A new biomarker that predicts colonic neoplasia outcome in patients with hyperplastic colonic polyps

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ABSTRACT

The most frequently occurring lesions in the colon are the hyperplastic polyps (HP). HP have long been considered as lesions with no malignant potential and colonoscopy for these patients is not recommended. However recent works suggest that HP may represent precursor lesions of some sporadic colorectal cancers. Until now, none biomarker allows to identify the subset of HP that may have a malignant potential. Because the hormone precursor, progastrin has been involved in colon carcinogenesis, we investigated whether its expression in HP predicts occurrence of colonic neoplasm after resection of HP. We retrospectively analyzed progastrin expression in HP from 74 patients without history of colorectal pathology. In our study, 41% of patients presenting an initial HP subsequently developed adenomatous polyps, recognized as precursor lesions for colorectal adenocarcinomas. Progastrin was overexpressed in the HP in 40% of the patients. We showed a significant association between progastrin overexpression and shortened neoplasm-free survival (P= 0.001). Patients with high overexpression of progastrin had a 5 year neoplasm-free survival of 38% as compared to 100% for the patients with low progastrin expression. In addition, we established a predictive test based on progastrin staining and patient’s age that predicts occurrence of neoplasm after developing a first HP with a sensitivity of 100% (95%CI: 79%-100%) and a specificity of 74% (51%-90%).

We show that progastrin expression evaluation in HP is an efficient prognostic tool to determine patients with higher risk of metachronous neoplasms who could benefit from an adapted follow-up.
INTRODUCTION

In the general population the most frequently occurring lesion in the colon is the hyperplastic polyp (HP), with a prevalence in western populations of 10% to 35% (1). HP have long been considered as innocuous lesions with no malignant potential, whereas adenomatous polyps were recognized as neoplastic precursor lesions for colorectal adenocarcinomas.

However, large HP (size > 1cm) and the presence of multiple HP (number > 5) in hyperplastic polyposis syndrome have been clearly associated with colorectal adenomas or adenocarcinoma (2-5). In addition to these cases, more recent studies have suggested a link between HP and sporadic colorectal cancer. Huangl (6) found that patients with HP on initial colonoscopic examination have an increased incidence of colorectal adenomas on follow-up colonoscopy. Other groups have proposed the idea that HP may represent precursors of some sporadic colorectal cancers, particularly in the serrated neoplasia pathway (7-10). Despite these new data, the colonoscopy for patients with HP is not recommended, except after resection of one large hyperplastic polyp (≥ 1 cm) and/or multiple polyps (n ≥5) or if there is a family history of HP, neither in the guidelines from the French National Agency for Accreditation and Evaluation in Healthcare, nor by the American College of Gastroenterology (11, 12).

Until now, there is no biomarker to identify the subset of HP that may have a malignant potential. However, data previously enounced strongly suggest that identifying a marker that could predict, with a good sensitivity and specificity, the occurrence of colonic neoplasm after resection of HP would be crucial for identifying
the subset of patients at risk after HP who could benefit from a more suitable follow up strategy.
The hormone precursor progastrin (PG) is now recognized as a growth factor playing an important role in colon carcinogenesis. Transgenic mice overexpressing progastrin present an increased proliferative index in colonic mucosa. They also have an increased risk of developing preneoplastic lesions and adenocarcinomas in colonic epithelium when treated with a carcinogen, azoxymethane (13, 14). The role of progastrin in proliferation, survival and migration of human colon cancer cells has also been clearly established (15-20). PG-growth promoting effects are mediated through the activation of several key signal transduction pathways (21, 22). In addition, several works including ours showed that depletion of endogenous progastrin produced by colon cancer cells, leads to an inhibition of tumour growth (23, 24). Moreover, high concentrations of PG are found in colon tumours and in blood of 80% of patients with colorectal cancer. Surgical resection of the tumour induces a dramatic decrease of PG levels in the serum, suggesting that the tumour itself is the source of PG (25-27). PG is also expressed in adenomatous polyps (26, 28), which represent neoplastic precursor lesions for colorectal adenocarcinomas. In contrast, this hormone precursor is absent from the healthy intestinal epithelium.

All these data and our previous results suggested that progastrin may be a key factor of colon carcinogenesis and that its expression may predict the clinical outcome of early colonic lesions such as HP. In consequence, we investigated whether progastrin is expressed in hyperplastic polyps and determined whether its expression in HP is associated to the occurrence of colorectal cancer. We also assessed the performance of a predictive test based on progastrin staining of HP.
MATERIALS AND METHODS

Study design

We conducted a single centre historical cohort study.

Sample size, patients and data collection

The required sample size for our study main endpoint, Neoplasm-free survival (NFS), was estimated for the logrank test using the method of Freedman and Schoenfeld, with STATA v11 software (STATAcorp, Texas, USA). Thirty-eight patients were required. To demonstrate an association between progastrin staining and neoplasm occurrence, our sample size was calculated to detect a 4-fold increase in the hazard of the case group (minimum hazard ratio (HR) of 4) with 80% power. Based on our preliminary data, the probability of free-neoplasm surviving to the end of the study was set at 0.65 in the control group and prevalence of strong expression of progastrin at 50%.

We reviewed the medical records of all cases of HP diagnosed in the Pathology department of Rangueil Hospital, from 1st January 2000 through 31th December 2001. We excluded cases with a preceding colorectal adenocarcinoma or adenoma, familial colorectal adenocarcinoma history, hyperplastic polyposis, chronic inflammatory bowel disease, insufficient colon site information or follow-up data. In addition, none of the patients in the cohort showed any evidence of colonic adenoma at the initial colonoscopy. The final study group included 74 patients. We also selected 14 normal colonic tissues from resected non-complicated diverticula, 8 adenomas and 6 colorectal adenocarcinomas. Clinical data were collected for all the patients, colonoscopic data for 39 patients. Follow up colonoscopies have been
performed for surveillance purpose without particular colorectal pathology (see the exclusion criteria above). Approval of an institutional research ethics committee was obtained in accordance with the precepts of the Helsinki Declaration.

**Immunohistochemistry**

For immunohistochemistry on the formaldehyde-fixed, paraffin embedded tissues, heat Induced epitope retrieval was performed in Tris/EDTA buffer and primary antibodies were applied overnight. Detection was done using the DakoCytomation Envision+ System-HRP. Anti-progastrin antibody (1137) (29) kindly provided by Pr Shulkes was used for all the study (dilution used 1:1000). In additional experiments (when mentioned), anti-progastrin antibody H-90 (Santa Cruz) was used at the dilutions recommended by the provider.

Progastrin staining was measured by percentage of stained epithelial cells in the whole polyp. All specimens were examined in a double blinded fashion by two pathologists. Because the inter-rater agreement was excellent (ICC=0.9), percentages were reported as the average between the two readers.

**Statistical analysis**

Univariate analysis was conducted to compare clinical and immunohistochemistry findings between the different study groups using the Chi² test or Fisher exact test (when required) for categorical variables and Wilcoxon-Mann-Whitney for quantitative variables. We calculated intra-class correlation in order to determine inter-rater agreement for immunohistochemistry staining.

For neoplastic-free survival analysis, only patients with colonoscopic follow up data were included (39 patients). We performed Kaplan-Meier curves and log rank test to
assess the association of progastrin expression with the occurrence of a new colorectal neoplastic event. The main end point was neoplasm-free survival. The time to event was determined as the time interval between the diagnosis of HP and the occurrence of metachronous colorectal adenomas in the same site (proximal or distal colon) as the first HP. Progastrin expression was recorded as low, moderate or high expression with the percentage of positive epithelial cells. The “normality” threshold of progastrin expression (low expression) was determined using the 95th percentile of percentage of stained cells in normal colonic tissue (<10%). Moderate expression of progastrin was defined as staining in 10% to 50% of polyp epithelial cells and high expression as staining in more than 50% of cells. When a patient had several polyps, the polyp with the strongest expression of progastrin was retained for evaluation. The log-rank test was also used to assess significance of clinical characteristics. Quantitative variables were recorded into two-class variables using the median. To demonstrate that progastrin was a prognostic factor independent from other clinical factors, cox proportional-hazards model was performed to test the simultaneous influence on disease free survival of all covariate with a p-value<0.20 in the univariate analysis. After a backward-stepwise selection, only significant variables (p<0.05) were kept in the final cox model.

Receiver operating characteristic analysis was then performed to select optimal “diagnostic” threshold for each significant quantitative variable after multivariate analysis. Using the ROC analysis results, we constructed a predictive test based on a composite score with the significant variables, in order to predict the occurrence of a neoplastic event among patient with HP. Performance of this test was measured by sensitivity (Se), specificity (Spe), positive predictive value (PPV) and negative predictive value (NPV). We also constructed a classification tree using the same
variables significantly associated with occurrence of a neoplastic event. To validate
the classification tree, a bootstrap validation (30, 31) (with 100 bootstrap samples)
was performed and the misclassification error rate estimated.

In our study, all tests were two-sided and statistical significance was set at a P value
of 0.05. Analyses were performed using STATA v11 (32) and R (with “tree” and
“ipred” packs).
RESULTS

Patient and polyps characteristics

Clinical and pathologic features of patients and their polyps are shown in table 1. For the whole cohort, the median age of patients was 65 year old (y.o.) (sd : 14 y.o) and 43% of the patients were female. All polyps measured less than 1 cm with an average diameter of 3 mm, and the number of polyps at diagnosis was less than 5 for all the patients. Twenty % of HP were localized in the proximal colon and 80% in the distal colon, which is consistent with previous observations. In our sample, none HP displayed the major histological features described for sessile serrated adenomas (SSA), including architectural abnormalities and loss of expression of MLH1 (4, 10, 33) The subset of patients with colonoscopic follow up was representative of the whole cohort since no statistical difference on clinical and immuno-histochemical features was found between patients with a colonoscopic follow up and the ones with a medical follow up. During the follow-up, an occurrence of adenomas at the same general area of the colon as the initial HP (proximal or distal colon) was found in 41% of the patients. The pathologic type of these metachronous adenomas was tubular adenomas except in one case where a tubulovillous adenoma was observed. Mainly low grade adenomas (38%) were diagnosed and 3% were high grade adenomas.

Progastrin expression in normal colon, colonic hyperplastic polyps, adenomas and adenocarcinomas

Representative pictures of progastrin staining, obtained with the anti-progastrin antibody 1137 (29), in the different colon tissues are shown in figures 1 and 2. Similar results have been obtained with the commercially available anti-progastrin H-90
antibody. Representative pictures with this antibody are shown in supplementary data 1. The percentages of progastrin-positive cells in normal colon, HP, adenomas, and adenocarcinomas are reported in figure 3A. Progastrin expression in HP was significantly different from the expression in normal colon (respectively, median: 45%, range: 0%-100%; versus 1%, range: 0%-10%, p=0.002). 100% of the tested adenomas or adenocarcinomas displayed an important expression of progastrin. As previously described (26, 28), progastrin expression was higher in adenomas and adenocarcinomas than in normal colon (low grade dysplasia adenomas, p=0.001; high grade dysplasia adenomas, p=0.0007; adenocarcinomas, p=0.0005).

In normal colon, average percentage of progastrin staining was 2.6% (sd: 3.7%, range: 0-10). We considered, that progastrin staining was low when the percentage of staining was less than the 95th percentile of the normal tissue (<10%). In the whole cohort, weak expression of progastrin (<10%) was found in 26% of the HP. Expression of the prohormone was moderate (10%-50% of staining) in 34% of the HP and high (>50% of staining) in 40% of them (Table 1). The results were not significantly different in the patients group with colonoscopic follow up (weak expression 18%, moderate expression 36%, high expression 46%, p=0.26).

**Patient neoplasm-free survival**

Survival analysis was performed using data from patients who had at least one colonoscopy during their follow up. As mentioned above, clinical and immunohistological characteristics of these patients did not significantly differ from the other patients of the cohort. The median for the follow up period was 5 years (range [2-10]). Only occurrence of metachronous adenomas at the same general area of the colon (proximal or distal colon) was considered as a recurrence event. The overall median of neoplasm-free survival (S50%) was 8 years and survival at 5
years was 67% (95%CI: 49%-80%) (Figure 3B). Univariate analysis (Table 2) showed a significant association between progastrin overexpression and shortened neoplasm-free survival (p=0.001). Patients with high expression of progastrin had a median neoplasm-free survival of 5 years, whereas during the 10 year following period, the median survival was not reached by patients with low expression of progastrin, since no recurrence occurred (Table 2, figure 3C). The 5 year-neoplasm-free survival (5y.S) for the patients with weak progastrin expression was 100%, this value decreases to 84% for the patients with moderate expression and to 38% for the patients with high expression. In the same way, age was significantly associated with occurrence of adenoma (p<0.0001, for patients who were less than 64 year old: 5y.S=95% and S50%=10 years vs 5y.S=26% and S50%=5 years for older patients, Table 2).

To demonstrate that the association between progastrin staining and neoplasm-free survival was independent from the subject’s age, HP localisation and HP size, we performed multivariate analysis (table 2). After adjustment, progastrin expression was still highly significantly associated with adenoma occurrences (p<0.0001). Taken together, these results show that progastrin staining in HP is an independent prognostic factor. As mentioned above, 41% of patients having an initial HP developed metachronous adenomas (Table 1) with 100% of these adenomas presenting a high staining of progastrin. Interestingly, the surrounding unaffected mucosa of the adenomas was also positive for progastrin. Representative pictures are shown in supplementary data 2.

**A predictive test for recurrence**

To assess whether a predictive test using progastrin and age can predict occurrence of neoplasm after developing a first HP, we performed the receiver operating
characteristic curves for the percentage of progastrin positive cells and for age (Figure 4A, 4B). The predictive performance of progastrin and age were estimated by the area under the curve (AUC), which were, respectively, 0.81 (95%CI: 0.68-0.94) and 0.86 (95%CI: 0.74-0.98). Using the ROC curves, we also determined the best “diagnostic” threshold (i.e. with a 100% sensitivity to detect all the patients at risk to develop an adenoma and an optimal specificity) for each parameter in order to construct a classification tree. For progastrin staining, we chose the 40% - threshold (Sensitivity (Se)=100% and specificity (Spe)=57%) and for age the 54 y.o- threshold (Se=100% and Spe=48%). The classification tree, calculated with R software, shows that all patients with less than 40% of progastrin positive HP cells can be considered “at low risk”. Among patients with more than 40% of PG staining in HP, only patients older than 54 y.o. can be considered as “at high risk” (Figure 4C). This composite test had a sensitivity of 100% (95%CI: 79%-100%), a specificity of 74% (51%-90%), a positive predictive value of 73% 95%CI: 50%-89%) and a negative predictive value of 100% (95%CI: 81%-100%). Misclassification error rate was 15% in our study sample. To estimate generalization error of classification, a bootstrap validation was performed. The out-of-bag misclassification error estimate was 25.6%. Among misclassification errors, no false negative was found and all neoplasm occurrences were detected. Therefore, we established a predictive test based on progastrin staining and patient’s age that predicts occurrence of colonic neoplasm after developing a first HP.
DISCUSSION

We show for the first time the expression of progastrin in HP. This supports the hypothesis that this hormonal precursor, known to be expressed in human colorectal adenomas and adenocarcinomas, could also play a pro-tumoral role at the very early stage of colonic carcinogenesis.

More importantly, we also showed that patients, whose HP displayed a strong expression of progastrin, were at higher risk to develop adenoma. These patients had a 5 year neoplasm free survival of 38% as compared to 100% for the patients with no/weak progastrin expression.

In our study, 41% of patients having HP developed metachronous adenomas. Only two patients showed recurrent hyperplastic polyps at follow up colonoscopy with a progastrin staining similar to the initial polyp. These results are in accordance with a previous study which reported a 2 times greater risk for subsequent colon neoplasia in patients having HP compared to HP free patients (6).

It is generally accepted that a subset of HP, named sessile serrated adenomas (SSA) by Torlakovic (4, 10), might be preneoplastic lesions. In our study, none HP displayed characteristics of SSA, such as architectural abnormalities and loss of MLH1 (33). The metachronous adenomas that we observed in our cohort were all tubular or tubulo-villous adenomas and did not display histological feature of serrated adenoma nor carcinoma. However, the existence of an association between HP and classical neoplasms is not surprising since patients with hyperplastic polyposis develop both serrated and classical carcinomas. Furthermore, HP are known to share the same risk factors as classical colorectal adenocarcinoma. Distinction between the serrated and classical pathway might not be as dichotomous as suggested by the existence of mixed polyps, in which serrated and non serrated features coexist.
Since the progastrin gene has been previously shown to be a target of the transcriptional complex β-catenin/TCF-4 (34, 35) we have analyzed the activation of the β-catenin pathway in the HP. None of the HP presented a delocalisation of β-catenin from the plasma membrane to the cytoplasm or the nucleus characteristic of the activation of the pathway (supplementary data 3). The Ras/Raf pathway is known to be activated in 50 to 80% of HP (36, 37) and some authors suggest its involvement in the development of hyperplastic lesions (6). Since several studies have suggested a correlation between the Ras/Raf pathway activation and progastrin gene expression (34, 38), this pathway might be responsible of the progastrin overexpression observed in the HP.

In our study, patients with HP presenting a high expression of progastrin were at higher risk to develop metachronous adenomas in the same localisation. This high expression of progastrin in HP might reflect an environmental background with greater risk for developing colorectal neoplasia. However, in our cohort three patients developed adenomas in another site than their original HP but progastrin was overexpressed (>50%) in only one of them while progastrin was overexpressed in all HP with occurrence at the same site. This result suggests that progastrin overexpression is not only a marker of pejorative background. Indeed, another hypothesis is the presence of molecular abnormality, such as progastrin expression, in these polyps but also in the adjacent colon. Interestingly, adjacent colonic epithelium of all HP with a high expression of progastrin also showed a strong expression of the hormonal precursor. In contrast, for all the hyperplastic polyps negative for progastrin, the surrounding unaffected mucosa was also negative. Representative pictures are shown in supplementary data 4. Similar observations have also been done on normal colonic epithelium adjacent to adenocarcinomas. This may suggest that the adjacent histologically normal colonic epithelium of both lesions also express early molecular abnormalities. “The molecular security margin”
might be lower than histological security margin. Therefore, HP endoscopic excision
might thus be not extensive enough to prevent a further neoplastic event. We
demonstrated that progastrin staining could be an efficient tool to detect patients with
higher risk of metachronous neoplasms. Based on our data, a test based on
progastrin staining and age would display 100% of sensitivity and 74% of specificity.
In our study, there was no false negative patient (NVP=0). Thus patient with no
progastrin overexpression, whatever their age, would have a low risk to develop
metachronous neoplasm, whereas patients in our high progastrin expression group
had a 73% risk of developing a neoplasm. Misclassification rate of our composite
predictive test was only 15%. In this study, the sample size with colonoscopic follow
up was restricted to 39 patients for several reasons. First, the guidelines from either
the French National Agency for Accreditation and Evaluation in Healthcare, or the
American College of Gastroenterology do not recommend follow up colonoscopies
for patients with "benign" HP. Second, to avoid potential bias in our study, we
excluded all the cases with a preceding colorectal adenocarcinoma or adenoma,
familial colorectal adenocarcinoma history, hyperplastic polyposis or chronic
inflammatory bowel disease, as well as insufficient colon site information or follow-up
data. Third, we included only the patients HP diagnosed in the Pathology department
of Rangueil Hospital, from 1st January 2000 through 31th December 2001. To have
up to 10 years follow up. Despite the relative small size of our sample Progastrin
expression predicts occurrence of neoplasm after developing a first HP with a high
sensitivity and specificity .and we obtained a very high and significant difference in
neoplasm free survival between patients with high expression of progastrin (5 year
neoplasm free survival of 38%) as compared to patients with no/weak expression (5
year neoplasm free survival of 100%). However, it might be interesting to complete
this study by a retrospective multicentre study including more patients or a
prospective study.
We performed a bootstrap cross-validation to estimate misclassification error. The out-of-bag misclassification rate estimate was 26%. Interestingly, none of the patients in our study cohort met the guidelines criteria for endoscopic surveillance, i.e. resection of one hyperplastic polyp $\geq 1$ cm and/or multiple polyps ($n \geq 5$) or a family history of hyperplastic polyps. Hence, a predictive test based on progastrin and age could be useful to select a subset of patients who would benefit from colonoscopic surveillance. In our study, none of the patients has developed colorectal adenocarcinoma during their follow up. Nevertheless, because these patients have benefited an endoscopic surveillance although current guidelines do not recommend it, they could have been treated at adenoma stage before adenocarcinoma transformation.

We show for the first time that progastrin expression in HP is a strong independent predictive factor of risk for developing an adenoma and we establish here and propose a classification tree to select the population of patients who need a colonoscopic follow-up. In summary, we have demonstrated that progastrin staining in HP could be an efficient tool to detect patients with higher risk of metachronous neoplasms.
TABLES

Table 1: Clinical and immunohistological features

<table>
<thead>
<tr>
<th>Variables</th>
<th>Whole cohort N=74</th>
<th>Patients with coloscopic follow up n=39</th>
<th>p-value</th>
</tr>
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<tr>
<td></td>
<td>% [95%CI]</td>
<td>% [95%CI]</td>
<td></td>
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<tr>
<td>Age (y.o)</td>
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<tr>
<td>Median</td>
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<tr>
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<tr>
<td>Male</td>
<td>57% [45-68]</td>
<td>59% [42-74]</td>
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<tr>
<td>Female</td>
<td>43% [32-55]</td>
<td>41% [26-58]</td>
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<tr>
<td>Number of polyps</td>
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<tr>
<td>1</td>
<td>59% [47-71]</td>
<td>62% [45-77]</td>
<td>0.89</td>
</tr>
<tr>
<td>2-4</td>
<td>41% [29-53]</td>
<td>38% [23-55]</td>
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<tr>
<td>Size of polyps (largest diameter, mm)</td>
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</tr>
<tr>
<td>Median</td>
<td>3</td>
<td>3</td>
<td>0.49</td>
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<tr>
<td>Range</td>
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<td>1-5</td>
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<tr>
<td>Localization</td>
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<tr>
<td>Proximal colon</td>
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<td>Distal colon</td>
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<tr>
<td>Occurrence of metachronous adenoma</td>
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<td>Loss of expression of MLH1</td>
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<tr>
<td>No/low expression (&lt;10%)</td>
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<td>Moderate expression (10%-50%)</td>
<td>24% [16-37]</td>
<td>18% [8-34]</td>
<td>0.26</td>
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<tr>
<td>High expression (&gt;50%)</td>
<td>34% [23-46]</td>
<td>36% [21-53]</td>
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<tr>
<td>Progastrin expression</td>
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<tr>
<td>No/low expression (&lt;10%)</td>
<td>26% [16-37]</td>
<td>18% [8-34]</td>
<td>0.26</td>
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<tr>
<td>Moderate expression (10%-50%)</td>
<td>34% [23-46]</td>
<td>36% [21-53]</td>
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<tr>
<td>High expression (&gt;50%)</td>
<td>40% [29-53]</td>
<td>46% [30-63]</td>
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</table>

Progastrin expression was recorded as “No/low moderate or high expression with the percentage of positive colonic epithelial cells. The “physiological” threshold of progastrin expression was determined using the 95th percentile of percentage of stained cells in normal colonic tissue (10% of positive cells).

Chi 2 tests (for categorical variables) and Student tests (for continuous variables) were performed to compare clinical and immunohistological features between patients who had a coloscopic follow up and those who had not.

95%CI: Binomial exact 95- confidence interval was calculated for each percentage.

* One-sided, 97.5% confidence interval.

NA: not available
NC: not calculable
Table 2: Neoplasm-free survival according to clinical and immunohistological features

<table>
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<th>Multivariate analysis</th>
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<td><strong>Age (y.o)</strong></td>
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<tr>
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<td>7</td>
<td>56% [27-78]</td>
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<tr>
<td><strong>Size of polyps</strong></td>
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<td>(largest diameter, mm)</td>
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<tr>
<td>&lt;3 mm</td>
<td>NR</td>
<td>76% [47-90]</td>
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<tr>
<td>3-6 mm</td>
<td>7</td>
<td>60% [35-78]</td>
<td></td>
</tr>
<tr>
<td><strong>Localization</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal colon</td>
<td>5</td>
<td>43% [10-73]</td>
<td>0.1378</td>
</tr>
<tr>
<td>Distal colon</td>
<td>10</td>
<td>74% [54-86]</td>
<td></td>
</tr>
<tr>
<td><strong>Expression of progastrin</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No/low expression (&lt;10%)</td>
<td>NR</td>
<td>100% [NC]</td>
<td>0.001</td>
</tr>
<tr>
<td>Moderate expression (10%-50%)</td>
<td>10</td>
<td>84% [50-96]</td>
<td></td>
</tr>
<tr>
<td>High expression (&gt;50%)</td>
<td>5</td>
<td>38% [15-61]</td>
<td></td>
</tr>
</tbody>
</table>

Neoplasm-free survival among patients with colonic hyperplastic polyps who had a coloscopic follow-up.

Multivariate analysis was performed using a cox model including all variables with a p-value<0.20 in the univariate analysis. Final model was obtained after backward stepwise selection, keeping variables with a p-value<0.05.

Y: year, 5y.S.: 5 years-Survival.
95% CI: 95% confident interval was calculated for all 5 years-Survival values.
NR: Not reached, NC: not calculable.
AKNOWLEDGMENTS

We thank Sophie Leguelec for her help in this project and Serge Estaque for his technical assistance.
REFERENCES

FIGURES LEGENDS

Figure 1. Progastrin staining in normal colon and hyperplastic polyps (HP).
A) Negative progastrin staining in normal colon (x40). B) Weak progastrin staining in the bottom of the crypt in normal colon (arrows)(x40). C, D) Progastrin negative HP (respectively, x20 and x40) E, F) HP overexpressing progastrin (respectively, x20 and x40). G) Positive HP-adjacent normal colon (x20). Anti-progastrin antibody 1137 (29) was used for all experiments.

Figure 2. Progastrin staining in colon adenomas and adenocarcinomas.
A, B) Strong staining in low grade (A, x20) and high grade dysplasia adenoma (B, x40). C to F) Strong progastrin staining in adenocarcinomas at different magnifications (x4, x20, x40 and x100). G) Adenocarcinoma-adjacent normal tissue positive for progastrin. Anti-progastrin antibody 1137 (29) was used for all experiments.

Figure 3. Progastrin expression in colonic tissues and Kaplan Meier survival curves
A) Percentage of progastrin positive cells for normal colon, Hyperplastic polyps (HP), low grade dysplasia tubular adenoma (TA), high grade dysplasia TA, and adenocarcinoma (ADC). Wilcoxon tests were performed to compare the percentage of progastrin positive cells in the different tissues to the percentage in normal colon. B) Overall neoplasm-free Survival among patients with a colonoscopic follow-up, C) neoplasm-free Survival among patients with a colonoscopic follow-up, according to progastrin staining. p-value corresponds to logrank univariate analysis.
Figure 4. A predictive test for recurrence.
A) Receiver operating characteristic curves for progastrin staining, B) Receiver operating characteristic curves for age. “a”-Threshold had a 100% sensitivity and the optimal specificity. Se: Sensitivity, Spe: specificity, PG: percentage of progastrin staining, y.o.: year old. C) Classification tree using variables significantly associated with neoplasm occurrence after multivariate analysis (Progastrin staining and age).

Supplementary data 1. Progastrin staining in hyperplastic polyps (HP), colon adenomas and adenocarcinomas.
A) Progastrin negative HP. B) HP overexpressing progastrin. C) Strong staining in adenomas. D) Strong progastrin staining in adenocarcinomas. Anti-progastrin antibody H90 was used for all experiments. (x20)

Supplementary data 2. Progastrin staining in colon adenomas and surrounding normal mucosa.
Progastrin staining of adenomas (A, C) developed by 2 patients presenting an initial hyperplastic polyp positive for progastrin and the corresponding surrounding normal mucosa (B, D) (x 20).

Supplementary data 3. β-catenin staining in hyperplastic polyps (HP) positive for Progastrin
Hyperplastic polyps of patients positive for progastrin showing a membrane localisation of β-catenin staining. (x20).

Supplementary data 4. Progastrin staining in hyperplastic polyps (HP) and the corresponding surrounding normal mucosa.
A, B) HP overexpressing progastrin and the corresponding surrounding normal mucosa (patient 1). C, D) HP overexpressing progastrin and the corresponding surrounding normal mucosa (patient 2) and E, F) Progastrin negative HP and the corresponding surrounding normal mucosa (patient 3).
Figure 1

A

B

C

D

E

F

G
Figure 2
Figure 3

A

Prolactin staining (percentage of positive epithelial cells)

Normal colon, HP, Low grade TA, High grade TA, ADC

P = 0.002, P = 0.001, P = 0.0007, P = 0.0005

B

Overall Kaplan-Meier survival estimate

neoplasm-free survival

Number at risk: 39, 39, 30, 19, 9, 1

Time (years)

C

Kaplan-Meier survival estimates - Progastrin expression

No/Low expression

Moderate expression

Strong expression

Number at risk:

Normal expression: 7, 7, 7, 6, 3, 0

Moderate expression: 14, 14, 11, 8, 5, 1

Strong expression: 18, 18, 12, 5, 1, 0

p = 0.001
Figure 4

A

Area under ROC curve = 0.8098

a. PG ≥ 40%: Se=100% Spe=57%
b. PG ≥ 50%: Se=81% Spe=65%
c. PG ≥ 55%: Se=75% Spe=74%

B

Area under ROC curve = 0.8614

a. Age ≥ 54 y.o.: Se=100% Spe=48%
b. Age ≥ 64 y.o.: Se=94% Spe=78%
c. Age ≥ 66 y.o.: Se=81% Spe=83%

C

PROGASTRIN STAINING

<40%  ≥ 40%

AGE

<54 y.o. ≥ 54 y.o.

Low risk patients

High risk patients

Misclassification rate 15%
(out-of-bag misclassification rate estimate=26%)
Cancer Prevention Research

A new biomarker that predicts colonic neoplasia outcome in patients with hyperplastic colonic polyps

Catherine Do, Claudine Bertrand, Julien Palasse, et al.

Cancer Prev Res  Published OnlineFirst February 24, 2012.

Updated version  Access the most recent version of this article at: doi:10.1158/1940-6207.CAPR-11-0408

Supplementary Material  Access the most recent supplemental material at: http://cancerpreventionresearch.aacrjournals.org/content/suppl/2012/02/24/1940-6207.CAPR-11-0408.DC1

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