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Rectal Mucosal Quantitative Galactose Oxidase-Schiff Reaction as an Early Detection Biomarker for Colorectal Cancer: Comparison to Fecal Occult Stool Blood Test

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Abstract

The galactose oxidase-Schiff (GOS) reaction detects D-galactose- β -[1,3]-N-acetyl-D-galactosamine. This is a T-antigen expressed in mucus from malignant cells and colonic mucosa adjacent to cancer but not in normal mucosa. Previous studies using a qualitative GOS assay proved to be of limited value for the detection of colorectal neoplasia. We used a newly developed quantitative GOS assay to determine its potential as an early detection biomarker for colorectal cancer. We completed a multi-center, prospective, cross-sectional cohort validation study consisting of 70 normal controls, 23 high-risk normal patients (polyp history or family history of colorectal cancer (CRC) with currently normal colonoscopy), 137 patients with adenomatous polyps, and 69 with colorectal cancers. Prior to colonoscopy, two samples of stool were collected via a rectal exam: one for FOBT, and one for GOS. The area under the ROC curve (AUC) for detecting colonic adenomas and cancer for normal colons, computed with logistic regression was 0.69 for GOS, 0.62 for FOBT, and 0.73 for GOS combined with FOBT. Adding GOS to FOBT did not significantly change the ROC of FOBT alone. GOS does not appear to be a suitable marker of colorectal neoplasia.

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Conflict of Interest: Michael J. Eveleigh was a former senior executive with PreMD Inc., the company that developed the GOS test.

Keywords

Colorectal Neoplasms/diagnosis; Colorectal Neoplasms/prevention & control; Carcinoma/diagnosis; Galactose Oxidase; Predictive Value of Tests

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Introduction

The galactose oxidase-Schiff (GOS) reaction detects D-galactose- β -[1,3]-N-acetyl-D-galactosamine, also known as the Thomsen-Friedenreich antigen. This is a T-antigen expressed in mucus from malignant cells and colonic mucosa adjacent to cancer but not in normal mucosa [1]. As detailed previously,[4] a qualitative assay has been developed to detect GOS in rectal mucus when applied and air dried on a membrane. A positive reaction was initially taken to be a magenta-colored stain, but the technique was subjective and not useful as a screen for colorectal cancer [2]. A new quantitative method was designed to assess the GOS reactivity of rectal mucus by measuring color hue using a portable absorbance spectrophotometer (PreMD, Inc, Toronto, Ontario). In a series of clinical cohort studies, GOS reactivity on 1787 subjects scheduled for colonoscopy found that a positive GOS reaction was significantly associated with cancer risk (odds ratio 3.83, 95% confidence interval 2.30–6.38, $p < 0.001$). Overall sensitivity for cancer was 49.1%, higher for early stage disease (Duke's A and B, 54% sensitivity) than later stage disease (Duke's C and D, 36% sensitivity) [3].

The current study aimed to assess performance of the new quantitative mucosal GOS test as a screening test for colorectal neoplasia and to compare the operating characteristics of the GOS assay to those of the fecal occult blood test (FOBT) and to the combination of the two.

Methods

This was a multi-center, prospective, cross-sectional validation study, consisting of subjects with 1) colorectal adenocarcinoma (cancer), 2) colorectal adenomas (adenoma), 3) adenoma history or 1st degree family history of cancer or hereditary non-polyposis colorectal cancer with currently normal colonoscopy (high-risk normal) or 4) normal colonoscopy with no history of adenoma and no family history of colorectal neoplasia, or adenomas on qualifying colonoscopy (normal controls). Cancer patients must have had a diagnosis of resectable CRC and be studied before surgery. Adenoma subjects must have had at least one adenoma still in the colorectum during baseline sample collections.

The baseline visit was conducted prior to a scheduled colonoscopy, at a pre-surgery visit for cancers and large adenomas, or two weeks post-colonoscopy if no lesions were seen (normal and high-risk normal). Informed consent and survey data (eligibility, demographics, family history, and prior colorectal cancer screening) were collected at the baseline visit. One stool collection for FOBT was obtained after the baseline visit but before any colonoscopy preparation procedure, colonoscopy, or removal of any colonic lesions. GOS collection via a rectal exam was obtained once at baseline or, if applicable, prior to beginning the colonoscopy.

Rectal Mucus Sample Procurement for Galactose Oxidase Schiff's (GOS)

A rectal mucus sample was obtained during a standard digital rectal examination using a GOS negative lubricant such as mineral oil. Two samples were applied to two cards and batch shipped to PreMD, Inc. in Hamilton, Ontario, Canada for blinded analysis, using their previously defined process [3].

FOBT Collection and Assay

Subjects were provided with a standard collection kit for Hemoccult II® SENSAR® Fecal Occult Blood Test (BeckmanCoulter Inc, Brea, CA) including detailed instructions on completion of the stool guaiac card (FOBT) using one stool specimen sampled from a different portion of the stool for each of three windows on the card. The FOBT card was shipped to the University of Michigan at room temperature and developed and read following manufacturer's instructions.

Analysis

The GOS scores for prepped participants were compared by one-way ANOVA. Logistic regression was used to classify participants into disease states based upon marker. Receiver operator curves (ROC) were constructed for GOS score alone, FOBT outcome alone, and both combined to detect normal and high-risk normal participants from participants with adenomatous polyps or colorectal cancer. The GOS scores for adenoma and cancer patients (groups with significant numbers of prepped and unprepped study participants) were compared by a two-way ANOVA to determine if prep had an effect.

Results

Samples and data were collected from 428 study participants. To avoid confounding of colonoscopy preparative procedure, analysis was restricted to the 299 study participants who had completed the full preparative procedure for colonoscopy and who had complete samples and data sets. The distribution across disease groups was 70 normal controls, 23 high-risk normal, 137 with adenomatous polyps, and 69 with CRC. The analysis sample consisted of 140 women and 159 men, of whom 146 were current smokers (49%), and 39 were diabetic (13%). The mean age was 60.1 years (range 29 to 88 years).

The GOS scores were significantly different ($p < 0.001$, $R^2 = 0.09$) with the high-risk normal significantly different from all other groups ($p < 0.001$ after Bonferroni adjustment) (Figure 1). The area under the curve (AUC) was 0.69 for GOS, 0.62 for FOBT, and 0.73 for GOS combined with FOBT; combining GOS score with FOBT did not significantly change the ROC over that for FOBT alone (Figure 2). The colonic preparative procedure significantly suppressed the GOS scores of the colorectal cancer participants (2.87 unprepped compared to 1.78 prepped, $p < 0.008$), but had an opposite, yet non-significant ($p = 0.4$) effect on the GOS score for participants with adenomatous polyps (1.50 unprepped compared to 1.86 prepped).

Discussion

The new quantitative GOS technique, which avoids the subjective interpretation of the previous assay,[4] had an ROC similar to that for one FOBT card. Combining GOS and FOBT did not improve the ROC. Colonic preparation appears to affect the GOS Score. Thus, new quantitative GOS score does not appear to be useful as an early detection biomarker for colorectal adenomas or cancer.

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Figure 1.
GOS scores by disease groups.

Figure 2.

ROC curves for GOS score and the combination of GOS score and FOBT to detect normal and high-risk normal participants from participants with adenomatous polyps or colorectal cancer.