The diagnostic accuracy of papanicolau staining in brush biopsy as an alternative to tissue biopsy in patients undergoing colonoscopy for non-obstructing colonic tumors at Vicente Sotto Memorial Medical Center from February 2016 to September 2017: A prospective study

Becaldo CL
Kuan AM
Ong LY

Department of Medicine
Vicente Sotto Memorial Medical Center
Cebu City, Philippines

Correspondence:
Dr. Clint L. Becaldo at
clintmt05@yahoo.com

Accepted for publication:
December 2019

Abstract

Background: In the Philippines in 2010, colorectal cancer combined ranked 4th for both sexes. Early detection of colorectal malignancy remains in the forefront of long-term management. *Objective:* The study was conducted to determine the diagnostic accuracy of papanicolau stain in detecting malignancy from brush biopsy specimens in patients undergoing colonoscopy. *Methodology:* A prospective, open label 19-month study from February 2016 to September 2017 was done on patients aged 19 years and above. On colonoscopy, brush biopsies around the four sides of tumors were sampled after the tissue biopsy procedure, smeared to slides, fixed in 95% ethyl alcohol, stained using papanicolau method and read by two independent pathologists. *Research Instrument:* Tabulated data sheets and statistical analysis using SPSS. *Results:* There were 60 subjects who underwent colonoscopy for colonic tumors, with average age of 55.60 years, 53.3% of whom were females. 50% were smokers, and 85% had history of colon cancer in the family. There was a significant association of papanicolau stain in detecting malignancy (p=0.002), with sensitivity of 79.2% and specificity of 66.6%. *Conclusion:* Papanicolau stain shows good sensitivity, correctly identifying 79.2% of patients who truly have colon cancer, with fair specificity at 66.6%.

Keywords: colorectal cancer, papanicolau stain, brush biopsy

Introduction

In 2010, colorectal cancer was second to lung cancer as a cause of cancer death in the United States, with 142,570 new cases and 51,370 deaths.[1-3] In the Philippines in 2010, cancers of the colon and rectum combined ranked 4th for both sexes, 3rd among males and 4th among females. There were 5,787 new cases in both sexes; 3,208 were noted in males and 2,579 in females.[4] Globally, incidence rate[4-8] and mortality[6,8-11] have decreased significantly during the past 20 years, likely due to enhanced and more compliantly followed screening practices.[3] The American Cancer Society suggests fecal hemoccult screening annually and flexible sigmoidoscopy every five years, beginning at the age of 50 for asymptomatic individuals having no colorectal cancer risk factors. The entire large bowel should be visualized endoscopically or radiographically for adenomatous polyps, since most colorectal cancers, regardless of etiology, arise from these polyps.[1] Colonoscopy has been shown to be superior and has a higher sensitivity for detecting cancers than the strategy employing occult fecal blood testing and flexible sigmoidoscopy.[1-3]

In the past, only the more proximal and distal portions of the gastrointestinal tract could be sampled by blind or direct visualization techniques, without the necessity of open surgery or external radiologic image-guided methods. Currently, most portions of the gastrointestinal tract may be sampled by upper and lower intestinal endoscopies with the use of available smaller fiberoptic tubes, allowing direct visualization of the lesions and endoscopic ultrasound-guided biopsy methods.

Increasing incidence of cancer in various organs has underscored the need for accurate diagnostic methods and quick results. A short turnaround time and timely communication of official results obviates further unnecessary investigation and helps relieve patient anxiety. This has been made possible by the use of newer instruments and techniques which have made it relatively easier to collect not only cytologic but also histologic specimens from most gastrointestinal sites, which may serve as complementary or adjunct specimens to the main tissue specimen. Cytologic techniques are particularly useful for preoperative diagnosis of gastrointestinal lesions that may otherwise be inaccessible. These techniques are also useful for lesions that may pose significant risks for complications during standard biopsy methods, such as bleeding, perforation, or tumor dissemination. Examples of cytologic samples are those taken from fine needle aspiration cytology (FNAC), imprint cytology (IC), frozen section (FS), scrape cytology, and papanicolau (pap) stain or smear.

Papanicolau stain was originally used to screen and detect human cervical cancer, but currently has also been applied for evaluation of sputum, urine, breast and other non-gynecologic specimens. At present, papanicolau stain is widely available, cheap, and has shorter turnaround time compared to biopsies.[12,13]

The setting of this study, the Vicente Sotto Memorial Medical Center (VSMMC) is a regional, tertiary government hospital receiving referrals from other institutions within the city and from nearby provinces of Cebu, Bohol, and Negros Oriental. Annually, this institution does an average number of 250 colonoscopies for colonic tumors.

This study investigates the usefulness of papanicolau staining as an alternative method to tissue biopsy and aims to determine its diagnostic accuracy, sensitivity and specificity in detecting malignancy from brush biopsy specimens in patients with non-obstructing colonic tumors undergoing colonoscopy. It also aims to describe the demographic profile of subjects included.

Review of Related Literature

Adenocarcinomas are, by far, the most common malignancy of the gastrointestinal tract. Most colorectal cancers, regardless of etiology, arise from adenomatous polyps. Clinically, the probability of an adenomatous polyp becoming a cancer depends on the size of the lesion, its gross appearance, and histologic features. Detection of an adenomatous polyp involves visualization of the entire bowel endoscopically or radiographically, since synchronous lesions are noted in about one-third of cases.[3]

Gastrointestinal malignancy may be suspected on clinical and serologic grounds (elevated CEA, AFP) and by imaging techniques (X-ray, ultrasound, computed tomography, magnetic resonance imaging and barium scans). However, cytohistologic sampling with morphologic evaluation of lesion is in most instances necessary to provide a definitive diagnosis before treatment is initiated. For generations, diagnosis has relied upon the acquisition of cellular material for ex-vivo microscopic analysis by pathologists. Cytologic techniques, depending on the tumor location and type, may be employed for primary diagnosis, prognosis, and prediction of tumor behavior as well as for secondary/recurrent diagnoses, and may also be used for staging purposes.[14] But given the limitations of biopsies, including cost, time delay, and risks to patients, new optical techniques are being evaluated for their ability to achieve diagnostic “biopsies” in situ.

Papanicolau method of staining, originally indicated for vaginal smears to detect cervical cancers, is a method of examining with a microscope a sample of superficial cells that line the inner wall of the uterine cervix to detect any abnormal cell for early diagnosis of cervical cancer. It was developed in 1928 by the Greek doctor George Nicholas Papanicolaou (1883-1962) at the Cornell Medical College of New York. He also developed the particular polychrome staining reaction designed to demonstrate variations of cellular maturity and metabolic activity. Cytoplasmic transparency is a function of high ethanol concentration of the stain, which is important in order to view multilayered cell aggregates.

Smears demonstrate excellent results when stained according to the papanicolau technique. Advantages of this staining procedure are: (1) transparent blue staining of cytoplasm attained due to the action of high alcoholic content of the cytoplasmic counterstain, allowing overlapping cells to be seen and identified; (2) excellent nuclear detail produced; (3) predictable color range, which is of great value in cellular identification and classification, producing good differential coloring of basophilic and acidophilic cells; and (4) stain is valuable in comparing cellular appearances in smears with their counterpart in similarly stained sections. The procedure, however, is a little complicated and does not give accurate acidophilic index due to factors such as (1) wide spectrum of red shades produced on superficial cells; (2) the need for rapid fixation to preserve nuclear features; (3) cells can float off the slide; and (4) thicker areas of the smear often artifactually take up orange stain.[11,12]

Papanicolau stain is also used for non-gynecologic (clinical) material. It has also been applied for evaluation of other specimens such as sputum, urine, breast, or other tissues containing squamous epithelial or similar cells. In the study of Ranjan et al., (India, 2013), 55 patients presenting as having tumor involving different organs and clinically diagnosed as malignant were included. The study included five specimens from lymph nodes, three from cervix, thirty-one from breast, four from gastrointestinal tract (GIT), three from parotid gland, five from ovary, one from uterine corpus, and three from skin. During the operation, smears for imprint cytology were made from cut surfaces of tumors. These were stained with either papanicolaou stain or hematoxylin and eosin (H&E) stain. The results of imprint cytology were compared with hematopathologic examination using H&E staining. They showed that the imprint cytology for benign and locally infiltrative tumors gave 100% accuracy and 97% (34/35) for malignant tumor. It was concluded that pap smear was useful for evaluating tumor and that the simplicity of technique can be used at small centers with low-level facility set-up.[15]

Operational Definitions

Sensitivity is defined as the ability of the test to correctly identify the proportion of subjects indicated by brush biopsy as having malignant colonic tumor as truly having the malignancy. This is represented mathematically by the equation:

$$\text{Sensitivity} = \frac{\text{true positive}}{\text{true positive} + \text{false negative}} x100$$

Specificity is the ability of the brush biopsy to correctly identify the proportion of subjects who tested negative and truly have no malignancy. Mathematically, this is represented by the following equation:

$$\text{Specificity} = \frac{\text{true negative}}{\text{true negative} + \text{false positive}} x100$$

Positive predictive value (PPV) is the probability that subjects identified to be positive for malignancy by papanicolau-stained brush biopsy truly have malignancy. This is represented by the following equation:

$$\text{PPV} = \frac{\text{true positive}}{\text{true positive} + \text{false positive}} x\ 100$$

Negative predictive value (NPV) is the probability that subjects identified to be negative for malignancy by papanicolau stain truly do not have malignancy. This is represented by the following equation:

$$\text{NPV} = \frac{\text{true negative}}{\text{true negative} + \text{false negative}} x\ 100$$

A positive likelihood ratio (LR) is the likelihood that a positive papanicolau stain result would be expected in a subject with malignancy compared to the likelihood that that same result would be expected in a subject without the malignancy. The higher the value, the more likely the patient has malignancy. For example, a likelihood ratio of greater than 1 indicates that the positive pap stain result is associated with malignancy. A likelihood ratio less than 1 indicates that the same result is associated with absence of malignancy. This is represented by the following equation:

$$\text{Likelihood Ratio (+)} = \frac{Sensitivity}{1 - Specificity}$$

Study Population

Sample Size

Based on 2010 Philippine data that cancer of the colon and rectum combined ranked 4th for both sexes (7%), 3rd among males (8%) and 4thamong females (6%),

having 5,787 new cases in both sexes; 3,208 in males and 2,579 in females,[4] and assuming that colonic tumors could occur in both sexes at a rate of 7%, estimated at precision of 6.5% with a confidence interval of 95%, the computed sample size of 59 subjects was presumed sufficient in this study.

Inclusion and Exclusion Criteria

Patients 19 years old and above undergoing colonoscopy for colonic tumors from February 2016 to September 2017 at Room 11 endoscopy unit of the Vicente Sotto Memorial Medical Center were included. Patients without consent and with signs and symptoms of obstruction such as abdominal pain and tenderness, vomiting, abdominal distention, diarrhea and/or constipation were excluded.

Ethical Considerations

This study was approved by the VSMMC Ethics Committee and Research Committee. Informed consent was taken after recruitment. Any foreseeable risks during the conduct of the study, such as bleeding, were deemed unlikely. Strict confidentiality and data privacy were ensured. Only the researcher and co-investigator had access to the data bank. No patient names were revealed.

Methodology

Data Collection Technique

After informed consent, data from all patients undergoing colonoscopy for colonic tumors at VSMMC from February 2016 to September 2017 were collected, including demography, clinical presentation, and risk factors. During colonoscopy, standard tissue biopsies were taken, after which, brush biopsies of the four sides of colonic tumors were performed. Specimens were submitted for histopathologic examination.

Maneuvers

Brushings were then smeared directly to slides and fixed in 95% ethyl alcohol. After fixation, slides were immediately transferred to 80% alcohol and passed in sequence through 50% alcohol, 40% alcohol and finally to distilled water. Thereafter, slides were stained with Harris hematoxylin staining solution for exactly 45 seconds, then immersed three times in distilled water using three separate containers, and then rinsed in 50% alcohol. This was followed by immersion in a solution of 1.5% ammonium hydroxide in 70% alcohol for 1 minute, then rinsed with 70% alcohol and passed in sequence through 80% and 95% alcohol. Slides were then stained in OG-6 staining solution for 1 ½ minutes and rinsed in three changes of 95% alcohol. Afterwards, these were exposed for three minutes in EA-65 or EA-50 staining solution. Thereafter, were rinsed in three changes of 95% alcohol, dehydrated and cleared by passing through the following solutions: absolute alcohol, equal parts of ether and absolute alcohol, and two changes of xylene. The stained slides were then put in the mounting medium (DPX) for reading and read separately by two independent pathologists, to be interpreted as either benign or malignant. The results were compared with the standard histopathologic reading which was used as the gold standard.

Data gathered were collated, tabulated and statistically analyzed using SPSS software.

Results

A total of 60 patients were enrolled in the study. The average age among the subjects was 55.60 years old. There were 53.3% females. Fifty percent of subjects were smokers. Eighty-five percent had positive family history of colorectal cancer (**Table 1**).

Table 1. Patient demographics

Mean (± SD)	N (%)
Age	55.6 (13.1)
Sex	
Females	32 (53.3%)
Males	28 (46.7%)
Family History	51 (85.0%)
Smoking	30 (50.0%)

Staining Results

Eighty percent of patients biopsied were positive for colorectal cancer using H&E (**Table 2)**.

Table 2. Staining results

Result	N (%)
Pap stain	
Negative	18 (30%)
Positive	42 (70%)
H&E Stain	
Negative	12 (20%)
Positive	48 (80%)

The cross-tabulation between papanicolau stain and H&E stain results is shown in **Table 3.**

Table 3. Pap stain and H&E stain cross-tabulation

	(+) H&E	(-) H&E	Total
(+) pap stain	38	4	42
(-) pap stain	10	8	18
Total	48	12	60

Sensitivity of the test is 79.2% and specificity is 66.6%. LR (+) is 2.30 (indicating that a positive test significantly increases the probability of having colon malignancy), and LR (-) is 0.31, indicating that a negative pap stain significantly decreases the probability of having colon malignancy.

The diagnostic accuracy of papanicolau stain in brush biopsy for colonic tumors is shown in the following equations:

$$\text{Sensitivity} = \frac{38}{40} x\ 100 = \ 79.2\%$$

$$\text{Specifity} = \frac{8}{12} x\ 100 = \ 66.6\%$$

$$\text{Positive Predictive Value} = \frac{38}{42} x\ 100 = \ 90.5\%$$

$$\text{Negative Predictive Value} = \frac{8}{18} x\ 100 = \ 44.4\%$$

$$\text{Likelihood Ratio (+)} = \frac{Sensitivity}{1 - Specificity} = \frac{79.2}{(100 - 66.6)} = \ 2.30$$

$$\text{Likelihood Ratio (-)} = \frac{1 - Sensitivity}{Specificity} = \frac{(100\text{-}79.2)}{66.6} = \ 0.31$$

The relationship between the two staining methods are shown in **Tables 4 and 5.**

Table 4. Chi-square tests

	Value	df	Asymptotic Significance (2-sided)	Exact Significance (2-sided)	Exact Significance (1-sided)
Pearson Chi-square	9.603[a]	1	.002		
Continuity Correction	7.545	1	.006		
Likelihood Ratio	8.900	1	.003		
Fisher's Exact Test				.004	.004
Linear-by-Linear Association	9.443	1	.002		
N of Valid Cases	60				

Table 5. Symmetric measures

		Value	Approximate Significance
	Phi	.400	.002
	Cramer's V	.400	.002
N of valid cases		60	

Discussion

The results in this study evaluating the diagnostic accuracy of papanicolau-stained brush biopsies in detecting colon malignancy correctly identified a high percentage of cancer cases, yielding a sensitivity of 79.2% and specificity of 66.6%. Compared to the results of Ranjan et al. (India, 2013)[15] whose smears showed 100% accuracy for benign tumors and 97% accuracy for malignant tumors, the results in our study have modest values. However, the test in our study shows a high PPV of 90.58%, which means that among patients who tested positive with pap stain there is high probability of them also having cancer. The high PPV may be attributed to particular characteristics of patients included in the study, who were mostly symptomatic, thereby increasing the probability of the disease.

The test's low negative predictive value in this study (44.4%) means that the test cannot accurately predict that a patient with a negative pap stain truly does not have cancer.

Conclusion

In the Philippines, standard hematopathological examination is used for biopsy specimens. In hospitals with a heavy patient load, turnaround time for biopsy results is most often prolonged. Hospitals catering to patients belonging to the low socio-economic group have the necessity to offer the least possible medical cost and shortened hospital stay. Whereas preoperative diagnostic accuracy of a tumor is an essential part in patients' work-up, attention has been drawn to the need for quick yet accurate diagnostic methods.

This research is the only known study in the Philippines evaluating the diagnostic accuracy of papanicolau stain for colonic tumors. And this investigation yields promising results for this modality, particularly for gastrointestinal lesions. Besides being easy to perform, it is cheap, costing only half the price of standard H&E staining. Moreover, papanicolau staining is expeditious, yielding rapid results that can be reported in as early as 24 hours after collection, enabling prompt therapeutic action.

No diagnostic test is perfect, and errors can occur in each step of a test procedure – physician skill, the manner by which the specimens are collected and processed, and the experience of the pathologist doing the interpretation. It is the better judgment of the practitioner to consider that what is ideal may not be practical and, more importantly, to be open to the realization that, resource-wise, no two hospital settings are the same.

Conflict of Interest

The authors declare no conflicts of interest.

References

1. Feldman M, Friedman L, Sleisenger and Fordtran's Gastrointestinal and liver disease, 10th ed. Philadelphia: Saunders Elsevier. 2016.
2. Turner J. The gastrointestinal tract. In: William Schmidt (ed.) Robbins and Cotran Pathologic Basis of Disease. 8th ed. Philadelphia: Saunders Elsevier. 2010; pp 763-829.
3. Mayer R. Gastrointestinal tract cancer. In: Shanahan J, Davis K (eds.) Harrison's principles of internal medicine. 18th ed. New York: McGraw Hill Press. 2013; pp 764-776.
4. Laudico AV, Medina M, Mirasol-Lumague MR, Mapua C, Redaniel MT, Valenzuela F, Pukkala E. Philippine cancer facts and estimates, 2010. Philippine Cancer Society. Available at https://www.academia.edu/4907961/2010_PHILIPPINE_CANCER_FACTS_AND_ESTIMATES_PHILIPPINE_CANCER.
5. Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, et al. Global, regional and national cancer incidence, mortality, years of life, years lived with disability and disability adjusted life-years for 32 cancer groups, 1990-2015: A systematic analysis for the global burden of disease study. JAMA Oncol. 2017; 3:524-48.
6. Winawer SJ, Zauber AG, Ho MN, O'Brien MJ, Gottlieb LS, Waye JD, et al. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. N Engl J Med. 1993; 329:1977-81.
7. Brenner H, Chang-Claude J, Seiler CM, et al. Protection from colorectal cancer after colonoscopy: a population-based, case-control study. Ann Intern Med. 2011; 154:22-30.
8. Canadian Cancer Society; Cancer Information / Diagnosis & Treatment / Managing Side Effects / Bowel Obstruction. http://www.cancer.ca/en/cancer-information/diagnosis-and-treatment/managing-side-effects/bowel-obstruction/?region=on
9. Nishihara R, Wu K, Lochhead P, Morikawa T, Liao X, et al. Long-term colorectal-cancer incidence and mortality after lower endoscopy. N Engl J Med Overseas Ed. 2013; 369:1095-105.
10. Baxter NN, Goldwasser MA, Paszat LF, Saskin R, Urbach DR, and Rabeneck L. Association of colonoscopy and death from colorectal cancer. Ann Intern Med. 2009; 150:1-8.
11. Zauber AG, Winawer SJ, O'Brien MJ, Lansdorp-Vogelaas I, Van Ballegooijen M, Hankey BF, et al. Colonoscopic polypectomy and long-term prevention of colorectal-cancer deaths. N Engl J Med Overseas Ed. 2012; 366:687-96.
12. Bruce-Gregorios J. Exfoliative Cytology. In: Bruce-Gregorios J (ed.) Histopathologic Techniques. 2nd ed. Makati City, Philippines: MG Reprograhics Supply & Services Inc. 1974; 215-237.
13. Dhurba G. Papanicolaou (PAP) staining: Introduction, principle, procedure and interpretation, cytopathology. Available at https://laboratoryinfo.com/papanicolaou-pap-staining-principle-procedure-interpretation.

14. Conrad R, Casteino-Prabhu S, Cobb C, Razad A. Role of cytopathology in the diagnosis and management of gastrointestinal tract cancers. J Gastrointest Oncol. 2012 Sep; 3(3):285-298. Available at http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3418535.
15. Ranjan A, Chandoke RK, Chauhan N. Study of tumors by imprint cytology. Indian Journal of Clinical Practice. October 2013; vol. 24, no. 5.