The serrated neoplasia pathway: investigating the role of serrated polyps in colorectal cancer development
Boparai, K.S.

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Hyperplastic polyposis syndrome: a pilot study for the differentiation of polyps using high resolution endoscopy, autofluorescence imaging and narrow-band imaging

K.S. Boparai
F.J.C. van den Broek
S. van Eeden
P. Fockens
E. Dekker

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ABSTRACT

Background: Endoscopic differentiation and removal of potentially premalignant sessile serrated adenomas (SSAs) may be an important step in preventing colorectal cancer (CRC) development in hyperplastic polyposis syndrome (HPS).

Objective: To assess the value of high resolution endoscopy (HRE), autofluorescence imaging (AFI) and narrow-band imaging (NBI) for differentiating polyps in HPS.

Design: A prospective polyp series.

Setting: Single tertiary referral center.

Patients and Interventions: 7 patients with HPS underwent colonoscopy using endoscopic trimodal imaging (ETMI), which incorporates HRE, AFI and NBI in one system. All detected polyps were analysed with AFI for colour and with NBI for Kudo pit pattern and vascular pattern intensity (VPI).

Main outcome measurements: The accuracy, sensitivity and specificity of AFI and NBI in differentiating detected polyps were determined by using histology as a gold standard.

Results: A total of 19 hyperplastic polyps (HPs), 32 SSAs and 15 adenomas were detected. For differentiating SSAs from HPs, AFI-colour, Kudo pit pattern and VPI resulted in a diagnostic accuracy of 55%, 55% and 52% respectively. For differentiating adenomas from HPs, this was 65%, 94% and 90% respectively. Macroscopically, the combination of size ≥3mm and proximal location resulted in the highest accuracy (76%) for differentiating SSAs from HPs.

Limitations: Small sample size.

Conclusion: Endoscopic differentiation between HPs and SSAs using ETMI proved unsatisfactory. Differentiation of adenomas from HPs was well possible with NBI but not with AFI.
INTRODUCTION

Hyperplastic polyposis syndrome (HPS) is a recently recognised condition characterised by the presence of multiple (>30) hyperplastic polyps (HPs) spread throughout the colon and has frequently been linked with colorectal cancer (CRC).\(^1\)-\(^4\) Besides multiple HPs, serrated adenomas are frequently seen in HPS as well.\(^1\)-\(^8\) In fact, the co-existence of sessile serrated adenomas (SSAs) has been considered by some as a characterizing feature of this condition.\(^9\)

Molecular research in SSAs strongly suggests that these polyps are precursor lesions which may lead to CRC.\(^10\)-\(^13\) Accordingly, authorities recommend that SSAs should be endoscopically managed like conventional adenomas.\(^14\) In this respect, endoscopic differentiation of SSAs from HPs and removal of SSAs may be an important step in preventing cancer development in HPS. However, HPs and SSAs, being both often small in size and sessile/flat in shape, are similar in appearance and therefore difficult to distinguish from each other when using standard endoscopy (figure 1).\(^15\)-\(^18\)

Novel endoscopic imaging techniques may aid in the differentiation of conventional adenomas and HPs with high accuracy.\(^19\)-\(^22\) Autofluorescence imaging (AFI) facilitates differentiation of adenomas from non-adenomatous polyps based on different fluorescence emission spectra.\(^23\)-\(^25\) The use of AFI in differentiating polyps in HPS patients has not been described before. Narrow band imaging (NBI) utilizes short wavelength visible light to provide improved details of the mucosal pit pattern and microvasculature. Pit pattern analysis by applying the Kudo classification has shown to be a reliable approach for distinguishing adenomas from non-adenomatous polyps and has also been used to describe serrated adenomas.\(^26\)-\(^31\)
Figure 1: Similar endoscopic appearance of a hyperplastic polyp (left) and a sessile serrated adenoma (right) using conventional white light endoscopy with corresponding haemotoxylin and eosin stains below.

However, at the times of these studies the diagnosis SSA was not yet in practice and Kudo pit patterns for serrated adenomas varied considerably from II to as high as IV.\textsuperscript{27, 28} In addition, the assessment of microvasculature and vascular pattern intensity (VPI) with NBI is believed to be a relatively easy method for differentiating adenomas from non-adenomatous polyps, but has not been evaluated in HPS.\textsuperscript{20, 29, 30, 32-34}

The aim of this study was to assess the diagnostic accuracy of high resolution endoscopy (HRE), AFI and NBI for the differentiation of polyps in patients with HPS.
PATIENTS AND METHODS

Study population

This study was conducted at the Academic Medical Center Amsterdam and was approved by the local medical ethics committee. Consecutive patients with HPS were invited to participate when fulfilling the criteria for HPS in accordance with the World Health Organisation (WHO): (1) at least five histologically confirmed HPs proximal to the sigmoid colon, of which two are greater than 10mm in diameter, or (2) more than 30 HPs distributed throughout the colon. Patients <18 years or patients with severe coagulopathy or insufficient bowel cleansing were excluded from this study.

Endoscopic equipment

All procedures were performed with the endoscopic tri-modal imaging (ETMI) system, which integrates HRE, AFI and NBI into one unit (XCV-260 HP, Olympus Inc., Tokyo, Japan). The endoscope (XCF-H240FZL) is equipped with a movable lens for optical magnification (up to 100×) and two high-quality charge coupled devices: one for HRE/NBI and one for AFI. The light source used in this system (XCLV-260HP) was of the type “sequential RGB-illumination”. The ETMI specifications have previously been described in detail. During colonoscopy, the endoscopist could easily switch between the three imaging modalities by pressing a button on the shaft of the endoscope. A high resolution monitor was used for all procedures.
**Colonoscopy procedure**

Patients were prepared with 4-6 L polyethylene glycol solution (Kleenprep; Norgine GmbH, Marburg, Germany) and underwent colonoscopy under conscious sedation with midazolam and/or fentanyl. The colonoscope was advanced until cecal intubation was confirmed by identification of the appendiceal orifice and ileocecal valve. Upon reaching the cecum, the level of bowel preparation was determined as good (100% of the mucosa visible), moderate (90-100%) or poor (<90%) after rinsing and suctioning.

During withdrawal of the endoscope, HRE was used to detect colonic polyps. All detected polyps were assessed for size (open biopsy forceps: 8mm), shape (Paris classification) and location. Subsequently, each polyp was assessed with AFI for polyp colour: green, ambiguous or purple. Purple and ambiguous colours were considered suspicious for adenoma and green was considered non-suspicious for adenoma. Hereafter, the Kudo pit pattern (I-V) was assessed with NBI. Kudo pit pattern III-V were considered suspicious for adenoma, while pit pattern I-II were non-suspicious. Still images (BMP-format) with all modalities were acquired after which the polyp was resected and harvested for histopathology. All procedures and instant assessments with AFI and NBI were performed by one experienced endoscopist (ED), who has performed >2,500 colonoscopies and >50 ETMI colonoscopies.

In addition, representative NBI images of all detected lesions were later assessed for vascular pattern intensity (VPI) as described by East et al. For this purpose, all sharp high-quality images were displayed in a random order to the same endoscopist who was blinded for final histopathology. Images were directly displayed on a personal
NBI increases polyp detection in HPS

computer in standard format (3.2 × 2.4 inch; 200 pixels/inch) without any post-processing. The VPI was scored as lighter (weak), the same (normal) or darker than (strong) the surrounding mucosa. Strong VPI was considered suspicious for adenoma, whereas weak/normal VPI was considered non-suspicious.

Reference standard

All polyp specimens were blindly evaluated by a gastrointestinal pathologist (SvE). Lesions were classified as HP, SSA, traditional serrated adenoma (TSA), mixed polyp or conventional adenoma based on the morphological features on H&E staining which was used as reference standard.\textsuperscript{13, 14, 39}

Statistical analysis

SSAs were regarded as adenomatous polyps, owing to their premalignant potential.\textsuperscript{10-14} Consequently, it was examined whether these polyps were suspicious on AFI (ambiguous/purple) and/or NBI-VPI (strong). In addition, NBI pit patterns of SSAs were expected to be comparable to HPs (Kudo II) as these polyps are described to be microscopically difficult to distinguish from each other.\textsuperscript{14, 18, 40, 40} TSAs, mixed polyps and conventional adenomas were expected to be suspicious on AFI and NBI as these polyps harbour neoplastic changes of the epithelium.

The sensitivity, specificity, and diagnostic accuracy plus 95%-confidence interval (95%-CI, using the Wilson procedure without correction for continuity) for differentiating SSAs, TSAs, mixed polyps and conventional adenomas (i.e. adenomatous group) from HPs were determined for each modality by comparing the endoscopic diagnosis
to final histopathology, which served as reference standard. Lesions histologically diagnosed as normal mucosa were excluded from the analysis. The size, location and shape of all lesions were summarized and compared between the different polyps using the Chi-Square test, Fisher's exact test, Kruskal-Wallis test or Mann-Whitney U test when appropriate. To make statistical comparisons and to calculate the 95%-CI, it is necessary to assume that results for individual polyps constitute statistically independent observations, even when there may have been more than one polyp assessed in individual patients. A p-value less than 0.05 from a single test was considered statistically significant, but it is recognized that there was multiple testing of outcome data arising from individual polyps. Examining the nominal p-values in light of correction for multiple testing using the method of correction of Bonferroni, it is suggested that only those nominal p-values less than 0.01 will retain significance after correction. The uncorrected p-values are presented with the warning that p-values between 0.01 and 0.05 should be considered as provisional. For reporting the results of this study, the STARD guidelines were used.

Results

From January 2005 to July 2006, 7 patients (5 male) who met the criteria for HPS, underwent colonoscopy with ETMI. The median age of all patients was 55.8 (range 54-71) years. At colonoscopy all patients had good to moderate bowel preparation. A total of 66 polyps (19 HPs, 32 SSAs and 15 tubular adenomas) were detected as well as 10 additional lesions displaying normal mucosa on histology (excluded from the analysis). Macroscopic polyp characteristics are summarized in table 1. Overall, SSAs were larger than HPs (p<0.001, Mann-
NBI increases polyp detection in HPS

Whitney U test) and adenomas (p<0.001, Mann-Whitney U test). There was no significant difference in size between HPs and adenomas.

Concerning the differentiation of SSAs from HPs, the odds ratio for predicting a polyp to be SSA was 2.3 (95%-CI: 1.2-4.4) for size (per mm increase), 4.9 (1.3-18.0) for flat shape and 3.9 (1.1-14.7) for proximal location. The combination of size ≥3mm and proximal location yielded the largest differential value (p<0.0001) between SSAs (21/32: 66%) and HPs (1/19: 5%) with a corresponding odds ratio of 34.4 (4.0-292). The sensitivity and specificity of this combination for differentiating SSAs from HPs would be 66% (95%-CI: 48-80%) and 95% (75-99%) respectively. The overall diagnostic accuracy would be 76% (62-87%).

**Autofluorescence imaging**

With AFI, 10/19 (53%) HPs displayed a green colour versus 14/32 (44%) SSAs and 3/15 (20%) adenomas (p=0.142). The sensitivity, specificity and diagnostic accuracy of AFI for discriminating SSAs from HPs based on colour were 56% (95%-CI: 39-72%), 53% (32-73%) and 55% (41-68%) respectively. Differentiation with AFI between adenomas and HPs had a sensitivity, specificity and accuracy of 80% (55-93%), 53% (32-73%) and 65% (48-79%).
Polyp characteristics

<table>
<thead>
<tr>
<th></th>
<th>All polyps (n=66)</th>
<th>HP (n=19)</th>
<th>SSA (n=32)</th>
<th>Adenoma (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median size mm</td>
<td>2 (2-5)</td>
<td>2 (1-2)</td>
<td>3 (2-8)</td>
<td>2 (1-3)</td>
<td>0.001*</td>
</tr>
<tr>
<td>(interquartile range)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.013**</td>
</tr>
<tr>
<td>Proximal colon</td>
<td>51 (77%)</td>
<td>11 (58%)</td>
<td>27 (84%)</td>
<td>13 (87%)</td>
<td></td>
</tr>
<tr>
<td>Distal colon</td>
<td>6 (9%)</td>
<td>1 (5%)</td>
<td>3 (9%)</td>
<td>2 (13%)</td>
<td></td>
</tr>
<tr>
<td>Rectum</td>
<td>9 (14%)</td>
<td>7 (37%)</td>
<td>2 (7%)</td>
<td>0 (0%)</td>
<td>0.048**</td>
</tr>
<tr>
<td>Shape</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-Ip</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>0-Ir</td>
<td>18 (38%)</td>
<td>9 (47%)</td>
<td>5 (16%)</td>
<td>4 (27%)</td>
<td></td>
</tr>
<tr>
<td>0-II</td>
<td>48 (62%)</td>
<td>10 (53%)</td>
<td>27 (84%)</td>
<td>11 (73%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 1: Clinicopathological characteristics of all detected polyps in 7 patients with hyperplastic polyposis syndrome. Proximal colon = cecum, ascending and transverse colon; distal colon = descending colon and sigmoid colon. *Kruskal-Wallis test (only SSAs differed significantly from HPs and adenomas on additional testing with the Mann-Whitney U test). **Pearson Chi-Square test. Note that Bonferroni correction for multiple testing removes statistical significance except where p<0.01 in this table.

Narrow band imaging

The sensitivity, specificity and diagnostic accuracy of the Kudo classification with NBI for differentiation of SSAs from HPs were 28% (95%-CI: 16-33%), 100% (83-100%) and 55% (41-68%). For differentiating adenomas from HPs, the obtained sensitivity, specificity and diagnostic accuracy were 87% (81-98%), 100% (83-100%) and 94% (62-96%) (table 2). During the subsequent NBI image evaluation, the VPI was assessed for 14 HPs, 28 SSAs and 15 adenomas. Images of the other detected polyps could not be included for analysis because VPI assessment was not possible (e.g. blurry images). The sensitivity, specificity and diagnostic accuracy of VPI for differentiating SSAs from HPs, were 36% (21-54%), 86% (6-96%) and 52% (38-67%) respectively. When adenomas were compared with HPs the sensitivity,
specificty and diagnostic accuracy were 93% (74-96%), 86% (60-96%) and 90% (70-98%).

<table>
<thead>
<tr>
<th>NBI</th>
<th>HP (n=19)</th>
<th>SSA (n=32)</th>
<th>Adenoma (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pit pattern</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kudo I-II</td>
<td>19 (100%)</td>
<td>23 (72%)</td>
<td>2 (13%)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Kudo III-V</td>
<td>0</td>
<td>9 (28%)</td>
<td>13 (87%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NBI</th>
<th>HP (n=14)</th>
<th>SSA (n=28)</th>
<th>Adenoma (n=15)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VPI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weak/normal</td>
<td>12 (86%)</td>
<td>18(64%)</td>
<td>1 (7%)</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Strong</td>
<td>2 (14%)</td>
<td>10 (36%)</td>
<td>14 (93%)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Kudo pit pattern classification and vascular pattern intensity (VPI) assessment of all detected polyps with NBI. *p-value for adenomas compared to HPs and SSAs.

Table 3 lists the sensitivities, specificities and diagnostic accuracies of the different endoscopic modalities for the grouped differentiation of both high-risk SSAs and conventional adenomas from low-risk HPs.

<table>
<thead>
<tr>
<th>SSA/adenoma vs. HP</th>
<th>Modality</th>
<th>AFI</th>
<th>NBI-pit pattern</th>
<th>NBI-VPI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(95%-CI)</td>
<td>30/47: 64%</td>
<td>22/47: 47%</td>
<td>24/43: 56%</td>
<td></td>
</tr>
<tr>
<td>Specificity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(95%-CI)</td>
<td>10/19: 53%</td>
<td>19/19: 100%</td>
<td>12/14: 86%</td>
<td></td>
</tr>
<tr>
<td>Diagnostic accuracy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(95%-CI)</td>
<td>40/66: 61%</td>
<td>41/66:62%</td>
<td>36/57: 63%</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Sensitivity, specificity and diagnostic accuracy of all endoscopic modalities for differentiating sessile serrated adenomas and conventional adenomas (high risk) from hyperplastic polyps (low risk) in patients with hyperplastic polyposis syndrome.
DISCUSSION

In the present study, SSAs were significantly larger and more often proximally located than HPs but the latter finding has its nominal significance removed by Bonferroni correction for multiple testing of data. This is in concordance with recent comparative studies, in which sporadic SSAs were also found to be larger than sporadic HPs and preferentially located in the right colon whereas HPs were more often found distally.\textsuperscript{12, 18, 42} Interestingly, in our study the combination of size \( \geq 3\text{mm} \) (median size of SSAs) and proximal location, showed a highly significant difference between SSAs and HPs but with a corresponding diagnostic accuracy of only 76%.

This pilot study demonstrated that the diagnostic accuracy of AFI was unsatisfactory for differentiating SSAs from HPs (accuracy 55%). The presence of epithelial dysplasia and hypervascularisation within adenomatous polyps are considered to be responsible for their different fluorescence emission spectra when compared to non-adenomatous polyps and hence lead to a different colour on AFI.\textsuperscript{24, 43} Although epithelial dysplasia has occasionally been described in advanced SSAs progressing to carcinomas \textsuperscript{44}, SSAs generally lack these histopathological features as confirmed by the present study (figure 2).
Figure 2 (left column). Green (A), ambiguous (B) and purple (C) coloured sessile serrated adenomas using autofluorescence imaging (AFI).

Figure 3 (right column). Variation of pit-pattern characteristics in sessile serrated adenomas using narrow-band imaging (NBI): Kudo I (A), Kudo II (B), Kudo III (C)

Therefore, as expected a-priori, AFI appeared insufficient to distinguish HPs from SSAs. Furthermore, the diagnostic accuracy of AFI for differentiating adenomas from HPs was also insufficient (65%).
This is in accordance with a previous study evaluating AFI for differentiation of neoplastic and non-neoplastic lesions in the colon, in which a similar diagnostic accuracy (68%) was obtained.\textsuperscript{45}

Kudo pit pattern analysis with NBI demonstrated an equally insufficient diagnostic accuracy for differentiation of SSAs from HPs as AFI (55%). SSAs are microscopically defined as polyps with irregular crypts displaying dilatation, branching and exaggerated serration, especially at the base of the crypts. However, despite these defined crypt characteristics, microscopic differentiation of SSAs from HPs remains difficult.\textsuperscript{14, 18, 40, 42} NBI pit pattern analysis, which describes the orifices of crypts, showed similar (Kudo II) phenotypes in HPs and SSAs, confirming that crypt characteristics of these polyps are not only microscopically but also endoscopically difficult to use for differentiation purposes (figure 3).

As HPs and SSAs mainly differ in histological anatomy at the base of the crypts, it was therefore expected a-priori that the pit pattern of these polyps, which is assessed at the luminal site of the crypts, was comparable. Differentiation of adenomas from HPs however was very well possible with NBI (accuracy 94%). This corresponds with previous studies in which the diagnostic accuracy of NBI for differentiation of adenomas from non-neoplastic polyps ranged from 77-99\%.\textsuperscript{19-21, 29, 30, 32, 33, 46}

Previous use of VPI in sporadic polyps suggested that this method has a comparable diagnostic accuracy in differentiating adenomas from non-adenomatous polyps as pit pattern analysis.\textsuperscript{20, 29, 46} Based on the principle of increased vascularization, adenomatous polyps would have a stronger VPI and thus have a darker colour when viewed with NBI.\textsuperscript{19, 20, 29, 30, 32-34, 46} Owing to the presumed premalignant potential of SSAs, we examined whether a darker colour due to
hypervascularization could be observed in these polyps. Overall only 10/28 (36\%) of SSAs displayed a darker colour than the surrounding mucosa, resulting consequently in a low diagnostic accuracy and insufficient differential value (figure 4).

Figure 4: Weak (A), normal (B) and strong (C) vascular pattern intensity (VPI) displayed in three different sessile serrated adenomas using narrow-band imaging (NBI).

NBI-VPI analysis did however show a similarly high diagnostic accuracy as NBI-pit pattern for differentiating adenomas from HPs. These high diagnostic accuracies observed with NBI (pit-pattern and
VPI) are interesting considering that the median size of all polyps in this study was only 2mm. This corresponds with results from previous studies evaluating NBI for differentiating diminutive (<10mm) polyps. 

This study was performed in HPS patients in a tertiary referral center by a single endoscopist specialized in HPS. This could explain the diminutive size of polyps detected in these patients as they undergo annual surveillance endoscopies with removal of most polyps, leaving only small ones in situ. However, typical HPs seldom exceed 5mm in size and in a recent prospective study of unselected consecutive patients undergoing colonoscopy, 83% of detected SSAs were ≤10mm and 36% were ≤5mm. These findings suggest that the predominant polyps in HPS, i.e. HPs and SSAs, are typically small. Nevertheless, HPS is associated with a significantly increased risk of developing CRC. Also in our personal (unpublished) experience of annual HPS surveillance, intra-mucosal carcinomas have been detected in serrated polyps as small as 4mm. Therefore, unlike in the general population, diminutive polyps should be considered clinically relevant in HPS.

A possible limitation of this study is that only HPS patients were selected for endoscopic differentiation of polyps using ETMI. Thus, our results for differentiating polyps can not by default be extrapolated to polyps in non-HPS patients, as these are not necessarily identical. Furthermore, one may question the generalizability of this pilot study since the sample size of SSAs was relatively small. However, as the accuracies of AFI, NBI-pit pattern and NBI-VPI were all far from acceptable (52-55%) in differentiating SSAs from HPs, and the upper limit of the 95%-confidence interval of these accuracies was 68% at best, a larger sample size is unlikely to alter the conclusion of this pilot
study. Moreover, during real-time polyp assessment, AFI was used first after which an assessment with NBI was performed. This may cause AFI-colour to influence the subsequent NBI assessment and thus cause bias. However, when differentiating adenomas from HPs, NBI results were markedly better than prior AFI results, suggesting that bias due to the order of assessment was minimal. Finally, in this study diminutive lesions displaying normal mucosa on histology (n=10) were excluded from analysis. When these lesions were grouped with HPs at additional analysis, diagnostic accuracies for the different endoscopic modalities remained largely unchanged with a maximum difference of 5% (range: 1-5%).

In summary, the diagnostic accuracy of AFI, NBI-pit pattern and NBI-VPI proved unsatisfactory for differentiating SSAs from HPs in patients with HPS. Differentiation of conventional adenomas from HPs was well possible using both NBI-pit pattern and NBI-VPI. Proximal colonic location combined with a size ≥ 3mm proved to be the most valuable for differentiating SSAs from HPs. Nevertheless, the diagnostic accuracy of this combination appears still too low for clinical use (76%).
REFERENCES


