

FOCUS

PAPANICOLAOU SOCIETY OF CYTOPATHOLOGY

Companion Society of the United States and Canadian Academy of Pathology

Dedicated to Clinical Practice, Clinical Education and Clinical Research



From the Editor's Desk



Dear colleagues,

It is time for our coming Focus issue!

We have exciting articles on recent topics potentially affecting our profession in the coming years. On lighter side we have images from Dr. Giorgadze. Please enjoy the music and join the fun!

The details about various benefits of joining PSC membership are highlighted on the last page. Please recommend to your colleague to join PSC membership by sending the membership form downloaded from <http://www.papsociety.org/docs/09/pscapp2009.pdf>.

Members and other readers are encouraged to send the articles or other contributions (eg. interesting images in cytology, book reviews, case reports, reviews etc) to me or any of the Focus editorial board members. We are accepting contributions for the December 2014 edition. The deadline for submitting the contributions are flexible, but we appreciate if your submissions are received at vshidham@med.wayne.edu prior to November 7, 2014.

Please enjoy the issue!

Sincerely,

Vinod B. Shidham, MD, FRCPATH, FIAC

President's Message

Zubair W. Baloch, MD, PhD



"Everyone has been made for some particular work and the desire for that work has been put in every heart".

~Mawlana Jalaludin Rumi

"Society with Big Heart" – these were the words I used in my first President's message in 2013 describing what PSC means to me; not surprising I am still holding this emotion as I write this communication. The close of my two year Presidency is only a small punctuation mark in my career with PSC and I look forward to continuing my work as an active member of my favorite society. I have to thank many of friends of PSC whose support and constructive criticism kept me aligned with the goals of the society and needs of the membership. I cannot begin to describe the immense support from the PSC executive board and various committee members that have enabled me to carry out society tasks for two years. The PSC committee chairs have worked

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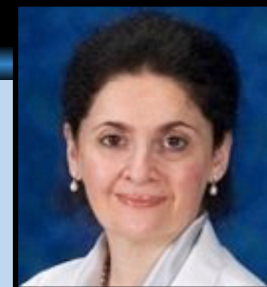
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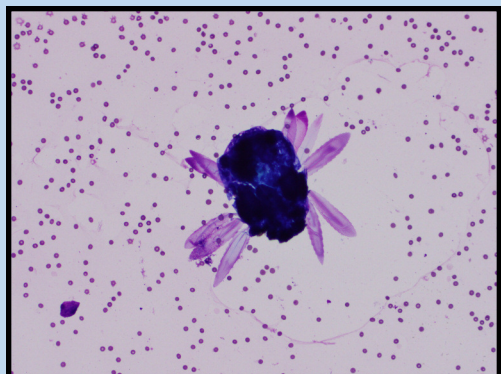
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FLIGHT OF THE BUMBLEBEE

We found this bumblebee-like artifact in a thyroid aspirate during our departmental conference. It immediately reminded us of the famous musical piece "Flight of the Bumblebee" by the great Russian composer Rimsky-Korsakov. In our opinion, adding a fragment from this musical piece rendered by the virtuoso American trumpeter Harry James enhances the impression of the "flight" and reflects a "bee-zzy" atmosphere of our departmental conference. Please click on the link to listen: www.papsociety.org/newsletters/2014/June-2014-Focus-bmbbeefinal.wav



From the Papanicolaou Cytology Laboratory
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Con't from page 1

From the President's Desk

tirelessly on an exceptional volume of projects, charges and tasks, either planned or unexpected to maintain the effectiveness of our society. My USA and international colleagues have been very creative in these past two years to keep the name and goals of PSC current at both national and international events. To name the few the Annual Papanicolaou Cytopathology Tutorial is being cosponsored by PSC for the past three-years. PSC is co-sponsoring a slide seminar with American Society of Cytopathology at the annual meeting of American Society of Clinical Pathology at Tampa, Florida. PSC was also a prominent sponsor of educational events at the recent meeting of European Federation of Cytology Societies held in Geneva, Switzerland.

Although cytopathologist don't always acknowledge this, in my view we have always occupied the most leveraged position in diagnostic medicine. We are situated in a space where the "rubber meets the road"; a cytopathologist is the face of pathology department to a patient during fine-needle aspiration (FNA) service and molecular analysis performed on a limited cellularity cytology specimen is now essential in the current era of personalized medicine. PSC from its inception has recognized the importance of our profession, and has devised educational sessions to bring forth and discuss current trends in cytopathology. In keeping with this tradition, the topic for the upcoming 2015 PSC scientific program to be given at the 104th USCAP annual meeting will focus on the diagnostic challenges and exciting new developments in the field of head and neck

pathology especially in reference to small biopsy and FNA specimens. The session has been developed by Dr. Mathew Zarka and is titled as **"Small Biopsy Specimens of Head and Neck with Emphasis on Cell Cytology and the Role of Special Studies."** Dr. William C. Faquin will address the diagnosis of salivary gland lesions by FNA including diagnostic pitfalls and the incorporation of ancillary studies as an aid to the diagnosis of these challenging lesions. The second presentation by Dr. Raja R. Seethala will focus on small biopsies of intraoral lesions and para-pharyngeal space lesions. Dr. Lester D.R. Thompson will discuss small biopsy specimens of sino-nasal lesions; and the final talk by Dr. Margaret S. Brandwein-Gensler will cover difficult squamo-proliferative lesions and variants of squamous cell carcinoma, and challenging benign and malignant mimics of head and neck squamous lesions.

Finally, the best is yet to come. The next president of PSC, Dr. Tarik Elshiekh, is one of the brightest and most dynamic surgical pathologist and cytopathologist I know, with strong interest in education and fostering relationship among various disciplines in pathology. He has been an active member of PSC and ASC executive board and holds a prominent position in USCAP. I am confident that PSC, under his leadership will continue to maintain its current place and gain prominence at both national and international levels. Yes, it has been a rewarding experience and pleasure serving PSC for the past two years. And yes, I am with PSC for the rest of my professional journey.



Immunocompromized Host Cytology

Dr. Michelle Pramick

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Clinical History

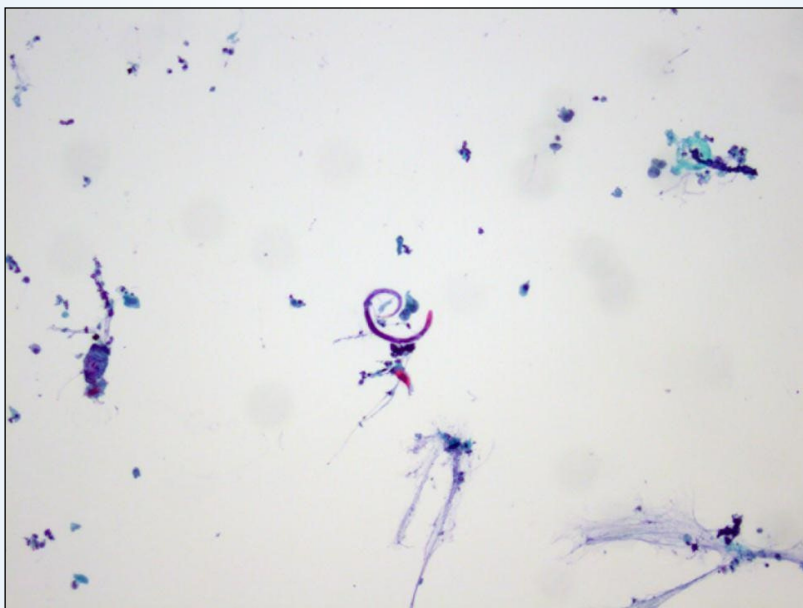
20 year-old male with HTLV-1 associated T-cell lymphoma presented as a left nasal cavity mass with status post hyper-CVAD and intrathecal methotrexate chemotherapy, was admitted for lower back pain. The initial concern was for spinal cord compression. He was found to have persistent lytic lesions in the lumbar spine. The patient was also found to have central diabetes insipidus. He remained an inpatient for his next round of chemotherapy after which he was planned to be discharged. His temperatures however, his temperature spiked to 102.9°F with nausea, vomiting, and diarrhea. At that time he was already on PO Flagyl for *C. difficile* prophylaxis. His fever continued to spike as he had a change in mental status. A cerebrospinal fluid culture was positive for *Enterococcus* and he was started on broad spectrum antibiotics.

Two weeks following the lumbar puncture, he developed worsening abdominal distention, pain, and constipation. A CT of the abdomen showed dilation of the proximal and mid small bowel with pneumatosis and portal venous gas. This was concerning for obstruction and possibly ischemic bowel. He was taken to surgery for an exploratory laparotomy; however, there was no evidence of perforation. He was then transferred to the intensive care unit where his course was complicated by hypoglycemia and seizures and he had to be intubated. He was noted to have severe constipation and a partial small bowel obstruction five days following surgery. The patient also had difficulty weaning from the ventilator. A bronchoalveolar lavage specimen was obtained.

Image Figures

1. ThinPrep slide, Bronchoalveolar lavage, Papanicolaou stain, 10x
2. ThinPrep slide, Bronchoalveolar lavage, Papanicolaou stain, 20x
3. ThinPrep slide, Bronchoalveolar lavage, Papanicolaou stain, 40x
4. ThinPrep slide, Bronchoalveolar lavage, Papanicolaou stain, 63x
5. ThinPrep slide, Bronchoalveolar lavage, Grocott's Methenamine Silver stain, 40x
6. ThinPrep slide, Bronchoalveolar lavage, Gram Weigert stain, 40x

Images

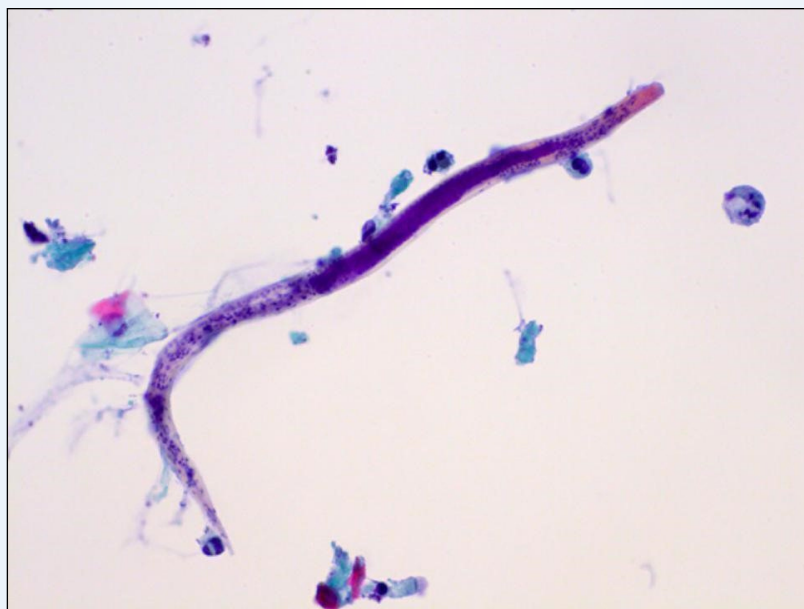


1. ThinPrep slide, Bronchoalveolar lavage, Papanicolaou stain, 10x

Quiz Case



2. ThinPrep slide, Bronchoalveolar lavage, Papanicolaou stain, 20x

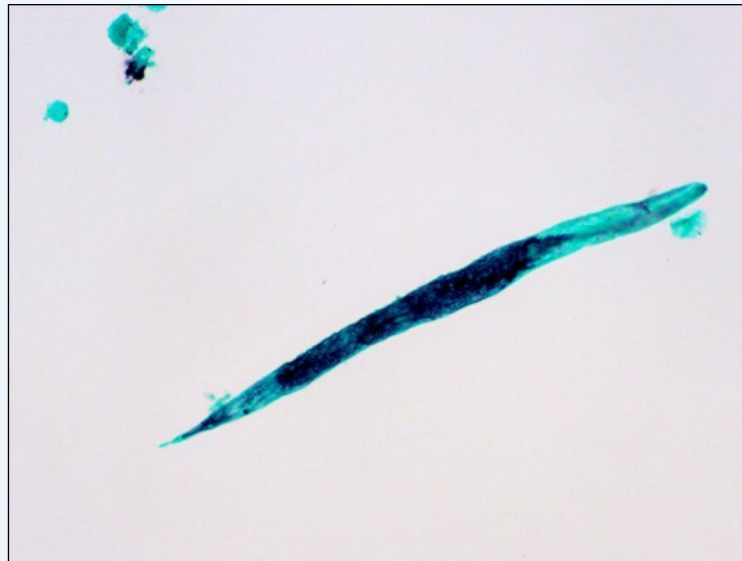


3. ThinPrep slide, Bronchoalveolar lavage, Papanicolaou stain, 40x

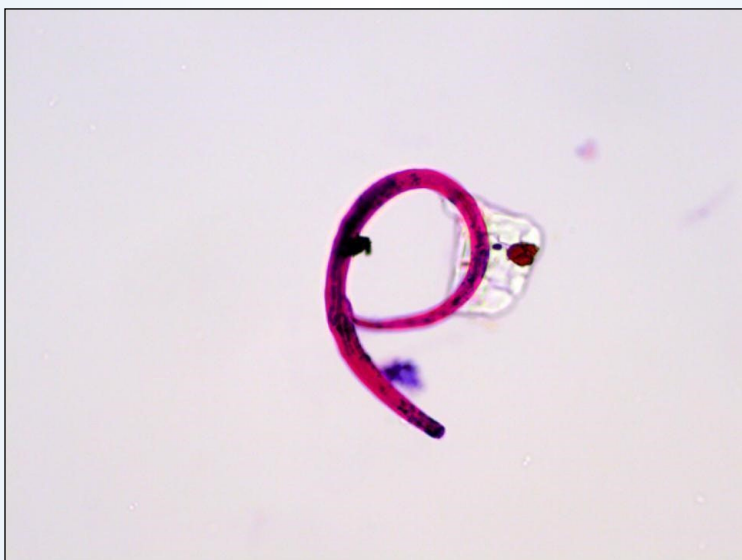
Quiz Case



4. ThinPrep slide, Bronchoalveolar lavage, Papanicolaou stain, 63x



5. ThinPrep slide, Bronchoalveolar lavage, Grocott's Methenamine Silver stain, 40x



6. ThinPrep slide, Bronchoalveolar lavage, Gram Weigert stain, 40x

Quiz Case

Questions

1. What is the diagnosis?
 - a. *Trichinella spiralis*
 - b. *Trichuris trichiura*
 - c. *Strongyloides stercoralis*
 - d. *Ascaris lumbricoides*
2. What is the most common way of becoming infected with *Strongyloides*?
 - a. by ingesting infected meat
 - b. by fecal-oral transmission
 - c. through mosquito bites
 - d. contacting contaminated soil
3. There is an association with *Strongyloides* infection with which virus?
 - a. Hepatitis C virus
 - b. Human T-Cell Lymphotropic Virus-1
 - c. Human herpesvirus 8
 - d. BK polyomavirus
4. What is the gold standard for the diagnosis of *Strongyloides*?
 - a. serial stool examination
 - b. enzyme-linked immunosorbent assay
 - c. indirect immunofluorescence assay
 - d. luciferase immunoprecipitation assay

Discussion

ThinPrep slides show the curved cylindrical shaped larval form of strongyloides. The slides demonstrate organisms with both blunted and a tapered ends amongst a relatively clean background. (Figures 1 and 2) On higher magnification, detailed internal structure can be appreciated. (Figures 3 and 4) The organism's internal structure stains with Grocott's Methenamine Silver (GMS) stain (Figure 5) and Gram Weigert stain (Figure 6).

A prior case report of *Strongyloides* in a cervical smear showed a more tightly coiled larva. In that case, the internal structures of the parasite took a deep purple color on a Papanicolaou-stained cervical smear.¹ In comparison, a case of *Strongyloides* in a bronchoalveolar lavage specimen processed as a ThinPrep slide had a less coiled appearance, which may be due to processing technique.² The clean background on ThinPrep, as well as the light stain of the larvae, and detail of the internal structure make the organisms readily apparent.

Strongyloidiasis is caused by an intestinal parasitic nematode (roundworm). The species, *Strongyloides stercoralis*, is the most prevalent and is clinically important. *Strongyloides stercoralis* is most common in tropical regions; however it occurs in a wide variety of climates. In the United States, it is most commonly reported among refugees and immigrants.³ Studies performed in the 1980s in rural southeastern United States reported prevalence estimates ranging from 1.2%–6.1%.^{4,5} Infection is often associated with agricultural activities; the most common way of becoming infected with *Strongyloides* is by contacting soil that is contaminated with *Strongyloides* larvae.³ Studies have shown an association with *Strongyloides* infection and Human T-Cell Lymphotropic Virus-1 (HTLV-1). People infected with HTLV-1 are more likely to become infected with *Strongyloides*, and are more likely to develop severe cases of strongyloidiasis.⁶

Quiz Case

The life cycle of *Strongyloides* is quite complex, with fluctuation between free-living and parasitic cycles, the potential for autoinfection, and multiplication within a host. In an autoinfection, the rhabditiform larvae become infective filariform larvae, which can penetrate either the intestinal mucosa (internal autoinfection) or the skin of the perianal area (external autoinfection) where they enter the circulatory system, and are carried successively to the lungs and penetrate the alveolar spaces. They are carried to the bronchial tree and pharynx where they are swallowed to eventually reach the small intestine where they mature into adults. They may also disseminate widely in the body.³

Infection with *Strongyloides* can occur as acute strongyloidiasis, chronic strongyloidiasis, hyperinfection syndrome, or disseminated strongyloidiasis. Symptoms vary widely based on the type of infection. Acute strongyloidiasis can be associated with a localized pruritic rash at the site of skin penetration. The patient may develop a dry cough as the larvae migrate from the lungs through the trachea. After the larvae are swallowed into the gastrointestinal tract, patients may experience gastrointestinal symptoms. Chronic strongyloidiasis is usually asymptomatic, but in patients with clinical disease gastrointestinal and cutaneous manifestations are the most frequent.³ Eosinophilia is present in 50% to 80% of patients with mild chronic infection.⁷

Hyperinfection syndrome and disseminated strongyloidiasis are most frequently associated with a subclinical infection in immunocompromised patients, including those receiving high-dose corticosteroids.^{3,8} There are numerous signs and symptoms associated with hyperinfection syndrome and disseminated strongyloidiasis, and a partial list includes: abdominal pain, nausea, vomiting, diarrhea, ileus, intestinal obstruction, bacterial sepsis, cough, wheezing, dyspnea, hoarseness, pneumonitis, hemoptysis, respiratory failure, diffuse interstitial infiltrates or consolidation on chest radiographs, aseptic or gram-negative meningitis, peripheral edema and ascites secondary to hypoalbuminemia from protein losing enteropathy, recurrent gram negative bacteremia/sepsis from larvae carrying bacteria that penetrate mucosal walls, syndrome of inappropriate secretion of anti-diuretic hormone (SIADH), recurrent maculopapular or urticarial rash. Patients may also develop larva currens (the pathognomonic serpiginous rash).^{3,9}

The gold standard for the diagnosis of *Strongyloides* is serial stool examination. However, up to seven stool exams to reach a sensitivity of 100% may be required. Frequently, larvae can be seen in fluid from bronchoalveolar lavage specimens. In addition, there are serologic tests available that are sensitive, but have the potential to cross-react with other parasites, decreasing their specificity.³

Acute and chronic strongyloidiasis are treated with oral Ivermectin for 1-2 days. Hyperinfection syndrome and disseminated strongyloidiasis are treated with oral Ivermectin until stool and/or sputum exams are negative for 2 weeks. If possible, immunosuppressive therapy should be stopped or reduced. In certain instances, Investigational New Drug (IND) exemptions for the veterinary subcutaneous formulation of Ivermectin have been granted by the FDA.³ Diagnosis of *Strongyloides stercoralis* hyperinfection requires clinical awareness. The mortality rate is 15% in hyperinfection syndrome⁷ and can reach up to 80% in disseminated disease.¹⁰ Considering that hyperinfection is associated with a higher mortality rate, it is important for the pathologist to convey the findings of the diagnosis to the clinical teams as soon as possible.

In the current case, *Strongyloides stercoralis* larvae were confirmed by morphology in an ova and parasite examination. The patient was treated with subcutaneous ivermectin for *Strongyloides* hyperinfection. He continued to have daily fevers and was also found to have *Pseudomonas* on sputum culture, for which he was treated with appropriate antibiotics. He was eventually weaned from the ventilator to a trachea collar. His small bowel obstruction resolved with nasogastric suction and he was transitioned back to an oral diet. He was discharged to home with the plan of continuing chemotherapy and starting radiation therapy for HTLV-1 associated T-cell lymphoma. Of note, this patient did not have any significant travel history.

Quiz Case

Answer Key

1. C

2. D

3. B

4. A

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CMAS[‡] - Pancreas - EUS-FNA Cytopathology (PSC guidelines) S1:3 of 5.

Standardized terminology and nomenclature for pancreatobiliary cytology: The Papanicolaou Society of Cytopathology Guidelines

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These PSC guideline articles published under Open Access charter may be disseminated by multiple journals/platforms.

All parts of these PSC guidelines are also available in print as CMAS (CytoJournal Monographs/Atlas Series) #1. Please check on CytoJournal home (www.cytojournal.com) under 'Monograph and Atlas' <http://www.cytojournal.com/monographs.asp>

Abstract

The Papanicolaou Society of Cytopathology has developed a set of guidelines for pancreatobiliary cytology including indications for endoscopic ultrasound (EUS) guided fine-needle aspiration (FNA) biopsy, techniques of EUS-FNA, terminology and nomenclature of pancreatobiliary disease, ancillary testing and post-biopsy treatment and management. All documents are based on the expertise of the authors, a review of the literature, discussion of the draft document at several national and international meetings over an 18 month period and synthesis of online comments of the draft document on the Papanicolaou Society of Cytopathology web site [www.papsociety.org]. This document selectively presents the results of these discussions and focuses on a proposed standardized terminology scheme for pancreatobiliary specimens that correlate cytological diagnosis with biological behavior and increasingly conservative patient management of surveillance only. The proposed terminology scheme recommends a six-tiered system: Non-diagnostic, negative, atypical, neoplastic [benign or other], suspicious and positive. Unique to this scheme is the "neoplastic" category separated into "benign" (serous cystadenoma) or "other" (premalignant mucinous cysts, neuroendocrine tumors and solid-pseudopapillary neoplasms (SPNs)). The positive or malignant category is reserved for high-grade, aggressive malignancies including ductal adenocarcinoma, acinar cell carcinoma, poorly differentiated neuroendocrine carcinomas, pancreatoblastoma, lymphoma and metastases. Interpretation categories do not have to be used. Some pathology laboratory information systems require an interpretation category, which places the cytological diagnosis into a general category. This proposed scheme provides terminology that standardizes the category of the various diseases of

the pancreas, some of which are difficult to diagnose specifically by cytology. In addition, this terminology scheme attempts to provide maximum flexibility for patient management, which has become increasingly conservative for some neoplasms.

Key words: Guidelines, nomenclature, pancreas, Papanicolaou Society of Cytopathology, terminology

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INTRODUCTION

Early detection of cancer whether it is a malignancy of the ductal, acinar, or neuroendocrine system is the key to patient survival. With the increased use of endoscopic ultrasound (EUS) guided fine-needle aspiration (FNA) for the evaluation of pancreatobiliary lesions, coupled with our improved understanding of premalignant lesions and the evolving management algorithm for patients with pancreatic cysts,^[1,2] it is clear that cytopathologists play a very important role in the diagnosis and management of patients with pancreatic solid or cystic lesions and pancreatobiliary strictures. Hampering patient management is the lack of standardized nomenclature for pancreatobiliary disease, especially for the premalignant cysts.

A standardized terminology and nomenclature system that provides intra- and inter-departmental guidance for diagnosis and which correlates with biological behavior and management recommendations is imperative for both FNA of pancreatic masses and cysts and brushing cytology of pancreatobiliary strictures. Interpretation categories do not have to be used. Some pathology laboratory information systems, however, require an interpretation category, which has been standard practice in cytology for decades. Such categories do aid in clinical and translational research, which is imperative for progress in the field. Below is a proposed terminology scheme with six categories including a category "neoplastic" that is divided into clearly "benign" neoplasms and "other" neoplasms with less definitive biologic behavior predictable by cytological features.

These proposed guidelines on standardized terminology for pancreatobiliary cytology specimens stems from the expertise of the authors, review of the literature, discussions with pathologists at several national and international meetings over an 18 month period and synthesis of online comments of the draft document on the Papanicolaou Society of Cytopathology web site [www.papsociety.org].

PROPOSED PANCREATOBILIARY TERMINOLOGY CLASSIFICATION SCHEME

- I Non-diagnostic
- II Negative (for malignancy)
- III Atypical
- IV Neoplastic: Benign or Other
- V Suspicious (for malignancy)
- VI Positive/malignant.

CATEGORY I: NON-DIAGNOSTIC

Background

Non-diagnostic specimens may be due to technical or sampling issues that preclude the pathologist from providing

any useful information from the FNA biopsy relative to the lesion sampled. The clinical and imaging context should be taken into consideration. The absence of "epithelial cells" in the sample does not necessarily make a specimen non-diagnostic. For example, a pseudocyst by definition lacks an epithelial cyst lining, and mucinous cysts may only have thick colloid-like mucin, or a fluid with elevated carcinoembryonic antigen (CEA), findings sufficient to support an interpretation of a neoplastic mucinous cyst even when an epithelial component is lacking.^[3-5]

Definition

A non-diagnostic cytology specimen is one that provides no diagnostic or useful information about the solid or cystic lesion sampled; for example, an acellular aspirate of a cyst without evidence of a mucinous etiology such as thick colloid-like mucus, elevated CEA or KRAS/GNAS mutation (see Category IV). Any cellular atypia precludes a non-diagnostic report.

Example cytological interpretations

Evaluation limited by preparation artifact

Non-diagnostic

Tissue entrapped in blood clot and fibrin precluding cytological evaluation.

Satisfactory for evaluation

Non-diagnostic

Gastrointestinal contamination only

Satisfactory for evaluation

Non-diagnostic

Normal acinar and ductal epithelium. The biopsy does not explain the well-defined pancreatic mass seen on imaging.

Evaluation limited by scant cellularity

Non-diagnostic

Non-specific cyst contents with insufficient cyst fluid volume for ancillary testing.

CATEGORY II: NEGATIVE (FOR MALIGNANCY)

Background

A negative cytology sample is synonymous with the absence of malignancy and any cellular atypia in the cytology sample. A negative cytology interpretation that is descriptive without a diagnosis of a specific condition such as chronic pancreatitis or pseudocyst is not synonymous with a benign lesion. A descriptive negative interpretation implies that the sample is adequately cellular and that no cytological atypia is identified in the evaluated cytology sample. This includes the presence of normal pancreatic tissue in the appropriate clinical setting such a vague fullness on imaging and no distinct mass lesion. The false negative rate of an FNA of a solid mass lesion averages

15% and in the setting of a clinically and radiologically suspicious mass with a presumed diagnosis of ductal adenocarcinoma, such an aspirate is presumed to be a false negative sample.^[6,7] The false negative rate for aspirates of cystic lesions is as high as 60% due to acellular or scantily cellular samples, in addition to the lack of defined nomenclature, criteria and experience in interpreting these lesions outside of major academic hospital settings.^[8] That being said, the absence of high-grade epithelial atypia in a pancreatic cyst aspirate has a very high negative predictive value for malignancy.^[9] Since not all centers provide biochemical or molecular analysis of cyst fluid and/or the results of such testing may not be available at the time of cytological interpretation, it is reasonable to report as “negative” cyst fluids with mucinous debris of uncertain origin (lesional versus gastrointestinal contamination) as such findings likely correlate with the clinical and imaging features of a low-grade branch-duct (BD) intraductal papillary mucinous neoplasm (IPMN). The clinician will find such a “negative” report much more helpful for patient management than a “non-diagnostic” report. See example cytological interpretations.

The false negative rate for the interpretation of pancreatobiliary brushing samples is also high due to the difficulty in obtaining diagnostic tissue that is often subepithelial, entrapped in desmoplastic stroma and/or markedly degenerated, coupled with the high threshold for a malignant interpretation due to the typical clinical setting of underlying inflammatory diseases such as primary sclerosing cholangitis and/or biliary stenting that can inherently cause marked reactive atypia.^[10]

Definition

A negative cytology sample is one that contains adequate cellular and/or extracellular tissue to evaluate or define a lesion that is identified on imaging. When using the negative category one should give a specific diagnosis when practical including:

- Benign pancreatobiliary tissue in the setting of vague fullness and no discrete mass
- Acute pancreatitis
- Chronic pancreatitis
- Autoimmune pancreatitis
- Pseudocyst
- Lymphoepithelial cyst
- Splenule/accessory spleen.

Example cytological interpretations

Satisfactory for evaluation

Negative for malignancy

Benign, reactive ductal epithelium and acinar tissue, acute and chronic inflammation and a background of necrotic, calcific debris consistent with chronic pancreatitis.

Evaluation limited by scant cellularity

Negative for malignancy

Cellular stromal fragments with lymphocytes and plasma cells suggestive of autoimmune pancreatitis.

Satisfactory for evaluation

Negative for malignancy

Cyst fluid with inflammation and histiocytes, yellow amorphous pigment and no cyst lining epithelial cells consistent with pseudocyst fluid. (If available, add results of cyst fluid analysis; for example “low cyst fluid CEA [10 ng/ml] and markedly elevated amylase level [50,000 U/L] supports the diagnosis”).

Satisfactory for evaluation

Negative for malignancy

Mucinous cyst debris of uncertain etiology. No high-grade epithelial atypia identified. Correlation with imaging and ancillary studies required

Satisfactory for evaluation

Negative for malignancy

Non-mucinous cyst fluid with hemosiderin-laden macrophages and no epithelial cells, suggestive of serous cystadenoma. Correlation with clinical and imaging required. (If available, add results of cyst fluid analysis; for example “low CEA and low amylase support the interpretation”).

CATEGORY III: ATYPICAL

Background

The interpretation category “atypical” is heterogeneous and includes cases with reactive changes, low cellularity, premalignant changes (dysplasia) and cases assigned to this category due to observer caution in diagnosis. In one study, the risk of malignancy in this category for pancreatic and bile duct brushings was approximately 44%^[10] and in another the risk of malignancy for atypical FNAs of pancreatic solid masses was approximately 82%.^[11]

This interpretation is used when a cytological specimen contains cellular or extracellular tissue that displays morphologic features beyond recognizable normal tissue components or reactive changes that can comfortably be interpreted as such and therefore classified as benign or “negative”. An atypical interpretation does raise the possibility of a neoplasm and in fact, may be suggestive of a low-grade neoplasm, but the cytological findings are insufficient to be suspicious for a high-grade malignancy and tissue is insufficient for confirmation of a specific diagnosis. Conservative interpretation of diagnostic samples is not uncommon due to the significance of the surgical intervention, often a pancreaticoduodenectomy.

The negative and atypical categories have historically been the categories containing premalignant mucinous cysts, with benign appearing low-grade dysplastic cysts (adenomas) being placed in the negative category and the higher grade dysplastic cysts being placed in the suspicious category. The lack of well-established criteria for the various grades of dysplasia in mucinous cysts has hampered a more standardized approach to classification. However, given the management algorithm for mucinous cysts which recommends a conservative approach for cysts at low risk for malignancy,^[1,2] it is imperative that the pathologist relate on the cytology report that a neoplastic mucinous cyst has been detected by FNA (e.g., Neoplastic: Other) and to relate the presence or absence of cytologically high-grade appearing epithelium (e.g., high-grade epithelial atypia that represents at least high-grade dysplasia and possibly invasive carcinoma^[12,13] (see Category IV).

Abundant cytoplasmic mucin in pancreatic ducts is an abnormal finding and indicates a neoplastic change. The differential diagnosis for glandular epithelium with mucinous cytoplasm includes pancreatic intraepithelial neoplasia (PanIN), biliary intraepithelial neoplasia (BilIN), IPMN, mucinous cystic neoplasm (MCN) and adenocarcinoma. PanIN is not an entity recognized by imaging, but it may be a source of atypia in aspirates of solid masses.^[14] Gastric epithelial contaminant is another source of mucin containing epithelium that may be confused with ductal epithelium with mucinous dysplasia.^[15] Of note, gastric epithelium may demonstrate some of the changes of pancreatic neoplasia, such as nuclear grooves and inclusions and subtle crowding. Duodenal enterocytes are non-mucinous with a brush border and in addition to this feature, can be recognized by the presence of scattered goblet cells and intraepithelial lymphocytes.

Premalignant lesions of the bile ducts have historically been called biliary dysplasia or atypical biliary epithelium. A new consensus classification of BilIN was published in 2007.^[16] Using biliary brushing cytology derived from patient's suffering from primary sclerosing cholangitis, choledochal cyst or hepatolithiasis, this proposal classified BilIN into a three-grade classification scheme, similar to that used in other organs such as the pancreas and prostate. The histopathological criteria are similar to those for intraductal lesions of the pancreas; however, the cytopathological criteria of these lesions have not been defined. It can be assumed that their cytological features will be similar to what has been described as dysplasia in the biliary tract^[17] with grades 1 and 2 lesions causing atypia of bile duct epithelium on brushings, previously referred to as low grade dysplasia.

Definition

The category of atypical should only be applied when there are cells present with cytoplasmic, nuclear, or architectural

features that are not consistent with normal or reactive cellular changes of the pancreas or bile ducts and are insufficient to classify them as a neoplasm or suspicious for a high-grade malignancy. The findings are insufficient to establish an abnormality explaining the lesion seen on imaging. Follow-up evaluation is warranted.

Examples of cytological interpretations

Evaluation limited by preparation artifact

Atypical

Atypical ductal cells obscured by crush artifact.

Evaluation limited by scant cellularity

Atypical

Scant population of small monomorphic polygonal cells of unclear origin: Normal acinar cells versus endocrine proliferation. Additional tissue is warranted for diagnosis of this 2 cm round mass lesion in the pancreatic tail.

Evaluation limited by scant cellularity

Atypical

Atypical bile duct epithelium with nuclear features suggestive of repair in a background of acute inflammation.

Evaluation limited by scant cellularity

Atypical

Atypical bile duct epithelium with mucinous metaplasia and mild nuclear atypia.

CATEGORY IV: NEOPLASTIC

Category IVA: Neoplastic: Benign

Background

A common benign neoplasm of the pancreas is serous cystadenoma. Histologically, serous neoplasms consist of fine fibrous septae lined by cuboidal, glycogen-rich cells without atypia. Fibrous septa include numerous small capillary structures. This dense vascularization explains the often hemorrhagic aspect of the cyst fluid as well as the presence of numerous hemosiderin-laden macrophages on cytological preparations. Such macrophages can be observed in up to 63% of cases, whereas they are almost always absent in cystic mucinous neoplasms.^[18] Macrophages can, however, only be considered as a surrogate marker of serous cystic neoplasms and cannot be used as a definitive cytological criterion. When coupled with cytological analysis and with appropriate clinical and imaging features, biochemical analysis of CEA level, typically less than 5 ng/ml and amylase levels, generally also very low relative to other pancreatic cysts support this diagnosis. Caution must be used because some mucinous cysts have very low CEA levels and conversely, serous neoplasms can, albeit rarely, present with elevated CEA levels, which can reach into the hundreds and rarely low thousands.^[3-5] Other benign neoplasms in the pancreas

such as cystic teratoma and schwannoma are extremely rare and are also placed in this category.

Definition: Neoplastic: Benign

This interpretation category connotes the presence of a cytological specimen sufficiently cellular and representative, with or without the context of clinical, imaging and ancillary studies, to be diagnostic of a benign neoplasm.

Example cytological interpretation

Evaluation limited by scant cellularity

Neoplastic: Benign

Scant non-mucinous cuboidal epithelium and scant hemosiderin-laden macrophages in a non-mucinous cyst fluid consistent with the clinical impression of a serous cystadenoma (if available add results of cyst fluid analysis; for example "low CEA (0.5 ng/ml) and amylase (150 U/L) levels support the diagnosis").

Category IIIB: Neoplastic: Other

Background

Aside from the clearly malignant neoplasms like conventional pancreatic ductal adenocarcinoma (PDAC) and the definitively benign neoplasms like serous cystadenoma, there are neoplasms (other) that are either pre-invasive, premalignant neoplasms (IPMN and MCN with low, intermediate or high grade dysplasia) or of low-grade malignant behavior (pancreatic neuroendocrine tumor [PanNET] and solid-pseudopapillary neoplasm [SPN]) that warrant distinction from aggressive, high-grade malignancies (most notably pancreatic ductal adenocarcinoma (PDAC)). The rationale for this distinction and classification is explained in more detail below for each neoplasm, but, in general, the rationale relates the desire to standardize the cytological nomenclature and terminology which correlates with the 2010 world health organization WHO classification and terminology. In addition, there was the need to remove the "malignant" classification from neoplasms diagnosed cytologically with uncertain or low-grade malignant potential, a move which provides a reasonable classification that correlates with the increasingly conservative approach to these neoplasms. The standard cytological categories of "atypical" and "suspicious for malignancy (SFM)" are categories that connote an indeterminate interpretation that does not provide for a definitive cytological interpretation of a neoplasm, which could lead to inappropriate patient management and possibly an unnecessary repeat diagnostic procedure.

All of these pancreatic tumors are clearly neoplastic and some low-grade malignant.^[19-21] As such, the heading "Neoplastic: Other" is a reasonable generic term that accurately reflects the pre-operative, cytological

terminology. The terminology "Neoplastic: Other" does not define the neoplasm as benign or malignant, nor does it correlate with a specific management algorithm.

Definition: Neoplastic: Other

This interpretation category defines a neoplasm that is either premalignant such as intraductal papillary neoplasm of the bile ducts (IPN-B), IPMN or MCN with low, intermediate or high-grade dysplasia by cytological criteria, or a low-grade malignant neoplasm such as well-differentiated PanNET or SPN. While mucinous epithelium in biliary brushing specimens may indeed represent a neoplastic change, given the lack of evidence-based literature on the cytological interpretation, histology and management of these lesions, low-grade mucinous change of biliary epithelium will remain in the "atypical" rather than "neoplastic" category.

PanNET

The current preferred nomenclature for this neoplasm is PanNET.^[22] Synonyms include pancreatic endocrine tumor and pancreatic endocrine neoplasm. The term "neuroendocrine tumor" is inferred to mean a well-differentiated neoplasm and is a term that should be used whether in the primary site or in a metastatic site (e.g., liver FNA with metastatic well-differentiated neuroendocrine tumor). In the WHO 2010 classification system, the term neuroendocrine carcinoma infers either high-grade large cell neuroendocrine carcinoma or small cell carcinoma. The cytological interpretation of PanNET infers a well-differentiated proliferation of the pancreatic endocrine cells creating a mass lesion greater than 0.5 cm that may or may not be functional by producing inappropriate levels of various hormones and that may or may not demonstrate aggressive features on histological examination.^[23] Although, it is now widely accepted that well-differentiated PanNETs all have malignant potential,^[23] albeit very slow growing and even curable if caught at an early stage, these neoplasms are placed in this more generic neoplastic category to distinguish them from highly aggressive malignant neoplasms and to offer management flexibility in elderly patients with small tumors where the risk to benefit ratio of surgery is high compared to conservative management.

SPN

SPN is a solid, secondarily cystic low-grade epithelial neoplasm with established clonal mutations in cancer-associated genes and an ability to metastasize. They typically occur in young females and demonstrate a variably solid and cystic appearance on imaging. It is a parenchymal-rich, stromal-poor proliferation of monotonous cells that defy prediction of biological

behavior based on cytological features. Although, this neoplasm is one that will almost always be resected due to the typical young age of the patient, like PanNET, it is considered a low-grade malignancy and as such, it is included in this category.^[24]

Neoplastic mucinous cysts of the pancreas (IPMN and MCN)

The two primary neoplastic mucinous cysts of the pancreas consist of IPMN and MCN. Understanding the clinical, imaging and cyst fluid analysis characteristics of IPMN and MCN is vital to the interpretation of the cytological specimen. Given that the cytological features of these two mucinous cysts are usually indistinguishable for all practical purposes, the cytological features will be presented together. The pathologist should correlate the clinical, imaging and cyst fluid analysis characteristics to make the most likely specific diagnosis.

Management guidelines have evolved over time and have become much more conservative given the prevalence of incidental, asymptomatic cysts identified in the general population and especially in the elderly. MCN, although mostly low-grade,^[25,26] are usually identified in young to middle-aged women in the body or tail of the pancreas that can be relatively easily removed with a distal pancreatectomy alleviating the need for expensive, life-long surveillance. Main-duct and combined-type IPMNs are all removed due to the inherent high risk of malignancy.^[27] BD-IPMNs are more often than not low-grade neoplasms identified in the pancreatic head of the elderly with co-morbid conditions making pancreaticoduodenectomy a high-risk procedure greater than the risk of the cyst progressing to malignancy.^[27] If a cyst is mucinous and there is no evidence of high-grade dysplasia or carcinoma, then conservative management is reasonable.^[1,2] The difficult position for the pathologist then becomes grading the epithelium of the cyst. It is quite difficult in other organ systems even on histology to stratify grades into four tiers: Low, moderate and severe dysplasia and carcinoma. This difficulty is exponential when interpreting just a few cells that have undergone partial degeneration in cyst fluid and that may be associated with gastrointestinal tract (GI) contamination. A high threshold for malignancy is in order. That being said, recognition of atypical epithelial cells and their distinction from low-grade dysplasia is vitally important to the recognition of a cyst with high-grade atypia that likely corresponds to at least moderate dysplasia and in a high proportion of cases, high-grade dysplasia or worse.^[28-30] Resection prior to invasion provides the patient with the best prognosis and high-risk imaging features such as a markedly dilated main pancreatic duct or a mural nodule in a cyst that lead to resection are very often signs of an invasive neoplasm. As such, aspiration of cysts without these features provides the best opportunity for early detection of carcinoma.

MCN

MCN of the pancreas is typically a multiloculated, mucin-producing epithelial neoplasm with subepithelial ovarian-type stroma that in almost all cases does not communicate with the pancreatic ductal system and in almost all cases occurs in women. Like IPMN, these neoplasms are stratified by the degree of cytological and architectural atypia into low-grade, intermediate-grade and high-grade dysplastic, pre-malignant (non-invasive neoplasms) and invasive carcinomas (invasive mucinous cystadenocarcinoma). The invasive carcinomas are usually of tubular type, but rare carcinomas such as undifferentiated carcinoma with osteoclast-type giant cells may also be seen.^[25,26] A similar neoplasm occurs in the biliary tract. The cytological features will be similar to its pancreatic counterpart.

IPMN

IPMNs are primarily intraductal proliferations of ductal epithelium creating a macroscopic lesion resulting in ductal dilatation, cyst formation and/or a mass lesion. Intraductal tubulopapillary neoplasms are included with IPMN as this neoplasm is not only rare, but would be cytologically indistinguishable from some IPMNs. Invasion of the duct or cyst wall occurs in about one-third of resected IPMN and is most common in IPMN of main-duct type. There are three main types of IPMN:^[27,31-34]

- **Main-duct IPMN:** In general, associated with diffuse dilatation of any portion of the main pancreatic duct or the entire pancreas. The definition of "dilatation" is variable in the literature. The 2006 Sendai guidelines define it as >6 mm, but the new 2012 guidelines define it as 10 mm or greater with >5 mm being "worrisome".^[2] Visualization of mucin extruding from the ampulla on EUS or endoscopic retrograde cholangiopancreatography (ERCP) is pathognomonic. The epithelial cell type most often associated with main-duct IPMN is intestinal type epithelium (MUC 5AC, MUC 2 and CDX2+) which, by definition, is at least of intermediate (moderate) grade dysplasia. Invasive carcinomas most often arising from intestinal-type IPMN are colloid carcinomas^[33,35]
- **BD-IPMN:** Cysts adjacent to a non-dilated main pancreatic duct, most often in the uncinate process, but occurring throughout the pancreas in one or more locations. Imaging features generally depict a thin-walled unilocular cyst that may or may not demonstrate a connection to the pancreatic ductal system. Small "raspberry-like" multiloculated cysts are also typical of BD-IPMN. The cyst lining is most often of gastric-foveolar type and although most are low-grade, this epithelial cell type can display intermediate and high-grade dysplasia. Invasive carcinomas arising from these cysts tend to be of the tubular type and have a prognosis similar to conventional pancreatic adenocarcinoma.^[36]

- Combined-type IPMN: Neoplasia involving both the main ducts and BDs of the pancreas typically represented on imaging by a dilated main pancreatic duct with one or more BD cysts.

Two other epithelial cell types may be seen in IPMN. Pancreatobiliary epithelium is relatively uncommon and by definition, is equivalent to high-grade dysplasia. Oncocytic epithelium is the least common epithelial cell type and is also considered high-grade. Oncocytic type epithelium is distinguished by the moderate amounts of dense, granular, oncocytic cytoplasm. While low-grade gastric-foveolar type epithelium is recognizable, it may not be distinguishable from gastric epithelial contamination in transgastric biopsies. It is generally not possible nor is it important to distinguish the epithelial cell types with intermediate to high-grade dysplasia.

Cyst fluid analysis

Analysis of the cyst fluid from pancreatic cysts is invaluable in accurate classification of the cyst as mucinous or non-mucinous. It is well-established that although each lab should establish their own cut-off value, that, CEA levels of ~200 ng/ml are strongly supportive of a neoplastic mucinous cyst.^[3,4] A low CEA level does not exclude a mucinous etiology. In addition, CEA levels do not distinguish between benign and malignant cysts.^[3,4] Amylase levels of cyst fluid are helpful in supporting the interpretation of a pseudocyst as such fluids typically have amylase levels in the thousands,^[5] but amylase levels do not distinguish between IPMN and MCN.^[37,38] Serous cystadenomas tend to have both low CEA and amylase levels as do cystic PanNETs.^[39,40]

Molecular analysis

KRAS testing may supplement CEA as the detection of KRAS supports a mucinous etiology.^[41] Although, the combination of KRAS, LOH and quality and quantity of deoxyribonucleic acid correlates with malignancy,^[42] a KRAS mutation in and of itself is not specific for malignancy. A recent study of pancreatic cyst fluid has shown that detection of GNAS supports a specific interpretation of IPMN, but does not distinguish pre-malignant from malignant (invasive) IPMN.^[43] See the report of Committee IV for a more detailed discussion of ancillary testing.

Approach to the cytological analysis of pancreatic cysts

The cytopathologist's approach to the interpretation of a pancreatic cyst should be to address two basic questions: (1) Is the cyst mucinous or non-mucinous; and (2) Is the cyst high-grade or not? Malignant is defined as unequivocal features of adenocarcinoma (see section on positive for malignant cells). Atypia less than overtly malignant is included in this category of Neoplastic: Other.

To answer the first question of a mucinous etiology, the first clue may come from the gastroenterologist who describes "thick, viscous or white, sticky" fluid upon aspiration. This type of fluid is generally thick enough to make a direct smear. Thinner fluids are best processed as a cytospin preparation in order to capture all of the cells and to preserve the characteristics of the cyst fluid. Placing the cyst fluid in a preservative attenuates the viscosity of the fluid and may make thin mucin difficult or impossible to appreciate. Contamination of the specimen with mucin from the gastrointestinal tract is also a consideration. Thick, colloid-like mucin is neoplastic (with rare exception such as in a gastrointestinal duplication cyst) and mucin with evidence of cellular cyst debris also supports origin from the cyst and not the GI tract.^[44] Conversely, thin mucous with naked grooved nuclei evoke GI contamination. Special stains for mucin may be helpful but should be interpreted with caution. A mucicarmine or Alcian blue positive thin film of a cytospin or thick wavy wisps of mucoid fluid that stains positively without significant GI epithelial contamination are stain outcomes that support a mucinous etiology. Negative mucin stains do not exclude a mucinous cyst. CEA elevation or detection of a KRAS mutation may be necessary to support a mucinous etiology, but, a non-elevated CEA or absent KRAS mutation does not exclude a mucinous cyst.

To answer the second question of high-grade, an evaluation of the epithelial component is required. Less than overt malignancy is best interpreted as either low-grade or high-grade atypia as the accuracy in distinguishing intermediate (moderate) from high-grade dysplasia is difficult if not impossible and the criteria to do so with any accuracy have not been established.^[45] GI contaminating epithelium needs to be recognized as such (see criteria under Category I). Cytological criteria distinguishing high-grade atypia from low-grade atypia has recently been described.^[12] Cells smaller than a 12 μ duodenal enterocyte showing an increased nuclear to cytoplasmic ratio, an abnormal chromatin pattern and background necrosis represent high-grade epithelial atypia placing the cyst at high-risk for malignancy.^[2,13,46-48]

Both mucin production and epithelial cells are not required for the diagnosis of a mucinous cyst. The aspirates of some mucinous cysts are acellular but are clearly mucinous from the visible thick, colloid-like extracellular mucin, elevated CEA or KRAS/GNAS mutation. Similarly, a cyst fluid with high-grade mucinous epithelial dysplasia or carcinoma may not demonstrate extracellular mucin or an elevated CEA.^[28,49]

Approach to the cytological evaluation of biliary tract cysts

The approach to evaluating cysts arising in the biliary tract has not been as formally studied as those of the pancreas. However, it can be surmised that IPN-B and MCN-B will have similar cytological features on aspiration. The role

of ancillary studies in these cysts, such as measurement of CEA, is not established.

IPN-B

IPN-B shares many clinical and pathological features with IPMN of the pancreas. It is a neoplastic proliferation growing within the bile ducts composed of a papillary proliferation of mucin containing neoplastic cells that may occur anywhere in the ductal system. It progresses from low, to high grade and eventually invasive carcinoma, just as IPMN of the pancreas does. Gastric, pancreatobiliary, intestinal and oncocytic subtypes have been described, but show a different distribution than observed in IPMN-P.^[50,51] These are more likely to be sampled by brushing cytology than by FNA. When they present as cystic masses, they may be aspirated and the cytological features of aspiration cytology will be similar to those of IPMN of the pancreas. The cytological features of brushing cytology for IPN-B are not described here. While there are no prospective or retrospective reports, these features are extrapolated from the histopathological features and are similar to what is encountered in brushing cytology of IPMN of the pancreas.

Gastrointestinal stromal tumor

GISTs are very rare as a primary pancreatic neoplasm (extra-gastrointestinal stromal tumor (EGIST)), however, they commonly occur in a peripancreatic location such as the omentum, mesentery, duodenum and stomach, thus mimicking a primary pancreatic neoplasm at times. GIST are spindle cell and/or epithelioid mesenchymal neoplasms with differentiation along the lines of the interstitial cell of Cajal that usually expression c-kit protein (CD117), DOG1 and CD34 by immunohistochemistry.^[52-54] There is variable expression of alpha-smooth muscle actin and essentially no reactivity for desmin. As with all spindle cell lesions, procuring cellblocks on such specimens will facilitate a definitive diagnosis.

Examples of cytological interpretations

Satisfactory for evaluation

Neoplastic: Other

Mucinous cyst fluid with low-grade dysplasia (see note)

Note: Benign-appearing mucinous epithelium is present from this transduodenal FNA in a background of abundant extracellular mucin. (If available, add CEA is elevated at 357 ng/ml supporting the diagnosis).

Satisfactory for evaluation

Neoplastic: Other

Cyst fluid with thick colloid-like extracellular mucin containing cyst debris consistent with a neoplastic mucinous cyst, favor MCN given the clinical and imaging findings of a 45-year-old female with a multiloculated cyst in the pancreatic tail. Scant benign appearing mucinous

epithelium is present of uncertain origin, favor gastric contamination. No high-grade epithelial atypia present.

Evaluation limited by scant cellularity

Neoplastic: Other

Mucinous cyst fluid with high-grade epithelial atypia (see note)

Note: No thick extracellular mucin is present, but cyst fluid CEA is 1267 ng/ml supporting the diagnosis. In addition, molecular analysis demonstrates a *KRAS* point mutation, which supports a mucinous etiology. The epithelial cells are most consistent with high-grade dysplasia, however, invasive carcinoma cannot be excluded. Correlation with imaging findings required.

Satisfactory for evaluation

Neoplastic: Other

Well-differentiated neuroendocrine tumor

Note: Tissue is not available for ancillary studies, however, the morphological features of endocrine differentiated are well-defined, or

Immunohistochemical stains on the corresponding cellblock confirm endocrine differentiation (synaptophysin and chromogranin are positive). Proliferation marker Ki-67 shows less than 2% nuclear staining suggestive of a grade 1 tumor.

Satisfactory for evaluation

Neoplastic: Other

Solid-pseudopapillary neoplasm.

CATEGORY V: SUSPICIOUS (FOR MALIGNANCY)

Background

The cytological interpretation category of "SFM" generally refers to pancreatic adenocarcinoma, but may be used with any malignant neoplasm and this terminology scheme recommends using it for high-grade, aggressive malignancies. "Suspicious for" is NOT "diagnostic of" and clinical and radiological information must be correlated with the suspicious cytological findings to justify surgical intervention. Like the "atypical" interpretation category, the "suspicious" category suffers from significant interobserver variability often stemming from varying experience of the pathologist in interpreting pancreatic cytology. Due to the high threshold of a malignant interpretation and thus the low false positive rate of pancreatic cytology, many samples are conservatively interpreted and may benefit from a second opinion by an experienced pancreatic cytopathologist to save the patient the potential of a repeat diagnostic procedure.

Aspirates insufficient to make a definitive diagnosis of at least a neoplasm such as PanNET or SPN should be placed in the atypical category with specification of the indeterminate interpretation in the diagnosis line.

However, for aspirates that produce a “solid-cellular” clearly neoplastic epithelial proliferation which includes PanNET, acinar cell carcinoma, pancreatoblastoma and SPN in the differential diagnosis, but which has insufficient tissue for confirmatory ancillary studies to make a specific diagnosis, the SFM category is an appropriate classification.

SFM is an indeterminate category resulting from three major challenges with interpretation of FNA specimens of the pancreas. The first challenge is the very high level of differentiation of certain pancreatic adenocarcinomas that may harbor very subtle cytologic abnormalities.^[55] The second challenge is scant cellularity. Pancreatic adenocarcinoma induces a tumor-associated sclerotic response that may contribute to this sparse cellularity.^[56] The third problem that cytologists must address is gastrointestinal contamination that, when substantial, may mask some scattered tumor cells and when injured and reactive, may mimic carcinoma. When these challenges are faced in a single case, a definitive diagnosis of malignancy may be impossible, but malignancy is probable. In these cases, where the degree of suspicion for malignancy is high enough to require therapeutic intervention, one may classify the lesion as “SFM”. This category has a very high positive predictive value for malignancy.^[56-58] The SFM diagnosis must be correlated with clinical symptoms and imaging characteristics. When a patient has a high clinical suspicion of pancreatic cancer and a pancreatic mass on imaging studies, the diagnosis of suspicious most likely indicates the presence of cancer.^[56-58] Autoimmune pancreatitis should be a clinical consideration as it is a well-known pitfall mimicker of PDAC clinically, radiologically and cytologically. The distinction between a positive diagnosis and an SFM diagnosis is based on both quantitative and qualitative criteria. Suspicious cases represent 5-12% of published cases,^[59] but most studies focus on pancreatic adenocarcinoma, so the number of cases that are considered as suspicious for PanNETs, acinar cell carcinomas or lymphomas is very difficult to establish.

As a category, the risk of malignancy for brushing specimens designated “SFM” is approximately 80% and 96% for the EUS-FNA specimens identified as SFM.^[60]

Definition

A specimen is SFM when some, but an insufficient number of the typical features of a specific malignant neoplasm are present, mainly pancreatic adenocarcinoma. The cytological features raise a strong suspicion for malignancy, but the findings are qualitatively and/or quantitatively insufficient for a conclusive diagnosis, or tissue is not present for ancillary studies to define a specific neoplasm. The morphologic features must be sufficiently atypical that malignancy is considered more probable than not.

Examples of cytological interpretations

Satisfactory for evaluation
Suspicious (for malignancy)
Rare markedly atypical epithelial cells suspicious for adenocarcinoma.

Satisfactory for evaluation
Suspicious (for malignancy)
Mucinous cyst with high-grade epithelial atypia and abundant coagulative necrosis suspicious for invasive carcinoma.

Satisfactory for evaluation
Suspicious (for malignancy)
Solid cellular neoplasm with features suspicious for acinar cell carcinoma. Tissue for confirmatory ancillary studies is not available.

CATEGORY VI: POSITIVE OR MALIGNANT

Background

Since 9 of 10 malignancies in the pancreas are conventional ductal adenocarcinoma, the “positive” or “malignant” category is often related to this category. Low-grade malignancies such as well-differentiated PanNET and SPN are included in the Neoplastic: Other category. Other high-grade malignancies are also included here such as acinar cell carcinoma, pancreatoblastoma, lymphoma and metastases. The specificity of a positive or malignant interpretation for both pancreatic FNA and biliary brushing is very high, >90-95% in most studies.^[6,7,10,57,61-65] Relying on strict criteria contributes to this high specificity at the expense of sensitivity. Rapid on site evaluation of solid mass lesion FNAs contributes to diagnostic yield.^[66-68]

Definition

A group of neoplasms that unequivocally display malignant cytologic characteristics and include PDAC and its variants, cholangiocarcinoma, acinar cell carcinoma, high-grade neuroendocrine carcinoma (small cell and large cell), pancreatoblastoma, lymphomas, sarcomas and metastases to the pancreas.

PDAC

PDAC is a malignant invasive gland (duct) forming epithelial neoplasm typically composed of classic tubular glands, but, in variants, with other morphologically diverse epithelial morphologies. PDAC, or infiltrating ductal adenocarcinoma, is the most common primary cancer of the pancreas which accounts for 85-90% of all pancreatic malignancies.^[27,34] High-grade tumors generally demonstrate overt features of malignancy that makes cytological diagnosis straight forward. Well-differentiated tumors can be extremely

challenging due to minimal deviation from normal ductal morphology making a definitive diagnosis of malignancy challenging.

Cholangiocarcinoma

The diagnostic criteria for invasive cholangiocarcinoma are the same as for ductal adenocarcinoma of the pancreas on FNA samples. Published diagnostic criteria for adenocarcinoma in a bile duct brushing specimen^[69,70] demonstrate variable predictive values.^[71] The presence of indwelling stents and the underlying inflammatory conditions that lead to bile duct stricture and the increased risk for malignancy are factors in and of themselves that contribute to the need for a high threshold for malignancy in these specimens. As such, the sensitivity for detecting malignancy in these specimens is low.^[10,61,62,65] An overall assessment for the presence of malignancy may be best in these samples. The addition of ancillary testing using FISH and other molecular methods may also improve sensitivity^[72,55] (see report from Committee IV on ancillary testing in pancreatobiliary specimens).

Major diagnostic pitfalls in the evaluation of bile duct brushings include obscuring of malignant epithelium by overlying benign epithelium, insufficient sampling, degeneration due to bile or duodenal contents, primary sclerosing cholangitis^[73] and atypical squamous metaplasia due to bile duct stones and stents. Correlation of the cytological findings with the clinical findings may help as a biliary stricture is more likely to be malignant in older male patients who are symptomatic and do not have a history of stones.^[10]

Colloid carcinoma (mucinous, non-cystic)

A carcinoma of ductal differentiation showing abundant extracellular mucin production, with at least 80% of the tumor on histology demonstrating large pools of extracellular mucin and cuboidal epithelial cells "floating" in the mucin. This uncommon variant accounts for 1-3% of PDAC and the vast majority arise in association with IPMN of intestinal type. Gender and age distribution is similar to PDAC; however, the prognosis appears to be significantly better.^[35]

Medullary carcinoma

A carcinoma characterized by poor histologic differentiation, syncytial growth pattern, pushing borders and an intense lymphoplasmacytic response. Medullary carcinoma is characterized by a special genetic profile with 69% of these tumors displaying wild-type *KRAS* genes and 22% of these tumors have microsatellite instability.

Adenosquamous carcinoma

A rare subtype with relative frequency of 3-4% and relatively poorer prognosis compared to the conventional ductal adenocarcinoma, this variant shows malignant glandular and

squamous components ranging from extensive glandular differentiation with focal squamous differentiation to predominantly squamous differentiation.^[74]

Undifferentiated carcinoma with osteoclast-like giant cells

Often admixed with ordinary PDAC, this tumor is a distinctive type of sarcomatoid carcinoma with the striking and unique cytohistologic features characterized by a prominent component of reactive osteoclast-like giant cells in a background of spindle cells. Often seen in association with MCN, these tumors may also arise with *in-situ* (PanIN III and invasive ductal carcinoma).^[75]

Undifferentiated carcinoma

Also known as anaplastic carcinoma this rare variant of PDAC has a relative frequency of 2-7%. It is a high-grade carcinoma composed of large, undifferentiated, markedly pleomorphic cells.^[76] Tumors with this morphology should prompt the pathologist to consider metastatic disease and to evaluate available tissue with ancillary studies to investigate the possibility.

Acinar cell carcinoma

A rare malignant epithelial neoplasm with exocrine acinar differentiation. Lipase hypersecretion syndrome is present in only 16% of the patients but serves as a clinical clue to the diagnosis. A significant proportion of the cases have small neuroendocrine component or scattered neuroendocrine cells within the tumor. Approximately 50% of the patients have metastatic disease at presentation, often restricted to the regional lymph nodes and liver.^[77-83]

Poorly-differentiated neuroendocrine carcinoma (small cell carcinoma or large cell neuroendocrine carcinoma)

Both small cell and large cell high-grade neuroendocrine carcinomas exhibit cytoarchitectural and clinicopathological features indistinguishable from their pulmonary (and extra pulmonary) counterparts. These carcinomas account for less than 1% of all primary pancreatic cancers and 2-3% of PanNETs. These carcinomas are extremely rare in the pancreas and the possibility of metastatic lung carcinoma or extension from the more common primary site of the ampulla (for large cell type) should always be excluded first.^[22,84]

Pancreatoblastoma

A rare neoplasm, primarily of childhood, characterized by acinar differentiation, endocrine differentiation and distinctive squamoid nests. Furthermore known as infantile pancreatic carcinoma, this is an extremely rare pancreatic tumor in childhood, comprising 0.5% of pancreatic non-endocrine tumors with rare occurrence

in adults. Pancreatoblastoma tends to be less aggressive in infants and children compared to adults. The cancer has been associated with alterations in the Wnt signaling pathway and chromosome 11p loss of heterozygosity, Beckwith-Wiedemann syndrome and familial adenomatous polyposis. Alpha-fetoprotein may be elevated in up to 68% of patients with pancreatoblastoma and can be used to follow patients for recurrence post-operatively.^[85]

Non-Hodgkin lymphoma

Hematopoietic malignancies in the pancreas are rare and usually involve the pancreas secondarily.^[86] Pancreatic lymphomas are most commonly non-Hodgkin lymphoma that can clinically mimic pancreatic adenocarcinoma. One of the advantages to FNA evaluation is that pre-operative diagnosis of lymphoma can preclude unnecessary surgery. Primary pancreatic lymphomas are most commonly large B-cell lymphomas.^[86] While the cytomorphological features may suggest lymphoid differentiation, there may be overlapping features with other neoplasms that produce a solid cellular smear pattern. Ancillary tests such as flow cytometry and immunohistochemistry are typically necessary for diagnosis and especially for subclassification.

Metastatic tumors

Secondary neoplasms involving the pancreas are rare and pancreatic involvement as the sole site of metastasis is even more uncommon. The common neoplasms that metastasize to the pancreas include melanoma, renal cell carcinoma and carcinomas from the lung, colorectum and breast.^[27] Direct extension from cancer of the stomach, duodenum, gallbladder, liver and retroperitoneum may also occur.

Renal cell carcinoma is notorious for giving rise to a late solitary metastasis, even decades following nephrectomy. Renal cell carcinoma is also the most likely malignancy to metastasize to the pancreas and mimic a primary neoplasm.^[87] The cytological findings of metastatic renal cell carcinoma are similar to those seen in the kidney with bland polygonal cells, round slightly eccentric nuclei, prominent nucleoli and vacuolated cytoplasm. Distinction from clear cell or lipid rich neuroendocrine tumor is warranted as the morphology of these two neoplasms may be indistinguishable.

Examples of cytological interpretations

Satisfactory for evaluation
Positive (for malignancy)
Adenocarcinoma.

Satisfactory for evaluation
Positive (for malignancy)
Malignant glandular and squamous cells consistent with adenosquamous carcinoma.

Satisfactory for evaluation

Positive (for malignancy)

Adenocarcinoma with morphological features consistent with renal primary.

COMPETING INTERESTS STATEMENT BY ALL AUTHORS

The authors declare that they have no competing interests.

AUTHORSHIP STATEMENT BY ALL AUTHORS

All authors of this article declare that we qualify for authorship as defined by ICMJE <http://www.icmje.org/#author>. Each author has participated sufficiently in the work and take public responsibility for appropriate portions of the content of this article. Each author acknowledges that this final version was read and approved.

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Research Article

Authors attain comparable or slightly higher rates of citation publishing in an open access journal (CytoJournal) compared to traditional cytopathology journals - A five year (2007-2011) experience

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Abstract:

Background: The era of Open Access (OA) publication, a platform which serves to better disseminate scientific knowledge, is upon us, as more OA journals are in existence than ever before. The idea that peer-reviewed OA publication leads to higher rates of citation has been put forth and shown to be true in several publications. This is a significant benefit to authors and is in addition to another relatively less obvious but highly critical component of the OA charter, i.e. retention of the copyright by the authors in the public domain. In this study, we analyzed the citation rates of OA and traditional non-OA publications specifically for authors in the field of cytopathology. **Design:** We compared the citation patterns for authors who had published in both OA and traditional non-OA peer-reviewed, scientific, cytopathology journals. Citations in an OA publication (CytoJournal) were analyzed comparatively with traditional non-OA cytopathology journals (Acta Cytologica, Cancer Cytopathology, Cytopathology, and Diagnostic Cytopathology) using the data from web of science citation analysis site (based on which the impact factors (IF) are calculated). After comparing citations per publication, as well as a time adjusted citation quotient (which takes into account the time since publication), we also analyzed the statistics after excluding the data for meeting abstracts. **Results:** Total 28 authors published 314 publications as articles and meeting abstracts (25 authors after excluding the abstracts). The rate of citation and time adjusted citation quotient were higher for OA in the group where abstracts were included ($P < 0.05$ for both). The rates were also slightly higher for OA than non-OA when the meeting abstracts were excluded, but the difference was statistically insignificant ($P = 0.57$ and $P = 0.45$). **Conclusion:** We observed that for the same author, the publications in the OA journal attained a higher rate of citation than the publications

in the traditional non-OA journals in the field of cytopathology over a 5 year period (2007-2011). However, this increase was statistically insignificant if the meeting abstracts were excluded from the analysis. Overall, the rates of citation for OA and non-OA were slightly higher to comparable.

Key words: Citations, impact, open access, publication

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INTRODUCTION

It has been more than a decade since the publication of The Budapest Declaration, a landmark article, which was the result of a meeting of key players including many Nobel-laureates from the Open Access (OA) movement^[1]. This declaration stated in part, "An old tradition and a new technology have converged to make possible an unprecedented public good." They were speaking of using the internet and OA principles to disseminate scientific knowledge obtained through research to more people than ever before.^[1-4]

In the time since this declaration, the scientific world has seen a steady increase in the acceptance of the OA publication charter as a robust and viable method of publication, thereby increasing the impact of OA on the scientific literature. This has increased the number of OA publications on the internet, which are available freely to anyone with internet access. Major societies, government agencies, top publishers, and consortiums in the scientific community have followed by publishing many additional declarations supporting the use of OA.^[5]

One reason for the growth of OA in the medical community is the known advantage this platform has for both the readers and the authors. In 2001, Steve Lawrence reported in *Nature* a sentinel publication after analyzing 119,924 articles and concluded that free online availability of scientific publications increased citation rates.^[6] Kurtz *et al.*,^[7] Harnad *et al.*,^[8] and others published similar results.^[5,9,10]

Other than increasing the citation rates^[11], an additional relatively less appreciated beneficial aspect of the OA charter is the retention of copyright by the intellectual property (IP) owner of the individual publication, that is its author/researcher^[2,12]. The efforts, time, skills, talent, and many more assets, including variety of public resources, contributed by the ethical owner, the author (s) of the individual publications, are very important and deserve further consideration. Not to lose this IP to any group with restricted benefits to general academia and the public is a major benefit of the OA charter, which is achieved by applying the Creative Commons Attribution License^[13], allowing retention of published material in the public domain. Authors are increasingly experiencing the benefits of this feature, which leads to more freedom in sharing and utilizing previously published unique materials such as images, figures, tables, etc., *The benefit is applied to numerous academic activities including but not limited to writing reviews, chapters, books, and other teaching material, simply by citing the source of the original information.*^[14]

Authors have a better chance of becoming a renowned expert on their given subject, by seamless global distribution of their effort to anyone in the world with internet access. An additional advantage gained by authors, readers and the medical community as a whole, and perhaps the most important benefit of OA, is the advances in discovery and treatment as purported by the translational research model, which are made possible by barrier free dissemination of scientific knowledge.

No studies to date have looked at the impact of Open Access publishing on the citation rate in a small subspecialty field like cytopathology, where the majority of journals have been traditional-type publications. Our hypothesis, based on the findings reported previously concerning open access publishing,^[5-10] is that for the same author publishing in both types of cytopathology journals, the publications in OA Cytopathology Journal such as *CytoJournal* under the Open Access charter will have a similar or higher citation rate (CR) as compared to the publications in the traditional non-OA cytopathology journals.

MATERIAL AND METHODS

The data in this study was collected solely from the Web of Science based on which impact factors (IF) are calculated.^[15] The traditional non-OA journals analyzed are *Acta Cytologica*, *Cancer Cytopathology*, *Cytopathology*, and *Diagnostic Cytopathology*. This was compared with similar data for the publications in *CytoJournal* as OA cytopathology journal. The five years, 2007-2011, chosen arbitrarily are closer to the current year of 2013 with reasonable time needed for generation of citations for most of the journals and publications.

The authors selected for this study were those who fit the following criteria:

- Those who published in *CytoJournal* at least two times within the time period of 2007- 2011
- Those who published in non-open access traditional cytopathology journals (*Acta Cytologica*, *Cancer Cytopathology*, *Cytopathology*, and/or *Diagnostic Cytopathology*) at least twice within the time period of 2007-2011
- Those who were not past or current editors/co-editors of the journals under study.

Each publication by these authors from the journals selected and the number of citations garnered by each publication as of August 22, 2013 was recorded using the Web of Science citation analysis site [Figure 1].^[15] Web of Science was chosen as the database for our study for several reasons. It is a very large database with over 37 million records, and it includes all of the relevant and credible journals in the field of cytopathology. The database is also

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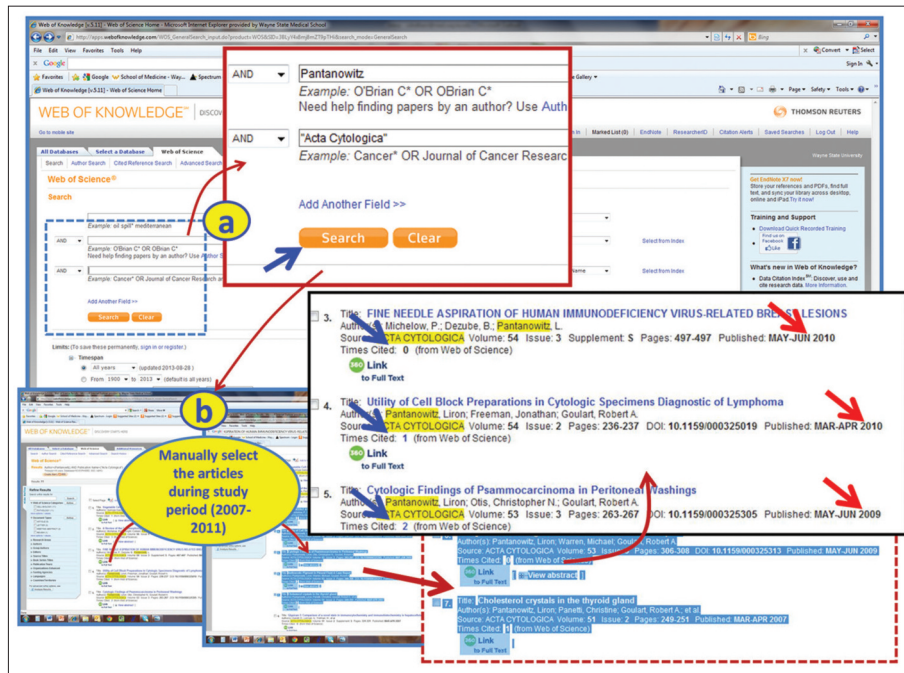


Figure 1: Use of the 'Web of Sciences Database' to harvest the raw data on number of citations for various publications for different authors publishing in cytopathology journals under study [Table 2]. (a) Add the name of the author and the journal. Click 'Search'; (b) Manually select the publications during 2007 through 2011. Note the citation numbers and reference details [Table 2]

publisher neutral" giving equal treatment to commercial, OA, societal and university publications.^[15] Citations of each publication for each author in both Open Access and traditional non-OA journals were noted and categorized by the publication year. The citations per publication (CPP) for the two journal types were compared.

We designed another value to take into account the influence of the time factor after the publication (i.e. giving more power to publications which have had less time since publication). This metric, the 'time adjusted citation quotient' (Q value) was defined and calculated as follows:

$$Q \text{ Value} = \sum_{i=2007}^5 [N(Yr)/C(Yr)]/X$$

i Starting year 2007.

N (Yr): Total number of citations for all publications under consideration in a specific year (Yr).

C (Yr): Average number of CPP in a specific year (Yr) by all cytopathology journals under study.

X: Total number of publications under consideration for that author from 2007 to 2011 for that journal category (OA or non-OA).

The Web of Science's citation analysis [Figure 2]^[15] was the source of the average number of CPP for that year in that journal i.e. *C* (2007), *C* (2008), *C* (2009), *C* (2010),

and *C* (2011). Upon completion of the search for each of the cytopathology journal, *C* (Yr), the mean value for each year was calculated [Table 1]. The *Q* value for each publication in CytoJournal as OA cytopathology journal as well as the traditional non-OA cytopathology journals was then calculated by above formula. Minitab software^[16] was used for statistical analysis.

To compare CRs for CytoJournal as an OA cytopathology journal *versus* non-OA cytopathology journals, by using freely available data on the web, a few of these authors were also analyzed arbitrarily by 'Publish or Perish' software which uses 'Google scholar' data.^[17,18]

RESULTS

A total of 28 authors were identified as per the criteria who published papers or meeting abstracts in both OA and non-OA journals. When meeting abstracts were excluded 25 authors were considered. Overall, a total of 314 publications in cytopathology journals during 2007-2011 were evaluated based on the data from web of science citation analysis site (Impact factor is calculated based on this data) [Table 2].^[19-32] Some publications were attributed to more than one author included in the study. The data shown in red in Table 2 indicate publications as meeting abstracts. Because OA cytopathology journal and some non-OA cytopathology journals did not publish meeting abstracts on regular basis and this feature may

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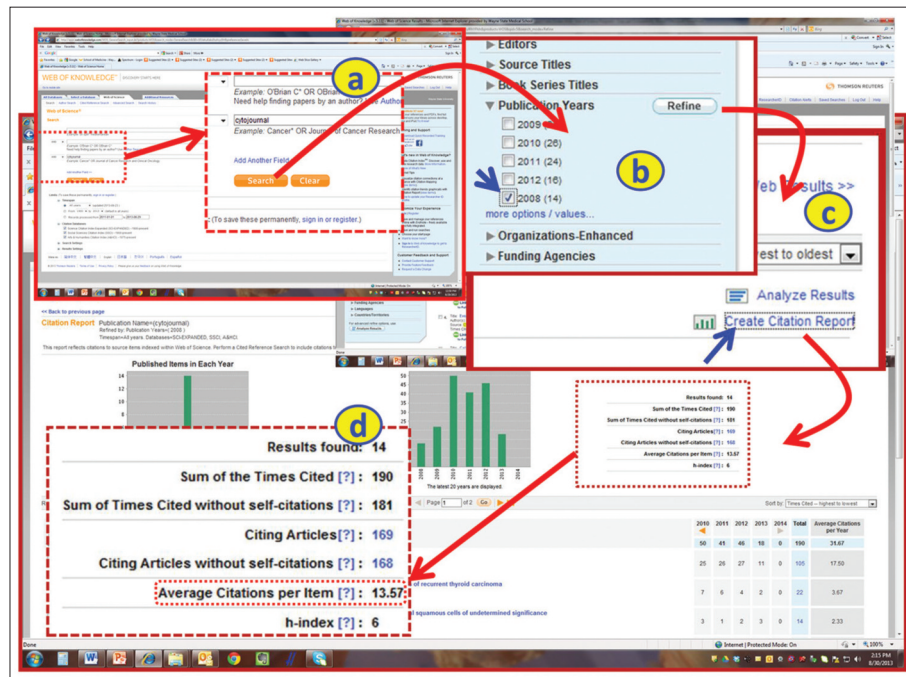


Figure 2: Use of 'Web of Sciences citation analysis tool' for finding the average number of citations per publication [C (Yr)] in a specific year for all the cytopathology journals under study. (a) Add the name of the journal; (b) Click 'publication years', choose the specific year of interest (2008); (c) Click 'Create Citation Report' link on the next screen; (d) Note the 'Average Citations per Item' C (Yr) for that journal for that year (2008) [Table 1]

Table 1. Average number of citations per publication in a specific year [C (Yr)] for various cytopathology journals

Year	Annual averages for various Cytopathology journals					C (Yr)
	CytoJournal	Acta cytologica	Cancer cytopathology	Cytopathology	Diagnostic cytopathology	
2007	NA*	1.02	4.87	5.44	5.99	4.330
2008	13.50	2.69	2.94	4.34	6.01	5.896
2009	3.35	1.90	2.82	3.62	3.63	3.064
2010	3.08	0.27	2.31	2.65	2.52	2.166
2011	2	2.15	3.94	1.85	1.65	2.318

* CytoJournal publications were indexed by ISI from 2008, so they were not indexed in 2007. C (Yr), Mean of annual averages of citations per publication for that year

potentially impact the final comparison, we analyzed the data in two ways: First on all the data including the meeting abstracts, and then repeating the analysis after excluding the meeting abstracts.

In the group in which meeting abstracts were included, the combined number of publications per author ranged from 4 to 41 with an average of 15.6 and a median of 15. The number of publications per author in CytoJournal ranged from 2 to 9 with an average of 3.1 and a median of 2. The number of publications per author in traditional non-OA journals ranged from 2 to 32 with an average of 12.5 and a median of 15.

In the group in which meeting abstracts were excluded, the combined number of publications per author ranged from 3 to 27 with an average of 11.6 and a median of 10.

The number of publications per author in CytoJournal ranged from 2 to 9 with an average of 3.1 and a median of 2. The number of publications per author in traditional non-OA journals ranged from 1 to 25 with an average of 8.4 and a median of 7.

The citations per publication (CPP) and the time adjusted quotations (Q values) were calculated for CytoJournal as an OA journal *versus* the traditional non-OA cytopathology Journals with the meetings abstracts included [Table 3]. Overall, the averages of both CPP and Q values were higher for OA Cytopathology Journal (cytojournal) than the traditional non-OA journals. To confirm our hypothesis, paired t-tests were run on both data sets (CPP and Q values) using Minitab software.^[16]

Table 2. Authors and citations (raw data)

Author name	Author# (See Table 3)	Open access journal number of citations ^{reference number} (T=Number of articles)					Non-open access journals number of citations ^{reference number} (T=Number of articles)					Average citations per article 2007-2011
		2007*	2008	2009	2010	2011	2007	2008	2009	2010	2011	
Shipra Agarwal	1	N/A	-	-	-	4 ¹⁹ , 2 ²⁰	2 ²¹	-	-	0 ²² , 0 ²³ , 0 ²⁴ , 0 ²⁵	0 ²⁶ , 0 ²⁷	2/7=0.286
Manon Auger	2	N/A	-	9 ²⁸ , 3 ²⁹	-	-	25 ³⁰ , 0 ³¹ , 0 ³² , 0 ³³	0 ³⁴ , 2 ³⁵ , 6 ³⁶	0 ³⁷ , 13 ³⁸ , 0 ³⁹	1 ⁴⁰ , 2 ⁴¹ , 2 ⁴² , 0 ⁴³ , 0 ⁴⁴	0 ⁴⁵	70/16=4.38
R. M. Austin	3	N/A	-	3 ⁴⁶ , 4 ⁴⁷	7 ⁴⁸	-	0 ⁴⁹ , 0 ⁵⁰ , 8 ⁵¹ , 0 ⁵² , 24 ⁵³ , 0 ⁵⁴	0 ⁵⁵ , 0 ⁵⁶ , 0 ⁵⁷ , 0 ⁵⁸ , 0 ⁵⁹ , 0 ⁶⁰ , 15 ⁶¹	9 ⁶² , 0 ⁶³	1 ⁶⁴ , 1 ⁶⁵ , 1 ⁶⁶	1 ⁶⁷ , 1 ⁶⁸ , 5 ⁶⁹	76/21=3.62
Zubair W. Baloch	4	N/A	24 ⁷⁰ , 110 ⁷¹	-	-	2 ⁷²	12 ⁷³	211 ⁷⁴ , 0 ⁷⁵	11 ⁷⁶ , 53 ⁷⁷ , 0 ⁷⁸	45 ⁷⁹ , 14 ⁸⁰	-	346/8=43.25
Oralia Barboza-Quintana	5	N/A	10 ⁸¹	3 ⁸²	-	-	3 ⁸³	-	-	0 ⁸⁴	-	3/2=1.5
Joel S. Bentz	6	N/A	-	3 ⁴⁶	5 ⁸⁵	-	0 ⁸⁶	1 ⁸⁷ , 0 ⁸⁸ , 0 ⁸⁹ , 10 ⁹⁰	0 ⁹¹ , 0 ⁹² , 0 ⁹³ , 0 ⁹⁴ , 4 ⁹⁵	0 ⁹⁶ , 8 ⁹⁷ , 0 ⁹⁸	3 ⁹⁹	26/14=1.86
Fadi Brimo	7	N/A	-	9 ²⁸ , 3 ²⁹	-	-	25 ³⁰	4 ¹⁰⁰	13 ³⁸ , 0 ³⁷	1 ⁴⁰ , 2 ⁴¹	-	45/6=7.5
Krista L. D'Amore	8	N/A	-	-	6 ¹⁰¹	3 ¹⁰²	-	-	0 ¹⁰³ , 0 ¹⁰⁴	-	-	0/2=0
Gregory G. Freund	9	N/A	-	3 ⁴⁶ , 1 ¹⁰⁵	-	-	0 ¹⁰⁶	-	0 ¹⁰⁷	0 ¹⁰⁸	-	0/3=0
Raquel Garza-Guajardo	10	N/A	10 ⁸¹	3 ⁸²	-	-	3 ⁸³	-	-	0 ⁸⁴	-	3/2=1.5
Robert A. Goulart	11	N/A	6 ¹⁰⁹	17 ¹¹⁰	4 ¹¹¹ , 0 ¹¹²	-	5 ¹¹³ , 40 ¹¹⁴ , 0 ¹¹⁵ , 1 ¹¹⁶ , 0 ¹¹⁷ , 0 ¹¹⁸ , 0 ¹¹⁹ , 0 ¹²⁰ , 0 ¹²¹ , 0 ¹²² , 0 ¹²³ , 0 ¹²⁴	0 ¹²⁵ , 0 ¹²⁶ , 0 ¹²⁷	2 ¹²⁸ , 0 ¹²⁹ , 0 ¹³⁰ , 1 ¹³¹ , 0 ¹³² , 0 ¹³³	1 ¹³⁴ , 0 ¹³⁵ , 0 ¹³⁶	0 ¹³⁷	50/25=2
Prabodh K. Gupta	12	N/A	24 ⁷⁰ , 2 ¹³⁸	-	9 ¹³⁹	4 ¹⁴⁰	12 ⁷³ , 3 ¹⁴¹	3 ¹⁴²	6 ¹⁴³ , 53 ¹⁴⁴ , 0 ¹⁴⁵	1 ¹⁴⁴	-	78/7=11.14

Cont....

Table 2. Contd...

Author name	Author#	Open access journal number of citations ^{reference number} (T=Number of articles)					Non-open access journals number of citations ^{reference number} (T=Number of articles)					Average citations per article 2007-2011
		2007*	2008	2009	2010	2011	2007	2008	2009	2010	2011	
Rana S. Hoda	14	N/A	14 ¹⁷¹	-	1 ¹⁷²	-	2 ¹⁷³ , 2 ¹⁷⁴ , 29 ¹⁷⁵ , 0 ¹⁷⁶	0 ¹⁷⁷ , 0 ¹⁷⁸	1 ¹⁷⁹ , 0 ¹⁸⁰ , 0 ¹⁸¹ , 2 ¹⁸²	1 ¹⁸³ , 0 ¹⁸⁵ , 0 ¹⁸⁶	2 ¹⁸⁷ , 0 ¹⁸⁸	39/15=2.6
Maryanne Hornish	15	N/A	6 ¹⁰⁹	17 ¹¹⁰	-	-	0 ¹¹⁵ , 0 ¹¹⁷ , 0 ¹¹⁹	0 ¹²⁵ , 0 ¹²⁶ , 0 ¹²⁷	0 ¹³⁰	0 ¹³⁶	-	0/8=0
Venkateswaran K. Iyer	16	N/A	-	2 ¹⁴⁵	3 ¹⁴⁶	4 ¹⁹ , 2 ²⁰	3 ¹⁸⁸	0 ¹⁸⁹	-	0 ²² , 5 ¹⁹⁰ , 1 ¹⁹¹ , 1 ¹⁹² , 0 ²⁴ , 0 ²⁵ , 0 ¹⁹³ , 0 ¹⁹⁴ , 1 ¹⁹⁵ , 0 ¹⁹⁶	0 ²⁶ , 0 ¹⁶⁹ , 0 ¹⁹⁷ , 1 ¹⁹⁸ , 0 ¹⁶⁷	12/18=0.67
Dharshana N. Jhala	17	N/A	-	-	5 ¹⁹⁹	0 ²⁰⁰	19 ²⁰¹ , 0 ²⁰²	3 ²⁰³ , 17 ²⁰⁴ , 0 ²⁰⁵ , 0 ²⁰⁶	0 ²⁰⁷ , 0 ²⁰⁸	3 ²⁰⁹	0 ²¹⁰ , 1 ²¹¹ , 0 ²¹²	43/12=3.58
Nirag C. Jhala	18	N/A	-	-	5 ¹⁹⁹	0 ²⁰⁰	19 ²⁰¹ , 0 ²⁰²	3 ²⁰³ , 17 ²⁰⁴ , 0 ²⁰⁵ , 0 ²⁰⁶	0 ²⁰⁷ , 0 ²⁰⁸	-	0 ²¹¹ , 1 ²¹² , 0 ²¹³	40/11=3.64
Walid Khalbuss	19	N/A	-	-	5 ²¹³ , 2 ²¹⁴ , 15 ²¹⁵	1 ²¹⁶ , 0 ²¹⁷ , 1 ²¹⁸ , 1 ²¹⁹ , 1 ²²⁰ , 0 ²²¹	1 ²²² , 2 ²²³ , 3 ²²⁴	0 ²²⁵ , 0 ²²⁶	0 ²²⁷ , 0 ²²⁸ , 0 ²²⁹	4 ²³⁰ , 0 ²³¹ , 0 ²³² , 0 ²³³ , 0 ²³⁴	1 ²³⁵ , 1 ²³⁶	12/15=0.8
Kamal K. Khurana	20	N/A	7 ²³⁷	3 ²³⁸	-	-	0 ²³⁹	0 ²⁴⁰	0 ²⁴¹ , 0 ²⁴²	0 ²⁴³ , 0 ²⁴⁴	-	0/6=0
Sandeep R. Mathur	21	N/A	-	2 ¹⁴⁵	3 ¹⁴⁶	-	3 ¹⁸⁸	5 ¹⁵² , 8 ¹⁵¹ , 0 ¹⁸⁹ , 2 ¹⁵⁰	4 ¹⁵⁸ , 0 ²⁴⁵	5 ¹⁹⁰ , 1 ¹⁶² , 0 ²⁴⁶ , 0 ¹⁹⁶ , 0 ¹⁹⁴ , 1 ¹⁹⁵ , 0 ²⁵ , 0 ²³	0 ¹⁹⁷ , 0 ¹⁶⁷ , 0 ²⁷ , 0 ¹⁶⁹	29/19=1.53
Pam Michelow	22	N/A	-	4 ²⁴⁷	2 ²⁴⁸	-	0 ²⁴⁹	10 ²⁵⁰	0 ²⁵¹ , 1 ²⁵² , 2 ²⁵³ , 1 ²⁵⁴ , 1 ²⁵⁵ , 0 ²⁵⁶	0 ²⁵⁷ , 3 ²⁵⁸ , 0 ²⁵⁹ , 0 ²⁶⁰ , 0 ²⁶¹ , 0 ²⁶²	3 ²⁶³	31/15=2.07

Cont....

Table 2. Contd...

Author name	Author#	Open access journal number of citations ^{reference number} (T=Number of articles)					Non-open access journals number of citations ^{reference number} (T=Number of articles)					Average citations per article 2007-2011
		2007*	2008	2009	2010	2011	2007	2008	2009	2010	2011	
Sara E. Monaco	23	N/A	-	-	5 ²¹³ , 2 ²¹⁴ , 15 ²¹⁵	1 ²¹⁶ , 0 ²¹⁷ , 1 ²¹⁸ , 1 ²¹⁹ , 1 ²²⁰ , 0 ²²¹	-	3 ²⁶⁴	6 ²⁶⁵ , 0 ²²⁷ , 0 ²²⁹ , 1 ²⁶⁶	4 ²³⁰ , 0 ²³¹ , 0 ²³² , 0 ²³³ , 1 ²⁶⁷	1 ²³⁵	16/11=1.45
Liron Pantanowitz	24	N/A	6 ¹⁰⁹	17 ¹¹⁰	4 ¹¹¹ , 3 ²⁵⁰	1 ²¹⁷ , 1 ²¹⁹ , 1 ²²⁰ , 1 ²²¹ , 0 ²²²	5 ¹¹³ , 40 ¹¹⁴ , 17 ²⁶⁹ , 0 ¹¹⁵ , 1 ¹¹⁶ , 0 ²⁷⁰ , 0 ¹¹⁹ , 0 ¹²⁰ , 0 ¹²¹ , 0 ²⁷¹ , 0 ¹²³ , 1 ²⁷² , 0 ¹²⁴	4 ²⁷³ , 0 ¹²⁵ , 0 ¹²⁶ , 0 ¹²⁷	2 ¹²⁸ , 0 ¹²⁹ , 0 ¹³⁰ , 1 ¹³¹ , 0 ²⁵⁶ , 0 ¹³² , 0 ¹³³ , 0 ²⁷⁴	3 ²⁵⁸ , 0 ²⁶⁰ , 1 ¹³⁴ , 0 ¹³⁶	1 ²³⁷ , 3 ²⁶⁴ , 0 ¹³⁷	79/32=2.47
Bharat Rekhi	25	N/A	-	-	1 ²⁷⁵	1 ²⁷⁶	0 ²⁷⁷ , 2 ²⁷⁸	0 ²⁷⁹ , 5 ²⁸⁰ , 3 ²⁸¹ , 1 ²⁸²	0 ²⁸³	0 ²⁸⁴ , 3 ²⁸⁵ , 4 ²⁸⁶ , 3 ²⁸⁷ , 1 ²⁸⁸	0 ²⁸⁹ , 0 ²⁹⁰	21/14=1.5
Husain A. Saleh	26	N/A	5 ³¹³ , 6 ³¹⁴	12 ²⁹³	-	-	2 ²⁹⁴ , 5 ²⁹⁵	0 ²⁹⁶ , 0 ²⁹⁷ , 0 ²⁹⁸ , 0 ²⁹⁹	0 ³⁰⁰ , 0 ³⁰¹ , 0 ³⁰² , 3 ³⁰³ , 2 ³⁰⁴ , 0 ³⁰⁵ , 8 ³⁰⁶ , 2 ³⁰⁷ , 7 ³⁰⁸ , 1 ³⁰⁹	2 ³¹⁰ , 0 ³¹¹ , 0 ³¹²	-	42/19=2.21
Torill Sauer	27	N/A	-	-	3 ³¹³ , 6 ³¹⁴ , 2 ³¹⁵	-	4 ³¹⁶ , 0 ³¹⁷ , 0 ³¹⁸	5 ³¹⁹ , 2 ³²⁰	-	4 ³²¹ , 0 ³²² , 0 ³²³ , 1 ³²⁴	-	28/9=3.11
Michael J. Thrall	28	N/A	14 ¹⁷¹	1 ³²⁵	-	-	4 ³²⁶ , 0 ³²⁷ , 0 ³²⁸	5 ³²⁹ , 0 ³³⁰ , 0 ¹⁷⁸	-	7 ³³¹ , 0 ³³²	-	16/8=2

*CytoJournal indexing with ISI started from 2008 onwards, so the data for 2007 was not applicable- N/A

**Traditional non-OA cytopathology journals (Acta. Cytoplogica, Cancer Cytopathology, Cytopathology, and Diagnostic Cytopathology). N/A: Not applicable

The data in red indicate publications as meeting abstracts.

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Table 3. Comparison of 'Citations per publication' and 'time adjusted citation quotients' (Q values) for CytoJournal as OA cytopathology journal versus non-OA cytopathology journals with meeting abstracts included [Figure 3]

Author#	Data with meeting abstracts							
	Author		Citation per article			Time adjusted citation quotient (Q)		
	Last name	First name, MI	OA	Non-OA	Difference	OA	Non-OA	Difference
1	Agarwal	Shipra	4	0.286	3.71	1.726	0.066	1.66
2	Auger	Manon	6	4.38	1.62	1.958	1.085	0.873
3	Austin	R. M.	4.67	3.62	1.05	1.839	1.028	0.811
4	Baloch	Zubair W.	51.67	43.25	8.42	10.596	10.836	-0.240
5	Barboza-Quintana	Oralia	6.5	1.5	5	1.338	0.346	0.991
6	Bentz	Joel S.	4	1.86	2.14	1.644	0.583	1.061
7	Brimo	Fadi	6	7.5	-1.5	1.958	2.013	-0.055
8	D'Amore	Krista L.	4.5	0	4.5	2.032	0	2.032
9	Freund	Gregory G.	2	0	2	0.653	0	0.653
10	Garza-Guajardo	Raquel	6.5	1.5	5	1.338	0.346	0.991
11	Goulart	Robert A.	6.75	2	4.75	2.103	0.483	1.621
12	Gupta	Prabodh K.	9.75	11.14	-1.39	2.573	3.384	-0.812
13	Gupta	Ruchika	2.5	3.6	-1.1	1.019	1.318	-0.299
14	Hoda	Rana S.	7.5	2.6	4.9	1.418	0.662	0.756
15	Hornish	Maryanne	11.5	0	11.5	3.283	0	3.283
16	Iyer	Venkateswaran K.	2.75	0.67	2.08	1.157	0.268	0.889
17	Jhala	Dharshana N.	2.5	3.58	-1.08	1.154	0.8	0.354
18	Jhala	Nirag C.	2.5	3.64	-1.14	1.154	0.747	0.408
19	Khalbuss	Wvalid	2.89	0.8	2.09	1.320	0.273	1.047
20	Khurana	Kamal K.	5	0	5	1.083	0	1.083
21	Mathur	Sandeep R.	2.5	1.53	0.97	1.019	0.777	0.241
22	Michelow	Pam	3	2.07	0.93	1.114	0.618	0.496
23	Monaco	Sara E.	2.89	1.45	1.44	1.320	0.503	0.817
24	Pantanowitz	Liron	3.78	2.47	1.31	1.28	0.625	0.655
25	Rekhi	Bharat	1	1.5	-0.5	0.447	0.505	-0.058
26	Saleh	Husain A.	7.67	2.21	5.46	1.927	0.701	1.227
27	Sauer	Torill	3.67	3.11	0.56	1.693	1.107	0.586
28	Thrall	Michael J.	7.5	2	5.5	1.350	0.625	0.725

OA: Open access

For CPP, the following methodology was used. To make sure valid paired t-tests could be used, an Anderson-Darling normality test was run on the differences between CPP for CytoJournal as OA cytopathology journal and for the traditional non-OA journals. The normality test was passed and the paired t-test was run. A 95% confidence interval for the mean difference between [CytoJournal citations per publication] - [traditional non-OA citations per publication] was generated with the interval being (1.406, 3.824) with a P value of 0.001.

The same methodology was used to analyze the Q values. The 95% confidence interval for the mean difference

between [CytoJournal Q value] - [traditional non-OA Q value] was (0.473, 1.084) with a P value of approximately 0.0001. The findings confirmed the hypothesis that the publications in OA cytopathology journal generated improved citation rate (CR) with higher CPP and Q values with statistically significant difference as compared to the publications in the traditional non-OA cytopathology journals [Figure 3a and b].

With the second set of data, without the inclusion of meeting abstracts [Table 4], the same paired t-tests were run, with the null and alternative hypothesis similar to that for the first set of data. CPP with Open Access versus Non-Open Access showed a 95% confidence interval

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Table 4. Comparison of 'Citations per publication' and 'time adjusted citation quotients' (Q values) for CytoJournal as OA cytopathology journal versus non-OA cytopathology journals without meeting abstracts [Figure 3c and d]

Author#	Data without meeting abstracts							
	Author		Citation per article			Time adjusted citation quotient (Q)		
	Last name	First name, MI	OA	Non-OA	Difference	OA	Non-OA	Difference
1	Agarwal	Shipra	4	0.4	3.71	1.726	0.092	1.633
2	Auger	Manon	6	7	1.62	1.958	1.690	0.268
3	Austin	R. M.	4.67	7.5	1.05	1.839	2.113	-0.274
4	Baloch	Zubair W.	51.67	57.67	8.42	10.596	14.448	-3.852
5	Barboza-Quintana	Oralia	6.5	3	5	1.338	0.693	0.645
6	Bentz	Joel S.	4	4.33	2.14	1.644	1.36	0.284
7	Brimo	Fadi	6	7.5	-1.5	1.958	2.013	-0.055
8	D'Amore	Krista L.	4.5	-	4.5	2.032	-	-
9	Freund	Gregory G.	2	-	2	0.653	-	-
10	Garza-Guajardo	Raquel	6.5	3	5	1.338	0.693	0.645
11	Goulart	Robert A.	6.75	4.9	4.75	2.103	1.174	0.929
12	Gupta	Prabodh K.	9.75	13	-1.39	2.573	3.948	-1.376
13	Gupta	Ruchika	2.5	3.6	-1.1	1.019	1.318	-0.299
14	Hoda	Rana S.	7.5	4.33	4.9	1.418	1.103	0.315
15	Hornish	Maryanne	11.5	0	11.5	3.283	0	3.283
16	Iyer	Venkateswaran K.	2.75	0.86	2.08	1.157	0.344	0.812
17	Jhala	Dharshana N.	2.5	5.38	-1.08	1.154	1.2	-0.045
18	Jhala	Nirag C.	2.5	5.71	-1.14	1.154	1.173	-0.019
19	Khalbuss	Walid	2.89	2	2.09	1.320	0.455	0.865
20	Khurana	Kamal K.	5	-	5	1.083	-	-
21	Mathur	Sandeep R.	2.5	1.7	0.97	1.019	0.457	0.562
22	Michelow	Pam	3	3.88	0.93	1.114	1.159	-0.044
23	Monaco	Sara E.	2.89	3	1.44	1.320	1.107	0.214
24	Pantanowitz	Liron	3.78	5.2	1.31	1.28	1.312	-0.032
25	Rekhi	Bharat	1	1.75	-0.5	0.447	0.589	-0.142
26	Saleh	Husain A.	7.67	3.23	5.46	1.927	1.024	0.904
27	Sauer	Torill	3.67	5.6	0.56	1.693	1.992	-0.299
28	Thrall	Michael J.	7.5	5.33	5.5	1.350	1.669	-0.317

OA: Open access

of (-1.038, 1.848) with a *P* value of 0.568. For the *Q* value, a 95% CI interval of (-0.309, 0.677) with a *P* value of 0.448. This analysis showed that CPP and *Q* values were also higher when meeting abstracts were taken out of the data set, but the difference was statistically insignificant [Figure 3c and d].

The results with 'Publish or Perish' software using 'Google scholar' data^[17,18] also showed comparable pattern with higher citation rates for the publications in OA cytopathology journal than the traditional non-OA cytopathology journals. This data was unfiltered and included citations by all sorts of publication types.

DISCUSSION

A citation is defined as "a quoting of an authoritative source for substantiation".^[11] As almost all authors would like to be seen as "authoritative source" and their work as "substantial," citations are a crucial metric in determining the success of both authors and journals. They are used in calculating such relied upon publication metrics in journalology as impact factor and H-factor, which are used critically by many in evaluating the worthiness of a journal or an author^[12]. Citations are indexed in several large databases on the World Wide Web, the largest of which is Thomson Scientific's Web of Science® which currently

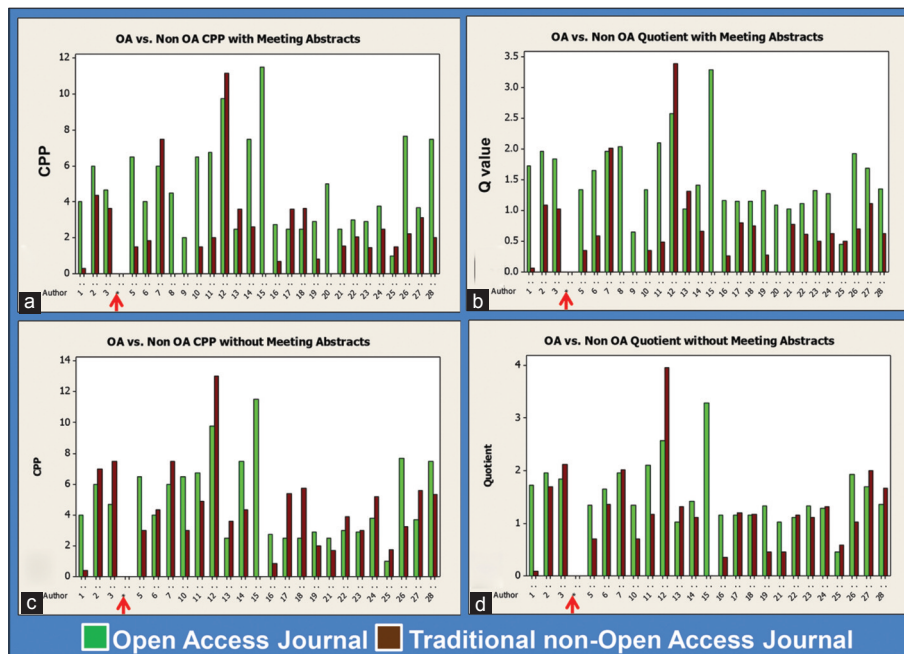


Figure 3: Comparison of citations per publication (CPP) and time adjusted citation quotient (Q value) for OA cytopathology journal (a and c) versus non-OA cytopathology journals (b and d) with (a and b) and without (c and d) inclusion of meeting abstracts in the analysis. (a and b) The publications in OA cytopathology journal generated relatively improved citation rate with higher CPP and Q values as compared to the publications in the traditional non-OA cytopathology journals if all publications were included in the analysis. c and d. Although the CPP and Q values continued to be higher for OA cytopathology journal when the meeting abstracts were taken out of the analysis, the difference was statistically insignificant
[Red Arrows : Author #4 is not included in these graphs. This author (also with higher CPP and Q value for OA journal (CytoJournal) as compared to non-OA cytopathology journals with meeting abstracts included) would have decreased the size of other bars and skewed the graph. For the data details on this author, please see Tables 2 and 3]

contains more than 40 million bibliographic records and 550 million citations from the past 100 years. We conducted the current study using the same data which is also used to calculate impact factor (IF)^[15].

Since the Budapest declaration, several studies have examined the impact of the Open Access model of publication on the rates of citation for publications/authors. In 2001 in *Nature*, Steve Lawrence was the first to publish that free online availability of a publication greatly increased its impact on the scientific community. He analyzed CRs for 119,924 conference articles in computer science and related disciplines and excluded self-citations. He demonstrated the relationship of online availability as a function of the number of citations per article and the year of publication. The results were quite dramatic, showing a direct relationship between the factors, specifically a 157% increase in citations for articles that were free online compared to those which were not available free online.^[6]

Our study showed that, in the field of cytopathology, authors who published in both OA cytopathology journal and traditional non-OA journals, accrued a relatively higher rate of citation per publication and time adjusted citation

quotient for their publications in the OA journal with statistical difference ($P < 0.01$) [Table 3 and Figure 3a, b]. However, if meeting abstracts were excluded from the analysis, increase in CPP and Q values was statistically insignificant [Table 4 and Figure 3c, d] This data supports the prior published conclusions that the OA model is a legitimate platform for publication with comparable or even higher citation rates to traditional journals.

Kurtz *et al.* studied the increased CRs in OA publications but noted a possibility of selection bias. The suggested bias was that the most prominent authors are more likely to make their publications available in an OA model, artificially increasing the rate of citations.^[7] As previously mentioned, cytopathology is a uniquely concise field, in which there are very limited numbers of Open Access journals, *CytoJournal* being one, but several traditional non-OA cytopathology journals. Because of this there are some authors who have publications in both OA and traditional journals, making comparison of the CRs possible while eliminating the above mentioned selection bias suggested by Kurtz *et al.* as a confounding factor.^[7]

Another type of variable is the 'early view bias,' wherein a publication that is posted on an OA platform before final

publication will have more time to accrue citations and thus skew the data towards citations in OA.^[10] The OA journal used in our project (*CytoJournal*) does not post in pre-publication form, and thus our study was controlled against this type of bias.

Kurtz *et al.* also discussed another type of bias for which we were not able to control and that might influence the CRs of OA publications. This is a different type of selection bias, wherein the individual author selects their most important (and thus citable) publications for OA.^[7] This type of bias is extremely subjective and difficult to prove or refute in a controlled study. However, it is important to highlight that the quality of the published material is generally the primary factor responsible for its overall impact and CR. Traditionally, the quality of published material is predominantly facilitated by the peer-review component of the editorial activity of the peer-reviewed journals. Thus, it is critical to understand and consider the quality of the peer-review process of any scientific journal irrespective of its OA status.

Some concerns have been raised in recent years regarding sprawling, low-quality journals (including some OA journals) which may have high turnover of many publications that have little relevance or contribution to today's scientific discoveries. This is an issue not applicable only to OA, but also to any journal irrespective of its status as an OA journal or traditional non-OA journal. The very core of any reputable scientific journal, with a quality-minded editorial board, is the high standard of the publications received and accepted after a vigorous peer review process with proactive participation by peer-reviewers.

We also evaluated, using a small cohort, the citation pattern of OA *versus* non-OA cytopathology publications with 'Publish or Perish,' software which uses 'Google Scholar' data on open, freely available platform.^[17,18] As compared to the Web of Science, the inclusion of citations by Google Scholar is wider and includes many journals and other platforms which may cite the original work with a relatively liberal approach. The initial analysis with 'Publish or Perish' based on 'Google Scholar' data showed comparable results with relatively higher rates of citation for *CytoJournal* practicing OA publication model as compared to traditional non-OA cytopathology journals (without statistical significance).

In summary, this study demonstrated that in the small subspecialty field of cytopathology, authors who published in both an Open Access journal (*CytoJournal*) and at least one traditional journal (*Acta Cytologica*, *Cancer Cytopathology*, *Cytopathology*, and/or *Diagnostic*

Cytopathology) accrued a comparable or slightly higher CR for OA publications as compared to the traditional non-OA cytopathology journals over a five year period from 2007-2011 [Figure 3].

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Abbreviations (in alphabetical order) used:

C, average number of CPP for a particular journal; C(Yr), C for a specific year; CPP, Citations Per Publication; CR, Citation Rate; IF, Impact Factor; IP, Intellectual Property; N/A, not applicable; OA, Open Access; Q value, time adjusted citation quotient; Yr, year.

COMPETING INTEREST STATEMENT BY ALL AUTHORS

No competing interest to declare by any of the authors.

AUTHORSHIP STATEMENT BY ALL AUTHORS

All authors declare that we qualify for authorship as defined by ICMJE <http://www.icmje.org/#author>.

ETHICS STATEMENT BY ALL AUTHORS

This study did not require approval from Institutional Review Board (IRB) (or its equivalent) as it is based on analysis of published data on web.

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