Volume 18 Number 1 June 2013

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

I am delighted to invite Tamar Giorgadze, MD, PhD as the guest editor for June 2013 issue of Focus. I thank all retiring editorial board members: Drs. Auger, Caraway, Fadare, & Nicosia for their excellent and proactive timely input in organizing past issues of Focus. I welcome Drs. Lastra, Lin, and Nassar as new editorial board members.

The publication committee members thank Dr. Auger for her contribution to the Humanities Corner in the Focus Newsletter until this issue. She has put forth extraordinary efforts to include interesting topics under this category for the past issues. From December 2013 onward, Dr. Giorgadze has agreed to contribute a new section, 'Images in Cytology'.

The Focus editorial board is also thankful to the PSC members for their timely contributions to the past Focus issues. We are looking forward to their future proactive contributions.

I thank Dr. Giorgadze for the excellent organization of this Focus issue and appreciate her hard work. Please enjoy the issue!

Sincerely,

Vinod B. Shidham, MD, FRCPath, FIAC

From the Guest Editor's Desk

Tamar Giorgadze, MD, PhD

First of all I would like to thank Dr. Vinod Shidham for inviting me to be a Guest Editor of the current issue of Focus Newsletter, and also express my sincere gratitude to all co-authors for their contributions. It has been a rewarding experience.

Con't on page 2

Presidents Message

Zubair W. Baloch, MD, PhD

Don't worry when you are not recognized, but strive to be worthy of recognition.

~ Abraham Lincoln

It is a great honor to serve as the president of Papanicolaou Society of Cytopathology (PSC). I define PSC as a society which represents who we are as professionals. Even though PSC has limited membership roster as compared to other esteemed pathology organizations; it continues to foster a tradition of no frills welcoming society. Based on personal experiences of 17-years I believe PSC is a society with ***"Big Heart"*** which has stood the test of time solely due to the ongoing volunteering of its officers and founding members. I consider myself lucky to be the president of PSC (2013-2015); as I am surrounded by amazing mentors and friends who have beyond recognition served this organization and continue to

Con't on page 2

In This Issue

Membership Application

(Please download, print and complete)
http://www.papsociety.org/docs/09/pscapp2009.pdf

Focus is published by the Papanicolaou Society of Cytopathology

Editor:
Vinod B. Shidham, MD, FRCPath, FIAC
Wayne State University School of Medicine
Karmanos Cancer Center, & DMC
vshidham@med.wayne.edu

Associate editor:
Adebowale Joel Adeniran, M.D.
Yale University School of Medicine
adebowale.adeniran@yale.edu

Editorial office:
Vinod B. Shidham, MD, FRCPath, FIAC
Professor & Vice-chair, Dept of Pathology,
Wayne State University School of Medicine,
Karmanos Cancer Center, & DMC
Old Hutzel Hospital (Dept of Cytology-Ground Floor),
4707 St. Antoine Blvd
Detroit, MI 48201
Ph: (313) 745 0831
(Kathy Rost Assistant to Dr. Shidham)
Fx: (313) 745 7158

Con't from page 1

From the Guest Editors Desk

We have made a couple of additions to this issue. Along with the established Timely Topic and The Humanity Corner sections, this issue features two new sections, namely Images in Cytology and Case Report.

Timely Topic section includes two reviews which have not been previously published in other journals. Dr. Diana Rossi of the Catholic University of Rome provided us with a comprehensive review of ancillary studies that could be useful for the preoperative diagnosis of thyroid lesions, evaluating pros and cons of their use, and shared her personal experience in utilizing ancillary techniques for liquid-based cytology specimens. Dr. Paul Tranchida of Wayne State University, contributed an up to date review of opportunities and challenges that digital imaging and telecytology present in our every day practice.

Dr. Manon Auger of McGill University, as always, delighted us with a witty quiz for The Humanity Corner section. This time Dr. Auger put together a quiz on early days of microscopic technique and microscopes. The first microphotogram that we have chosen for publication in the Images in Cytology section appears to be in the spirit of Dr. Auger's quiz theme and was contributed by Dr. Nora Frisch, Cytopathology Fellow at Wayne State University and me. The Case Report, also in a quiz format, was provided by Dr. Pulinthanathu and Dr. Shi, Fellows in Cytopathology, New York University Medical Center. We hope that these two new sections of Focus will be well-liked by in-training pathologists, members of PSC and other readers, and will encourage them to make their own contributions to the Newsletter.

The last three of our issue highlight new benefits offered to the PSC members. Please spread the word about PSC to your colleagues and trainees and encourage them to join the society (www.papsociety.org).

Finally, I would like to thank Dr. Nora Frisch for copy-editing, and Dr. Shidham and Dr. Simsir for their effort in putting together this issue of Focus Newsletter.

Sincerely,

Tamar Giorgadze, MD, PhD, MIAC
Guest Editor

Con't from page 1

President's Message

do with utmost dedication. These include founding members, past presidents and current members serving either on PSC executive board or committees. If I am asked to name a few individuals who have really left their mark on PSC and will continue to do so; I will no doubt mention without hesitation Dr. Carlos Bedrossian one of the founding members of PSC and Andrea Abati who infused new life into PSC. During Andrea's presidency PSC had the most successful scientific program at USCAP 2006 on thyroid FNA which eventually led to Bethesda thyroid FNA conference and well-known Bethesda Classification Scheme for Thyroid FNA interpretation.

The 2013 annual PSC meeting was held in Baltimore, MD during the 102th USCAP Annual Meeting. On behalf of the current PSC leadership and members, I would like to thank the officers and committee chairs and members for their 2011-2013 terms of service to the PSC. Special thanks go to the past presidents, Drs. Martha Pitman and Lester Layfield. The officers for the 2013-2015 term include myself as President, Dr. Tarik Elsheikh as President-Elect, Dr. Britt-Marie Ljung as treasurer and Dr. David Chhieng as Secretary. The committee roster for 2013-2015 is available on the PSC website.

The 2013 PSC presentations began with the International Relations Committee Afternoon Session moderated by Dr. Eric Suba. The presentations included, **A Cervical Cancer Prevention Project in Guatemala given** by Dr. Anne Ruch, SewHope, Toledo OH. Dr. Rajan Dewar spoke on **Cervical Cancer Prevention Project in India** followed by Dr. Eric Suba highlighting the **Global Delphi Exercise for Cervical Cancer Prevention**. These informative presentations were followed by the Annual Business Meeting and cocktail reception. The PSC then presented its Companion Society Evening Session.

Prior to the scientific presentations, a brief awards ceremony occurred in which Jan Silverman, MD was honored with the **Yolanda Oertel Interventional Cytopathologist of the Year Award,** Michael Cohen, MD received the **L.C. Tao Educator of the Year Award** and Sudha Kini, MD received the **PSC Lifetime Achievement Award.** Thanks were given to Drs. Oertel, Tao and Abati for their support of these awards.

The Scientific Program titled "Algorithmic Approach to Pancreaticobiliary Cytology: A Step Toward Recommendations and Guidelines" was arranged in collaboration with American Society of Cytopathology. I do want to brag that this effort was spearheaded by two PSC past Presidents Drs. Lester Layfield and Martha Bishop Pitman. This is in keeping with the tradition of PSC being the leader in commencing the discussion and formulating guidelines/recommendation for cytopathology practice. The session commenced with speaker introduction and moderation by Mathew Zarka, MD. , Dr. William R. Brugge gave the presentation entitled, ***Indications and techniques for cytologic sampling of pancreatic and bile duct lesions.*** This was followed by the presentation entitled, ***Cystic lesions of the pancreas: Diagnostic criteria with emphasis on pitfalls*** given by Barbara A. Centeno, MD. Dr. Syed Ali then discussed and illustrated ***the cytomorphology and pitfalls in diagnosis of solid lesions of pancreas.*** The scientific session concluded with a very interesting presentation by Ralph H. Hruban regarding ***the interactive and education smartphone and IPAD app for pancreatic pathology*** developed by Johns Hopkins Hospital.

The Humanities Corner

Manon Auger, MD, FRCS (C), McGill University Health Center, Montreal, Quebec, Canada
manon.auger@mcgill.ca

Having read recently about the history of cytology, I considered that it might be opportune to present a short quiz to gauge your knowledge about the early days of our field.

Question 1

Who invented the compound microscope?

a) Anton van Leuwenhoeck
b) Zacharias Jansen
c) Hans Jannsen
d) Hans Lippershey

Question 2

Using a microscope, what were the first natural objects drawn and published?

a) bacteria
b) yeasts
c) bees
d) worms

Question 3

Who coined the word "cell"?

a) Johannes Muller
b) Robert Hooke
c) Anton van Leuwenhoeck
d) Pierre Borel

Please see answers to questions on page 10

Con't from page 2

President's Message

The topic for 2014 PSC Scientific Program to be given at the 103rd USCAP meeting will focus on the challenges as well as the exciting new developments in the field of cytopathology. The session has been developed by Dr. Mathew Zarka and carries a provocative title of ***"Reinventing Our Profession: Challenges and Experiences".*** Presentations will be given by Drs. Britt-Marie Ljung and Susan Rollins on the topic of ultrasound-guided FNA performed by pathologist in both academic and community settings. Drs. Yuri Nikiforov and Dara Aisner will discuss the emerging technologies and new molecular markers applied to thyroid and lung cytology specimens. The session will end with a presentation from Dr. Liron Pantanowitz on next generation sequencing and current biomedical informatics challenges.

The Papanicolaou Society continues to foster relationships with both national and international pathology societies. PSC has developed exciting new sponsored events with other societies which include American Society of Clinical Pathology, American Society of Cytopathology, Binford-Dammin Infectious Diseases Society and European Federation of Cytopathology. As I mentioned earlier that I am a "lucky president"; this all cannot be accomplished without the diligent workings of scientific program committee chaired by Mathew Zarka and international scientific program committee and relations committee chaired by Dr. Fernando Schmitt. To further the global mission of PSC Dr. Andrew Field with his colleagues Drs. Britt-Marie Ljung, Eric Suba and Matthew Zarka continue to reach out internationally with particular emphasis in sub Saharan Africa.

I must also thank the other PSC committees. The ***membership committee chaired by Dr. Momin Siddiqui*** with national and international committee members has worked effortlessly to update PSC membership list and distribute membership flyers at national and international meetings. I am hopeful that this diligent campaigning will lead to an increase in our membership. The ***education committee under the guidance of Dr. Aylin Simsir*** is ready for your educational contributions to be published in focus. ***Dr. Vinod Shidham serving as the chair of the newsletter and publication committee*** continues to provide his professional editorial experience in putting together the issues of "Focus" for PSC website. ***Dr. Dan Kurtycz as chair of the website committee*** with his new cast of members and help from website manager Mr. Earle Barnes continues to improve the PSC website to present as a portal to happenings and educational material for PSC members. ***The research committee chaired by Dr. Claire Michael*** will work diligently at every USCAP meeting to select the best cytopathology abstract for research award.

I cannot conclude this communication without sharing two more exciting news; "Cancer Cytopathology" has been added to the list of scientific journals being offered at a discounted rate to PSC members, and our own Dr. Eric Suba will moderate a session on global pathology at annual meeting of American Society of Clinical Pathology .

On a personal level I am nervous and excited to carry the torch of the founding members and past presidents. I thrive on feedback and hope to receive the same from membership to serve you better and be what you want and need PSC to be; the society with ***"Big Heart".***

ANCILLARY TECHNIQUES IN THE EVERY DAY PRACTICE OF THYROID FOLLICULAR LESIONS

Esther Diana Rossi MD, PhD, MIAC
Division of Anatomic Pathology and Histology-Catholic University of Sacred Heart Rome, Italy
Email: esther.rossi@rm.unicatt.it

ABSTRACT:

BACKGROUND: Follicular patterned (FP) lesions are a challenging category for which the morphologic evaluation is not often not sufficient to define the correct nature. Some ancillary techniques have been developed to make a diagnosis achievable. In recent years, immunocytochemistry (ICC) has been shown to be helpful in differentiating low and high risk FP lesions as well as molecular testing for *BRAF-1* activating mutations, which have been identified in 29-69% of papillary thyroid cancers (PTC). The use of Liquid based cytology (LBC) might facilitate the application of these techniques.

OBJECTIVE: Our aim is to discuss the role of LBC in the application of ICC and BRAF mutation in predicting the outcomes and determining the management options of patient with FP. We report our experience in the field of LBC cytological cases with ICC and *BRAF* mutational analysis.

CONCLUSIONS: Immunocytochemistry and *BRAF* gene mutation can be successfully carried out on LBC processed material. A cytological combination of morphology and ancillary techniques may show a significant improvement of the diagnostic accuracy and allow better prediction of malignancy mainly for FP.

REVIEW:

The aims of this article are: a) to review the efficacy and feasibility of LBC for the application of ICC and *BRAF* molecular analysis in thyroid lesions; b) to identify the situations in which application of these techniques will be useful in defining a more specific preoperative diagnosis, and also predict possible aggressive tumor behavior correlated to the thyroid lesion classification; c) to propose a clinical-diagnostic use of ICC and molecular applications on LBC of thyroid FP lesions in order to better define their nature and management.

Thyroid nodules are found worldwide in the general population and comprise both non-neoplastic and neoplastic entities. The distinction between benign and malignant neoplasms often represents a grey area with much controversy (1-3).

Fine-needle aspiration cytology (FNAC) represents the most important, and generally the first diagnostic tool for evaluating thyroid lesions. It has found a worldwide application because of its simplicity, safety and cost-effectiveness. FNA leads to a correct diagnosis in more than 70% of cases, and to a correct clinical approach in more than in 90% of patients (1).

It is important to emphasize that approximately 10-30% of FNAC samples are signed out with an "indeterminate" diagnosis belonging to a "grey zone" of follicular proliferations. Management of these lesions is a high number of unnecessary thyroidectomises with additional morbidity and higher health care costs. This indeterminate cytological category includes several subcategories (follicular neoplasms-FN, suspicious for malignancy-SM, atypical cells of undetermined significance-AUS) which are difficult to accurately define, because of the poor reproducibility. Each subcategory carries a different risks of malignancy, and these lesions are usually surgically excised. This rate of unnecessary thyroidectomies may be considered to be too high, as the final expected rate of overall malignancies is 20-30% (5-6).

An articulate debate on this category has been emphasized also by a number of new classifications systems for reporting thyroid cytopathology, leading to a breakdown of the indeterminate category to include following 3 subcategories with different risks of malignancy (ranging from 5-15%, 15-30% and 60-75%, respectively) : 1) follicular lesions of undetermined significance or atypia of undetermined significance (FLUS/AUS); 2) Follicular neoplasms and Hurtle cell neoplasms; 3) Suspicious for malignancy (SM) (6, 7, 25, 26).

For this reason, a growing number of experts in the field have increasingly emphasized the need to identify specific markers able to discriminate between malignant or benign lesions. The morphological pitfalls and drawbacks of thyroid follicular proliferations have induced a rising enthusiasm for the possible role of immunocytochemistry (ICC) and molecular tests on FNAC samples. However, at the conclusion of the Bethesda conference no recommendations for the use of ancillary techniques were made for this category, mainly due to the possibility of false positive or false negative immunocytochemical results (27).

The expression of immunomarkers has been used as an additional tool for diagnosing malignant thyroid tumors regardless of the presence of capsular and/or vascular invasion. HBME-1 and Galectin-3 have reached the highest specificity and sensitivity in malignant lesions although none of the

immunomarkers studied, have shown a diagnostic accuracy sufficient for using them as single antibody characteristic of malignancy (2, 3, 8-11).

One of our recent experiences has highlighted the application of an immunocytochemical panel consisting of HBME-1 and Galectin-3 as the best choice to discriminate between low and high risk of malignancy in follicular proliferations, showing a 81% overall diagnostic accuracy, which increased to 92% when a concordant positive panel was applied (28).

There are few papers in the literature which address the use of ICC in thyroid FNAC, including one series of 20 FNAC in which ICC was performed on cell-blocks yielding 100% sensitivity and specificity, and a recent paper by Cochand-Priolett et al who found identical results applying an ICC panel of HBME-1 and Cytokeratin 19 to 150 LBC thyroid specimens (8, 29). The results for indeterminate cytological cases favored malignant or benign disease with sensitivity, specificity, and negative and positive predictive values of 100%, 85.2%, 100%, and 86.2%, respectively (8).

Recent scientific data have shown that molecular alterations of specific pathways play a pivotal role in some types of thyroid cancer and, importantly, arise early in the tumorigenic process, justifying the use of these as markers of malignancy. In particular, papillary carcinoma, the most common thyroid malignancy, may carry *BRAF, RET/PTC* or *N-RAS* mutations (12-15). The promising diagnostic role of *BRAF* mutation analysis has been studied in some prospective and retrospective studies, defining the presence of any mutation as a strong predictor of cancer and reporting 100% accurate results in the follicular lesion of indeterminate significance category (17, 30, 31-33).

The *V600E BRAF* mutation is found in 29-69% of classical PTC and in 80% of tall cell variant (TCV), while it is less commonly identified in the follicular variant of papillary carcinoma (FVPC) (16). The evidence that the presence of a *BRAF* mutation could portend tumor aggressiveness and in some cases to a progression to poorly differentiated carcinoma with a less favorable prognosis has led to *BRAF* testing playing critical role on thyroid FNAC samples, providing a basis for preoperative risk stratification of thyroid lesions. Several studies have referred to the feasibility and simplicity of the *BRAF* molecular testing for cytological diagnosis of thyroid FP lesions including not only indeterminate proliferations but also suspicious for malignancy lesions, as well as its use as a prognostic indicator in the category of malignant lesions (12, 13, 17,30)

The cytological diagnostic utility of molecular testing was studied by Nikiforov et al in several papers analyzing a panel of molecular mutations including *BRAF.* Their prospective study of 1056 indeterminate FP lesions including follicular neoplasm group and the SM- suspicious for malignancy group, found, respectively, a 87% and 95% rate of malignancy in the *BRAF* mutated cases (13).

In this regard Mathur et al postulated the use of a scoring model including cytology and N-RAS mutational analysis for correctly classifying FP lesions as benign or malignant in 91% of all samples (34). Recently, Niemeier et al. proposed a combined molecular-pathologic score for a cohort of 403 micro-papillary thyroid carcinomas, supported by the evidence that the combined use of histologic criteria and *BRAF* status increased sensitivity from 77% to 96% and specificity from 68% to 80% (35).

It is well known that the majority of thyroid carcinomas are well-differentiated and display indolent behavior. They are usually treated with total thyroidectomises, although some of them, presenting with a more aggressive course, would require a more extensive surgical treatment, including lymph-node evaluation (36-37).

In many reports, *BRAF* mutations, mainly involved in the activation of MAPK-Kinase pathway, have been correlated with aggressive thyroid tumor behavior such as extra-thyroidal extension, advanced tumor stage at presentation and lymph node or distant metastases. This mutation brings about the final impairment of the function of the sodium-iodide symporter (NIS) and of other genes metabolizing iodide (30, 37-39).

Consequently, the new literature regarding ancillary testing is being reflected in the revised management guidelines for patients with thyroid nodules and differentiated thyroid cancer, recently published by the American Thyroid Association. They suggest utilization of a molecular panel (including *BRAF,* RAS, *RET/PTC* and *PAX8-PPAR*) for patients with indeterminate FNA cytology to help in guiding their management based on cytological diagnoses (39-40).

The use of these special techniques (e.g. molecular markers and immunocytochemistry) in FNAC has two main problems/barriers to solve: 1) the difficulties in its application on conventional cytology specimens and 2) the non diagnostic role of a single antibody. To solve these problems, especially in terms of specificity, Liquid Based Cytology (LBC), originally developed for cervical smears, has gained popularity as an alternative technique for collection and preparation of cytological specimens with application to fine-needle cytology and particularly to thyroid lesions with good results (1-3). This method involves the collection of cells into a methanol-based preservative solution, followed by processing with a semi-automated device, leading to an almost complete elimination of the background interference from blood, and easily visible cells laid out into a small 20-mm round area (18-21).

However, there are conflicting opinions and controversial data regarding the efficacy of LBC, in spite of fact that there are multiple positive aspects to its use including cost-effectiveness, time-sparing and most importantly the simple application of ancillary techniques such as ICC and molecular biology (3- 7, 22, 23).

Several authors stated that the application of these special techniques (e.g. ICC and molecular analysis) may be more easily performed when the liquid based method is used, pointing out LBC as a promising approach for routine use, mainly owing to the ease of application of ICC and molecular testing for common somatic mutations, which has been shown in some other papers from our group (1-2, 40-44).

A recent paper by Chang et al studied the application of *BRAF* molecular mutation analysis on liquid based cytology with similar results, but only in the group diagnosed as positive for malignancy group or papillary carcinoma. They did not evaluate the indeterminate FP lesions. They showed 84.9% sensitivity regardless of the cytological method applied (45).

Regarding the technical aspects, in our experience, the application of both immunocytochemistry and molecular analyses on LBC is feasible, highly reproducible and provides high yield with a 100% informative immunocytochemical and molecular results (28). Furthermore, we also found a complete concordance with the results of the immunocytochemistry and *BRAF* mutational analysis between cytology and surgical specimens. Our challenge was to combine the morphology with the use of LBC method with application of ICC and *BRAF* analysis (especially for the groups of FN, AUS-FLUS and SM) and create a valid diagnostic approach in thyroid follicular patterned lesions (46).

Our results proved the significant role of an immunocytochemical panel (made up by HBME-1 and Galectin-3) in the detection of malignancy in FN/AUS-FLUS category (p=0.0002) and its role also in the SM category (p=0.0007). Based on our previously published results, in the category of SM, characterized by a high rate of malignancy (about 60-75%), the ICC panel was negative in all benign lesion and positive in 84.6% of malignancies. The expression of *BRAF* mutation turned out to be of prognostic significance, as it was present in 39% of multifocal cancers and 32% of cases with extra thyroidal extension and nodal positivity (43-44).

For the positive for malignancy cases in which morphological features are clearly conclusive for malignant diagnosis, we suggested the detection of *BRAF* mutation for prognostic value with a 0% false positive rate (43-44).

A comparison between the immunocytochemical results and molecular detection in each group showed that for FN/AUS-FLUS entity, the assessment of an ICC panel is much more useful in achieving a diagnosis than *BRAF* analysis, especially because of the great percentage of wild type *BRAF* for this category. In our FN/AUS-FLUS group, very few cases showed the presence of *BRAF* mutation, despite the use of a highly sensitive method, likely consequence of the 87.5% cases diagnosed as FVPC, whereas Nikiforov and Ohori reported a high incidence of *BRAF* mutation in the group of atypical cases-follicular lesions, ranging from 25 to 33% (12, 13, 17).

The differences may be accounted for by the criteria used in the different classifications systems, as well as in the small number of our FN/AUS-FLUS, and high prevalence of FVPC diagnosed on follow-up histology (12,17).

In our opinion, patients with an indeterminate cytological FN/AUS-FLUS diagnosis and concomitant negative ICC panel and *BRAF* wild type may be followed up conservatively with repeated FNAC in 6-12 months, whereas in the presence of ICC negative and *BRAF* molecular mutation expression, a surgical approach with total thyroidectomy is strongly suggested. In the same category, a positive immunopanel should be followed by a lobectomy or total thyroidectomy depending substantially on the *BRAF* expression pattern.

For the group of suspicious for malignancy (SM/TIR4), regardless of the ICC panel, *BRAF* mutation expression leads to a definitive 100% diagnosis of cancer. Regarding the SM/TIR4 lesions, our result showed 5 benign adenomas which yielded false-positive results on the ICC panel, most likely due to the chosen cut off of 50% for immunocytochemical expression (44). In the group of suspicious for malignancy (SM), the detection of a *BRAF* mutation has a 100% histological correlation with a diagnosis of thyroid carcinoma (p=0.0353) and also significantly matched with all the three aggressive parameters chosen, suggesting a more aggressive surgical treatment is appropriate for these cases. Furthermore, the *BRAF V600E* mutation has been highly associated with PTC and more often with the aggressive variants (e.g. tall cell variant and columnar ones) with much lower rate of *BRAF* mutations in FVPCs.

We found a strong association between *BRAF* expression and thyroid cancer and also a significant correlation for papillary carcinoma (more often mutated) versus FVPC (less often mutated). Our data showed a significant correlation between *BRAF* expression and all three aggressive behavior parameters, lymph-node metastases (p<0.0001), extra capsular invasion (p=0.03) and multi-focality (p=0.0003), for this category (43, 46).

In our setting, we believe patients with a positive ICC panel and *BRAF* mutation evidence, independently from the morphologic category to which they belong, should be addressed to total thyroidectomies with also neck dissection meaning a consequent reduction of incidence of second surgery or intra-operative pathology in more than 40% of cases.

In conclusion, we encourage combining the application of ICC and molecular analysis, particularly for the worrisome category of indeterminate follicular patterned lesions (FN/AUS-FLUS) but also for suspicious for malignancy (SM) in which the use of morphology alone is no longer the only option for achieving a definitive correct diagnosis.

REFERENCES

1) Rossi ED, Morassi F, Santeusanio G et al. Thyroid fine-needle aspiration cytology processed by Thin Prep: An additional slide decreased the number of inadequate results Cytopathology 2010; 21-6.

2) Rossi ED, Raffaelli M., Minimo C et al. Immunocytochemical evaluation of thyroid neoplasms on thin-layer smears from fine-needle aspiration biopsies. Cancer Cytopathol 2005; 105: 87-95

3) Cochand-Priollet B, Prat JJ, Polivka M et al. Thyroid fine needle aspiration: the morphological features on ThinPrep slide preparations. Eighty cases with histological control. Cytopathology 2003; 14: 343-9.

4) Ravetto C, Colombo L, Dottorini ME Usefulness of fine- needle aspiration in the diagnosis of thyroid carcinomas. A retrospective study in 37,895 patients. Cancer Cytopathol 2000 90: 357-63.

5) Poller DN, Ibrahim AK, Cummings MH et al Fine-needle aspiration of the thyroid. Importance of an indeterminate diagnostic category. Cancer Cytopathol 2000; 90: 239-44.

6) Baloch ZW, LiVolsi VA, Asa SL et al. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Fine-needle aspiration state-of-science Conference Diagn Cytopathol 2008; 36: 425-437

7) Fadda G, Basolo F, Bondi A et al. Cytological classification of thyroid nodules. Proposal of the SIAPEC-IAP Italian consensus working group. Pathologica 2010; 102:405-8.

8) Cochand-Priollet B, Dahan H, Laloi-Michelin M et al. Immunocytochemistry with cytokeratin 19 and HBME-1 increases the diagnostic accuracy of thyroid fine-needle aspirations. Preliminary report of 150liquid –based fine needle aspirations with histological control. Thyroid 2011; 21: 1067-73.

9) Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa S. Immunohistochemical diagnosis of papillary thyroid carcinoma. Mod Pathol 2001; 14 338-342.

10) Herrmann ME, LiVolsi VA, Pasha TL et al. Immunohistochemical expression of Galectin-3 in benign and malignant thyroid lesions. Arch Pathol Lab Med 2002; 126 710-713.

11) Dabbs D, Abendroth CS, Grenko RT, Wang X, Gail ER. Immunocytochemistry on the Thin-Prep Processor. Diagn. Cytopathol 1997; 17 388-392

12) Nikiforova MN and Nikiforov Y.Molecular diagnostics and predictors in thyroid cancer. Thyroid 2009; 19:1351-61.

13) Nikiforov YE, Steward DL, Robinson-Smith TM et al. Molecular testing for mutations in improving the fine needle aspiration diagnosis of thyroid nodules. J.Clin.Endocrinol.Metab 2009; 94:2092-98.

14) Soares P, Trovisco V, Rocha AS et al, BRAF mutations and RET/PTC rearrangements are alternative events in the ethiopathogenesis of PTC. Oncogene 2003; 22: 4578-80.)

15 Cheung CC, Carydis B, Ezzat S, Bedard YC, Asa SL. Analysis of ret/PTC gene rearrangements refines the fine needle aspiration diagnosis of thyroid cancer. J.Clin. Endocrinol. Metab 2001; 86: 2187-90.

16) Puxeddu E, Durante C, Avenia N, Filetti S Russo D. Clinical implications of BRAF mutation in thyroid carcinoma. Trends Endocrinol Metab 2008; 19(4):138-45.

17) Ohori NP, Nikiforova MN, Schoedel KE et al. Contribution of molecular testing to thyroid fine needle aspiration cytology of "Follicular lesion of undetermined significance/Atypia of undetermined significance". Cancer Cytopathol 2010; 118; 17-23.

18) Tulecke MA and Wang HH. ThinPrep for cytologic evaluation of follicular thyroid lesions: correlation with histologic findings. Diagn. Cytopathol 2003; 30: 7-13.

19) Scurry JP and Duggan MA. Thin layer compared to direct smear in thyroid fine-needle aspiration. Cytopathology 2000; 11: 104-15.

20) Afify AM, Liu J, Al-Khafaji BM. Cytologic artefacts and pitfalls of thyroid fine needle aspiration using Thin Prep. Cancer Cytopathol 2001; 93: 179-86.

21) Malle D, Valeri RM, Pazaitou-Panajiotou K, Kiziridou A, Vainas I, Destouni. Use of thin layer technique in thyroid fine needle aspiration. Acta Cytol 2006; 50: 23-7.

22) Fadda G, Rossi ED, Raffaelli M et al. Fine-Needle aspiration biopsy of thyroid lesions processed by thin-layer cytology: one-year institutional experience with histologic correlation. Thyroid 2006; 16: 975-981.

23) Saleh HA, Hammoud J, Zakaria R, Khan AZ. Comparison of Thin-Prep and cell block preparation for the evaluation of Thyroid epithelial lesions on fine needle aspiration biopsy. Cytojournal 2008; 25; 5:3.

24) Faquin WC, Baloch ZW. Fine needle aspiration of follicular patterned lesions of the thyroid. Diagnosis, management and follow-up according to national cancer institute (NCI) recommendations. Diagn.Cytopathol 2010; 38; 731-9

25) British Thyroid Association. Guidelines for the Management of Thyroid Cancer. Second Edition 2007.

26) Ali SZ and Cibas ES. The Bethesda System for Reporting Thyroid Cytopathology. New York, NY: Springer 2010.

27) Baloch ZW, LiVolsi VA, Asa SL et al. Diagnostic terminology and morphologic criteria for cytologic diagnosis of thyroid lesions: a synopsis of the National Cancer Institute Fine-needle aspiration state-of-science Conference Diagn Cytopathol 2008 36 425-437.

28) Fadda G,Rossi ED, Raffaelli M et al. Follicular thyroid neoplasms can be classified as low and high risk according to HBME-1 and Galectine 3 expression on liquid based fine needle cytology. Eur J Endocrinol 2011; 165: 447-453.

29) Nga ME, Lim GS, Soh CH et al. HBME-1 and CK19 ARE higly discriminatory in the cytological diagnosis of papillary thyroid carcinoma. Diagn Cytopathol 2008; 38; 550-6

30) Moses W, Weng J, Sansano I et al. Molecular testing for somatic mutations improves the accuracy of thyroid fine needle aspiration biopsy. World J.Surg 2010; 34: 2589-94.

31) Schmitt F, Barroca H. Role of ancillary studies in fine needle aspiration from selected tumors. Cancer Cytopathol. 2012; 25: 145-60

32) Schmitt F. Molecular cytopathology and flow cytometry: pre-analytical procedures matter. Cytopathology 2011; 22: 355-7

33) Longatto-Filho A, Goncalves AE, Martinho O et al, Liquid based cytology in DNA-based molecular research. Anal Qunat Cytol Histol. 2009; 31: 395-400

34) Mathur A, Weng J, Moses W. A prospective study evaluating the accuracy of using combined clinical factors and candidate diagnostic markers to refine the accuracy of thyroid fine needle aspiration biopsies. Surgery 2010; 148: 1176-7.

35) Niemeier LA, Kuffner Akatsu H, Song C et al. A combined molecular-pathologic score improbe risk stratification of papillary micro carcinoma. Cancer 2011; 118: 2069-77

36) Yip L, Farris C, Kabaker AS et al. Cost Impact of Molecular Testing for Indeterminate Thyroid Nodule Fine-Needle Aspiration Biopsies. J Clin Endocrinol Metab 2012; Mar 14. [Epub ahead of print]

37) Cisco RM, Shen WT, Gosnell JE Extent of surgery for papillary thyroid cancer: preoperative imaging and role of prophylactic and therapeutic neck dissection. Curr Treat Options Oncol 2012; 13 :1-10

38) Xing M. Braf mutationin papillary thyroid cancer:pathogenic role,molecular bases,and clinical implications. Endocr.Rev 2007; 28:742-62.

39) Oler G, Camacho CP, Hojaij FC, Michaluart P Jr, Riggins GJ, Cerutti JM. Gene expression profiling of papillary thyroid carcinoma identifies transcripts correlated with BRAF mutational status and lymph node metastasis. Clin Cancer Res 2008; 14:4735-42.

40) American Thyroid Association (ATA) Guidelines Taskforce on Thyroid Nodules and Differentiated Thyroid Cancer, Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, Mazzaferri EL, McIver B, Pacini F, Schlumberger M, Sherman SI, Steward DL, Tuttle RM. Revised management guidelines for patients with thyroid nodules and differentiated thyroid cancer. Thyroid 2009; 19: 1167-1214.

41) Musholt TJ, Fottner C, Weber M et al. Detection of papillary carcinoma by analysis of BRAF and RET/PTC1 mutations in fine needle aspiration biopsies of thyroid nodules. World J Surg 2008; 34: 2595-603.

42) Park SY, Park YJ, Lee YJ et al. Analysis of differential BRAF (V600E) mutational status in multifocal papillary thyroid carcinoma.Evidence of independent clonal origin in distinct tumor foci. Cancer 2006; 107:1831-38.

43) Rossi ED Rossi ED, Martini M, Capodimonti S et al. BRAF (V600E) mutation analysis on lbc-processed aspiration biopsies predicts bilaterality and nodal i involvement in papillary thyroid microcarcinoma. Cancer Cytopathol 2012. (Ahead of Pubmed)

44) Rossi ED, Martini M, Capodimonti S et al. Diagnostic and prognostic value of immunocytochemistry and BRAF mutation analysis on liquid based biopsies of thyroid neoplasms suspicious for carcinoma. Eur J Endocrinol. 2013 (Ahead of Pubmed)

45) Chang H, Lee H, Yoon SO, Kim H, Kim A, Kim BH. BRAF (V600E) mutation analysis of liquid-based preparation-processed fine needle aspiration sample improves the diagnostic rate of papillary thyroid carcinoma. Human Pathol 2011; 43: 89-95

46) Rossi ED, Martini M, Straccia S et al. The sequential application of immunocytochemistry, braf-1 and n-ras mutation analysis identifies malignant follicular thyroid neoplasms on liquid-based cytology Mod Pathol 2013; 26: 102A

DIGITAL IMAGING AND CYTOLOGY

Paul Tranchida, M.D.
Deparment of Pathology, Wayne State University
Email: ptranchi@med.wayne.edu

Ever since the first digital camera was invented in 1975, digital photography has made significant advances in terms of speed, resolution and affordability to the point that it has now been a decade since digital cameras first outsold film-based camera. 1). In an image-oriented field such as cytopathology, any digital representation of biologic material needs to have a high enough degree of nuclear and cytoplasmic detail so that an accurate diagnosis can be rendered. For that reason, digital imaging was not adopted until relatively recently where the quality of the captured image is now sufficient to convey an acceptable amount of morphologic detail. The marked increase in network bandwidth, defined as the rate of transmission of data over networks,has allowed high-resolution images to be transmitted reasonably rapidly over large distances not only within an institution but beyond its boundaries via the Internet. Since computers have also become more powerful and affordable during this time period, many pathology departments have completely transitioned from using traditional film photography to now capturing all their images digitally. In this article I will discuss the potential uses for digital imaging in the setting of a cytopathology department, and the different setups that are currently available and how they can be implemented.

Uses for Digital Imaging

There are a number of applications where digital imaging can be a helpful tool for practicing cytopathology. Cases with educational, medicolegal, or research value can now be archived and shared numerous times within and outside the department, without fear of the slide being broken, lost or having it deteriorate (2). Computer-assisted screening of Pap tests has led to increased productivity and sensitivity in detecting squamous intraepithelial lesions as compared to manual screening, by identifying slides that are more likely to contain abnormalities. (Figure 1) (3). Imaging also enables the practice of telecytology, broadly defined as practicing cytopathology over a distance. Some possible scenarios where this could be used are triaging specimens on-site obtained through fine needle aspiration (FNA) biopsies, and also obtaining remote consultation, in real time or asynchronously, for challenging cases. An example of real-time consultation would be videoconferencing through a computer network where two or more users able to visualize a slide and make annotations simultaneously. Telecytology has evolved from its origins as static images captured through a digital camera to whole slide imaging (WSI) where a glass slide is completely scanned, converted digitally and stored, and later retrieved and manipulated for evaluation. The following sections discuss in more detail the different types of hardware and software setups that can be utilized for cytologic imaging.

Static Images

The simplest and least expensive setup for digital imaging is using a digital camera mounted on a microscope that takes single static snapshots of only one field at a time at one focal plane. There are many digital cameras available which can be mounted on either compatible microscopes or through the use of adaptors, the latter choice being an important consideration if upgrading the departmental microscopes is an expensive proposition. If the users only need to take still images for the purposes of illustrating important findings for the purposes of teaching, generating images for journal articles, and similar static-image uses, the digital camera option provides adequate functionality for minimal investment.

Dynamic Imaging

In order to practice real-time examination of cytologic images, such as in on-site FNA triaging or remote consultation, a variety of setups can be used. One option is robotic microscopy, where a glass slide is loaded and then viewed in real-time through a video camera where the stage is controlled robotically. First iterations of robotic microscopes utilized a normal microscope where the slide was placed on the stage manually and the stage

controls and objectives/focus were remotely controlled over a network. More recent models such as the Nikon Coolscope (Figure 2) are more self-contained units where the slide is loaded into the machine and then navigated via a computer interface. This option may be the best for departments that need to be able to practice real-time microscopic examination at a remote location where generally only a few slides are examined at any one time but do not have the need or the resources for investing in a WSI system due to the cost and data storage considerations (more on this below).

Another setup involves real-time projection of live digital images from a digital camera, similar to the 'webcams' that are available for personal computers and are built into smartphones. This type of setup has been in use at the University of Pennsylvania where it has shown to be effective in triaging FNA specimens (4, 5). Advantages to this configuration are predominantly portability, relatively low expense and flexibility of choosing the hardware and software components. The ability to examine different planes of focus is of particular benefit to the cytopathologist, as frequently specimens will contain three-dimensional clusters where the individual cells need to be examined. However, one obvious disadvantage is that the remote pathologist cannot navigate or focus the slide directly and therefore the microscope operator needs to be instructed remotely.

WSI, also referred to as virtual microscopy, combines the advantages of working with a digital representation of a glass slide with the ability to navigate it akin to operating a traditional microscope. It essentially consists of taking multiple snapshots of all the fields of a slide and creating a large file that combines all these fields together so that a user can browse different areas of the slide and zoom in and out, and the appropriate images and magnifications will be displayed seamlessly (Figure 3). WSI technology has recently evolved in its performance where scanning of one or multiple slides is rapid enough where it can be accomplished within short time frames; depending on the resolution of the image it can be as fast as 1-2 minutes. In addition, it is possible to scan the slide at multiple levels of focus, thus allowing the operator to be able to examine three-dimensional clusters. At the medical school affiliated with our institution (Wayne State University, Detroit, Michigan), the pathobiology courses have utilized WSI technology for a few years and have completely obviated the use of traditional microscopes with glass slides. The challenges to deploying these systems are the fact that they tend to be a costly platform in comparison to the other systems discussed, not just for the hardware itself but due to the fact that slide scanning generates large amounts of data, which then necessitates investments in adequate data storage space (6).

Considerations for Implementation

In addition to the practical and cost issues discussed above, any system that would be implemented in a department should undergo a validation process (7). This involves designing a system that best fits the needs of the user through careful planning and then once the components have been identified, obtained and configured, confirming that it is able to perform adequately in the appropriate setting. For example, in the FNA biopsy environment, testing the setup with previously performed cases that reflect the more common diagnostic scenarios that will be encountered allows the users to confirm that the practical and technical considerations for utilization of the system are being met. In any setups that require networking, whether it is wired or wireless, being able to ensure secure transmission of the images between different destinations is of paramount importance. Finally, having users be adequately trained to not only utilize but troubleshoot and maintain the system as needed is also essential.

Summary

There are a number of configurations available to a cytopathology department that wishes to incorporate digital imaging into their workflow. It is important to clearly identify the needs of the cytopathologists and/or cytotechnologists, and then see which of the aforementioned systems discussed above offer the desired features at an acceptable price. As these systems continue to evolve while their costs go down, there surely will be a point where their contribution to the cytopathology workflow will be great enough that they will become a standard feature in all departments. In response, cytopathology departments should provide training for cytopathologists and cytotechnologists so that these technologies can be utilized most effectively (8).

IMAGES:

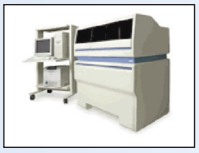


Figure 1: BD FocalPoint™ Slide Profiler performs computer-assisted screening of liquid-based Pap tests.

Figure 2: Nikon Coolscope is an example of a robotic microscope

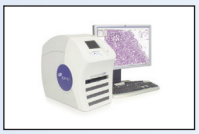


Figure 3: Aperio ScanScope CS2 is a whole-slide imaging system that can load up to five slides and scan them at 20X and 40X magnifications.

References:

1. Graham, J. (2003, February 27). Film fades to digital in camera sales. USA TODAY, Retrieved from www.usatoday.com
2. Donnelly AD, Mukherjee MS, Lyden ER, Radio SJ. Virtual microscopy in cytotechnology education: Application of knowledge from virtual to glass. Cytojournal. 2012;9:12. Epub 2012 Apr 30.
3. Pantanowitz L. The Cytopathology & Informatics Partnership: Current and Future Roles of Informatics in Cytopathology. Presentation at 57th Annual Scientific Meeting. American Society of Cytopathology (2009).
4. Goyal A, Jhala N, Gupta P. TeleCyP (Telecytopathology): real-time fine-needle aspiration interpretation. Acta Cytol. 2012;56(6):669-77.
5. Gupta PK. University of Pennsylvania aspiration cart (Penn-A-Cart): an innovative journey in fine needle aspiration service. Acta Cytol. 2010 Mar-Apr;54(2):165-8.
6. Thrall M, Pantanowitz L, Khalbuss W. Telecytology: Clinical applications, current challenges, and future benefits. J Pathol Inform 2011;2:51
7. Collins BT. Telepathology in cytopathology: challenges and opportunities. Acta Cytol. 2013;57(3):221-32. doi: 10.1159/000350718. Epub 2013 Apr 25.
8. Pantanowitz L, Hornish M, Goulart RA. Informatics applied to cytology. Cytojournal. 2008 Dec 29;5:16.

(Con't from page 4)

Answers to The Humanities Corner

Manon Auger, MD, FRCS (C), McGill University Health Center, Montreal, Quebec, Canada

Answer to Question 1: (B)

Zacharias Jansen is credited with the invention of the microscope, circa the 1590s, although the story begins much earlier. During the 1st century CE, the Romans were experimenting with glass, looking at objects through it. Different shapes of clear glass were tried, and one of their samples was thick in the middle and thin on the edges. They realized that if you held one of these "lenses" over an object, the object would look larger. The word "lens" is derived from the Latin word "lens" for lentil, because their shape resembled a lentil bean. Little use was made of lenses, however, until the end of the 13th century when the makers of spectacles produced lenses to be worn as glasses. The Italian Salvino D'Armate (c. 1258-1312) is credited with producing the first eye glasses.

Although credit of the first microscope is given to Zacharias Jansen (c. 1580-1638), a Dutch spectacle-maker from Middelburg, this attribution is much debated. Many scholars believe that his father Hans must have played an important role in the creation of the instrument, since the father and son worked together as spectacle-makers. There has also been some dispute as to whether Hans Lippershey, who was also a spectacle-maker living near the Jansens, could have invented the microscope, although he is most often credited with the invention of the first telescope. To complicate matters further, Zacharias Jansen also laid claim to the invention of the telescope.

The Jannsen's microscope consisted of three draw tubes with lenses inserted into the ends of the flanking tubes. The eyepiece lens was bi-convex and the objective lens was plano-convex, representing a very advanced compound design for that time period. The focusing of this hand-held microscope was achieved by sliding the draw tube in or out while observing the sample. The microscope was capable of magnifying objects approximately 3-10 times and though rudimentary, the Jansen microscope was an important advance from single lens magnification. By the end of the 17th century, further developments were made to microscopes, notably by Anton van Leeuwenhoek and Robert Hooke. Credit for coining the term "microscope" goes to Giovanni Faber, who gave that name to Galileo Galilei's compound microscope in 1625.

Anton van Leeuwenhoek (1632-1723), considered the "father of microscopy", was in fact an apprentice in a dry goods store where magnifying glasses were used to count the threads in cloth. He developed innovative methods for grinding and polishing lenses of great curvature which could give magnifications up to 270 times, the greatest known at that time, and these enabled him the build his microscopes. Anton van Leeuwenhoek was the first to visualize and describe bacteria and yeasts, and the circulation of red blood cells in capillaries. Throughout his life, he used his lenses to observe both biological organisms and inanimate objects. He reported his findings in over a hundred letters to the Royal Society of England and the French Academy.

Answer to Question 2: (C)

In 1630 Rome, Francesco Stelluti, using a microscope, drew and published the first depiction of three enlarged honeybees: these were the first published microscopic drawings and probably the only ones before those of Robert Hooke's famous Micrographia in 1665.

Why did Stelluti choose bees? The answer lies more in politics than sciences. In 1623, Maffeo Barberini had just been elected Pope (Urban VIII), and the coat of arms of the Barberini family was a shield bearing three bees. The Academy of the Lynx, a society promulgating new discoveries through meetings and publications, and to which Stelluti and Galileo belonged, wanted to do something to ingratiate itself to the new pope. The members of the Academy of the Lynx (the lynx being chosen as their emblem because of its proverbial curiosity and keen eyesight) decided to offer Urban VIII a beautiful print of three bees complete with the fine details as seen through the newly invented microscope. The Academy of the Lynx was obviously not entirely successful in gaining the good favors of Pope Urban VIII: indeed, he allowed the Inquisition to silence Galileo shortly afterwards…

Answer to Question 3: (B)

Robert Hooke is the one who coined the word "cell" to describe the small boxes he saw when he examined thin shavings of cork with a compound microscope, as illustrated in his book Micrographia or, Some physiological descriptions of minute bodies made by magnifying glasses: with observations and inquiries thereupon, published in 1665 under the auspices of the Royal Society of London. The "cells" he described in cork were, in reality, only the cellulose walls from which the living cells had long since vanished. Furthermore, he had no conception of the cell as a basic structural unit of plants and animals, an idea that developed only in the ensuing 200 years. Robert Hooke chose the word "cell" because the orderly arrangement of the cork bark reminded him of monk's rooms (cells) in a monastery.

References

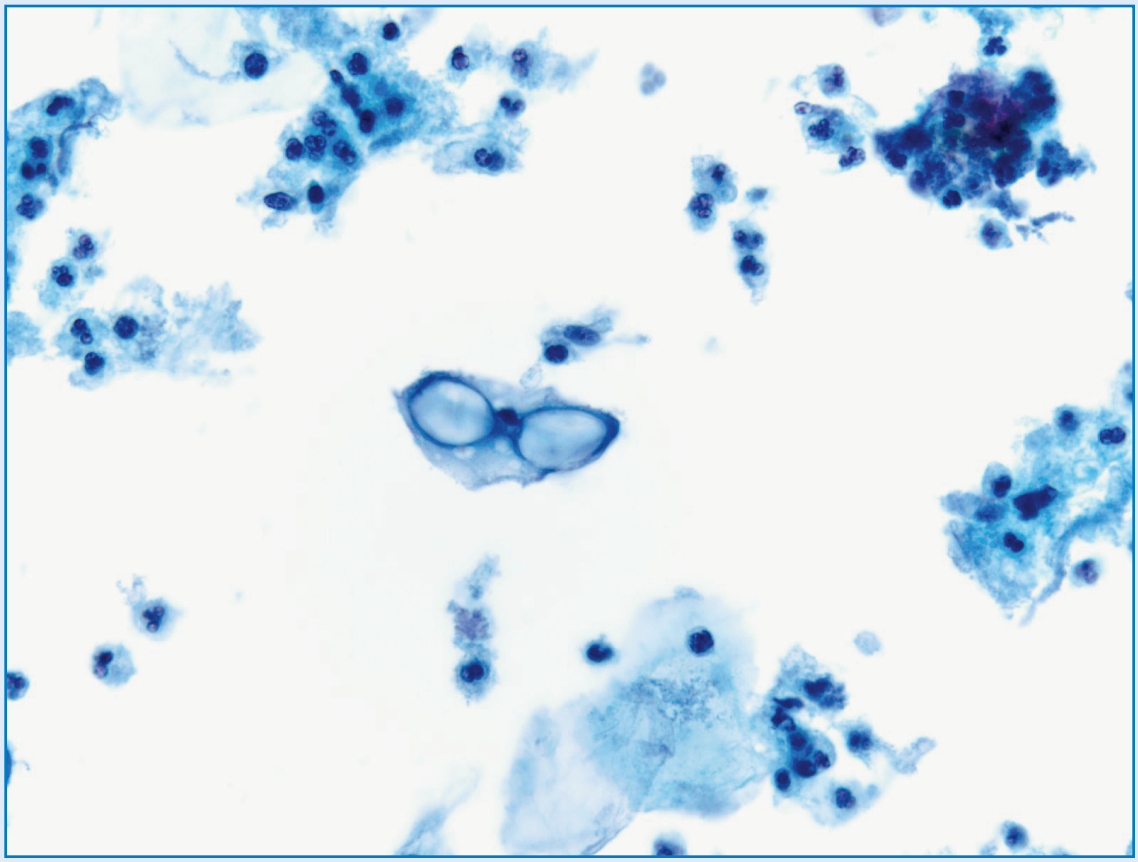
- Hajdu SI. Cytology from antiquity to Papanicolaou. Acta Cytol 1977;21:668-676.
- Gall JG. A pictorial history-views of the cell. ASCB, 1996, 128p.
- www.history-of-the-microscope.org (accessed on May 3rd, 2013)

Tamar Giorgadze, MD, PhD, MIAC and Nora K. Frisch MD, ABP, FCAP
Department of Pathology, Wayne State University

SPECTACLES UNDER THE MICROSCOPE

You could not see the cells in this photomicrograph using only the spectacles designed by Salvatore D'Amarte. For this task we used a modern microscope. However, these cells from a ThinPrep cervicovaginal Pap smear are a fun reminder of the precursor of our marvelous technology. The cytoplasmic inclusions in the cells create a striking resemblance to the convex glass of spectacles, the first invention used for the magnification of objects.

Tamar Giorgadze, MD, PhD, MIAC and Nora K Frisch MD, ABP, FCAP

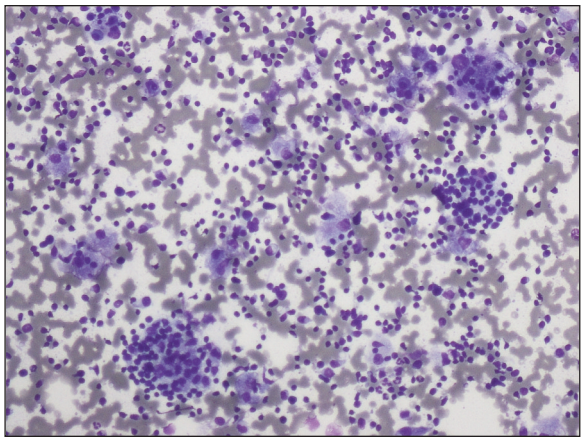


Rajiv Pulinthanathu, MD and Yan Shi, MD, PhD
NYU School of Medicine, Department of Pathology, New York, NY

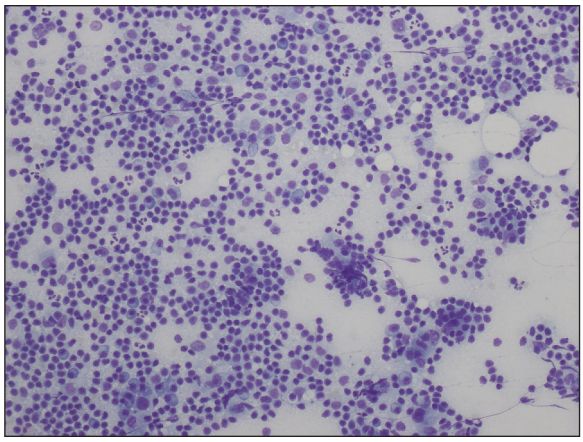
CASE HISTORY

57-year-old female presented to the FNA clinic with a 6 x 6 cm right posterior neck mass. The mass was firm, fixed and non-tender. The patient denied dysphagia, odynophagia, shortness of breath, fever or chills. The patient had no history of smoking or alcohol intake.

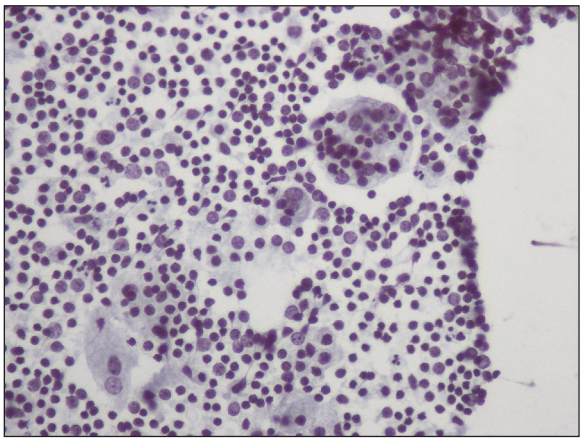
A.



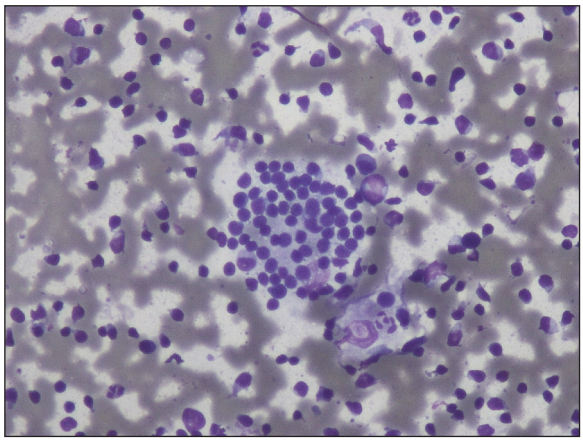
B.



C.



D.



Figures: A. FNA cytology smear of right neck lymph node, Diff-Quik stain, x 20
B. FNA cytology smear of right neck lymph node, Diff-Quik stain, x 20
C. FNA cytology smear of right neck lymph node, Pap stain, x 40
D. FNA cytology smear of right neck lymph node, Diff-Quik stain, x 40

QUESTIONS:

1. ***What is the most likely cytologic diagnosis?***
   A. Rosai-Dorfman disease
   B. Reactive lymphoid hyperplasia with sinus histiocytosis
   C. Gaucher's disease
   D. Malignant Histiocytosis
   E. Lymphoma

2. ***Which of the following immunophenotypical profile is most consistent with this entity?***
   A. S100 protein (+), CD68 (+), CD1a (+)
   B. S100 protein (+), CD68 (-), CD1a (+)
   C. S100 protein (+), CD68 (+), CD1a (-)
   D. S100 protein (-), CD68 (+), CD1a (-)
   E. S100 protein (-), CD68 (+), CD1a (+)

3. ***What is the most characteristic morphological feature of this entity?***
   A. Fibrosis
   B. Bean-shaped nuclei
   C. Emperipolesis
   D. Smudge cells
   E. Tart cells

4. ***Which of the following cells can be present in a large number in Langerhans cell histiocytosis?***
   A. Smudge cells
   B. Tart cells
   C. Plasma cells
   D. Eosinophils
   E. Mast cells

(For answers, go to page 13)

(Discussion and Answers)

Rajiv Pulinthanathu, MD and Yan Shi, MD, PhD
NYU School of Medicine, Deparment of Pathology, New York, NY

Discussion:
Rosai-Dorfman disease (RDD), also known as Sinus histiocytosis with massive lymphadenoapthy (SHML), is a rare nonmalignant proliferative disorder first described by Destombes in 1965 and was recognized as a distinct clinicopathologic entity by Rosai and Dorfman in 1969 in a report of four cases that were each originally diagnosed as "malignant reticuloendotheliosis".

RDD is a disease of childhood and early adulthood. Clinically the mean age of onset is second decade. It presents as prominent bilateral, massive, painless cervical lymphadenoapthy; other signs and symptoms include low grade fever, weight loss, leukocytosis, elevated erythrocyte sedimentation rate and hypergammaglobinemia. 43% of the cases the patients have at least one site of extra nodal involvement. The most frequent extranodal sites in decreasing order are skin, nasal cavity and paranasal sinuses, soft tissue, eyelid and orbit, and bone.

The exact nature of the RDD cells and the disorder is unknown. While macrophages are CD68(+), S-100(-), and CD1a(-), and many types of dendritic cells are CD68(-), S100 protein(+), and CD1a(+), the RDD cells are CD68(+), S100 protein (+), and usually CD1a(-). They also lack reactivity for R4/23, a monoclonal antibody specific for follicular dendritic cells. They express antigens associated with phagocytosis (such as the Fc receptor of IgG) and lysosymal activity (such as lysosome alpha-1-antitrypsin), antigens associated with early inflammation (Mac-387), and antigens found on monocytes but not tissue macrophages (OKM5 and CD15). They also express "activation" antigens, and receptors for transferrin and interleukin. These cells have been demonstrated to be polymorphic. The RDD cells have been called "true, functionally activated macrophages that may derive from circulating monocytes". Two studies have demonstrated an association with human herpesvirus-6. One case report suggested a relationship with or histologic continuum between RDD and an inflammatory pseudotumor.

The characteristic cytomorphology of this entity is the presence of a large number of histiocytes with round vesicular nuclei and abundant frothy cytoplasm.(Figure A) Many of these histiocytes engulf variable number of intact lymphocytes within their cytoplasm; a phenomenon referred to as lymphophagocytosis or emperipolesis (Figures C, D). Sometimes other cell types, such as plasma cells and red blood cells can also be observed within the cytoplasm. The background of RDD typically contains many plasma cells, lymphocytes and occasional neutrophils (Figure B).

Although the cytomorphologic features are well described, diagnostic difficulties can sometimes arise, especially on fine needle aspiration. The main differential diagnosis includes reactive lymphoid hyperplasia with sinus histiocytosis and Langerhans cell histiocytosis.

Lymphoid hyperplasia with sinus histiocytosis can have abundant histiocytes proliferation. The lymphohistiocytic aggregates in reactive lymphoid hyperplasia may mimic emperipolesis on smears with many small lymphocytes overlying or commingle with histiocytes. Meanwhile, emperipolesis in RDD sometimes can be difficult to appreciate, because too many lymphocytes engulfed in the histiocytes may obscure the underlying histiocyte nucleus. However, in RDD, the histiocytes proliferation is usually much more extensive than reactive lymphoid hyperplasia. The presence of emperipolesis is not just an occasional finding. It can be observed in the areas away from lymphohistiocytic aggregates and presents as a single-lying histiocyte with abundant engulfed lymphocytes in the cytoplasm (Figure C,D). In addition, immunohistochemical study can be helpful to differentiate these two entities. RDD cells are S100 protein (+), CD68 (+) and CD1a (-); while the histiocytes in reactive lymphoid hyperplasia are S100 protein (-), CD68 (+) and CD1a (-).

Langerhans cell histiocytosis is a neoplastic proliferation of Langerhans cells, mostly occurring in childhood. It enters into the differential diagnosis because Langerhans cells have abundant vacuolated cytoplasm which at low magnification may mimick histiocytes. However, Langerhans cells are characterized by indented, grooved, reniform nuclei, unlike the round vesicular nuclei in RDD. In addition, Langerhans cell histiocytosis lack emperipolesis and they are positive for both S100 protein and CD1a. Increased number of eosinophils is usually observed in the background of Langerhans cell histiocytosis but not present in RDD.

It is important to keep in mind that RDD-like changes in lymph node occasionally can be observed as part of the presentation in other diseases, such as Hodgkin's and non-Hodgkin's lymphoma. Complete specimen evaluation is critical to avoid mistakes.

Answers

1. A **2. C** **3. C** **4. D**

References:

1. Rosai J, Dorfman RF. Sinus histiocytosis with massive lymphadenoapthy: a newly recognized benign clinical entity. *Arch Pathol.* 1969;87:63-70.

2. Madhusmita Jena. Diagnosis of Rosai-Dorfman Disease by Fine Needle Aspiration Cytology in a case with Cervical Lymphadenopathy and Nasal Mass *Online Journal of Health and Allied Sciences* 2011;10(2)1-3.

3. Yuquan Shi, Adrienne Carruth Griffin, Paul JL Zhang, James N Palmer, Prabodh Gupta. Sinus histiocytosis with massive lymphadenoapthy *(Rosai-Dorfman Disease):* A case report and review of 49 cases with fine needle aspiration cytology. *CytoJournal* 2011,8:3.

4. Sujata Gaitonde. Multifocal, Extranodal Sinus Histiocytosis with Massive Lymphadenopathy. *Arch Pathol Lab Med.* 2007;131:1117-1121.

5. Foucar E, Rosai J, Dorfman RF. Sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease): a review of the entity. *Seminars in Diagnostic Pathology* 1990;7:19-73.

6. Levine PH, Jahan N, Murari P et al. Detection of HHV-6 in tissues involved by sinus histiocytosis with massive lymphadenopathy (Rosai-Dorfman disease). *Journal of Infectious Disease* 1992;166:291-295.

7. Govender D, Chetty R. Rosai-Dorfman disease of the breast. *Histopathology* 1997; 31(5): 483-484.

8. Wakely PE and Cibas ES. Lymph nodes. in: Cibas ES and Ducatman BS, editors. Cytology: Diagnostic principles and clinical correlates. 2nd ed. Saunders; 2003. p319-320.

PAPANICOLAOU SOCIETY OF CYTOPATHOLOGY

Dedicated to Clinical Practice - Clinical Education - Clinical Research

George N. Papanicolaou

1883-1962

JOIN THE PSC!

Benefits to PSC members:

- Annual subscription to Diagnostic Cytopathology
- CytoJournal OA Steward Plus' Benefits, including:
  - Free e-mail communications with electronic Table of Contents (e-TOC) of published articles at selected periodicity with links for PDF downloads.
  - Waiver of $1500 Article Publication Charge.
  - 50% discount towards most of the Cytopathology Foundation publications, including CytoJournal monographs and hard copy of CytoJournal.
- Annual subscription to Cancer Cytopathology at additional discounted rate of $60.00
- Focus Newsletter
- Special companion society scientific sessions at USCAP
- Image Atlas Access
- Membership Directory

The Membership Committee

APPLY TODAY: http://www.papsociety.org/members.html


PAPANICOLAOU SOCIETY OF CYTOPATHOLOGY
Dedicated to Clinical Practice - Clinical Education - Clinical Research
George N. Papanicolaou
1883-1962
JOIN THE PSC!
NEW Benefits to PSC members:
Special annual subscription rate of $60 to the journal "Cancer Cytopathology"
Will include the print journal (12 issues in 2014) as well as electronic access
Effective with Volume 122/Publishing year 2014
Discount $50 subscription rate for paper copy of "CytoJournal" (vs. regular $375)
The Membership Committee
APPLY TODAY: http://www.papsociety.org/members.html

PSC Membership - Apply Today!

http://www.papsociety.org/members.html

Membership..

MEMBERS ONLY

Download Membership Directory

Lost Username or Password?

Benefits to PSC members

- **Focus** Newsletter
- **Diagnostic Cytopathology** subscription
  (may donate the copy to pathologist in underserved country)
- **'CytoJ OA Stewards- Plus' benefits :-**
  Waiver of $1500 Article Publication Charge,
  FREE PDF of all **CytoJournal** articles,
  Optional print copy of **CytoJournal** at nominal annual subscription,
  & many other benefits http://www.cytojournal.com/OASteward.asp
- Representation at the **USCAP**
- Representation at the **European Congress of Cytopathology**
- **E-learning** education initiatives
- **Image atlas** access
- **Membership directory**

x all materials to:

Papanicolaou Society of Cytopathology (PSC) is
'CytoJournal OA Stewards'

CYTOJOURNAL
www.cytojournal.com
Peer-reviewed, Open access, Pub-Med indexed
Scholarly Cytopathology Journal

All PSC members entertain 'CytoJ OA Stewards' benefits
(for details visit http://www.cytojournal.com/OASteward.asp).

of Cytopatho

Dedicated to Clinical Practice □ Clinical Research
George N. Papanicolaou
1883-1962

By these letters let it be known that

Join today by downloading and filling the membership form from http://www.papsociety.org/docs/09/pscapp2009.pdf

NAME

is a member of the Papanicolaou Society of Cytopathology
YEAR

You will be emailed a certificate as shown here

Martha Bishop Pitman, M.D.
President, PSC

(Please forward this to your colleagues)

Eric Suba, M.D.
Treasurer, PSC