 23, 2015. The recommendations prepared by the guideline development group were circulated to professional associations and patient representatives (Table 1).

Each association was asked to assign one or two key representatives to act as external reviewers of the draft guideline. All expert referees made declarations of interest.

Globally, 10 external experts and/or patient representatives were involved in the external review of the clinical recommendations. All invited panellists received the scientific report for all research questions and were asked to indicate their level of agreement with the recommendation, with a score of '1' indicating 'completely disagree', '2' 'somewhat disagree', and '3' 'completely agree' (the panellists were also able to answer 'not applicable' if they were not familiar with the underlying evidence). If panellists disagreed with the recommendation, they were asked to provide an explanation supported by appropriate evidence and to suggest a more appropriate formulation. Scientific arguments reported by these experts were used to adapt the formulation or the strength of the clinical recommendations. In Appendix 8, an overview is provided of how their comments were taken into account.

**Table 1 – List of professional and patient associations invited.**

- Belgian Group of Digestive Oncology
- Belgian Group of Endoscopic Surgery
- Belgische Vereniging voor Radiotherapie-Oncologie - Association Belge de Radiothérapie-Oncologie
- Belgian Society of Medical Oncology
- Belgian Society of Surgical Oncology
- Kom op tegen Kanker
- Fondation contre le cancer
- Zelfhulpgroep NET & MEN kanker
- VHL Family Alliance Belgium

# 3 CLINICAL RECOMMENDATIONS

## 3.1 Multiple Endocrine Neoplasia type 2 (MEN2)

### *3.1.1 Evidence from indexed literature*

One recent HTA report of good quality was identified.[1] The objective of the report was to determine whether there is sufficient evidence of clinical need, safety, effectiveness and cost-effectiveness to recommend the public funding (in Australia) of genetic testing for hereditary mutations in the RET gene for (1) patients with symptoms of MEN2, and (2) a family member of a patient with a known pathogenic RET mutation. Since the literature search in the MSAC 2013 report was considered to be sufficiently rigourous (up to August 2012), no attempt was made to identify more recent primary studies.

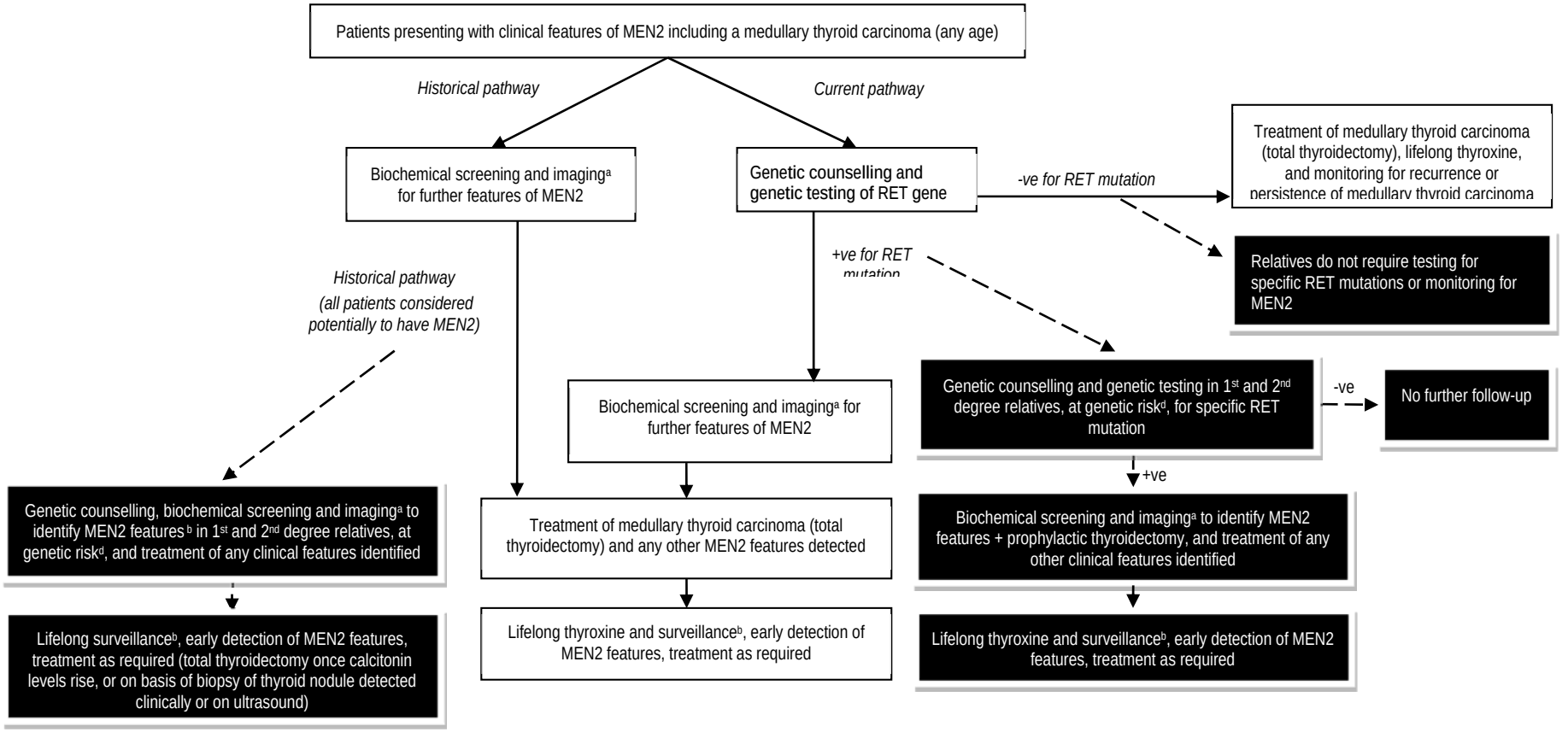
For the evaluation of the safety, effectiveness and cost-effectiveness of RET mutation testing, MSAC proposed two management algorithms as a basis, one for patients presenting with a medullary thyroid carcinoma (MTC) plus their first degree relatives (Figure 1) and the second for patients presenting with phaeochromocytoma or hyperparathyroidism, and their first degree relatives (Figure 2).[1] These pathways both include the 'historical' setting on the one hand (i.e. the investigations to be used in the absence of RET mutation testing), and the scenario with RET mutation testing being standard clinical practice on the other hand. With these two pathways in mind, MSAC defined PICO questions for the following four populations:

- Patients presenting with medullary thyroid carcinoma (MTC);
- Patients presenting with adrenal phaeochromocytoma (under 50 years of age);
- Patients presenting with hyperparathyroidism plus a diagnosis of MTC or phaeochromocytoma in a close relative;
- First-degree relatives of patients with a diagnosis of MEN2 or a known pathogenic RET mutation.

For all four populations a pathway including RET mutation testing (and targeted interventions depending on the outcome of RET mutation testing) was compared with the 'historical' setting, using long-term clinical assessment (ideally over the life-time of the patient) as the reference standard.

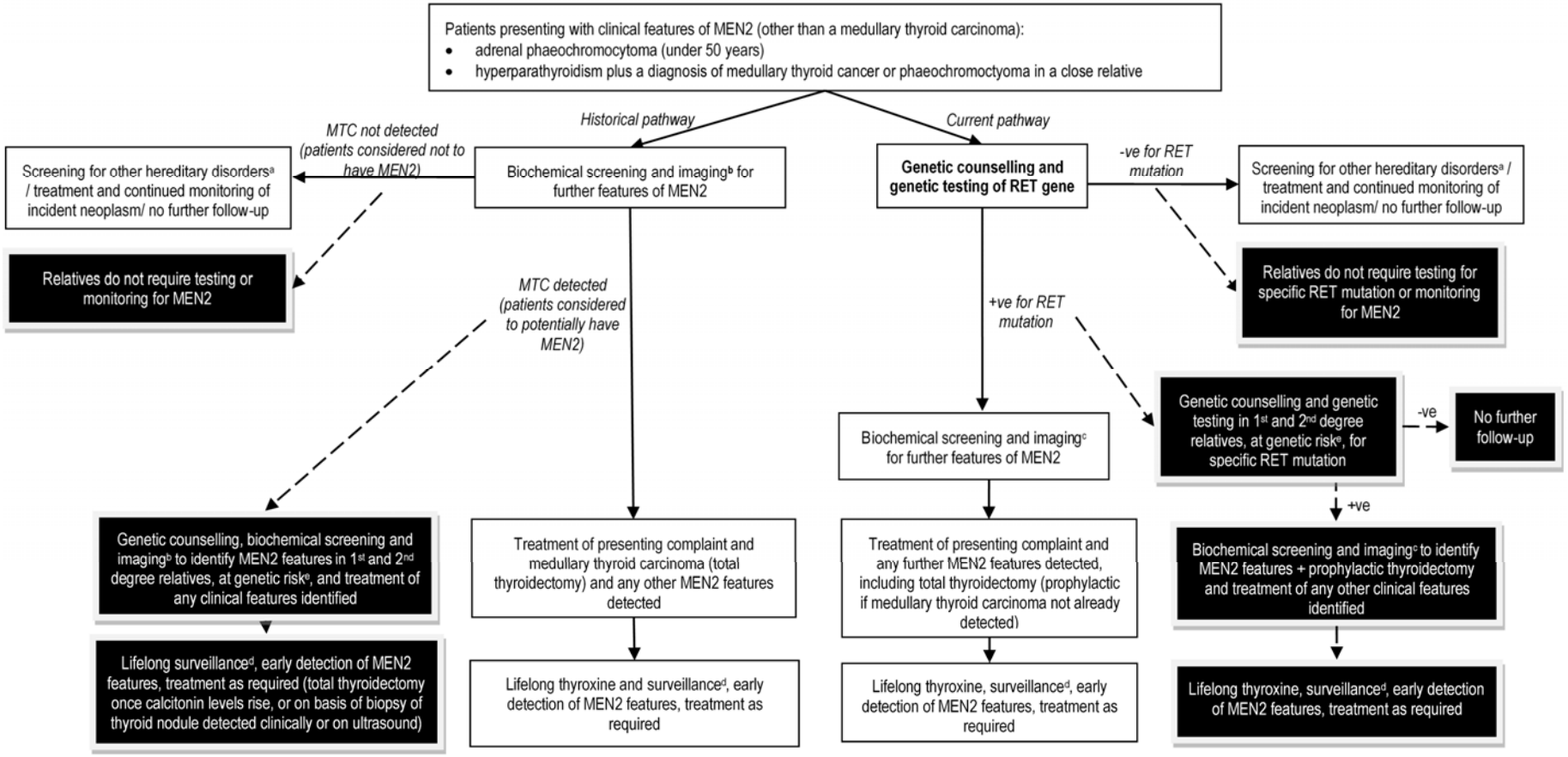
However, this reference standard is imperfect, since persons with a pathological RET mutation associated with MEN2 prior to the development of MTC would ideally undergo a prophylactic thyroidectomy, making it impossible to determine whether the individual actually would have developed an MTC or not.[1]

**Figure 1 – Management algorithm for use of a RET mutation test to diagnose and predict MEN2 (MTC identified in index case prior to genetic testing).[1]**




*[a] Biochemical screening and imaging for further features of MEN2: plasma or urine catecholamine (and adrenal imaging e.g. adrenal CT scan or MRI and/or MIBG scan if these are elevated) to assess for phaeochromocytoma, serum calcium (and parathyroid hormone if elevated) to assess for hyperparathyroidism; [b]Surveillance in those who have had a total thyroidectomy: Annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca2+) and calcitonin and carcinoembryonic antigen to detect persistence or recurrence of MTC; [c]Historical surveillance in those at risk of MEN2 who have not had a total thyroidectomy: Annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca2+); pentagastrin-stimulated serum calcitonin and neck ultrasound to assess for a medullary thyroid carcinoma; [d]2nd degree relatives would only be considered to be at genetic risk if 1st degree relatives have a RET mutation, clinical features of MEN2, or if information regarding 1st degree relatives is unavailable*

**Figure 2 – Management algorithm for use of a RET mutation test to diagnose and predict MEN2 (no MTC identified in index case prior to genetic testing).[1]**




*[a] Screening for other hereditary disorders: genetic testing of the VHL gene for von Hippel Lindau disease, genetic testing for SDHB, SDHC and SDHD mutations; if serum calcium and parathyroid hormone are elevated then additional testing for features of MEN1 (serum gastrin, serum insulin, serum glucagon, serum pancreatic polypeptide, serum vasoactive intestinal peptide, serum prolactin, growth hormone, and adrenocorticotrophic hormone (ACTH)); [b]Historical biochemical screening and imaging for further features of MEN2: pentagastrin-stimulated serum calcitonin and neck ultrasound to assess for a medullary thyroid carcinoma; plasma or urine catecholamine (and adrenal imaging e.g. adrenal CT scan or MRI and/or MIBG scan if these are elevated) to assess for phaeochromocytoma if not the presenting clinical feature, or serum calcium (and parathyroid hormone if elevated) to assess for hyperparathyroidism if not the presenting clinical feature; [c]Current biochemical screening and imaging for further features of MEN2: plasma or urine catecholamine (and adrenal imaging e.g. adrenal CT scan or MRI and/or MIBG scan if these are elevated) to assess for phaeochromocytoma if not the presenting clinical features, or serum calcium (and parathyroid hormone if elevated) to assess for hyperparathyroidism if not the presenting feature;*

*[d]Historical surveillance: Annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca2+); plus pentagastrin-stimulated serum calcitonin and neck ultrasound to assess for a medullary thyroid carcinoma or calcitonin and carcinoembryonic antigen after surgery for medullary thyroid carcinoma; Current surveillance: Annual general clinical examination, examination of thyroid (or thyroid bed if post-thyroidectomy), biochemical screen for phaeochromocytoma, screen for hyperparathyroidism (total and ionised serum Ca2+) ± calcitonin and carcinoembryonic antigen after surgery for MTC*

#### *3.1.1.1 Effectiveness*

MSAC identified nine historical controlled studies (eight at high risk of bias, one at moderate risk of bias because of very good reporting) that provided direct evidence showing that health outcomes are likely to be better for patients diagnosed with the addition of RET mutation testing:[1]

- One historical controlled study reported on the rate of death following RET mutation testing and subsequent treatments. Of those patients diagnosed and treated without knowledge of their RET mutation status, 31% died from distant metastases, compared with no patients diagnosed since the use of RET mutation testing. However, because of the lack of details in the article and the high risk of bias inherent in the study design (different length of follow-up in the two study arms), no strong conclusions were drawn by MSAC.
- Six historical controlled studies reported on the percentage of patients who were free of disease or who had residual or recurrent disease following total thyroidectomy. The six studies were consistent in the direction of effect, indicating that fewer patients who had been diagnosed with RET mutation testing subsequently had residual disease, recurrent disease or died, compared with those who were diagnosed without knowledge of RET mutations (RR=0.28, 95%CI 0.17 to 0.45). Again, these results are probably biased because of the different length of follow-up in the two study arms.
- Seven historical controlled studies reported on the incidence and severity of MTC in patients who underwent total thyroidectomy in the era prior to RET mutation testing compared with the era subsequent to the introduction of RET mutation testing. Those diagnosed and treated since RET mutation testing became available had almost half the risk of having an MTC at the time of surgery, compared with those whose treatment decisions were based on biochemical screening in the pre-RET mutation testing era (RR=0.53, 95%CI 0.32 to 0.90). It is unknown whether any clinical benefit has occurred in index patients, or whether all the benefits found have been due to more effective management of family members.
- One historical controlled study reported that age at diagnosis reduced for patients with MEN2A and FMTC between two surveys in Japan, one performed in 1996 (capturing data prior to the availability of RET mutation testing) and the other in 2002. Age at diagnosis in patients with MEN2B increased marginally, likely just through chance given the small sample. However, the MEN2B phenotype is more clearly diagnosed than the MEN2A, so genetic testing has probably had less impact on patients and their family members with or suspected of having MEN2B than MEN2A.
- Five historical controlled studies reported that the introduction of RET mutation testing allowed the age to significantly reduce at the time of total thyroidectomy. One Australian study reported that the mean age decreased from 32 years to 16 years.

Assessment of indirect (diagnostic accuracy) evidence supported the conclusions based on direct evidence of the impact of testing on patient health outcomes.[1] One historical controlled study and 3 case series reported instances of false positive results based on calcitonin levels, which led to patients either undergoing total thyroidectomy or being scheduled for surgery that was subsequently cancelled after a negative RET mutation status was identified. One single case of an individual free from RET mutations, in a family with known mutations, who had an MTC was noted. It is unknown whether this could be considered a false negative RET mutation test or a coincidental finding of a spontaneous MTC in a RET-mutation-negative family member of an FMTC kindred. Although a true comparison of accuracy was not able to be performed given the lack of long-term clinical follow-up data to use as a reference standard for MEN2 diagnosis, the limited evidence available would suggest that diagnoses made with the addition of RET mutation testing are likely to be more accurate than those made on the basis of biochemical screening. As the treatment option (thyroidectomy) is the same, irrespective of early or late identification of MEN2, and has proven effectiveness, it is unlikely that studies assessing the

comparative effectiveness of thyroidectomy in an 'earlier (RET-mutation-tested)' versus 'later (non-RET-mutation-tested)' MEN2 diagnosed population are necessary or will be conducted.

Patients who are asymptomatic gene carriers are likely to undergo prophylactic total thyroidectomy on the basis of this knowledge. Prophylactic surgery is associated with having a lower stage of MTC disease at time of surgery, compared with surgery performed on the basis of calcitonin levels.

#### *3.1.1.2 Safety*

MSAC did not identify studies that mentioned any safety concerns regarding RET mutation testing or surveillance for features of MEN2.[1] One historical controlled study reported one death from surgical complications in the pre-RET mutation testing era, compared with one death from surgical complications after diagnosis by RET mutation testing, with similar numbers of patients treated in both scenarios (N=29 in pre-RET mutation testing era; N=31 in RET mutation testing era). No further details on the nature of these deaths or information on confounding factors were reported.

Twelve case series reported on rates of adverse events due to total thyroidectomy, performed after RET mutation testing identified the patients as having (N=2), or being at risk of having (N=10), MEN2.[1] Transient hypoparathyroidism was the most commonly reported adverse event, mentioned in 8 studies, with rates between 5.0% and 36.4%. Permanent hypoparathyroidism, mentioned in 4 studies, occurred in 5.9-13.6% of patients. Temporary laryngeal nerve palsy occurred in 4.5-5.9% of patients, and one case of permanent laryngeal nerve palsy was reported (1.3% in 1 study).

Other complications following total thyroidectomy were one case of arterial bleeding requiring re-operation, one case of permanent unilateral Horner's syndrome, and one paediatric case with fluctuating thyroid function test results despite good thyroxine replacement compliance at 1-year follow-up.[1]

#### *3.1.1.3 Cost-effectiveness*

MSAC conducted an economic evaluation for both (1) RET mutation testing in potential index cases – MTC or phaeochromocytoma under 50 years of age – and (2) RET mutation testing in index cases and additional familial genetic testing in first- or second-degree relatives of identified RET-mutation-positive index cases.[1] With respect to the economic evaluation of genetic testing in potential index cases alone, a cost analysis (cost-minimisation) approach was used, as there is no evidence to suggest that health outcomes within the index case will be affected by genetic testing. On the contrary, with respect to familial testing, a cost-utility analysis was undertaken, as the ability to identify RET-mutation-positive family members via testing allows for prophylactic thyroidectomy treatment and therefore both health costs and outcomes are affected.

The cost-minimisation analysis of genetic testing in potential index cases demonstrated that cost savings occur within 5 years of testing.[1] Over the course of 30 years, savings of approximately $535 per MTC patient tested, or $1 458 per phaeochromocytoma patient under 50 years of age tested, would be expected compared with a scenario where testing was not available.

With respect to the cost-utility analysis of genetic testing of potential index cases and family members of patients identified as RET-mutation-positive, the results indicated that availability of genetic testing 'dominates' (i.e. it results in both improved health outcomes and cost savings), compared with the alternative scenario where testing is not available.

Sensitivity analyses suggested that the base-case economic conclusions are relatively robust.

Based on these findings MSAC concluded with reasonable certainty that RET mutation testing and subsequent targeted surveillance (in comparison with broader and increased reliance on imaging/biochemical surveillance) is cost-effective.[1]

### 3.1.2 Overview of published guidelines

Two consensus-based guidelines contain recommendations about RET mutation testing in patients with the MEN2 syndrome or their relatives.[5, 6] Both guidelines lack a good description of their methodology, although the ATA 2009 guideline was based on a Medline search. None of the guidelines contains detailed quality appraisal results of the included studies or evidence tables.

Both guidelines recommend RET mutation testing for patients with MTC or MEN2 (Table 2). Furthermore, both guidelines also recommend RET mutation testing in first-degree relatives of known mutation carriers before 5 years of age (exact age depending on the type of RET mutation).

In addition, the ATA 2009 guideline contains some more specific recommendations about RET mutation in patients with intestinal ganglioneuromatosis, lichen planus amyloidosis or pruritis in the central upper back.

**Table 2 – Overview of published guidelines on RET mutation testing for the MEN2 syndrome.**

| Guideline | Recommendation | AGREE II score 'Methodology' |
|---|---|---|
| **ATA 2009** | All patients with a personal medical history of primary C cell hyperplasia, MTC, or MEN2 should be offered germline RET testing | 25.0% |
| | The differential diagnosis in patients with intestinal ganglioneuromatosis should include MEN2B, which together with their history and physical examinations, family history, and ganglioneuromatosis histology may prompt germline RET testing | |
| | All people with a family history consistent with MEN2 or FMTC, and at risk for autosomal dominant inheritance of the syndrome, should be offered RET testing. For MEN2B this should be done shortly after birth. For MEN2A and FMTC this should be done before 5 years of age | |
| | Lichen planus amyloidosis or pruritis in the central upper back may indicate the presence of a 634 codon mutation and should prompt genetic testing | |
| | Pre- and post-test genetics counseling by a genetics counselor, or other qualified professional, should be offered to all patients undergoing RET testing | |
| | Once a germline RET mutation has been identified in a family, RET mutation analysis should be offered to all first-degree relatives of known mutation carriers which should be done before the age of recommended prophylactic thyroidectomy whenever possible | |
| **STOET 2010** | RET mutation analysis in<br>- Patients with MEN2 syndrome;<br>- Patients with sporadic MTC;<br>- Patients with sporadic pheochromocytoma aged < 50 years | 6.3% |
| | In case of known RET mutation: RET mutation analysis in first-degree relatives at young age (0-5 years) | |

**Conclusions**

Overall, clinical management with the addition of RET mutation testing would appear to have superior effectiveness and at least non-inferior safety, compared with diagnosis and treatment of MEN2 without knowledge of RET mutation status.

***Other considerations***

| Factor | Comment |
|---|---|
| **Balance between clinical benefits and harms** | Clinical management with the addition of RET mutation testing would appear to have superior effectiveness and at least non-inferior safety, compared with diagnosis and treatment of MEN2 without knowledge of RET mutation status |
| **Quality of evidence** | The direct evidence is limited to observational studies (historical controlled studies) |
| **Costs (resource allocation)** | • RET mutation testing is billed using the nomenclature code 565515 – 565526 (€353.30 anno 2014).<br>• Several recognized Belgian genetic centres provide RET mutation testing.<br>• MSAC concluded with reasonable certainty that RET mutation testing and subsequent targeted surveillance (in comparison with broader and increased reliance on imaging/biochemical surveillance) is cost-effective in an Australian context. |
| **Patients values and preferences** | According to the patient representatives, patients with MEN2 and their relatives should be clearly informed about the risk for medullary thyroid carcinoma and other typical manifestations. They should also be informed about the necessary surveillance for new manifestations, therapeutic options (including prophylactic thyroidectomy) and fertility planning (if applicable). Psychosocial support should also be offered. |
| **Comments** | • In some cases, more specifically RET mutations with a low penetrance (ATA risk level A)[5], RET mutation analysis of first-degree relatives can be postponed until after the age of 5 years. These relatives can be followed-up clinically (calcitonin and neck ultrasonography) and treated with total thyroidectomy in case of abnormal findings.<br>• Treatment (including surgery) should not be delayed because of genetic testing. |

| Recommendations | Strength of Recommendation |
|---|---|
| • Pre- and post-test genetic counselling should be offered to all patients with a clinical diagnosis of MEN2 (see box) or a sporadic MTC. | Strong |
| • All patients with a clinical diagnosis of MEN2 (see box) or a sporadic MTC, and selected patients with a phaeochromocytoma * should be offered germline RET testing. | Strong |

| Recommendations | Strength of Recommendation |
|---|---|
| • Once a germline RET mutation has been identified in a proband, RET mutation analysis should be offered to all first-degree relatives[#], preferably before the age of 5 years. | Strong |

[*] *See chapter 1.1.*

[#] *Or first-degree relatives of patients with clinical MEN2 who died before genetic testing was carried out.*

**Criteria for clinical diagnosis of MEN2**

- MEN2A: individual with (1) MTC and at least one family member with primary hyperparathyroidism and/or phaeochromocytoma, or (2) with at least two of the three major manifestations (MTC, phaeochromocytoma, primary hyperparathyroidism)
- MEN2B: individual with MTC, phaeochromocytoma and other characteristic features (i.e. mucosal ganglioneuromas, gastrointestinal ganglioneuromas, eye abnormalities including corneal nerve thickening, and/or skeletal abnormalities including marfanoid body habitus)
- Familial MTC: family with at least 4 members diagnosed with MTC (in the absence of pheochromocytoma or parathyroid adenoma/hyperplasia)

## 3.2 Multiple Endocrine Neoplasia type 1 (MEN1)

### *3.2.1 Evidence from indexed literature*

#### *3.2.1.1 Diagnostic accuracy of individual clinical and tumour characteristics for the detection of mutations*

Eight studies contained sufficient information to calculate the diagnostic accuracy of several clinical features for the detection of MEN1 mutation in patients with MEN1 phenotype or MEN1-related state (Table 3).[7-14] None of these clinical features appeared to have a good diagnostic accuracy. Having a parathyroid tumour was found to be the most sensitive clinical feature both for patients with the MEN1 phenotype (pooled sensitivity 99%, 95%CI 93-100%) as for patients with a MEN1-related state (1 small study, sensitivity 100%). However, with an estimated pre-test probability of 47% and on 1 000 evaluated patients, 392 patients with a parathyroid tumour would wrongly be predicted as having a MEN1 mutation.

**Table 3 – Diagnostic accuracy of clinical features for the detection of MEN1 mutation in patients with MEN1 phenotype or MEN1-related state. ***

| Population | Clinical feature | N studies | N patients | Median prevalence | Sensitivity * | Specificity * | Median PPV | Median NPV | Results per 1 000 patients evaluated | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Prevalence $ | False negatives | False positives |
| **MEN1 phenotype** | Familial | 4 | 191 | 61% | **71%** | **87%** | 97% | 64% | 47% | 137 | 69 |
| | Three major lesions | 5 | 382 | 47% | **31%** | **96%** | 79% | 62% | 47% | 324 | 21 |
| | Parathyroid tumour | 4 | 298 | 44% | **99%** | **26%** | 49% | 99% | 47% | 5 | 392 |
| | Pituitary tumour | 4 | 298 | 44% | **53%** | **35%** | 42% | 45% | 47% | 221 | 344 |
| | Pancreatic tumour | 3 | 146 | 47% | *82%* | *67%* | 64% | 82% | 47% | 85 | 175 |
| | Minor lesions | 2 | 126 | 47% | *20%* | *87%* | 63% | 55% | 47% | 376 | 69 |
| **MEN1-related state** | Familial | 4 | 532 | 29% | **76%** | **76%** | 54% | 89% | 8% | 19 | 221 |
| | Parathyroid tumour | 1 | 13 | 8% | *100%* | *42%* | 13% | 100% | 8% | 0 | 534 |
| | Pituitary tumour | 1 | 13 | 8% | *0%* | *75%* | 0% | 90% | 8% | 80 | 230 |
| | Pancreatic tumour | 1 | 13 | 8% | *0%* | *83%* | 0% | 91% | 8% | 80 | 156 |
| | Minor lesions | 1 | 13 | 8% | *0%* | *25%* | 0% | 75% | 8% | 80 | 690 |

* *Italic = medians, bold = pooled.* $ *Medians from unselected populations.*

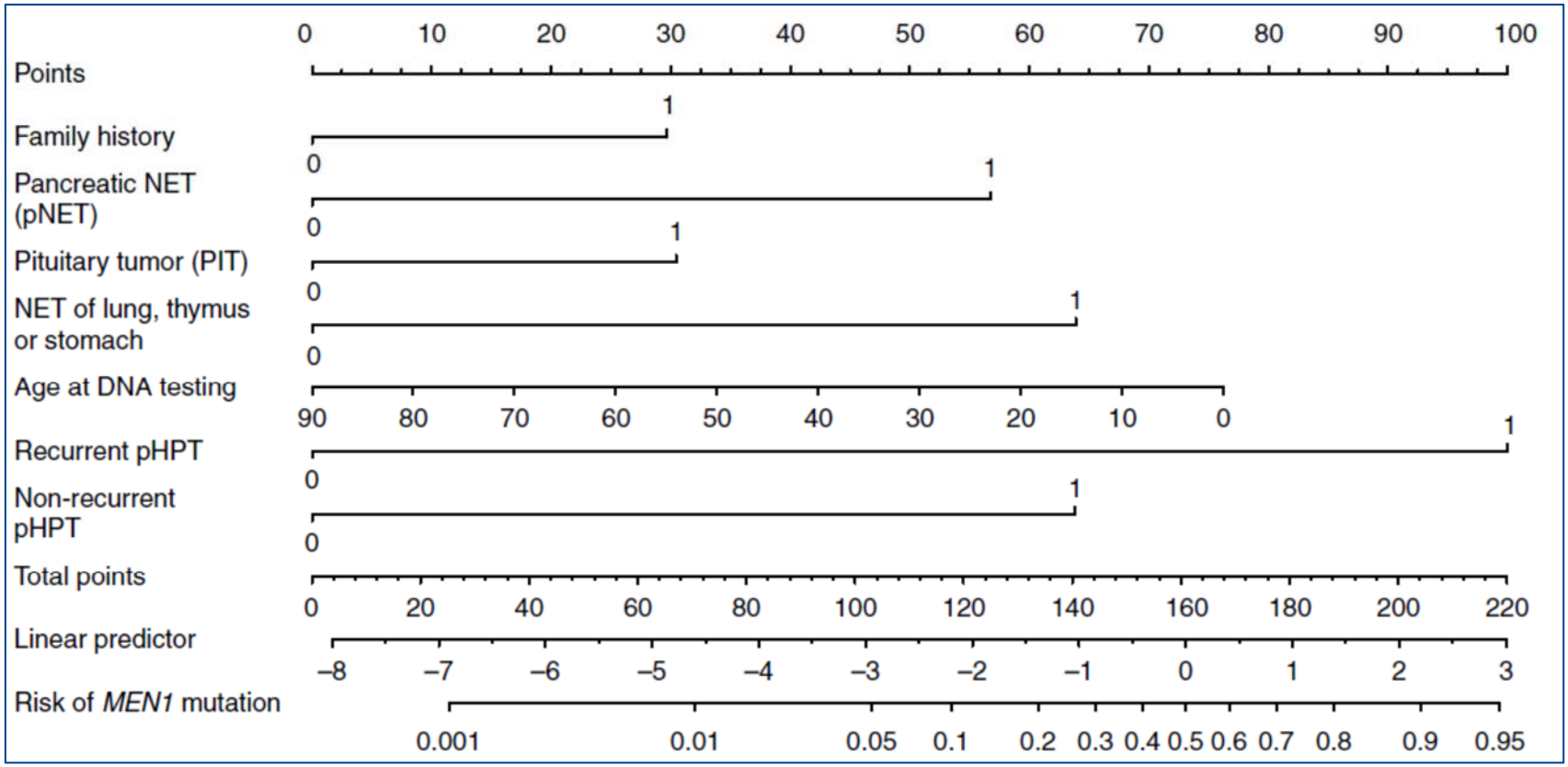
### *3.2.1.2 Published models and proposed screening algorithms*

No models were found for MEN1 mutation testing in patients with clinical MEN1. However, de Laat et al. published a model predicting the risk of having a MEN1 mutation in patients with sporadically occurring endocrine tumours.[15] From 1 185 MEN1 mutation analyses performed between 1998 and 2010, 365 patients were retrospectively included. Patients with clinical MEN1, a known MEN1 family member, missing clinical information or erroneous indication for ordering a MEN1 mutation analysis were excluded. The following variables were considered candidate predictors for a MEN1 mutation: patients' age at the moment of DNA testing, recurrent or multiglandular disease primary hyperparathyroidism, non-recurrent primary hyperparathyroidism, neuroendocrine tumours of the pancreas and duodenum, pituitary tumours, neuroendocrine tumours of stomach, lung, or thymus, adrenal hyperplasia or adenomas, and a family history of endocrine tumors. Based on a multivariate analysis, the following predictors were retained in the model: recurrent or multiglandular disease primary hyperparathyroidism (OR 162.4, 95%CI 30.36 to 868.55), non-recurrent primary hyperparathyroidism (OR 25.78, 95%CI 8.10 to 82.12), neuroendocrine tumours of the pancreas and duodenum (OR 17.94, 95%CI 5.86 to 54.87), pituitary tumours (OR 4.71, 95%CI 1.86 to 11.97), neuroendocrine tumours of stomach, lung, or thymus (OR 25.84, 95%CI 4.40 to 151.80), family history of MEN1-related endocrine tumors (OR 4.53, 95%CI 1.93 to 10.61), and age (OR 0.96, 95%CI 0.94 to 0.98). The diagnostic accuracy of the model was good, with an area under the curve (c-statistic) of 0.86 (95%CI 0.81-0.90). The model was externally validated in a Swedish cohort of 144 patients with sporadically occurring endocrine tumours. The c-statistic in this cohort was 0.77 (95%CI 0.66-0.88).

Based on the validated model, a nomogram was constructed (Figure 3). Appendix 6.2 provides some guidance on how to use the nomogram.

A limitation of the study is that the included patients underwent MEN1 mutation testing because the referring physician suspected the MEN1 syndrome, without having uniform referral criteria (>30% did not meet the Dutch 2001 testing criteria). This can have created a selection bias.

**Figure 3 – Nomogram to predict MEN1 mutation in patients with sporadically occurring endocrine tumours, proposed by de Laat et al.[15]**




*NET: neuro-endocrine tumours; pHPT: primary hyperparathyroidism; PIT: pituitary tumour.*

#### 3.2.1.3 Genetic screening of relatives

Six studies provided information on the use of MEN1 screening of relatives. Balogh et al. tested 21 first-degree relatives.[7] All of the six symptomatic relatives tested positive, while one out of fifteen (7%) asymptomatic relatives tested positive. Lairmore et al. tested 56 at-risk relatives from 9 unrelated MEN1 kindreds.[16] Seven patients (13%) tested positive. Hypercalcemia was either present at the time of genetic diagnosis or developed during the period of follow-up in 6 patients. One patient has not yet developed hyperparathyroidism after a mean follow-up 35.8 months. Lourenco et al. included 141 at-risk relatives from 13 unrelated index cases.[17] Thirty-nine relatives (28%) tested positive for a MEN1 mutation. Of these, 28 were symptomatic and detected through clinical screening. Eleven patients were asymptomatic. Of the 102 relatives without a MEN1 mutation, one had a sporadic primary hyperparathyroidism (MEN1 phenocopy). Tham et al. tested 169 relatives, and found a MEN1 mutation prevalence of 18% in the presymptomatic relatives and 94% in the symptomatic relatives.[13]Tso et al. found a prevalence of 19% of MEN1 mutations in 47 relatives.[14] The nine mutation carriers were all clinically affected. Finally, Waterlot et al. included 91 members from a MEN1 family.[18] Of these, 54 were clinically screened in the period 1992-1995, and 14 were found to be affected. From 1995 onwards, genetic screening was performed. All 14 clinically affected family members tested positive for a MEN1 mutation. Thirty-four asymptomatic family members were also genetically screened, and six were found to be positive. The 28 mutation-negative family members were excluded from annual clinical screening, while ten of these were annually screened before their mutation-status was known.

Two studies reported on the age-related penetrance of MEN1. Bassett et al. calculated the age-related penetrance in 320 members of 43 unrelated probands.[19] Two-hundred and one MEN1 mutant-gene carriers were identified, of which 155 were clinically affected. The age-related penetrance was 0% at <5y, 52% at 20y and 100% at 60y. Schaaf et al. analyzed data of 419 individuals, including 306 MEN1 patients.[20] The age-related penetrance was 10%, 35%, 67%, 81% and 100% at 20, 30, 40, 50 and 65y, respectively.

#### 3.2.1.4 Impact of genetic screening on outcomes

One retrospective study compared the outcomes of clinically versus genetically detected cases. Pieterman et al. included 74 patients with a clinical or genetic diagnosis of MEN1.[21] More patients with a clinical diagnosis had three manifestations at the time of MEN1 diagnosis (3 vs. 0) or at the end of follow-up (6 vs. 4). Furthermore, more patients with a clinical diagnosis had metastases (10 vs. 0) or died during follow-up (10 vs. 0). Five of these deaths were MEN1-related. However, the sample was not consecutive: 22 patients with an uncertain diagnosis of MEN1 and 4 patients with insufficient information were excluded. Also, the median follow-up was longer in the group with a clinical diagnosis (11y vs. 3y).

### 3.2.2 Overview of published guidelines

One consensus-based guideline contains recommendations about mutation testing in patients with the MEN1 syndrome or their relatives.[6] This guideline lacks a good description of its methodology. Furthermore, the guideline does not contain detailed quality appraisal results of the included studies or evidence tables. The recommendations are presented in Table 4. According to the STOET 2010 guideline a clinical diagnosis of MEN1 is made when 3/5 typical MEN1 tumours are present (parathyroid tumours, neuroendocrine tumours of pancreas/duodenum, anterior pituitary tumours, adrenocortical tumours and neuroendocrine tumours of the stomach, lungs or thymus) or when 1/5 typical MEN1 tumour is present in combination with a first-degree family member with MEN1.

**Table 4 – Overview of published guidelines on mutation testing for the MEN1 syndrome.**

| Guideline | Recommendation | AGREE II score 'Methodology' |
|---|---|---|
| **STOET 2010** | MEN1 mutation analysis in<br>• Patients with clinical MEN1 syndrome;<br>• First-degree relatives of MEN1 mutation carriers;<br>• First-degree relatives of patients with clinical MEN1 syndrome;<br>• Suspicion of MEN1 syndrome:<br>o 1/5 MEN1 tumours and <35y<br>o Multiple MEN1 tumours in one organ<br>o 2/5 MEN1 tumours | 6,3% |
| | In case of known MEN1 mutation: mutation analysis in first-degree relatives starting from 5y | |

**Conclusions**

- No validated models / algorithms exist for genetic testing of patients with clinical MEN1 syndrome. One validated model exists for MEN1 mutation testing in patients with sporadically occurring endocrine tumours.
- No single clinical feature has a good diagnostic accuracy to guide mutation testing.
- Genetic screening of relatives can preclude mutation-negative relatives from annual clinical screening.
- Evidence from one study of very low quality suggests that genetic screening for MEN1 mutations is associated with better outcomes than clinical screening.

***Other considerations***

| Factor | Comment |
|---|---|
| **Balance between clinical benefits and harms** | No evidence was found on harms associated with genetic testing. Only one study of very low quality suggests that genetic screening for MEN1 mutations is associated with better outcomes than clinical screening. The expected benefits of MEN1 genetic testing in patients with a clinical diagnosis or suspicion of MEN1 are (1) a confirmation of the diagnosis as such, (2) the identification of the need for targeted surveillance for MEN1-associated tumours, and (3) the identification of the need of first-degree relatives to undergo genetic counselling and testing.<br>Genetic screening of relatives can preclude mutation-negative relatives from annual clinical screening. Mutation-positive relatives will enter surveillance programmes targeted at early diagnosis of MEN1-associated tumours. |
| **Quality of evidence** | Direct evidence is based on one study of very low quality. No validated algorithms are available. |
| **Costs (resource allocation)** | • MEN1 mutation testing is billed using the nomenclature code 565515 – 565526 (€353.30 anno 2014). |

| Factor | Comment |
|---|---|
| | • Several Belgian genetic centres provide MEN1 mutation testing. |
| **Patients values and preferences** | According to the patient representatives, patients with MEN1 and their relatives should be clearly informed about the risk for typical manifestations. They should also be informed about the necessary surveillance for new manifestations, therapeutic options and fertility planning (if applicable). Psychosocial support should also be offered. |
| **Comments** | Treatment (including surgery) should not be delayed because of genetic testing. |

| Recommendations | Strength of Recommendation |
|---|---|
| • Pre- and post-test genetic counselling should be offered to all patients with a clinical diagnosis or suspicion of MEN1 (see box). | Strong |
| • All patients with a clinical diagnosis of MEN1 (see box) should be offered MEN1 genetic testing. | Strong |
| • In patients with a clinical suspicion of MEN1 (see box) MEN1 genetic testing may be considered. | Weak |
| • MEN1 mutation analysis should be offered to all first-degree relatives of MEN1 mutation carriers*. | Strong |

** Or first-degree relatives of patients with clinical MEN1 who died before genetic testing was carried out.*

**Criteria for clinical diagnosis of MEN1**

- At least two of the three major MEN1-associated tumours (parathyroid tumours, neuroendocrine tumours of pancreas/duodenum, anterior pituitary tumours).
- One of the three major MEN1-associated tumours in a first-degree relative of a case with a clinical diagnosis of MEN1.

**Criteria for clinical suspicion of MEN1**

- One of the three major MEN1-associated tumours in combination with one minor MEN1-associated tumour (adrenocortical tumours or neuroendocrine tumours of the stomach, lungs or thymus).
- Multiple MEN1-associated tumours in one organ.
- One MEN1-associated tumour at an age < 35 years and a family member with a different MEN1-associated tumour.

## 3.3 von Hippel-Lindau (VHL) syndrome

### *3.3.1 Evidence from indexed literature*

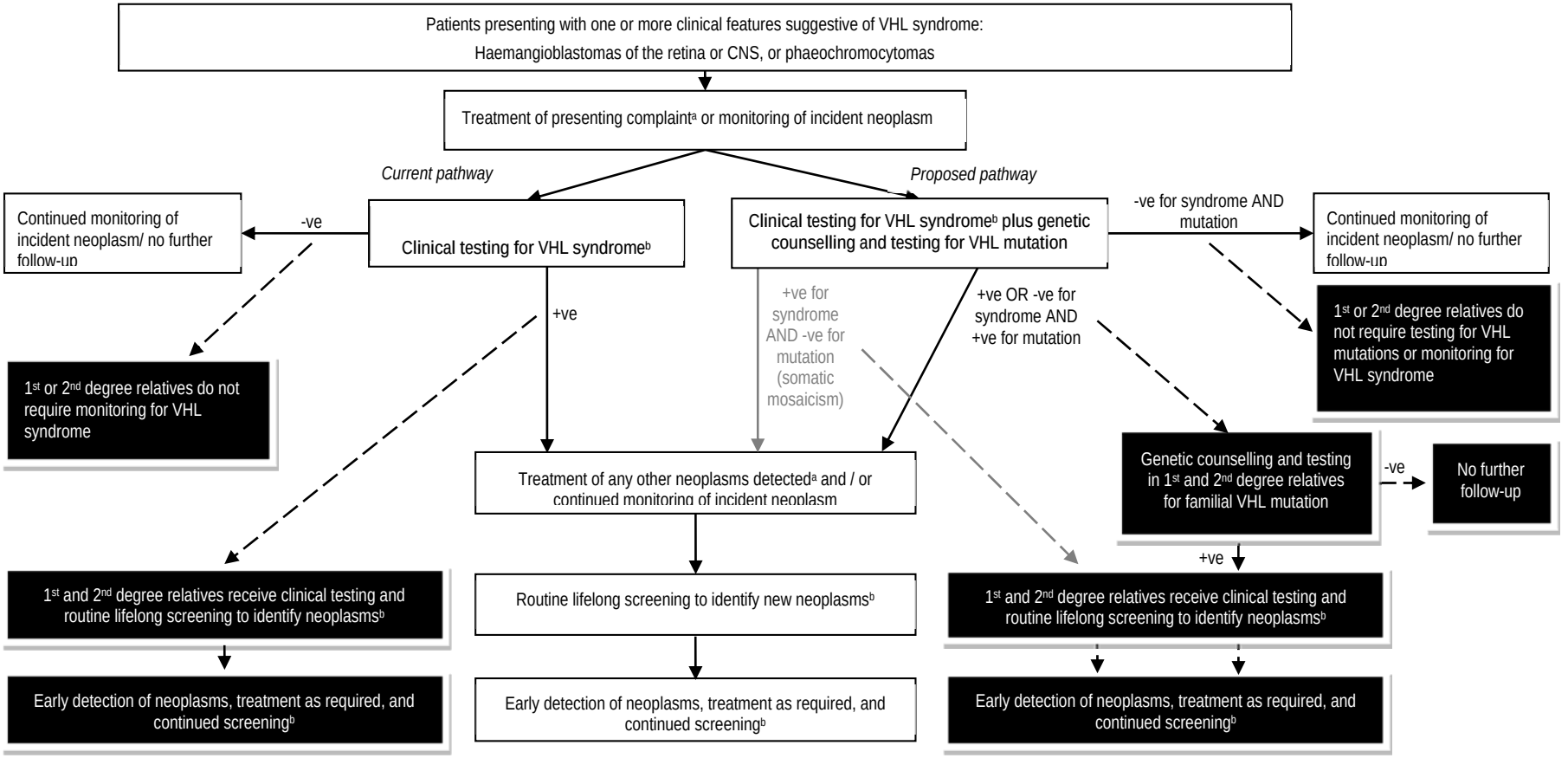
One recent HTA report of good quality was identified.[3] The objective of the report was to determine whether there is sufficient evidence in relation to clinical need, safety, effectiveness and cost-effectiveness to recommend public funding for genetic testing for hereditary mutations in the VHL gene for (1) patients with symptoms of VHL syndrome and (2) a family member of a patient with a known VHL mutation. Since the literature search in the MSAC 2011 report was considered to be sufficiently rigourous (up to May 2011), no attempt was made to identify more recent primary studies.

For the evaluation of the safety, effectiveness and cost-effectiveness of VHL mutation testing, MSAC proposed a management algorithm as a basis (Figure 4).[3] This pathway includes the 'historical' setting on the one hand (i.e. the investigations to be used in the absence of VHL mutation testing), and the scenario with VHL mutation testing being standard clinical practice on the other hand. With this pathway in mind, MSAC defined PICO questions for the following two populations:

- Patients presenting with one or more clinical features suggestive of VHL syndrome;
- Clinically unaffected first- or second-degree family members of patients with clinically diagnosed VHL syndrome and/or a diagnosed VHL genetic abnormality.

For both populations a pathway including VHL mutation testing (and targeted interventions depending on the outcome of VHL mutation testing) was compared with the 'historical' setting, using long-term clinical assessment (ideally over the life-time of the patient) as the reference standard.[3]

**Figure 4 – Management algorithm for use of VHL genetic testing in patients who present with clinical features suggestive of VHL syndrome, as well as their first- and second-degree relatives.[3]**




*1st degree relatives are parents, offspring and siblings that share 50% of their genes; 2nd degree relatives are grandparents, grandchildren, uncle, auntie, nephew, niece, half-sibling that share 25% of their genes; [a] Surgical resection, radiotherapy, laser therapy, anti-VEGF therapy; [b]Clinical testing = CT, MRI, ultrasound, urine and blood tests, family history, clinical history, other tests as appropriate to identify any signs of disease other than presenting complaint; biopsy and histopathology of any neoplasms; [c]Screening = CT, MRI, ultrasound, urine and blood tests; CNS=central nervous system*

#### *3.3.1.1 Effectiveness*

**Index cases**

No direct evidence was identified by MSAC comparing patient health outcomes following genetic testing in addition to usual clinical diagnosis versus usual clinical diagnosis alone in patients suspected of having VHL syndrome, or for assessing the effectiveness of VHL genetic testing when used as a triage test for life-long screening of family members.[3] Given that health benefits are derived from reduced morbidity and mortality due to annual screening for early detection of newly developed neoplasms, and that the annual screening protocol is identical for all patients clinically diagnosed with VHL syndrome, irrespective of their VHL mutation status, the lack of comparative data was predictable. It is therefore unclear what impact, if any, the addition of VHL genetic testing to clinical diagnosis has on the health outcomes of patients suspected of VHL syndrome.

According to MSAC, the VHL genetic testing methods of direct DNA sequencing of PCR products from all three exons of the VHL gene, plus a method to detect large deletions of the VHL gene such as MLPA, appeared to be the most accurate of the modalities available (Table 5).[3] This dual testing methodology is highly accurate, with median 100% sensitivity, specificity, positive predictive and negative predictive values. However, despite this accuracy, a false negative rate of 10.2% and a false positive rate of 4.2% were observed. The false negative rate of 10.2% suggests that detection of a germline mutation is not yet possible for some patients with VHL syndrome. Thus, according to MSAC, VHL genetic testing should not be used as a stand-alone test for the diagnosis of VHL syndrome in patients presenting with VHL-related neoplasms, but as a confirmatory test after clinical diagnosis in the index case. The false positive rate of 4.2% was expected, as there will always be a few patients who do not meet the criteria for clinical diagnosis of VHL syndrome but have an underlying VHL mutation. In these patients the disease may not yet have progressed sufficiently to obtain a positive clinical diagnosis.

**Table 5 – Median (and range of) diagnostic accuracy data from studies with a low–medium risk of bias for different genetic testing methodologies.[3]**

| Genetic testing methodology | Sensitivity | Specificity | PPV | NPV |
|---|---|---|---|---|
| **Pre-screened DNA sequencing** | 66.9% (51.8-87.5) | 95.0% (88.9-100) | 97.8% (85.7-100) | 72.2% (30.3-100) |
| **Direct DNA sequencing** | 76.9% (44.4-91.4) | 100% (57.1-100) | 100% (36.0-100) | 80.9% (14.3-100) |
| **Deletion detection (DD) methods** | 17.4% (3.9-36.6) | 100% (100-100) | 100% (100-100) | 17.1% (4.8-52.4) |
| **Pre-screened DNA sequencing plus DD** | 74.6% (14.3-100) | 94.9% (50.0-100) | 97.1% (54.2-100) | 80.0% (12.5-100) |
| **Direct DNA sequencing (no prescreening) plus DD** | 100% (70.0-100) | 100% (50.0-100) | 77.8% (77.8-100) | 100% (33.3-100) |

Genetic diagnosis of a VHL mutation was more accurate in patients with phaeochromocytoma than in any other patient group (100% sensitivity in 7/8 studies).[3] This was due to missense VHL mutations (detected by DNA sequencing) being the most common cause of phaeochromocytoma in VHL syndrome. Patients with phaeochromocytoma (syndromic or sporadic) had an overall 7% probability of having an underlying germline VHL mutation, whereas patients with familial phaeochromocytoma had a 50% probability of having a VHL mutation that is indicative of type 2C VHL syndrome.

**First- or second-degree family members**

Once an index case has a pathogenic VHL mutation identified, their close relatives need only be tested for that specific mutation, using a testing methodology known to be able to detect that type of mutation.[3] Therefore, contrary to testing of the index case, the diagnostic accuracy of genetic testing within family members did not vary to any great extent by specific genetic testing methodology. Every included study reporting accuracy data for relatives of someone with a known VHL mutation reported a sensitivity of 100%. This indicates that, as expected, patients who met the clinical diagnostic criteria for VHL syndrome carried the familial VHL mutation. The median specificity of 83.3-85.0% and the false positive rates of 16.9-23.5% reflect the difference in the timeframe required for a positive clinical diagnosis compared with a positive genetic test.[3] Younger relatives are more likely to receive a positive genetic test before any clinical signs of disease can be detected by clinical screening.

The 100% negative predictive value indicates that a negative genetic test result is likely to reflect the true disease status of the patient.[3] However, the median positive predictive value of 69.4% for first-degree relatives and 47.8% for first- and second-degree relatives reflects both the potential lag between a genetic and clinical diagnosis and the greater prevalence of VHL mutation carriers among first-degree relatives compared with second-degree relatives. That is, it reflects the imperfect nature of the reference standard at predicting which relatives would likely develop clinical symptoms over time, rather than reflecting poorly on the accuracy of the genetic test.

Approximately 4 out of 10 of all first- and second-degree relatives, and 2-3 out of 10 asymptomatic first- and second-degree relatives, who undergo VHL genetic testing were identified as carriers of the familial VHL mutation.[3]

**Change in patient management**

MSAC identified minimal evidence regarding patient management following diagnosis of VHL syndrome using genetic testing in combination with clinical diagnosis.[3] No study provided a direct comparison between patients with a known VHL mutation and those that had not been tested. Therefore, due to the lack of an appropriate comparator in these studies, no conclusions were drawn by MSAC about the change in patient management from genetic testing.

Knowledge of a specific germline VHL mutation in a patient with a clinical diagnosis of VHL syndrome may provide some information about the VHL syndrome type, which then determines the types of neoplasms that are likely to develop in a particular patient. Thus, management of patients with a known VHL mutation could be tailored to ensure early detection of the neoplasms most likely to occur. Although no difference in patient management is expected for patients presenting with the same VHL-associated neoplasms, based on the method of diagnosis, the VHL genetic test is expected to change patient management for asymptomatic relatives when used as a triage test for lifelong screening. Relatives with a negative genetic test result would not require lifelong screening, saving potential anguish and unnecessary use of healthcare resources. Lifelong screening programs can then be targeted towards relatives who have inherited the VHL mutation and are likely to develop VHL-associated neoplasms.

While 88.0-97.0% of clinically diagnosed VHL patients agreed to genetic testing in the evidence-base, only 58.5-65.8% of at-risk relatives agreed.[3] Additionally, relatives aged over 20 years were more likely to undergo genetic testing than children aged less than 5 years, suggesting that parents are reluctant to have very young children genetically tested. This reluctance appears to diminish with increasing age of the child. Only 38.9% of patients with a VHL mutation continued screening after 5 years.[3] Symptomatic patients were much more likely to continue than asymptomatic patients. Patients who have symptoms or have a neoplasm detected early are more aware of the personal risks involved than patients who have not developed any detectable neoplasms, and thus may be complacent. Whether compliance with annual screening is higher with knowledge of a VHL mutation than without could not be determined from the available evidence.

#### 3.3.1.2 Safety

MSAC did not identify studies that could inform an assessment of the safety of genetic testing in the diagnosis of VHL syndrome or for identification of family members with a VHL mutation.[3]

#### 3.3.1.3 Cost-effectiveness

MSAC searched the literature for existing cost-effectiveness analyses, but did not identify relevant cost-effectiveness or cost-utility analysis evaluating the cost-effectiveness of the use of genetic testing for VHL mutations in addition to usual clinical diagnosis in patients suspected of having VHL syndrome, or when used as a triage test for lifelong screening of family members.[3] One study compared the costs and benefits of the use of clinical screening only with genetic testing plus clinical screening, and 4 studies reported on the costs of clinical screening and VHL genetic testing, and found cost savings attributable to the reduction in the number of at-risk family members that required clinical screening. The first study also found that the use of genetic screening resulted in beneficial psychosocial outcomes, for example reduced levels of anxiety associated with the use of VHL screening programs and an associated reduction in the likelihood of early death of family members.

In the absence of direct evidence for the increased effectiveness of the addition of genetic testing to clinical testing, a conservative assumption of at least equal effectiveness was made and a cost comparison was performed by MSAC. The analysis considered the costs associated with an individual suspected of having VHL syndrome (the index case) and the costs associated with testing and monitoring (annual screening) their first- and second-degree relatives (who are at risk of having the VHL mutation). The first part of the analysis delivers individuals or family members into either monitoring or no-monitoring health states based upon the best information known from either genetic and clinical testing or clinical testing alone. A proportion of family members are assumed to refuse genetic testing (40%) and a proportion to refuse monitoring (60%). This non-compliance is a more realistic situation than 100% adoption of either testing or monitoring, and is important to consider because it will tend to dilute the cost savings associated with the genetic testing arm. Those who are genetically positive (whether this status is known or unknown) but refuse monitoring will transit to a monitoring state once they become symptomatic.

Due to the high sensitivity and specificity of the genetic test compared with a clinical diagnosis, there was very little difference in costs associated with managing the index case between the two arms, except for the cost of the VHL diagnostic test and the genetic counselling. However, when applied to family members, who have an assumed likelihood of carrying the VHL mutation of 26%, there is a marked decrease in monitoring among those who do not require monitoring (22.1%). Costs of monitoring are assumed to be accrued over a lifetime. Treatment costs are assumed to be equivalent in both arms.

The overall cost saving (through avoided inappropriate monitoring) of a single index case and their family over their lifetimes is $7 749 in discounted costs and $20 783 in undiscounted costs. As there are many uncertainties in the analysis, several sensitivity analyses have been performed by MSAC. The cost comparison is most sensitive to the prevalence of VHL syndrome among patients who are suspected of having it, and the uptake of genetic testing and monitoring among family members. In most sensitivity analyses, a cost saving remains following the introduction of VHL genetic testing. Furthermore, if monitoring and genetic testing rates among family members increase, the cost saving associated with genetic testing will markedly increase. The cost comparison is not sensitive to moderate changes in the proposed reimbursement for VHL genetic testing.

### 3.3.2 Overview of published guidelines

Two consensus-based guidelines contain recommendations about VHL mutation testing in patients with the VHL syndrome or their relatives.[6, 22] Both guidelines lack a good description of their methodology. None of the guidelines contains detailed quality appraisal results of the included studies or evidence tables.

Both guidelines recommend VHL mutation analysis in patients with a clinical diagnosis of VHL syndrome (Table 6). Furthermore, VHL mutation analysis is recommended in first-degree relatives of patients with a known VHL mutation, and this at a young age.

**Table 6 – Overview of published guidelines on VHL mutation testing for the von Hippel-Lindau syndrome.**

| Guideline | Recommendation | AGREE II score 'Methodology' |
|---|---|---|
| **Binderup 2013** | VHL mutation analysis in patients with VHL syndrome | 0.0% |
| | Full genetic work-up of the family of patients with suspected VHL syndrome | |
| **STOET 2010** | VHL mutation analysis in patients with VHL syndrome | 6.3% |
| | In case of known VHL mutation: VHL mutation analysis in relatives at young age | |

**Conclusions**

- No direct evidence was identified by MSAC comparing patient health outcomes following genetic testing in addition to usual clinical diagnosis versus usual clinical diagnosis alone in patients suspected of having VHL syndrome, or for assessing the effectiveness of VHL genetic testing when used as a triage test for life-long screening of family members.
- No study provided a direct comparison between patients with a known VHL mutation and those that had not been tested.
- MSAC did not identify studies that could inform an assessment of the safety of genetic testing in the diagnosis of VHL syndrome or for identification of family members with a VHL mutation.

***Other considerations***

| Factor | Comment |
|---|---|
| **Balance between clinical benefits and harms** | There is an absence of evidence on harms and benefits. |
| **Quality of evidence** | The evidence is limited to studies on diagnostic accuracy (indirect evidence). |
| **Costs (resource allocation)** | • VHL mutation testing is billed using the nomenclature code 565515 – 565526 (€353.30 anno 2014).<br>• Several recognized Belgian genetic centres provide VHL mutation testing.<br>• MSAC calculated that the overall cost saving (through avoided inappropriate monitoring) of a single index case and their family over their lifetimes is $7 749 in discounted costs and $20 783 in undiscounted costs (Australian context). |
| **Patients values and preferences** | According to the patient representatives, patients with VHL and their relatives should be clearly informed about the risk for renal cell carcinoma and other typical manifestations. They should also be informed about the necessary surveillance for new manifestations, therapeutic options and fertility planning (if applicable). Psychosocial support should also be offered. |
| **Comments** | Treatment (including surgery) should not be delayed because of genetic testing. |

| Recommendations | Strength of Recommendation |
|---|---|
| • Pre- and post-test genetic counselling should be offered to all patients with a clinical diagnosis or suspicion of VHL (see box). | Strong |
| • All patients with a clinical diagnosis of VHL (see box) should be offered VHL genetic testing. | Strong |
| • In patients with a suspected phenotype of VHL (see box), VHL genetic testing may be considered. | Weak |
| • Once a germline VHL mutation has been identified in a proband, VHL mutation analysis should be offered to all first-degree relatives as soon as possible*. | Strong |

** Or first-degree relatives of patients with clinical VHL who died before genetic testing was carried out.*

**Criteria for clinical diagnosis of VHL**

An individual with no known family history of VHL disease presenting with two or more characteristic lesions:

- Two or more hemangioblastomas of the retina, spine, or brain or a single hemangioblastoma in association with a visceral manifestation (e.g., multiple kidney or pancreatic cysts)
- Renal cell carcinoma (typically of the clear cell subtype)
- Adrenal or extra-adrenal pheochromocytoma
- Less commonly, endolymphatic sac tumour, papillary cystadenoma of the epididymis or broad ligament, or neuroendocrine tumour of the pancreas

An individual with a positive family history of VHL disease in whom one or more of the following disease manifestations is present:

- Retinal angioma
- Spinal or cerebellar hemangioblastoma
- Adrenal or extra-adrenal pheochromocytoma
- Renal cell carcinoma (typically of the clear cell subtype)
- Multiple renal and pancreatic cysts

**Criteria for clinical suspicion of VHL**

- Isolated central nervous system hemangioblastoma
- Isolated endolymphatic sac tumour
- Isolated renal cell carcinoma (typically of the clear cell subtype) at an age < 40 years
- Multiple renal cell carcinomas (typically of the clear cell subtype)
- Renal cell carcinoma (typically of the clear cell subtype) and a first- or second-degree relative with a typical VHL-tumour
- Phaeochromocytoma or paraganglioma (if no SDH mutation)
- Isolated papillary cystadenoma of the epididymis
- Bilateral epididymal cysts
- Two or more pancreatic serous cystadenomas
- Two or more pancreatic neuroendocrine tumours
- Pancreatic serous cystadenoma or neuroendocrine tumour, and first- or second-degree relative with a typical VHL-tumour
- Multiple pancreatic cysts and another typical VHL-tumour

## 3.4 Paraganglioma and phaeochromocytoma

### *3.4.1 Evidence from indexed literature*

Thirty-seven primary studies were included: 18 studies with paraganglioma patients,[23-40] 6 studies with phaechromocytoma patients[41-46] and 13 studies with a mixed population of paraganglioma and phaeochromocytoma patients.[47-59] Thirty-five studies reported on clinical and tumour characteristics and their relation with the genotype, three studies reported on genetic screening of relatives.[23, 29, 31]

#### *3.4.1.1 Diagnostic accuracy of individual clinical and tumour characteristics for the detection of mutations*

**Familial disease**

Twenty-one studies (published in 21 papers) including 6 197 patients contained sufficient information to calculate the diagnostic accuracy of familial disease for the detection of mutations (Table 7).[23, 26, 28, 30, 33, 34, 38-41, 47-55, 57, 58] For the detection of mutations in patients with paraganglioma and/or phaeochromocytoma, familial disease has a high specificity (pooled 97%, 95%CI 95-99%) and PPV (median 91%), but a low sensitivity (pooled 43%, 95%CI 33-54%) and NPV (median 76%). With an estimated pre-test probability of 30% and on 1 000 evaluated patients, 171 patients without familial disease would wrongly be predicted as having no mutation, while 21 patients with familial disease would wrongly be predicted as having a mutation.

For the detection of SDH mutations in the general population of paraganglioma and/or phaeochromocytoma patients, the diagnostic accuracy results are similar to the general results, with false negatives of around 100 per 1 000 evaluated patients (Table 7). In more specific populations, specificity remains high, but the NPV low, resulting in more false negative results.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD, VHL or RET), the specificity remains moderate to high, while the NPV becomes moderate to high too. This is accompanied by lower false negative results for most subpopulations (Table 7).

**Table 7 – Diagnostic accuracy of 'familial disease' for the detection of mutations in patients with paraganglioma and/or phaeochromocytoma. [*]**

| Genetic test | Population | N studies | N patients | Median prevalence | Sensitivity * | Specificity * | Median PPV | Median NPV | Results per 1 000 patients evaluated: Prevalence [$] | False negatives | False positives |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **General** | PHEO/PGL | 22 | 6197 | 38% | **43%** | **97%** | 91% | 76% | 30% | 171 | 21 |
| **SDH** | PHEO/PGL | 9 | 1715 | 51% | **43%** | **98%** | 99% | 71% | 19% | 108 | 16 |
| | PGL only | 7 | 1392 | 42% | **54%** | **99%** | 99% | 71% | 48% | 221 | 5 |
| | HNPGL | 3 | 641 | 51% | *40%* | *97%* | 97% | 58% | 65% | 390 | 11 |
| | Cervical PGL | 2 | 71 | 38% | *55%* | *100%* | 100% | 78% | 38% | 171 | 0 |
| | Malignant PHEO/PGL | 1 | 54 | 43% | *9%* | *90%* | 40% | 57% | 43% | 391 | 57 |
| **SDHB** | PHEO/PGL | 11 | 3496 | 17% | **32%** | **88%** | 36% | 90% | 10% | 68 | 108 |
| | PGL only | 4 | 1155 | 24% | **38%** | **80%** | 33% | 82% | 21% | 130 | 158 |
| | PHEO only | 1 | 989 | 7% | *19%* | *97%* | 33% | 94% | 7% | 57 | 28 |
| | HNPGL | 2 | 686 | 19% | *44%* | *79%* | 33% | 87% | 10% | 56 | 189 |
| | Malignant PHEO/PGL | 1 | 54 | 43% | *9%* | *90%* | 40% | 57% | 43% | 391 | 57 |
| | Parasympathetic PGL | 1 | 24 | 33% | *63%* | *81%* | 63% | 81% | 33% | 122 | 127 |
| **SDHC** | PHEO/PGL | 4 | 1504 | 3% | *6%* | *88%* | 2% | 97% | 1% | 9 | 119 |
| | PGL only | 2 | 1043 | 4% | *18%* | *83%* | 4% | 96% | 4% | 33 | 163 |
| | HNPGL | 1 | 598 | 4% | *12%* | *89%* | 4% | 96% | 4% | 35 | 106 |
| **SDHD** | PHEO/PGL | 11 | 3499 | 9% | **52%** | **89%** | 36% | 95% | 8% | 38 | 101 |
| | PGL only | 5 | 1212 | 29% | **56%** | **90%** | 66% | 75% | 23% | 101 | 77 |
| | PHEO only | 1 | 989 | 3% | *11%* | *96%* | 7% | 97% | 3% | 27 | 39 |
| | HNPGL | 2 | 686 | 25% | *53%* | *82%* | 58% | 83% | 51% | 240 | 88 |
| | Parasympathetic PGL | 2 | 81 | 43% | *51%* | *85%* | 69% | 70% | 43% | 211 | 85 |
| **VHL** | PHEO/PGL | 5 | 2018 | 5% | **28%** | **86%** | 13% | 96% | 6% | 43 | 132 |
| | PHEO only | 1 | 989 | 6% | *0%* | *96%* | 0% | 94% | 8% | 80 | 37 |
| **RET** | PHEO/PGL | 4 | 1605 | 3% | **52%** | **88%** | 6% | 99% | 4% | 19 | 115 |
| | PHEO only | 1 | 989 | 3% | *0%* | *96%* | 0% | 97% | 5% | 50 | 38 |

[*] *Italic = medians, bold = pooled.* [$] *Medians from unselected populations.*

### Multiple tumours

Seventeen studies including 3 965 patients contained sufficient information to calculate the diagnostic accuracy of having multiple tumours for the detection of mutations (Table 8).[23, 26, 28, 30, 33, 34, 37-39, 41, 45, 51, 53, 56-59] For the detection of mutations in patients with paraganglioma and/or phaeochromocytoma, having multiple tumours has a high specificity (pooled 94%, 95%CI 90-96%), a moderate PPV (median 83%), but a low sensitivity (pooled 41%, 95%CI 33-50%) and NPV (median 75%). With an estimated pre-test probability of 30% and on 1 000 evaluated patients, 177 patients with a single tumour would wrongly be predicted as having no mutation, while 42 patients with multiple tumours would wrongly be predicted as having a mutation.

For the detection of SDH mutations in the general population of paraganglioma and/or phaeochromocytoma patients, the diagnostic accuracy results are similar to the general results, with false negatives of

around 100 per 1 000 evaluated patients (Table 8). In more specific populations, specificity remains moderate to high, but the NPV low, resulting in more false negative results.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD, VHL or RET), lower specificities are found (with the exception of SDHD), while the NPV becomes moderate to high. This is accompanied by lower false negative results for most subpopulations (Table 8), but often also more false positive results.

**Table 8 – Diagnostic accuracy of 'multiple tumours' for the detection of mutations in patients with paraganglioma and/or phaeochromocytoma. [*]**

| | | | | Median | | | Median | Median | Results per 1 000 patients evaluated | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Genetic test | Population | N studies | N patients | prevalence | Sensitivity * | Specificity * | PPV | NPV | Prevalence [$] | False negatives | False positives |
| **General** | PHEO/PGL | 17 | 3965 | 35% | **41%** | **94%** | 83% | 75% | 30% | 177 | 42 |
| **SDH** | PHEO/PGL | 7 | 1635 | 41% | **52%** | **92%** | 92% | 78% | 19% | 91 | 65 |
| | PGL only | 6 | 1366 | 41% | **53%** | **92%** | 94% | 73% | 48% | 226 | 42 |
| | HNPGL | 3 | 850 | 41% | *73%* | *80%* | 83% | 78% | 65% | 175 | 70 |
| | Cervical PGL | 2 | 71 | 38% | *61%* | *100%* | 100% | 81% | 38% | 148 | 0 |
| **SDHB** | PHEO/PGL | 12 | 3738 | 9% | **21%** | **82%** | 8% | 92% | 10% | 79 | 162 |
| | PGL only | 5 | 1353 | 20% | **18%** | **72%** | 2% | 78% | 21% | 172 | 221 |
| | PHEO only | 2 | 1260 | 6% | *14%* | *87%* | 6% | 94% | 7% | 60 | 121 |
| | HNPGL | 2 | 833 | 7% | *31%* | *58%* | 6% | 92% | 10% | 69 | 378 |
| | Parasympathetic PGL | 1 | 24 | 33% | *0%* | *69%* | 0% | 58% | 33% | 330 | 208 |
| **SDHC** | PHEO/PGL | 5 | 2005 | 2% | **17%** | **83%** | 0% | 97% | 1% | 8 | 168 |
| | PGL only | 2 | 1043 | 4% | *25%* | *80%* | 5% | 96% | 4% | 30 | 192 |
| | HNPGL | 1 | 598 | 4% | *19%* | *87%* | 6% | 96% | 4% | 32 | 192 |
| **SDHD** | PHEO/PGL | 13 | 3795 | 9% | **58%** | **90%** | 33% | 94% | 8% | 34 | 92 |
| | PGL only | 6 | 1410 | 29% | **61%** | **94%** | 89% | 88% | 23% | 90 | 46 |
| | PHEO only | 2 | 1260 | 3% | *49%* | *88%* | 13% | 98% | 3% | 15 | 116 |
| | HNPGL | 2 | 833 | 49% | *67%* | *75%* | 79% | 61% | 51% | 168 | 122 |
| | Parasympathetic PGL | 2 | 81 | 43% | *59%* | *96%* | 94% | 73% | 43% | 176 | 23 |
| **VHL** | PHEO/PGL | 6 | 2116 | 6% | **46%** | **88%** | 18% | 97% | 6% | 32 | 113 |
| | PHEO only | 2 | 1260 | 8% | *50%* | *90%* | 33% | 95% | 8% | 40 | 92 |
| **RET** | PHEO/PGL | 6 | 2116 | 3% | **50%** | **87%** | 10% | 98% | 4% | 20 | 125 |
| | PHEO only | 2 | 1260 | 4% | *55%* | *88%* | 16% | 98% | 5% | 22 | 114 |

[*] *Italic = medians, bold = pooled.* [$] *Medians from unselected populations.*

### Bilateral tumours

Six studies including 1 083 patients contained sufficient information to calculate the diagnostic accuracy of having bilateral tumours for the detection of mutations (Table 9).[37, 40, 48, 50, 51, 59] For the detection of mutations in patients with paraganglioma and/or phaeochromocytoma, having bilateral tumours has a good pooled specificity (91%, 95%CI 67-98%), but a low sensitivity (pooled 37%, 95%CI 23-53%), PPV (median 79%) and NPV (median 71%). With an estimated pre-test probability of 30% and on 1 000 evaluated patients, 189 patients without bilateral tumours would wrongly be predicted as having no mutation, while 63 patients with bilateral tumours would wrongly be predicted as having a mutation.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD, VHL or RET), lower specificities are found (with the exception of VHL and RET), while the NPV becomes moderate to high. This is accompanied by lower false negative results for most subpopulations (except for SDHB) (Table 9), but often also more false positive results.

**Table 9 – Diagnostic accuracy of 'bilateral tumours' for the detection of mutations in patients with paraganglioma and/or phaeochromocytoma. [*]**

| | | | | Median | | | Median | Median | Results per 1 000 patients evaluated | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Genetic test | Population | N studies | N patients | prevalence | Sensitivity * | Specificity * | PPV | NPV | Prevalence [$] | False negatives | False positives |
| **General** | PHEO/PGL | 6 | 1083 | 32% | **37%** | **91%** | 79% | 71% | 30% | 189 | 63 |
| **SDH** | HNPGL | 1 | 26 | 62% | *81%* | *80%* | 87% | 73% | 65% | 123 | 70 |
| **SDHB** | PHEO/PGL | 4 | 1004 | 12% | **0%** | **74%** | 0% | 82% | 10% | 100 | 234 |
| | Sporadic PGL | 1 | 51 | 20% | *0%* | *85%* | 0% | 78% | 20% | 200 | 120 |
| **SDHC** | PHEO/PGL | 2 | 639 | 0,5% | *0%* | *59%* | 0% | 99% | 1% | 10 | 406 |
| **SDHD** | PHEO/PGL | 4 | 1004 | 7% | **17%** | **83%** | 5% | 91% | 8% | 66 | 156 |
| | Sporadic PGL | 1 | 51 | 18% | *56%* | *98%* | 83% | 91% | 18% | 79 | 16 |
| **VHL** | PHEO/PGL | 3 | 953 | 6% | *68%* | *89%* | 20% | 97% | 6% | 19 | 103 |
| **RET** | PHEO/PGL | 3 | 953 | 5% | *69%* | *90%* | 27% | 98% | 4% | 12 | 96 |

[*] *Italic = medians, bold = pooled.* [$] *Medians from unselected populations.*

### Malignant tumours

Sixteen studies (published in 15 papers) including 3 656 patients contained sufficient information to calculate the diagnostic accuracy of having malignancy for the detection of mutations (Table 10).[23, 28, 30, 32, 34, 39, 41, 43, 44, 48, 51, 53, 55, 58, 59] For the detection of mutations in patients with paraganglioma and/or phaeochromocytoma, having malignancy has a high specificity (pooled 94%, 95%CI 88-97%), but a low sensitivity (pooled 11%, 95%CI 6-18%), PPV (median 36%) and NPV (median 70%). With an estimated pre-test probability of 30% and on 1 000 evaluated patients, 267 patients without malignancy would wrongly be predicted as having no mutation, while 42 patients with malignancy would wrongly be predicted as having a mutation.

For the detection of SDH mutations in the general population of paraganglioma and/or phaeochromocytoma patients, the diagnostic accuracy results are similar to the general results, with false negatives of 167 per 1 000 evaluated patients (Table 10). In more specific populations, specificity remains moderate to high (with the exception of sympathetic paraganglioma), but the NPV low, resulting in more false negative results.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD, VHL or RET), also moderate to high specificities are found, while the NPV becomes moderate to high too. This is accompanied by lower false negative results for most subpopulations (Table 10), and often acceptable false positive results.

**Table 10 – Diagnostic accuracy of 'malignant tumours' for the detection of mutations in patients with paraganglioma and/or phaeochromocytoma. [*]**

| Genetic test | Population | N studies | N patients | Median prevalence | Sensitivity * | Specificity * | Median PPV | Median NPV | Results per 1 000 patients evaluated: Prevalence [$] | False negatives | False positives |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **General** | PHEO/PGL | 16 | 3656 | 32% | **11%** | **94%** | 36% | 70% | 30% | 267 | 42 |
| **SDH** | PHEO/PGL | 5 | 900 | 41% | *12%* | *97%* | 52% | 59% | 19% | 167 | 24 |
| | HNPGL | 3 | 850 | 41% | *2%* | *100%* | 83% | 59% | 65% | 637 | 0 |
| | Cervical PGL | 1 | 23 | 35% | *13%* | *87%* | 33% | 65% | 38% | 331 | 81 |
| | Sympathetic PGL | 1 | 27 | 41% | *55%* | *38%* | 38% | 55% | 43% | 193 | 353 |
| **SDHB** | PHEO/PGL | 11 | 3546 | 11% | **25%** | **94%** | 29% | 91% | 10% | 75 | 54 |
| | PGL only | 4 | 945 | 19% | **6%** | **97%** | 17% | 82% | 21% | 197 | 24 |
| | PHEO only | 2 | 1071 | 7% | *55%* | *86%* | 23% | 96% | 7% | 31 | 130 |
| | HNPGL | 3 | 921 | 11% | *4%* | *97%* | 33% | 91% | 10% | 96 | 27 |
| | Parasympathetic PGL | 1 | 24 | 33% | *0%* | *94%* | 0% | 65% | 33% | 330 | 40 |
| **SDHC** | PHEO/PGL | 3 | 1291 | 1% | *0%* | *94%* | 0% | 99% | 1% | 10 | 59 |
| | HNPGL | 1 | 598 | 4% | *0%* | *94%* | 0% | 95% | 4% | 40 | 58 |
| **SDHD** | PHEO/PGL | 10 | 3464 | 9% | **5%** | **92%** | 7% | 89% | 8% | 76 | 74 |
| | PGL only | 4 | 645 | 32% | **4%** | **97%** | 30% | 67% | 23% | 221 | 23 |
| | PHEO only | 1 | 989 | 3% | *4%* | *91%* | 1% | 97% | 3% | 29 | 87 |
| | HNPGL | 3 | 921 | 35% | *3%* | *96%* | 33% | 65% | 51% | 495 | 20 |
| | Parasympathetic PGL | 1 | 24 | 29% | *0%* | *94%* | 0% | 70% | 43% | 430 | 34 |
| **VHL** | PHEO/PGL | 7 | 2534 | 6% | **6%** | **88%** | 4% | 93% | 6% | 56 | 113 |
| | PHEO only | 2 | 1004 | 6% | *4%* | *88%* | 2% | 93% | 8% | 77 | 110 |
| **RET** | PHEO/PGL | 6 | 2121 | 4% | *0%* | *91%* | 0% | 95% | 4% | 40 | 86 |
| | PHEO only | 2 | 1004 | 3% | *0%* | *91%* | 0% | 97% | 5% | 50 | 86 |

[*] *Italic = medians, bold = pooled.* [$] *Medians from unselected populations.*

### Metastatic disease

Four studies including 534 patients contained sufficient information to calculate the diagnostic accuracy of having metastatic disease for the detection of SDH mutations (Table 11).[24, 25, 40, 57] For the detection of SDH mutations in patients with paraganglioma and/or phaeochromocytoma, having metastatic disease has a high specificity (median 93%, range 80-100%), but a low sensitivity (median 13%, range 11-31%), PPV (median 71%) and NPV (median 70%). With an estimated pre-test probability of 19% and on 1 000 evaluated patients, 165 patients without metastatic disease would wrongly be predicted as having no SDH mutation, while 57 patients with metastatic disease would wrongly be predicted as having a SDH mutation.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD), a high specificity and NPV are found for SDHC and SDHD (1 study), but a low to moderate specificity and a low NPV for SDHB. This is accompanied by lower false negative results and acceptable false positive results for SDHC and SDHD (Table 11), but not for SDHB.

**Table 11 – Diagnostic accuracy of 'metastatic disease' for the detection of SDH mutations in patients with paraganglioma and/or phaeochromocytoma.** *

| Genetic test | Population | N studies | N patients | Median prevalence | Sensitivity * | Specificity * | Median PPV | Median NPV | Results per 1 000 patients evaluated: Prevalence $ | False negatives | False positives |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **SDH** | PHEO/PGL | 3 | 490 | 32% | *13%* | *93%* | 71% | 70% | 19% | 165 | 57 |
| | HNPGL | 2 | 221 | 47% | *21%* | *90%* | 86% | 56% | 65% | 513 | 35 |
| **SDHB** | PHEO/PGL | 2 | 313 | 24% | *29%* | *82%* | 34% | 77% | 10% | 71 | 162 |
| | Malignant PGL | 1 | 44 | 41% | *33%* | *69%* | 43% | 60% | 41% | 275 | 183 |
| **SDHC** | PHEO/PGL | 1 | 269 | 8% | *0%* | *92%* | 0% | 92% | 1% | 10 | 79 |
| **SDHD** | PHEO/PGL | 1 | 269 | 5% | *0%* | *92%* | 0% | 95% | 8% | 80 | 74 |

* *Italic = medians, bold = pooled.* $ *Medians from unselected populations.*

### Recurrence

Four smaller studies including 176 patients contained sufficient information to calculate the diagnostic accuracy of recurrent disease for the detection of mutations (Table 12).[33, 37, 39, 59] For the detection of mutations in patients with paraganglioma and/or phaeochromocytoma, recurrent disease has a high specificity (pooled 92%, 95%CI 77-97%), but a low sensitivity (pooled 12%, 95%CI 3-38%), PPV (median 57%) and NPV (median 61%). With an estimated pre-test probability of 30% and on 1 000 evaluated patients, 264 patients without recurrence would wrongly be predicted as having no mutation, while 56 patients with recurrence would wrongly be predicted as having a mutation.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD, VHL or RET), also moderate to high specificities are found, but the NPV remains low (with the exception of VHL and RET, 1 small study). This is accompanied by high false negative results for most subpopulations (again with the exception of VHL and RET, 1 small study) (Table 12).

**Table 12 – Diagnostic accuracy of 'recurrent disease' for the detection of mutations in patients with paraganglioma and/or phaeochromocytoma. [*]**

| Genetic test | Population | N studies | N patients | Median prevalence | Sensitivity * | Specificity * | Median PPV | Median NPV | Results per 1 000 patients evaluated | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Prevalence [$] | False negatives | False positives |
| **General** | PHEO/PGL | 4 | 176 | 39% | **12%** | **92%** | 57% | 61% | 30% | 264 | 56 |
| **SDH** | Cervical PGL | 1 | 48 | 42% | *35%* | *86%* | 64% | 65% | 38% | 247 | 87 |
| **SDHB** | PHEO/PGL | 3 | 128 | 20% | *0%* | *94%* | 0% | 78% | 10% | 100 | 54 |
| | PGL only | 2 | 75 | 26% | *6%* | *90%* | 25% | 73% | 21% | 197 | 79 |
| | Parasympathetic PGL | 1 | 24 | 33% | *13%* | *94%* | 50% | 68% | 33% | 287 | 40 |
| **SDHC** | - | | | | | | | | | | |
| **SDHD** | PHEO/PGL | 4 | 185 | 23% | **12%** | **92%** | 25% | 76% | 8% | 70 | 74 |
| | PGL only | 3 | 132 | 29% | *13%* | *88%* | 50% | 73% | 23% | 200 | 92 |
| | Parasympathetic PGL | 2 | 81 | 43% | *13%* | *91%* | 54% | 58% | 43% | 374 | 51 |
| **VHL** | PHEO/PGL | 1 | 53 | 2% | *100%* | *98%* | 50% | 100% | 6% | 0 | 19 |
| **RET** | PHEO/PGL | 1 | 53 | 4% | *0%* | *96%* | 0% | 96% | 4% | 40 | 38 |

[*] *Italic = medians, bold = pooled.* [$] *Medians from unselected populations.*

### Extra-adrenal disease

Nine studies including 2 379 patients contained sufficient information to calculate the diagnostic accuracy of having extra-adrenal disease for the detection of mutations (Table 13).[30, 41, 43-45, 48, 53, 55, 59] For the detection of mutations in patients with paraganglioma and/or phaeochromocytoma, having extra-adrenal disease has a moderate specificity (pooled 89%, 95%CI 82-93%), but a low sensitivity (pooled 26%, 95%CI 16-38%), PPV (median 44%) and NPV (median 77%). With an estimated pre-test probability of 30% and on 1 000 evaluated patients, 222 patients without extra-adrenal disease would wrongly be predicted as having no mutation, while 77 patients with extra-adrenal disease would wrongly be predicted as having a mutation.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD, VHL or RET), mostly moderate specificities are found (with the exception for the head and neck paragangliomas), while the NPV becomes high. This is accompanied by lower false negative results for most subpopulations (Table 13), but the false positive results remain above 100.

**Table 13 – Diagnostic accuracy of 'extra-adrenal disease' for the detection of mutations in patients with paraganglioma and/or phaeochromocytoma.***

| Genetic test | Population | N studies | N patients | Median prevalence | Sensitivity * | Specificity * | Median PPV | Median NPV | Results per 1 000 patients evaluated: Prevalence $ | False negatives | False positives |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **General** | PHEO/PGL | 9 | 2379 | 25% | **26%** | **89%** | 44% | 77% | 30% | 222 | 77 |
| **SDH** | HNPGL | 1 | 157 | 89% | *7%* | *100%* | 100% | 12% | 65% | 604 | 0 |
| **SDHB** | PHEO/PGL | 8 | 2392 | 8% | **56%** | **86%** | 30% | 95% | 10% | 44 | 126 |
| | PHEO only | 3 | 1344 | 7% | *63%* | *87%* | 28% | 97% | 7% | 26 | 121 |
| | HNPGL | 1 | 157 | 5% | *0%* | *93%* | 0% | 95% | 10% | 100 | 63 |
| | Malignant PHEO/PGL | 1 | 54 | 43% | *70%* | *71%* | 64% | 76% | 43% | 129 | 165 |
| **SDHC** | - | | | | | | | | | | |
| **SDHD** | PHEO/PGL | 6 | 2254 | 4% | **31%** | **85%** | 11% | 97% | 8% | 55 | 138 |
| | PHEO only | 2 | 1260 | 3% | *49%* | *87%* | 12% | 98% | 3% | 15 | 126 |
| | HNPGL | 1 | 157 | 81% | *8%* | *100%* | 100% | 20% | 51% | 469 | 0 |
| **VHL** | PHEO/PGL | 6 | 2112 | 6% | **22%** | **81%** | 6% | 93% | 6% | 47 | 179 |
| | PHEO only | 3 | 1275 | 7% | *13%* | *86%* | 6% | 92% | 8% | 70 | 129 |
| **RET** | PHEO/PGL | 5 | 1699 | 5% | *0%* | *82%* | 0% | 95% | 4% | 40 | 173 |
| | PHEO only | 3 | 1275 | 5% | *0%* | *85%* | 0% | 95% | 5% | 50 | 142 |

* *Italic = medians, bold = pooled.* $ *Medians from unselected populations.*

### Secretory tumours

Four studies including 568 patients contained sufficient information to calculate the diagnostic accuracy of having a secretory tumour for the detection of mutations (Table 14).[23, 39, 40, 58] For the detection of mutations in patients with paraganglioma and/or phaeochromocytoma, having a secretory tumour has a low specificity (pooled 78%, 95%CI 36-96%), sensitivity (pooled 24%, 95%CI 6-62%), PPV (median 50%) and NPV (median 51%). With an estimated pre-test probability of 30% and on 1 000 evaluated patients, 228 patients without a secretory tumour would wrongly be predicted as having no mutation, while 154 patients with a secretory tumour would wrongly be predicted as having a mutation.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD, VHL or RET), mostly low specificities are found, while the NPV becomes high (with the exception of SDHB and SDHD). This is accompanied by lower false negative results (Table 14), but the false positive results remain high.

**Table 14 – Diagnostic accuracy of 'secretory tumours' for the detection of mutations in patients with paraganglioma and/or phaeochromocytoma. [*]**

| Genetic test | Population | N studies | N patients | Median prevalence | Sensitivity * | Specificity * | Median PPV | Median NPV | Results per 1 000 patients evaluated: Prevalence [$] | False negatives | False positives |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **General** | PHEO/PGL | 4 | 568 | 51% | **24%** | **78%** | 50% | 51% | 30% | 228 | 154 |
| **SDH** | HNPGL | 2 | 43 | 51% | *21%* | *90%* | 75% | 52% | 65% | 513 | 35 |
| **SDHB** | PHEO/PGL | 3 | 579 | 33% | *83%* | *21%* | 5% | 64% | 10% | 17 | 711 |
| | Parasympathetic PGL | 1 | 24 | 33% | *0%* | *88%* | 0% | 64% | 33% | 330 | 80 |
| | Malignant PHEO/PGL | 1 | 54 | 43% | *83%* | *6%* | 40% | 33% | 43% | 73 | 536 |
| **SDHC** | PHEO/PGL | 1 | 501 | 1% | *25%* | *21%* | 0% | 97% | 1% | 7 | 782 |
| **SDHD** | PHEO/PGL | 2 | 525 | 19% | *35%* | *56%* | 28% | 76% | 8% | 52 | 405 |
| | Parasympathetic PGL | 1 | 24 | 29% | *14%* | *94%* | 50% | 73% | 43% | 370 | 34 |
| **VHL** | PHEO/PGL | 1 | 501 | 10% | *96%* | *23%* | 12% | 98% | 6% | 2 | 724 |
| **RET** | PHEO/PGL | 1 | 501 | 5% | *100%* | *22%* | 7% | 100% | 4% | 0 | 749 |

[*] *Italic = medians, bold = pooled.* [$] *Medians from unselected populations.*

### Head-and-neck location

Seven studies including 2 012 patients contained sufficient information to calculate the diagnostic accuracy of a head-and-neck location for the detection of mutations (Table 15).[26, 33, 50, 51, 53, 55, 57] For the detection of mutations in patients with paraganglioma and/or phaeochromocytoma, a head-and-neck location has a low specificity (pooled 70%, 95%CI 32-92%), sensitivity (pooled 45%, 95%CI 23-70%), PPV (median 49%) and NPV (median 64%). With an estimated pre-test probability of 30% and on 1 000 evaluated patients, 165 patients without a head-and-neck location would wrongly be predicted as having no mutation, while 210 patients with a head-and-neck location would wrongly be predicted as having a mutation.

For the detection of SDH mutations in the general population of paraganglioma and/or phaeochromocytoma patients, the diagnostic accuracy results are contradictory to the general results, with false negatives of 47 per 1 000 evaluated patients (but more false positives) (Table 15). In more specific populations, specificity and NPV remain low.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD, VHL or RET), also low to moderate specificities are found, while the NPV becomes high (with the exception of SDHB). This is accompanied by lower false negative results for most subpopulations (Table 15), but still high false positive results.

**Table 15 – Diagnostic accuracy of 'head-and-neck location' for the detection of mutations in patients with paraganglioma and/or phaeochromocytoma.** *

| Genetic test | Population | N studies | N patients | Median prevalence | Sensitivity * | Specificity * | Median PPV | Median NPV | Results per 1 000 patients evaluated: Prevalence $ | False negatives | False positives |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **General** | PHEO/PGL | 7 | 2012 | 39% | **45%** | **70%** | 49% | 64% | 30% | 165 | 210 |
| **SDH** | PHEO/PGL | 3 | 762 | 42% | *75%* | *27%* | 42% | 48% | 19% | 47 | 591 |
| | PGL only | 2 | 493 | 48% | *83%* | *14%* | 47% | 48% | 48% | 82 | 380 |
| | Cervical PGL | 1 | 48 | 42% | *90%* | *0%* | 39% | 0% | 38% | 38 | 620 |
| **SDHB** | PHEO/PGL | 6 | 1964 | 15% | *23%* | *82%* | 17% | 85% | 10% | 77 | 162 |
| | PGL only | 1 | 445 | 22% | *43%* | *17%* | 12% | 52% | 21% | 120 | 656 |
| **SDHC** | PHEO/PGL | 4 | 1353 | 1% | **75%** | **75%** | 4% | 99% | 1% | 2 | 248 |
| | PGL only | 1 | 445 | 4% | *88%* | *26%* | 4% | 98% | 4% | 5 | 710 |
| **SDHD** | PHEO/PGL | 6 | 1964 | 8% | **79%** | **82%** | 29% | 97% | 8% | 17 | 166 |
| | PGL only | 1 | 445 | 29% | *98%* | *36%* | 38% | 97% | 23% | 5 | 493 |
| **VHL** | PHEO/PGL | 4 | 1220 | 5% | **3%** | **81%** | 0% | 94% | 6% | 58 | 179 |
| **RET** | PHEO/PGL | 3 | 749 | 3% | *0%* | *79%* | 0% | 97% | 4% | 40 | 202 |

* *Italic = medians, bold = pooled.* $ *Medians from unselected populations.*

### Age

Seven studies including 2 442 patients contained sufficient information to calculate the diagnostic accuracy of different age cut-offs for the detection of mutations (Table 16).[26, 34, 39, 41, 44-46] Only the age categories <18y and <20y appear to have a moderate to high specificity in combination with a moderate to high NPV, but an ideal combination of false negative and positive results is never available.

**Table 16 – Diagnostic accuracy of different age cut-offs for the detection of mutations in patients with paraganglioma and/or phaeochromocytoma. [*]**

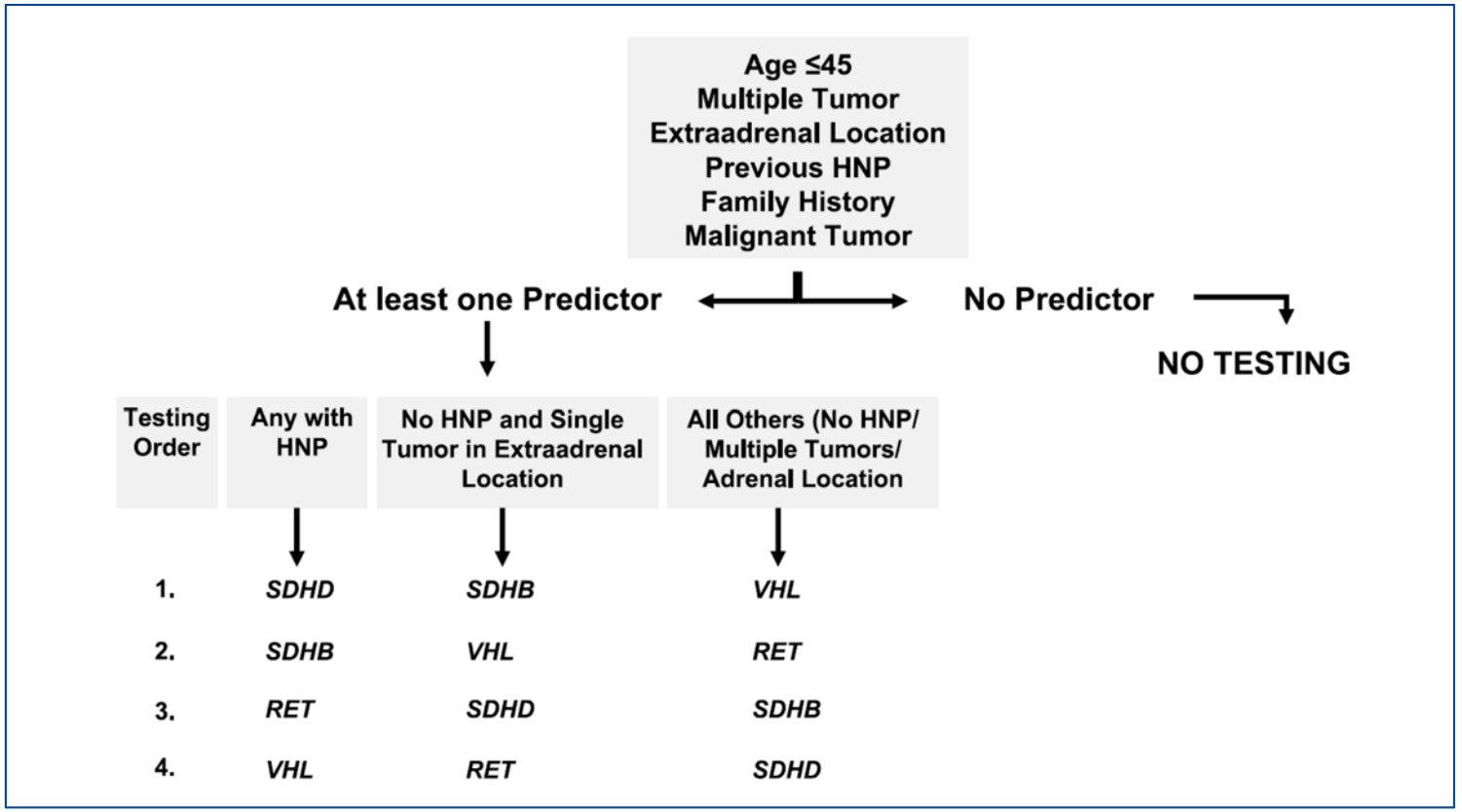
| | | | | | Median | | | Median | Median | Results per 1 000 patients evaluated | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Genetic test | Age cut-off | Population | N studies | N patients | prevalence | Sensitivity * | Specificity * | PPV | NPV | Prevalence [$] | False negatives | False positives |
| **General** | <18y | PHEO only | 1 | 271 | 24% | *41%* | *90%* | 56% | 83% | 24% | 142 | 76 |
| | <20y | PHEO/PGL | 1 | 100 | 8% | *13%* | *98%* | 33% | 93% | 30% | 261 | 14 |
| | <40y | PHEO/PGL | 2 | 124 | 35% | *58%* | *37%* | 33% | 55% | 30% | 126 | 441 |
| | <45y | PHEO only | 2 | 1004 | 19% | *59%* | *69%* | 32% | 89% | 24% | 98 | 236 |
| **SDH** | <35y | PGL only | 1 | 445 | 54% | *55%* | *83%* | 80% | 61% | 48% | 216 | 88 |
| | <40y | HNPGL | 1 | 598 | 31% | *59%* | *79%* | 55% | 81% | 65% | 266 | 74 |
| **SDHB** | <18y | PHEO only | 1 | 271 | 4% | *33%* | *83%* | 8% | 96% | 7% | 47 | 158 |
| | <35y | PGL only | 1 | 445 | 22% | *50%* | *66%* | 29% | 83% | 21% | 105 | 269 |
| | <40y | PGL only | 2 | 622 | 22% | *59%* | *44%* | 23% | 72% | 21% | 86 | 442 |
| | <45y | PHEO only | 1 | 989 | 7% | *77%* | *49%* | 11% | 96% | 7% | 16 | 474 |
| **SDHC** | <35y | PGL only | 1 | 445 | 4% | *38%* | *63%* | 4% | 96% | 4% | 25 | 355 |
| | <40y | HNPGL | 1 | 598 | 4% | *58%* | *69%* | 8% | 97% | 4% | 17 | 298 |
| **SDHD** | <18y | PHEO only | 1 | 271 | 4% | *27%* | *83%* | 6% | 96% | 3% | 22 | 165 |
| | <35y | PGL only | 1 | 445 | 29% | *60%* | *72%* | 47% | 81% | 23% | 92 | 216 |
| | <40y | PGL only | 2 | 622 | 22% | *67%* | *48%* | 29% | 79% | 23% | 76 | 400 |
| | <45y | PHEO only | 1 | 989 | 3% | *96%* | *49%* | 5% | 100% | 3% | 1 | 495 |
| **VHL** | <18y | PHEO only | 1 | 271 | 11% | *67%* | *88%* | 42% | 96% | 8% | 26 | 110 |
| | <45y | PHEO only | 2 | 1004 | 6% | *47%* | *64%* | 5% | 96% | 8% | 42 | 331 |
| **RET** | <18y | PHEO only | 1 | 271 | 5% | *0%* | *81%* | 0% | 94% | 5% | 50 | 180 |
| | <45y | PHEO only | 2 | 1004 | 8% | *62%* | *66%* | 19% | 95% | 5% | 19 | 323 |

[*] *Italic = medians, bold = pooled.* [$] *Medians from unselected populations.*

### *3.4.1.2 Published models and proposed screening algorithms*

Three articles report on the development of an algorithm for genetic screening. Erlic et al. retrospectively included 989 patients with apparently non-syndromic phaeochromocytoma, and tested them for germline mutations in the genes SDHB, SDHC, SDHD, VHL and RET.[41] Clinical parameters (age, gender, tumour number, tumour biology [benign vs. malignant], tumour location [adrenal, extra-adrenal, and concomitant adrenal with extra-adrenal], previous head-and-neck paraganglioma, and family history for paraganglial tumours) were analyzed as potential predictors for finding mutations by multiple logistic regression, validated by bootstrapping. Predictors for the presence of any mutation were age <45 years (OR 5.37, 95%CI 3.34 to 8.62), multiple pheochromocytoma (OR 8.78, 95%CI 5.47 to 14.08), extra-adrenal location (OR 4.93, 95%CI 3.00 to 8.10), and previous head-and-neck paraganglioma (OR 11.95, 95%CI 3.15 to 45.32). If the presence of any one predictor was used as indicative of proceeding with genetic testing, then 342 (34.6%) patients would be excluded, and only 8 carriers (4.3%) would be missed. The performance of the model was: sensitivity 95.7%, specificity 41.6%, PPV 23.0% and NPV 97.7%. Based on their analysis the authors also proposed an algorithm including a priority of genes to be tested according to specific clinical features (Figure 5). Importantly, this model has not been validated in an independent cohort.

**Figure 5 – Genetic testing algorithm for apparently non-syndromic phaeochromocytoma, proposed by Erlic et al.[41]**



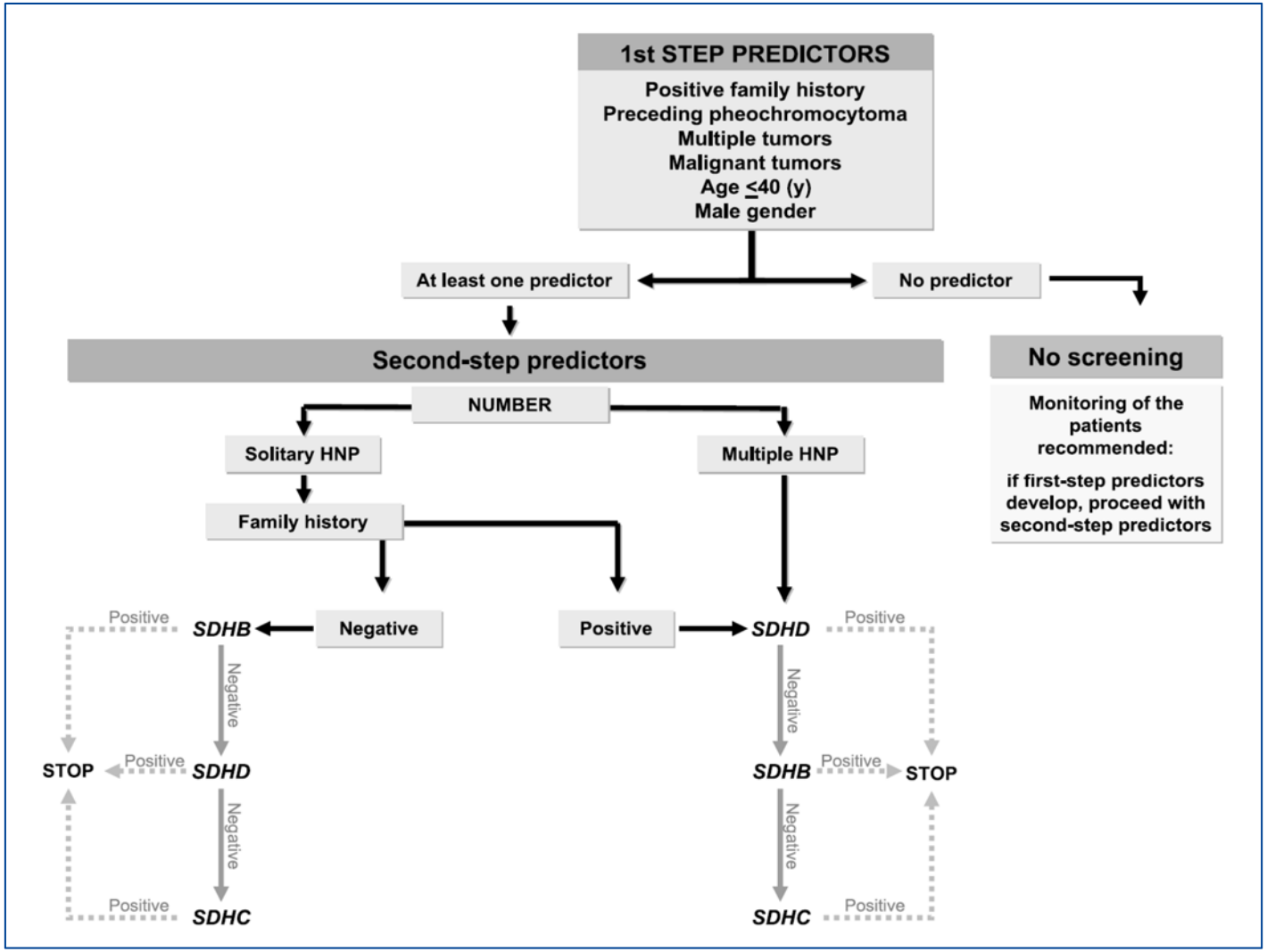

*HNP: head-and-neck paraganglioma.*

Jafri et al. prospectively included 501 patients with non-syndromic phaeochromocytoma, paraganglioma and head-and-neck paraganglioma.[55] Patients were tested for germline mutations in the genes SDHB, SDHD and VHL. Mutation detection rates were highest in those with a positive family history (62%), malignancy (53%), multiple tumours (33%) or paraganglioma (44%). Jafri et al. developed three models, each including positive family history, multiple tumours, extra-adrenal location and/or malignant disease, but with different age cut-offs (45y, 50y and 60y). While the model including the 45y cut-off achieved an overall mutation detection rate of 69% (97% of SDHD mutations, 95% of VHL mutations and 76% of SDHB mutations), the detection rate increased to 69.1% with the 50y cut-off and 76.4% with the 60y cut-off. However, this was at the cost of an increased number of tests undertaken. Again, this model was not validated in an independent cohort.

Finally, Neumann et al. included 598 patients with head-and-neck paraganglioma and tested them for germline mutations in the genes SDHB, SDHC and SDHD.[34] Six variables (age, gender, number of tumors [solitary or multiple], tumour biology [malignant or benign), previous paraganglial tumours, and family history for head-and-neck paraganglioma or pheochromocytoma) were included in a logistic regression model. Based on the model an algorithm was developed to identify which patients should be genetically tested for mutations. Bootstrap analysis was performed to assess the performance of the logistic regression model and the predictive ability of the testing algorithm, and to calculate the cost-reduction of our proposed testing strategy. Multiple logistic regression analysis indicated that 5 of the 6 variables (not tumour biology) included in the model were independently associated with presence of a germline mutation in one of the SDHx genes. If the presence of any one predictor was used as indicative of proceeding with genetic testing, then 272 (45.5%) patients would be excluded, and 15 carriers (8.2%) would be missed. The performance of the model was: sensitivity 91.8%, specificity 61.9%, PPV 51.5% and NPV 94.5%. The proposed algorithm is presented in Figure 6. This model was also not validated in an independent cohort.

Several other authors proposed genetic testing algorithms, although not based on multivariate analysis and modelling, but only based on univariate analysis (see appendix 6).[43, 48, 53, 54, 58] Most of these algorithms include as a first step the distinction between familial and/or syndromic cases versus sporadic cases. In cases with a familial and/or syndromic presentation, targeted genetic testing is recommended based on clinical features (e.g. RET testing in case of medullary thyroid carcinoma, VHL testing in case of hemangioblastoma, etc). For the apparently sporadic cases, the algorithms show some differences. For example only two algorithms include an age cut-off (30y [53] and 35y [43]). Most other clinical variables (head-and-neck location, malignant disease, extra-adrenal disease, multiple tumours, recurrence, etc) are not consistently used.

**Figure 6 – Genetic testing algorithm for apparently non-syndromic phaeochromocytoma, proposed by Neumann et al.[34]**




*HNP: head-and-neck paraganglioma.*

#### 3.4.1.3 Genetic screening of relatives

Bacca et al. screened 17 relatives of 4 unrelated patients with a non-syndromic head-and-neck paraganglioma and a known mutation of the SDHB, SDHC, SDHD, SDHAF2, VHL and RET genes.[23] Ten cases (58.8%; age range 17-71y) were found to carry a SDHD mutation, four of these (belonging to two families with the same type of mutation) were clinically affected. The remaining seven relatives were disease-free, but no indication was given about the duration of follow-up.

Hensen et al. included 243 relatives of a seven-generation family with head-and-neck paragangliomas and a D92Y missense mutation in the SDHD gene.[29] Of the 211 family members that were alive (6th and 7th generation), 189 accepted genetic testing. Of these, 63 tested positive (33%) and one other relative (that could not be tested) was found to be an obligate carrier (because of an affected offspring). In total, 53 paternal and 11 maternal mutation carriers were found. Using a Kaplan-Meier analysis, the authors calculated an overall clinical penetrance of 57% (30 symptomatic paragangliomas) with a maximum reached at 47y. The overall penetrance was 68% (an additional six paragangliomas detected with MRI screening), increasing to 87% at 70y.

Finally, Hes et al. included 19 relatives of an index patient with an extra-adrenal paraganglioma and a confirmed SDHB mutation.[31] Fourteen relatives (73.7%) were found to be mutation carriers. Of these eleven underwent clinical screening and two were identified with subclinical vagal paragangliomas. Penetrance, calculated with a Kaplan-Meier analysis, was 26% at 48y.

#### 3.4.1.4 Impact of genetic screening on outcomes

No studies were found that reported on the impact of genetic screening on clinical outcomes of patients with phaeochromocytoma and/or paraganglioma and their relatives.

#### 3.4.1.5 SDH immunohistochemistry

Five studies including 386 patients with phaeochromocytoma and/or paraganglioma evaluated the diagnostic accuracy of SDHB immunostaining for the detection of SDH mutations.[60-63] Two studies, a retrospective and a prospective, were reported in one article.[63] For the detection of SDH mutations in general, SDHB immunostaining has a high sensitivity (median 100%) and specificity (median 93%, range 84-97%), a moderate PPV (median 82%, range 67-96%) and a high NPV (median 100%) (Table 17). With an estimated pre-test probability of 20% and on 1 000 evaluated patients, no patient with positive SDHB immunostaining would wrongly be predicted as having no SDH mutation, while 56 patients with negative SDHB immunostaining would wrongly be predicted as having a SDH mutation.

When specific mutations are considered (i.e. SDHB, SDHC, SDHD), also high sensitivities and NPV are found, but the specificities and PPV become clearly lower. This is accompanied by no false negative results (Table 17), but higher false positive results.

**Table 17 – Diagnostic accuracy of SDH immunohistochemistry for the detection of SDH mutations in patients with paraganglioma and/or phaeochromocytoma. [*]**

| | | | | Median | | | Median | Median | Results per 1 000 patients evaluated | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Genetic test | Population | N studies | N patients | prevalence | Sensitivity * | Specificity * | PPV | NPV | Prevalence [$] | False negatives | False positives |
| **SDH** | PHEO/PGL | 5 | 386 | 22% | *100%* | *93%* | 82% | 100% | 20% | 0 | 56 |
| **SDHB** | PHEO/PGL | 4 | 341 | 12% | **100%** | **80%** | 42% | 100% | 10% | 0 | 180 |
| **SDHC** | PHEO/PGL | 2 | 233 | 2% | *100%* | *66%* | 6% | 100% | 1% | 0 | 337 |
| **SDHD** | PHEO/PGL | 4 | 341 | 8% | **100%** | **78%** | 33% | 100% | 8% | 0 | 202 |

[*] *Italic = medians, bold = pooled.* [$] *Medians from unselected populations.*

### 3.4.2 Overview of published guidelines

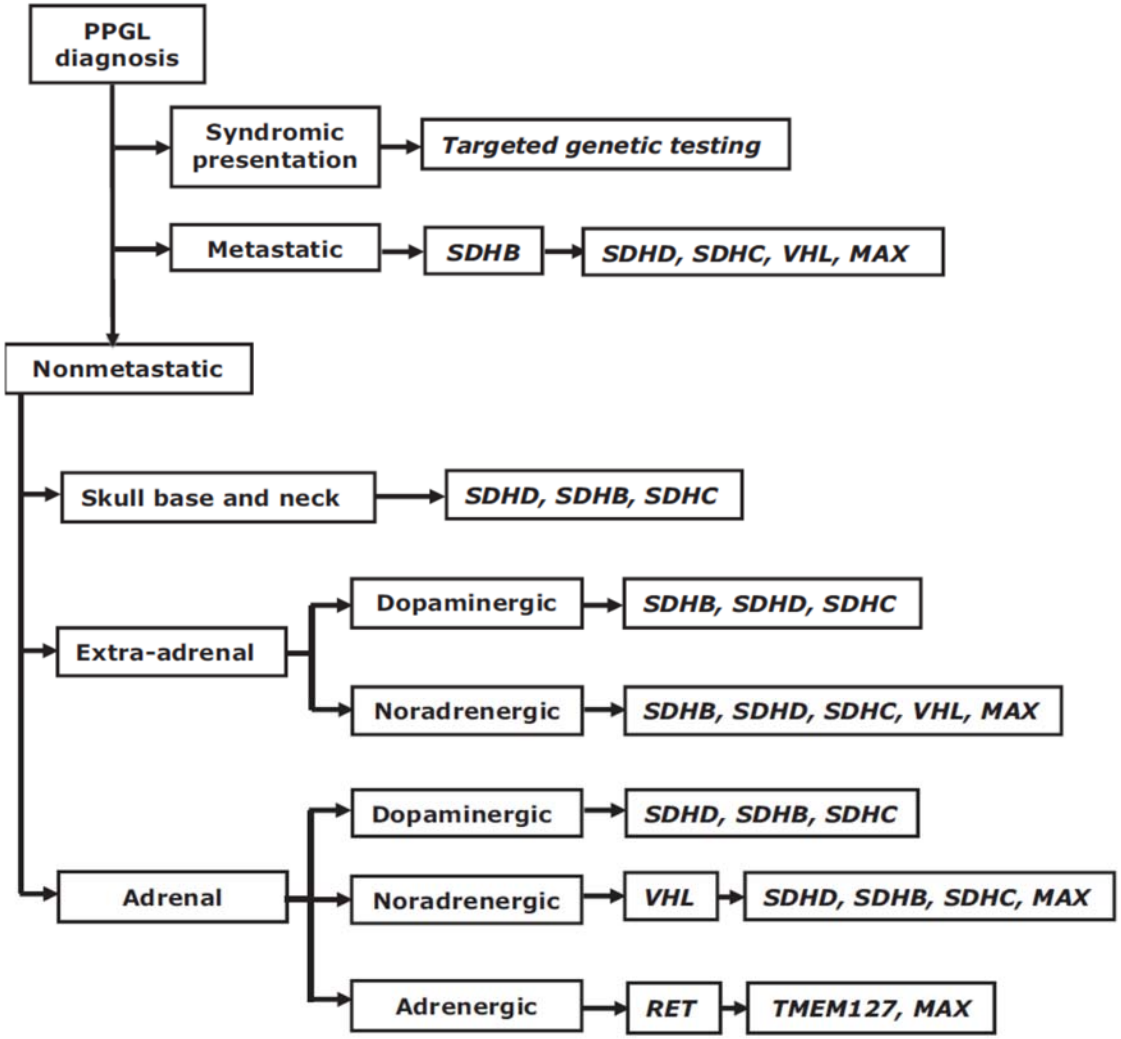
Three consensus-based guidelines contain recommendations about mutation testing in patients with phaeochromocytoma / paraganglioma or their relatives.[4, 6, 64] The AACE 2009 and STOET 2010 guidelines lack a good description of their methodology. The Endocrine Society 2014 guideline contains a more detailed description of the used methodology, but still clearly is consensus-based. None of the guidelines contains detailed quality appraisal results of the included studies or evidence tables.

Both the Endocrine Society 2014 and STOET 2010 guidelines recommend the use of clinical features to guide mutation testing (Table 18). However, only the Endocrine Society 2014 guideline contains a specific and clear algorithm (Figure 7). Similar to previously mentioned algorithms, targeted genetic testing is recommended in case of a syndromic presentation. However, clinical features that are not used in previously mentioned algorithms are metastatic disease and biochemical presentation.

**Table 18 – Overview of published guidelines on mutation testing for phaeochromocytoma / paraganglioma.**

| Guideline | Recommendation | AGREE II score 'Methodology' |
|---|---|---|
| **Endocrine Society 2014** | We recommend that all patients with PPGLs should be engaged in shared decision making for genetic testing | 29.2% |
| | We recommend the use of a clinical feature-driven diagnostic algorithm to establish the priorities for specific genetic testing in PPGL patients with suspected germline mutations | |
| | We suggest that patients with paraganglioma undergo testing of SDH mutations and that patients with metastatic disease undergo testing for *SDHB* mutations | |
| | We recommend that genetic testing for PPGL be delivered within the framework of health care. Specifically, pretest and post-test counseling should be available. All tests for PPGL genetic testing should be performed by accredited laboratories | |
| **AACE 2009** | About one-quarter of patients with a pheochromocytoma will have associated familial syndromes caused by mutations in the *RET* gene (multiple endocrine neoplasia type 2), *VHL* gene (von Hippel-Lindau disease), or succinate dehydrogenase genes; genetic study and counseling should be performed, especially for young patients or patients with an extra-adrenal pheochromocytoma | 16.7% |
| **STOET 2010** | **SDH**<br>SDH mutation analysis in patients with:<br>• Head-and-neck paraganglioma<br>• Phaeochromocytoma and familial head-and-neck paraganglioma / phaeochromocytoma<br>• Sporadic phaeochromocytoma < 50y | 6.3% |
| | Order of testing: SDHD, SDHB, SDHC (driven by clinical features: extra-adrenal and malignant phaeochromocytoma first SDHB) | |
| | In case of known SDH mutation: SDH mutation analysis in relatives at young adult age | |
| | **RET**<br>RET mutation analysis in patients with sporadic phaeochromocytoma <50y | |
| | In case of known RET mutation: RET mutation analysis in first-degree relatives at young age (0-5 years) | |
| | **VHL**<br>VHL mutation analysis in patients with VHL syndrome and phaeochromocytoma | |
| | In case of known VHL mutation: VHL mutation analysis in relatives at young age | |

**Figure 7 – Genetic testing algorithm for phaeochromocytoma and paraganglioma, proposed by the Endocrine Society.[4]**




*PPGL: phaeochromocytoma / paraganglioma.*

**Conclusions**

- No validated models / algorithms exist for genetic testing of patients with phaeochromocytoma and/or paraganglioma.
- The available algorithms generally agree on the use of clinical features to guide mutation testing. However, they disagree on which clinical features to be used in priority. In case of a syndromic presentation, targeted genetic testing is recommended by most algorithms.
- No single clinical feature has a good diagnostic accuracy to guide mutation testing.
- SDHB immunostaining has a high sensitivity and negative predictive value for the detection of SDH mutations in patients with phaeochromocytoma and/or paraganglioma.
- The evidence on genetic screening of relatives is limited.
- No studies were found that reported on the impact of genetic screening on clinical outcomes.

***Other considerations***

| Factor | Comment |
|---|---|
| **Balance between clinical benefits and harms** | No evidence was found on harm from genetic testing of patients with phaeochromocytoma and/or paraganglioma. However, also no evidence was found on the impact of genetic screening on clinical outcomes. No validated models were found either.<br>The expected benefits of genetic testing in patients with phaeochromocytoma / paraganglioma are (1) a confirmation of the diagnosis as such, (2) the identification of the need for targeted surveillance for other syndrome-associated tumours (mainly in case of a syndromic presentation), and (3) the identification of the need of first-degree relatives to undergo genetic counselling and testing.<br>Genetic screening of relatives can preclude mutation-negative relatives from annual clinical screening. Mutation-positive relatives will enter surveillance programmes targeted at early diagnosis of these tumours (and/or other syndrome-associated tumours in case of a syndromic presentation). |
| **Quality of evidence** | Only indirect evidence of very low quality is available. |
| **Costs (resource allocation)** | • VHL and RET mutation testing are billed using the nomenclature code 565515 – 565526 (€353.30 anno 2014). SDH testing (i.e. SDHB, SDHC and SDHD) is billed using the nomenclature code 565530 – 565541 (€552.47 anno 2014). SDH immunohistochemistry is billed using the nomenclature code 588070 – 588081 (€26.02 anno 2014).<br>• Several recognized Belgian genetic centres provide these tests. |

| Factor | Comment |
|---|---|
| **Patients values and preferences** | No specific comments were received, but in general the comments made for MEN2, MEN1 and VHL also apply to patients with phaeochromocytoma / paraganglioma. |
| **Comment** | (1) This report focused on genetic testing for SDHx genes (SDHD + SDHB + SDHC), VHL and RET. However, in patients with phaeochromocytoma / paraganglioma and clinical features (i.e. age < 35 years, metastatic disease, recurrent disease, bilateral tumours and/or familial disease) suggestive of a mutation who test negative for SDHx, VHL and RET, further genetic testing may be considered (as part of research). The following list contains other potentially affected genes:<br>• NF1<br>• SDHA<br>• SDHAF2<br>• TMEM127<br>• MAX<br>• EPAS1 (test not available in Belgium)<br>• FH (test not available in Belgium)<br>(2) As genetic technologies develop rapidly, panel-based tests are becoming more common. This is especially true for diseases with large genetic heterogeneity (i.e. when mutations in a number of genes can cause the same disease). Panel-based genetic tests allow to obtain results for multiple genes in one session. This will likely influence genetic testing of phaeochromocytoma / paraganglioma in the future.<br>(3) Because of a lack of evidence and because of clarity reasons, it was opted not to include functionality in our recommendations.<br>(4) Treatment (including surgery) should not be delayed because of genetic testing. |

| Recommendations | Strength of Recommendation |
|---|---|
| • Pre- and post-test genetic counselling should be offered to all patients with phaeochromocytoma / paraganglioma. | Strong |
| • In patients with phaeochromocytoma / paraganglioma and syndromic features, targeted genetic testing (e.g. for MEN2 and VHL) should be offered. | Strong |
| • All patients with phaeochromocytoma / paraganglioma that lack syndromic features should be offered genetic testing for SDHx genes (SDHD + SDHB + SDHC), VHL and RET (in this order). | Strong |

| Recommendations | Strength of Recommendation |
|---|---|
| • If tumour tissue is available, SDHB immunohistochemistry testing could be considered as a triage test before proceeding with genetic testing for SDHx genes. | Weak |
| • In patients with phaeochromocytoma / paraganglioma and clinical features suggestive of a mutation (i.e. age < 35 years, metastatic disease, recurrent disease, bilateral tumours and/or familial disease), who test negative for SDHx, VHL and RET, further genetic testing may be considered. | Weak |
| • Once a germline mutation has been identified in a proband, mutation analysis should be offered to all first-degree relatives irrespective of age. | Strong |

# 4 IMPLEMENTATION AND UPDATING OF THE GUIDELINE

## 4.1 Implementation

### *4.1.1 Multidisciplinary approach*

In this report we focused on the effectiveness of specific diagnostic interventions. In clinical practice, a multidisciplinary approach by different health care professionals should be encouraged. This approach should not only cover the medical needs of the patients but also their psychosocial needs.

### *4.1.2 Patient-centered care*

The choice of an intervention, e.g. germline mutation analysis, should not only consider medical aspects but also psychosocial consequences and patient preferences. Patients' representatives ask that a correct and understandable information be provided to individuals at increased genetic risk (within the philosophy of the Belgian law on patients rights of 26 September 2002). Continued support in decision-making is important during the different phases of the process (referral, testing, steps after a positive or a negative test). It is important to clearly explain figures about the increased risk of cancer. Balanced and understandable information about the pros and cons of the various decisions has to be provided (e.g. about clinical surveillance or prophylactic surgery). There is a need for psychosocial support (by professionals and by fellow patients if possible) when making choices, when informing children and family members about the genetic predisposition or with respect to fertility planning. A uniform policy followed by all Genetic Centres in Belgium is essential. It is important that general practitioners / oncologists / endocrinologists / psychologists are well informed about where to refer patients with these rare syndromes and tumours. According to the patients' representatives, a lot of people are currently not referred or do not receive the correct information about various mutations due to a lack of knowledge of these professionals.

### *4.1.3 Barriers and facilitators for implementation of this guideline*

During the stakeholders meeting on February 23rd 2015, the potential barriers and facilitators related to the use of this guideline were discussed.

The new billing codes for genetic tests (article 33) and the agreement on genetic testing consultation are in line with the recommendations in this guideline, and will facilitate its implementation.

The development of patient leaflets and information based on the present guideline will provide the means to clearly inform patients and their relatives about the indications for genetic testing and its consequences. These leaflets can be developed in collaboration with patients' representatives and organisations, such as the Flemish Cancer League, Fondation contre le cancer, Zelfhulpgroep NET & MEN kanker and VHL Family Alliance Belgium.

An important barrier is that these recommendations concern rare diseases for which the care is not necessarily centralised. Furthermore, the evidence is mainly of low to very low quality, and clinicians may be reluctant to implement recommendations based on such evidence.

### *4.1.4 Actors of the implementation of this guideline*

Clinical guidelines provide a tool for physicians to consult at different stages of the patient management pathway: screening, diagnosis, treatment and follow-up. They are developed according to standardised principles, based on scientific information regularly updated from the international literature. KCE formulates recommendations addressed to specific audiences (clinicians, decision-makers, sickness funds, NIHDI, professional organizations, hospital managers,...). KCE is not involved in the decision making process itself, nor in the execution of the decisions.

The implementation of this guideline will be facilitated by tools developed the College of Human Genetics and the College of Oncology. In addition, the content of this guideline is intended to be disseminated to caregiver groups by scientific and professional organisations using diverse channels such as websites and sessions of continuing education.

## 4.2 Monitoring the quality of care

This guideline should be considered as a starting point to develop quality improvement programs that targets all caregivers concerned.

It can be used as a tool to support health policies to improve the quality of care, e.g. through the support of actions to increase caregivers' awareness and to improve their practice, or through the development (or revision) of sets of process and outcome quality indicators.

## 4.3 Guideline update

In view of the rapidly evolving evidence due to the dynamic nature of this field, the clinical introduction of the routine analysis of a broad panel of germline DNA tests in at risk subjects will be monitored by the authors of this report. This guideline should be updated when sufficient new evidence is available. If, in the meantime, important new evidence would become available, this should be taken into consideration in the medical decision making.

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