Efficiency of the revised Bethesda guidelines (2003) for the detection of mutations in mismatch repair genes in Austrian HNPCC patients

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The clinical diagnosis of hereditary nonpolyposis colorectal cancer (HNPCC) is based on the Amsterdam II criteria (ACII). The purpose of using the Bethesda guidelines (BG) is to select tumours for microsatellite analysis. Recently, the modified Amsterdam criteria (ACmod) and Bethesda guidelines (BGmod) were proposed to simplify definitions. We evaluated the efficiency of the ACmod and BGmod to identify patients with germ-line mutations in MLH1 and MSH2 in 81 unrelated Austrian HNPCC families. Microsatellite (MS) analysis was performed in 55 tumours. The new criteria included more families than the old ones: BGmod, n = 81; BG, n = 72; ACmod, n = 52 and ACII, n = 35. The more stringent old criteria tended to show greater positive predictive value for association with a germ-line mutation than the corresponding new criteria: BGmod, 23%; BG, 26%; ACmod, 31% and ACII, 37%. The larger number of patients analysed in the ACmod group resulted in greater sensitivity compared to the ACII. The increased workload for BGmod was not associated with greater sensitivity. Microsatellite instability (MSI) significantly enhanced specificity in all subgroups. If the BG are used, we suggest that BG be given preference over BGmod, as the former signify a lesser workload.  

Key words: hereditary nonpolyposis colorectal cancer; microsatellite instability; Amsterdam criteria; Bethesda guidelines

Hereditary nonpolyposis colorectal cancer (HNPCC) has been linked to germ-line mutations in human DNA mismatch repair (MMR) genes.1–3 Proteins involved in the MMR complex include MSH2, MLH1, MSH6, MSH3, PMS1, PMS2, MLH3, EXO1, helicase and polymerase.2,4 The most frequently defective genes are MSH2 and MLH1, which are mutated in 75% of HNPCC families.5 The oncogenic impact of defects in the MMR system is based on a 100-fold higher mutation rate in cells with nonfunctional proteins than in normal cells.5,6 The altered function of the MMR system preferentially affects repetitive DNA sequences and results in microsatellite instability (MSI).7 MSI may also be used as a prescreening marker in HNPCC.1,5,6  

The management of HNPCC and related cancers involves very careful investigation of patients who inherited the germ-line mutation in this autosomal dominant syndrome, and also the provision of a suitable surveillance programme for the patients and their families. Testing for germ-line mutations of MMR genes in HNPCC is important because it allows the exclusion of healthy family members, bearing the wild-type allele from the recommended surveillance programme. It has been shown that frequent examination of mutation carriers leads to early detection and removal of premalignant lesions, and is associated with a higher probability of cure in cases of malignancies.8 A number of different inclusion criteria have been proposed to define patients with a high probability for MSI tumour DNA or families with a germ-line MMR mutation. The current definition of HNPCC is based on the Amsterdam criteria (AC II; Table I), which rely solely on clinical parameters.9 These criteria were recently modified to include small families as well (ACmod; Table II).10 The Bethesda guidelines are less stringent (BG; Table III). This clinical score was initially introduced as a tool for deciding whether genetic testing and/or microsatellite (MS) analysis should be performed.11 BG initially proved to be highly sensitive, but were considered too indefinite and unsuitable for primary sequence analysis of MMR genes.12 The modified Bethesda guidelines (BGmod; Table IV) were published recently.13 The purposes of revising the BG were the following: better identification of patients at risk for hereditary colorectal cancer, inclusion of a complete spectrum of colonic and extracolonic cancers, and better detection of MLH1 and MSH2 germ-line mutation carriers in patients with cancers, independently of the AC. None of the guidelines were devised for the selection of mutation carriers. Since evidence of a germ-line mutation is the sole means of diagnosing HNPCC with certainty, efficient criteria must be used to select patients for this analysis. We compared the original and the revised guidelines with regard to their efficiency in detecting the rate of MSI and germ-line mutations in MLH1 and MSH2.  

The aim of the study was to evaluate the revised Amsterdam criteria (ACmod) and Bethesda guidelines (BGmod) in a regional cohort of Austrian colorectal cancer patients suspected of having HNPCC. The original and the revised guidelines were compared with regard to their efficiency in detecting the rate of MSI and germ-line mutations in MLH1 and MSH2. Besides, for the purpose of detecting germ-line mutations in MLH1 and MSH2, we investigated the usefulness of MSI in addition to clinical criteria. To our knowledge, published data comparing BGmod and ACmod with the traditional selection criteria for genetic testing for HNPCC are scarce.

Material and methods

Patients

Eighty-one index patients from unrelated Austrian families were included in the study. The patients were selected retrospectively from individuals referred to our department for suspected hereditary colorectal cancer from 2000 to 2003. Only patients meeting at least one of the following groups of criteria were selected: BGmod (revised Bethesda guidelines 2003); BG (original Bethesda guidelines 1997); ACmod (modified Amsterdam crieri...
TABLE I – AMSTERDAM II CRITERIA (ACII)1

Three or more relatives with HNPCC-associated cancer (colorectal cancer or cancer of the endometrium, small bowel, ureter or renal pelvis) plus all of the following:
1. One affected patient should be a first-degree relative of the other two
2. Two or more successive generations should be affected
3. Cancer in one or more affected relatives should be diagnosed before the age of 45 years
4. Familial adenomatous polyposis should be excluded in any case of colorectal cancer
5. Tumours should be verified by pathological examination

1Ref. 9

TABLE II – MODIFIED AMSTERDAM CRITERIA (ACmod)1

Just one of these criteria need to be met:
1. Very small families, which cannot be further expanded, can be considered to have HNPCC with only two colorectal cancer in first-degree relatives if at least two generations have the cancer and at least one case of colorectal cancer was diagnosed by the age of 55 years
2. In families with two first-degree relatives affected by colorectal cancer, the presence of a third relative with an unusual early-onset neoplasm or endometrial cancer is sufficient

1Ref. 10

TABLE III – BETHESDA GUIDELINES (BG)1

Just one of these Criteria Need to be Met:
1. Individuals with cancer in families that fulfil the Amsterdam criteria
2. Individuals with two hereditary non-polyposis colorectal cancer (HNPPC)-related cancers, including synchronous and metachronous colorectal cancers or associated extracolonic cancers (which include endometrial, ovarian, gastric, hepatobiliary or small-bowel cancer or transitional-cell carcinoma of the renal pelvis or ureter)
3. Individuals with colorectal cancer and a first-degree relative with colorectal cancer and/or HNPPC-related extracolonic cancer and/or colorectal adenoma; one of the cancers must have been diagnosed before the age of 45 years and the adenoma diagnosed before the age of 40 years
4. Individuals with colorectal cancer or endometrial cancer that was diagnosed before the age of 45 years
5. Individuals with right-sided colorectal cancer with an undifferentiated pattern (solid/cribiform) on histopathology, which was diagnosed before the age of 45 years
6. Individuals with signet-ring-cell-type colorectal cancer that was diagnosed by the age of 45 years
7. Individuals with adenomas that were diagnosed by the age of 40 years

1Ref. 11

TABLE IV – REVISED BETHESDA GUIDELINES (BGmod)1

Just one of these criteria need to be met:
1. Individuals diagnosed with colorectal cancer before the age of 50 years
2. Synchronous or metachronous colorectal or other HNPCC-related tumours (which include stomach, bladder, ureter, renal pelvis, biliary tract, brain (glioblastoma), sebaceous gland adenomas, keratoacanthomas and carcinoma of the small bowel), regardless of age
3. Colorectal cancer with a high-microsatellite instability morphology that was diagnosed before the age of 60 years
4. Colorectal cancer with one or more first-degree relatives with colorectal cancer or other HNPCC-related tumours. One of the cancers must have been diagnosed before the age of 50 years (this includes adenoma, which must have been diagnosed before the age of 40 years)
5. Colorectal cancer with two or more relatives with colorectal cancer or other HNPCC-related tumours, regardless of age

1Ref. 13

DNA and RNA extraction

Nuclear DNA was isolated from paraffin-embedded tissue after histological verification by an experienced pathologist (F.W.). In 10 µm tissue sections, the paraffin was removed with xylene (Merck, Darmstadt, Germany), and the xylene with ethanol (Merck, Darmstadt, Germany). Cells were lysed with 250 µl digestion buffer (50 mM TRIS-HCL pH 8.0; Merck, Darmstadt, Germany) and 50 µg proteinase K (Roche Diagnostics, Mannheim, Germany). After denaturation of the enzyme at 95°C for 10 min, DNA was directly used for PCR amplification. DNA extraction from blood was performed with the QIAamp® DNA blood kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations.

Microsatellite instability assay

For microsatellite analysis, two groups, of 5 markers each, were selected: Group 1 consisted of D5S346, HSCAP53L, D2S123, Bat26 and D18S34 while group 2 consisted of D5S82, D2S134, D13S175, D11S904 and Bat25. The efficiency of this marker panel in detecting MSI was evaluated in a multi-centre study, which revealed a similar sensitivity as that for the National Cancer Institute (NCI) panel.1,14 The fragments were PCR amplified with primers that were ABI dye labelled on 5’. PCR was performed in 30 µl reaction mixtures using AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA) under standard conditions. A final extension step at 72°C for 1 hr was added before cooling to room temperature. Detailed protocols are available from the authors upon request. After amplification, 1 µl of each product of the corresponding group of markers was mixed. Of this mixture, 1 µl was added to 15 µl formamide (Amresco, Solon, Ohio) and 0.3 µl GeneScan™,500 ROX™ Size Standard (Applied Biosystems, Foster City, CA). After denaturation at 92°C for 2 min, the PCR products were separated on an ABI Prism® 310 Genetic Analyzer and analysed with the GeneScan® software package (Applied Biosystems, Foster City, CA). In the event of instability, additional smaller fragments were identified in the tumour sample compared to the corresponding normal tissue. If only one of the markers in the first group showed instability, 5 further markers (group 2) were used. The degree of instability was evaluated according to the percentage of markers showing band shifts. MSI-high was considered to exist when at least 30% of the analysed markers were unstable; any lower degree of instability or no instability was interpreted as microsatellite stable (MSS). MSI analysis was performed on all tumour samples included in the study.

Sequence analysis

The exons of MLH1 and MSH2 as well as the promoter regions of each gene were PCR amplified from genomic DNA with
AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA) and oligonucleotide primers, as described elsewhere. Detailed protocols are available from the authors upon request. To remove excess primers and dNTPs, 5 μl of each PCR product was incubated with 2.5 U exonuclease I (Amersham Pharmacia Biotech AB, Uppsala, Sweden) and 2.5 U shrimp alkaline phosphatase (Amersham Pharmacia Biotech AB, Uppsala, Sweden) at 37°C for 1 hr. Enzymes were inactivated by incubation at 70°C for 15 min. For sequence analysis, 1–2 μl of the purified PCR product was used with 2–3 pmol primers in the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) according to the manufacturer’s instructions. Separation and analysis of the sequencing reaction products were performed on an ABI Prism® 310 Genetic Analyzer, using standard protocols. All 55 tumour and 26 blood samples from index patients were subjected to this analysis. If DNA from tumour tissue was available, analysis of DNA from corresponding normal tissue or peripheral blood was performed on fragments containing a mutation. If no mutation was found in the tumour, sequence analysis was performed with DNA from normal tissue or peripheral blood.

Statistics
Data were analyzed using the SPSS for Windows Release 10.0 software package (SPSS, Chicago, IL) and the Clinical Calculator from VassarStats (http://faculty.vassar.edu/lowry/clin1.html). The identification of HNPCC families with germ-line mutations based on each of the defined criteria was tested in two-by-two contingency tables to determine sensitivity and specificity. The sensitivity of the test is the probability that the condition (germ-line mutation) is absent. The specificity of the test is the conditional probability that the test (selection criteria) will be negative if the condition (germ-line mutation) is absent. The positive predictive value of a test is the probability that the condition (germ-line mutation) is present when the test is restricted to those patients who test positive (fulfil the selection criteria). The negative predictive value of a test is the probability that the condition (germ-line mutation) will be absent when the test is restricted to all patients who test negative (do not fulfil the selection criteria). The associated 95% confidence intervals (95% CI) were calculated according to the efficient-score method for each of the estimates of sensitivity and specificity. A Chi-Square test was performed to compare the efficient-score method for each of the estimates of sensitivity and specificity. The negative predictive value of a test is the probability that the condition (germ-line mutation) will be absent when the test is restricted to all patients who test negative (do not fulfil the selection criteria). The positive predictive value of a test is the probability that the condition (germ-line mutation) is present when the test is restricted to those patients who test positive (fulfil the selection criteria). The negative predictive value of a test is the probability that the condition (germ-line mutation) will be absent when the test is restricted to all patients who test negative (do not fulfil the selection criteria). The associated 95% confidence intervals (95% CI) were calculated according to the efficient-score method for each of the estimates of sensitivity and specificity.

Results

Frequency of selection criteria for familial colorectal cancer in the Austrian cohort

All of the 81 patients included in the study met the BGmod criteria. Seventy-two patients (88.9%) fulfilled the BG, fifty-two (64.2%) met the ACmod and 35 (43.2%), the ACII criteria (Fig. 1).

Frequency of mutations in MLH1 and MSH2
Sequence analysis for mutations in MLH1 or MSH2 was performed independently of the results of MS analysis. This revealed germ-line mutations in 19/81 patients (23.5%). Twelve of the 19 (63.2%) mutations were located in MLH1 and 7 (36.8%) in MSH2. A detailed description of the mutations is given in Table V. All mutations have been described previously either by ourselves or others (http://www.insight-group.org).

In the ACI subgroup 13/35 (37.1%), patients had a pathogenic germ-line mutation. The other 6 mutation carriers did not comply with the ACII. Among 52 ACmod patients, 16 (30.8%) had a mutation. Three patients with a germ-line mutation did not fulfil

TABLE V – MUTATIONS IN AUSTRIAN HNPCC FAMILIES

| Gene | Exon | Description1 | Predicted changes1 | Family | Criteria2 |
|------|------|--------------|--------------------|--------|-----------|
| MLH1 | 1    | c.67G>A; c.70delG | [p.E23K; p.E23fsX35] | FH49   | BGmod/ACmod |
| MLH1 | 6    | c.531_532GG>CT | [p.L177F; p.E178X] | FH21   | BGmod/ACII |
| MLH1 | 10   | c.851T>A      | p.L284X            | 1254   | BGmod/ACII |
| MLH1 | 12   | c.1343delA    | p.L449fsX490       | FH50   | BGmod/ACII |
| MLH1 | 13   | c.1410_1413delAAAG | p.I407fsX506 | FH29   | BGmod/ACII |
| MLH1 | 13   | c.1489dupC    | p.P496fsX502       | FH1    | BGmod/ACII |
| MLH1 | 14   | c.1609C>T     | p.Q537X            | 509    | BGmod/ACII |
| MLH1 | 17   | c.1919_1920insT | p.L639fsX644       | FH27   | BGmod/ACII |
| MLH1 | IVS13| c.1559-1G>C   | Splice defect      | FH26   | BGmod/ACII |
| MLH1 | IVS5 | c.588+5G>A    | Splice defect      | FH43   | BGmod/ACII |
| MLH1 | IVS7 | c.588-1G>T; [r. = ., .588_589ins589-8_589-1] | p.K196fsX203 | FH11   | BGmod/ACII |
| MSH2 | 1    | c.110delT     | p.L366fsX63        | FH2    | BGmod/ACII |
| MSH2 | 4    | c.687delA     | p.K228fsX245       | FH45   | BGmod/ACII |
| MSH2 | 7    | c.1222_1223insT | p.Q407fsX589       | FH47   | BGmod/ACmod |
| MSH2 | 13   | c.2160_2163delAGGA | p.K720fsX506 | 1081   | BGmod/ACII |
| MSH2 | IVS2 | c.367-1G>A; [r. = , r.367delg] | p.K122fsX173 | FH12   | BGmod/ACII |
| MSH2 | IVS5 | c.942+3A>T    | Splice defect      | FH22   | BGmod/ACII |
| MSH2 | IVS6 | c.1076+1G>T   | Splice defect      | FH28   | BGmod/ACII |

1Del, deletion; ins, insertion; dup, duplication; fs, frameshift; nomenclature for mutation description according to den Dunnen et al.19–20

FIGURE 1 – Groups of patients. AC, Amsterdam Criteria; ACmod, modified Amsterdam Criteria; BG, Bethesda Guidelines; BGmod, modified Bethesda Guidelines.

1–2Least and most stringent criteria met by the family.
the ACII or ACmod, although family information spanning at least 2 generations was available. Seventy-two patients met the BG; 19 of them (26.4%) had a mutation. No patient who met the BGmod, but not the BG, had a mutation. The detection of a germ-line mutation was significantly correlated with the fulfilment of the ACII and the ACmod (p = 0.011 and p = 0.038, respectively), but not to the fulfilment of the BG criteria (p = 0.078).

The difference between the rates of mutations detected in the groups ACII 13/35 (37.1%), ACmod 16/52 (30.8%), BG 19/72 (26.4%) and BGmod 19/81 (23.5%) was not statistically significant (p = 0.462). When ACII, ACmod or BG were compared separately with the BGmod, no statistically significant difference was found (p = 0.13, p = 0.35 and p = 0.675, respectively).

Since BGmod were the required inclusion criteria for all patients (n = 81), we were able to determine sensitivity and specificity for the groups BG, ACmod and ACII in comparison to BGmod (Table VI). The largest subgroup were those patients who fulfilled the BG (n = 72). All patients with a germ-line mutation were found in this subgroup, which signifies a sensitivity of 100%. As expected, the specificity was low (14.5%). Inclusion of patients according to the ACII (n = 52) was associated with a specificity of 84.2% and a specificity of 41.9%. Application of the most stringent criteria (ACII n = 35) would have caused 6 mutations to be missed; thus, the ACII had the lowest sensitivity of 68.4%, and a specificity rate of 64.5%.

Microsatellite instability rate

Microsatellite analysis could be performed for all tumour samples (n = 55). Twenty-six patients had no tumour tissue of suitable quality for DNA examination. Of 55 BGmod patients, 50 also met the BG, 34 the ACmod and 24 the ACII criteria. MS analysis revealed 22 (40%) cases of high instability and 33 (60%) stable tumours. The proportion of tumours with MSI in the DNA was highest in the ACII (16/24 66.7%) and lowest in the BGmod group (22/55 40%); the difference was statistically significant (p = 0.029). In the ACmod group, 17/34 (50%) and in the BG, 22/50 (44%) tumours had MSI. The difference between the number of MSI tumours in the ACmod or BG compared to the BGmod was not statistically significant (p = 0.356 and p = 0.678, respectively).

Correlation of microsatellite detection rate and sequence analysis

The detection of a pathogenic germ-line mutation correlated highly significantly with MSI in the 55 tumour samples examined (p < 0.001). Thirteen of 22 (59.1%) MSI, but 0/33 (0%) MSH2 tumours were accompanied by a pathogenic mutation in MLH1 or MSH2. Evaluation of all patients subjected to MS analysis (55 BGmod patients) revealed that MSI was associated with a high sensitivity (100%, 95% CI 71.7; 100) and specificity (78.6%, 95% CI 62.8; 89.2) when used as a selection criterion for DNA sequencing to detect a germ-line mutation in MLH1 and MSH2.

Combining the clinical inclusion criteria with MSI significantly increased the specificity for detecting a germ-line mutation as compared to the use of clinical criteria alone (Table VII). The p-value for the detection of a mutation was <0.001 for BG+MSI or ACmod + MSI and 0.001 for ACII+MSI patients. The positive predictive values in the groups defined by MSI in combination with the clinical inclusion criteria were 64.7% for ACmod and 56.3% for ACII.

Discussion

As the incidence of HNPCC is low, screening the general population would be inefficient. Instead, it would be meaningful to identify families at specific risk. Several clinical diagnostic guidelines for HNPCC have been used for this purpose. The ACII have been used as the primary criteria for selecting patients who should be offered genetic diagnosis for HNPCC. The main point of criticism raised against the ACII is their stringency; particularly small families nearly always failed to fulfill the criteria. The BG were proposed to select tumours for MS analysis, and were initially shown to be sensitive (94%) criteria for identifying individuals with pathogenic mutations in MMR genes, but were not sufficiently specific (25%).

A recent publication indicated that BG and AC still missed 5 of 23 MMR mutation carriers within a cohort of 1,066 patients, nevertheless those criteria are still valuable in situations where sequence analysis of a large number of patients is not possible.

In a recent consensus paper, Umar et al. proposed modified Bethesda guidelines and Amsterdam criteria to overcome these deficiencies. To the best of our knowledge, little has been published about the frequency of the MMR mutation in connection with the new criteria. We analysed this subject in a cohort of 81 Austrian families fulfilling at least one of the new or old inclusion criteria and compared these subgroups with regard to the frequency of MMR germ-line mutations.
All families included in the study fulfilled the modified Bethesda guidelines (BGmod). The BG were met by 11% fewer families than the BGmod. This indicates that the BGmod substantially broaden the BG. However, the absolute numbers of detected mutations were equal in both groups. Thus, the use of BGmod as inclusion criteria signified a greater workload but was not associated with a larger number of detected mutations. The definition of BGmod appears to be less complicated than that of the BG, which signifies an obvious advantage for the management of HNPCC. As the target audience for the BGmod are oncologists, gynaecologists and gastroenterologists, the value of the guidelines for medical staff members who are unfamiliar with the characteristics of HNPCC seems to be questionable. The BGmod might be applied as inclusion criteria for referral for genetic counselling, but are possibly too indefinite for final selection of patients for further genetic analysis.

Primary sequence analysis of BG is too inefficient in view of its low specificity rate of 14.5%. Obviously, these criteria require a further prescreening method such as MSI analysis. The value of the latter assay in this process was confirmed by our data. Among the Austrian families investigated in the present study, no patient with microsatellite-stable tumour DNA had a germ-line mutation, while 60% of patients with MSI had a pathologically altered MMR gene. If only those patients who fulfilled the new or old BG and who had an MSI tumour were screened by genetic analysis, the workload for the old BG and the new BG would have been comparable in our patients. MSI as a selection criterion for the detection of mutation was associated with a specificity of 78.6% and a sensitivity of 100% in the BGmod group. Among BG patients, MSI had a specificity of 75.7% (95% CI 58.4; 87.6) and a sensitivity of 100% (95% CI 71.7; 100) for identifying patients with a germ-line mutation (data not shown). In our opinion, the quality of both sets of results justify the expensive technology of MSI analysis as a prescreening method to exclude familial cancer from general practice.

On the basis of the data of the Austrian cohort, the modified Amsterdam criteria (ACmod) proved to be very interesting. Primary sequence analysis in these patients was associated with a fairly high sensitivity rate (84.2%) compared to the BGmod group. Specificity (41.9%) was significantly higher than that was with the old BG (p = 0.001). By applying the ACmod, we would have missed only 3 of the 19 patients with a germ-line mutation, but the workload would have been much less. The ACmod as inclusion criteria might be of specific interest when there is no tumour tissue available for MSI analysis. MSI in addition to the ACmod increased specificity to 85.7%, but 8 mutation carriers were missed. Therefore, we suggest that these criteria be primarily used when MSI analysis is not available or MSI analysis is uneconomical because of the nonavailability of suitable tumour material. However, further studies on larger patient populations will have to prove the efficiency of the ACmod as sole selection criteria for mutation analysis.

The ACII proved to be extremely stringent, as they were associated with an underdiagnosis rate of 30% of the mutation carriers. We consider this percentage too high compared to the sensitivity rate of the other subgroups. The ACII criteria were advantageous in that the group comprised a small number of patients (33% less than the ACmod group). The addition of MSI to the ACII led to significantly better specificity (83.3%; p = 0.036) with regard to the presence of a mutation in the MMR genes, but 10 mutation carriers were missed. Like the ACmod, the ACII appear to be stringent enough to serve as inclusion criteria for patients being analysed by means of primary sequence analysis without any prescreening (Fig. 2).

MSI was confirmed as a very sensitive prescreening method to select patients for sequence analysis with regard to mutations in MLH1 or MSH2. Umar et al. suggest that this method should be used only in conjunction with the BG. The limitation of MSI as a surrogate marker of HNPCC is the fact that some family members have stable tumour DNA; certain sporadic cancers are associated with epigenetic silencing of MLH1 and may also lead to MSI. In our experience, the main limitation of MSI analysis is that it requires tumour tissue from one affected family member. Tumour samples could not be obtained from 26 patients for different reasons. A germ-line mutation was detected in 6/26 (23.1%) patients of this group. Values for sensitivity and specificity disregard these facts, which should be considered when the quality of MSI analysis as a prescreening method is assessed. MSI analysis can be performed in all patients suspected of having HNPCC, as it still signifies a reduction of the workload: only 50% of ACmod patients or 67% of ACII patients need to be examined.

Immunohistochemistry is being increasingly used as a prescreening method for the protein products of MLH1 and MSH2. In view of the potential advantage of immunohistochemistry in guiding the investigator to the correct gene for genetic testing, the method has been established as an aid for diagnosing HNPCC in our department. However, the final analyses are still pending for the majority of the patients reported here. The detected rate of mutations in the ACII families in this study is quite low (37.1%); this might be due to the fact that only DNA sequence analysis of MLH1 and MSH2 was used and no other method of analysis was employed. A proportion of families may bear large genomic deletions or mutations in other MMR genes.

Knowledge of HNPCC in general and its implications for surveillance is still not widespread. The main question is which of the clinical definitions should serve as a basis for the referral of patients to a genetic centre. Referring physicians might be confused when confronted with complex inclusion criteria; this might result in the exclusion of familial cancer from general practice. Too many inclusion criteria would have a similar effect. However, certain inclusion criteria should be used to select families for the clinical screening programme (colonoscopy, urological or gynaecological counselling), independently of the possibility of genetic testing. In our opinion, the ACmod criteria, being simple but associated with reasonable sensitivity, should be given preference. We suggest that these criteria be communicated to referring general physicians as the basic clinical diagnostic inclusion criteria for HNPCC.
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