Competency-Based Medical Education in Pathology

Egg Donor Pregnancy: A Potential Pitfall in the Diagnosis of Placental Molar Disease
COURSE DIRECTORS
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WHO SHOULD ATTEND?
This course is open to all Canadian trainees, US trainees, CAP-ACP resident members as well as non-members and pathologists. Priority will be given to PGY5 and PGY4 residents who are members of the CAP-ACP. Limited availability is also extended to non MD Laboratory Professionals at a fee per day.

OBJECTIVES
An experienced faculty team has been assembled to collaborate on the topics and presentation of the course material to provide attendees with a practical review of major topics in the specialty of Anatomical Pathology. Your faculty of skilled pathologists will focus on what you need to know in preparation for the examination.

At the end of the meeting participants will be able to:
1. Review and update core medical knowledge in preparation for the Royal College of Physicians and Surgeons of Canada certification examinations in Anatomic Pathology, General Pathology, and Hematological Pathology.
2. Develop strategies for optimal preparation and exam performance in the written, practical, and oral components of the examination, and understand examination formats
3. Discuss and assess the significance of new findings and observations in the context of current literature
4. Demonstrate competence in the non-medical expert CanMEDS roles, in particular laboratory management and quality assurance

ACCREDITATION
The Canadian Association of Pathologists’/Association canadienne des pathologistes Residents Review Course is accredited by the Canadian Association of Pathologists. This event is an Accredited Group Learning Activity (Section 1) as defined by the Maintenance of Certification program of The Royal College of Physicians and Surgeons of Canada.

For more information and to register: www.cap-acp.org/RRC.php
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Submission of Manuscripts
The manuscript must be sent by e-mail attachment to the Editor-in-Chief, Dr. George Yousef at: info@cap-acp.org Only electronic submissions will be acknowledged and sent out for peer review.
INVITED EDITORIAL

Will Theranos Perform a Pilot Study and Publish Their Results?

Recently, direct-to-consumer laboratory testing has become commercially available, but its effectiveness in diagnosing or preventing disease is speculative and without evidence. One such direct-to-consumer testing provider is Theranos ($9 billion valuation), which is offering laboratory testing on fingerprick blood in pharmacies in some USA jurisdictions.

Recently, the CEO of Theranos, Ms. Elisabeth Holmes, suggested in a Wall Street Journal article that people should be able to get any lab test on their own and that by doing so they will take control of their own health and work with physicians to detect diseases early. She concluded that, with such strategies, “fewer people have to say goodbye too soon.”

We previously argued that people’s blood self-testing at asymptomatic stages will not only fail to effectively identify early disease (due to the low positive predictive value of most current lab tests), but might also lead to overdiagnosis (uncovering indolent diseases that do not require treatment) and to many false positive findings, which will likely trigger additional costly and potentially harmful interventions. However, due to the speculative nature of these arguments, we here suggest that Ms. Holmes test her hypothesis with an imperfect, but potentially very useful, experiment.

Similar to the 100-person wellness project, which conducted a pilot for 9 months before launching (www.systemsbio.org/research/100k-wellness-project; findings are yet to be published), we suggest that Ms. Holmes offer free testing in her facilities (at Walgreen pharmacies) to 10,000 interested people. This would not be an expensive trial, given the company’s valuation at $9 billion. At $100–$1,000 per participant in testing costs, this would be a $1–10 million project. Participants should be allowed to select their test menu from the one currently available by Theranos (about 100 tests or so). The results can either be self-interpreted (with the help of Google), or with the aid of a physician.

The following, and maybe additional findings, should be recorded and published:
1. How many diseases were uncovered that could have been effectively treated or prevented?
2. How many patients benefitted and how?
3. How many of the findings were false positives? And how were these verified?
4. Did this testing uncover indolent disease (that is, disease that poses no threat to the patient’s life and need not be treated)?
5. How many participants were stressed or harmed by unnecessary interventions?

Without at least some of the information mentioned above, Ms. Holmes’ suggested benefits of direct-to-consumer testing are speculative and should not be implemented until some preliminary evidence is provided.

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Theranos acceptera-t-elle d’effectuer une étude pilote et de publier ses résultats?

Les analyses de laboratoire sont récemment devenues accessibles directement au public, mais leur efficacité pour le diagnostic ou la prévention des maladies reste encore à démontrer. La société Theranos (évaluée à 9 milliards de dollars) offre ce type de services; dans certains États américains, elle propose d’analyser en laboratoire une goutte de sang prélevée au bout du doigt, en pharmacie.

Dans un article récent du Wall Street Journal, Elisabeth Holmes, directrice générale de Theranos, avançait que chacun devrait pouvoir obtenir des résultats d’analyse par lui-même, et reprendre ainsi le contrôle de sa propre santé pour collaborer ensuite avec les médecins afin de détecter plus rapidement d’éventuelles maladies. Pour elle, de telles stratégies éviteraient que « tant de gens nous quittent trop tôt ». Nous l’avons déjà souligné : non seulement une analyse de sang effectuée en l’absence de symptômes ne permet pas d’identifier adéquatement une maladie de façon précoce (en raison de la faible valeur prédictive de la plupart des analyses de laboratoire), mais elle peut aussi donner lieu à un faux positif ou à un surdiagnostic (la découverte d’une maladie indolente qui ne nécessite pas de traitement), et donc entraîner des interventions coûteuses et potentiellement dangereuses. Cependant, étant donné le caractère spéculatif de ces arguments, nous proposons à Mme Holmes de vérifier sa propre hypothèse à l’aide d’une expérience qui, bien qu’imparfaite, pourrait s’avérer très utile.

Un peu comme pour le 100K Wellness Project, un projet pilote qui s’étend sur 9 mois (et dont les résultats ne sont pas encore publiés), nous proposons à Mme Holmes d’offrir des analyses gratuites dans ses installations – les pharmacies Walgreen – à 100 000 personnes. Étant donné la valeur de la compagnie, estimée à 9 milliards de dollars, les coûts seraient raisonnables; en effet, les analyses coûteraient entre 100 $ et 1 000 $ par participant, et les dépenses totales totaliseraient donc entre 1 et 10 millions de dollars. Chaque participant pourrait choisir les analyses à réaliser dans le menu actuellement offert par Theranos (environ 100 analyses possibles). Les résultats seraient interprétés par les participants eux-mêmes (avec l’aide de Google), qui pourraient demander l’aide de leur médecin.

Il faudrait consigner et publier les résultats suivants, entre autres :

1. Combien a-t-on découvert de maladies qui peuvent être efficacement traitées ou prévenues?
2. Combien de patients en ont bénéficié, et comment?
3. Combien de résultats sont des faux positifs? Comment a-t-on vérifié?
4. Les analyses ont-elles permis de découvrir des maladies indolentes (c’est-à-dire qui ne menacent pas la vie du patient et n’ont pas besoin d’être traitées)?
5. Combien de participants ont subi un stress ou des préjudices à cause d’une intervention inutile?

Tant qu’on ne dispose pas de ce type de renseignements, les arguments de Mme Holmes quant aux avantages des analyses offertes directement au consommateur ne sont que des spéculations; ces analyses ne devraient pas être disponibles avant que l’on aie au moins des données préliminaires.

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The medical literature on adverse events shows that most of them are not directly related to a lack of medical expertise. They are usually related to systems failure and inadequate communication.\(^1,\^2\) Regardless of the cause, a growing societal pressure for accountability and better outcomes in the health care system in Canada prompted the Royal College of Physicians and Surgeons of Canada (RCPSC) to initiate reform in both medical practice and medical education.\(^3\) Medical training in North America has traditionally been knowledge-based, with a strong emphasis on structure and process, and the principles of residency training have not changed in essence since Flexner.\(^4\) However, educational theory and practice have evolved and new principles have been applied to many domains.

Miller’s often-quoted pyramid establishes that knowledge is only the first educational step and should progress through “knows how” and “shows how” in order to achieve the most expected outcome: “does.”\(^5\) In other words, the desired professional is not someone who knows everything about it, but someone who does it properly.\(^6,\^6\) That is why the RCPSC, as well as other governing bodies around the world, decided to incorporate the principles of competency-based education.

Competency-Based Medical Education

General Competencies and Activities

The balance of knowledge, skills, and attitudes is more important for a practicing physician than having a hypertrophic knowledge base. Knowing the ten most common causes of headache, how to distinguish them and recognize red flags, developing a management and follow-up plan with the patient, and orchestrating transfer of care will probably lead to a better outcome than listing 53 causes of headache and not knowing what to do with the aching soul in front of you.

The attributes necessary for a physician to effectively interact with patients and address their needs and complaints are varied and belong to different spheres. Traditionally, they have always been considered as common sense, abilities that you either possess or do not. However, you cannot teach what you cannot name, and that is the work of the educationalist: to deconstruct these intrinsic human abilities that are seamlessly integrated into tangible elements that can be named and taught. Competency frameworks, like the Royal College CanMEDS, provide a visual schema that separates these competencies into thematic domains for educational purposes. These are the physician roles that, in our daily activities, cannot be
individualized, as summarized by ten Cate and Scheele viewpoint from 2007 (which I strongly suggest reading):

“In the day-to-day work environment, these (CanMEDS) roles are not automatically recognized because the competencies they represent are indeed so general. Residents are not asked to play a health advocate role on Monday, be a communicator on Tuesday, a collaborator on Wednesday, and an expert on Thursday. All roles are intertwined in a complex way that makes them less visible and measurable.”

Perhaps the biggest advantage of having a well-developed competency framework is to map the abilities required for doing the work, develop learning objectives and teaching activities that will cover them all, have an assessment matrix to evaluate them, and identify weaknesses and strengths of the trainee and the program. However, as noted, in daily work these competencies are indivisible and run by default, and thus are difficult to measure. The most important question, then, is not whether the resident possesses each individual competency, but whether they are able to do the work. From a practical perspective, it is also much easier to observe their daily activities and assess the outcomes, which should reflect a balance among their knowledge, skills, and attitudes.

Although competencies and activities are essential parts of competency-based programs, they belong to two different realms: the former is the theoretical structure, the subject of clinician educators, and the later is the practical component, a matter of clinician teachers. So the questions are, “What daily activities form the core of a specialty?” and “What tasks should a physician be able to accomplish to provide safe and qualified patient care?” The term “entrustable professional activities” (EPAs) was coined to define such activities. In 2007, ten Cate and Scheele used this definition (slightly adapted):

“EPAs are those professional activities that together constitute the mass of critical elements that operationally define a profession. If we think of a competent [pathologist], we should be able to list those activities that form the core of that profession . . . “ Patients’ and instructors’ trust in a trainee and their entrustment of responsibility to that trainee are essential concepts in this approach, because they reflect the most important outcome of postgraduate training: a trainee’s readiness to bear professional responsibility.”

Entrustable Professional Activities

Although at first glance it looks like a complex and abstract concept, the process of entrustment of a trainee is the basis of apprenticeship models of professional training and something we routinely do in our practice. It is the moment we trust in a trainee to perform a task without our supervision, whether it is grossing a surgical specimen, ordering ancillary tests, discussing the case with the referring physician, or writing the final report.

To make this more palatable, I like the analogy with something everyone can relate to—entrustable childhood activities (ECAs). As children grow up, they become progressively able to do different activities, which usually follow a chronological sequence: climbing stairs, riding a bike, staying home alone, and driving a car. This exercise also helps us define the EPAs in our profession. We should avoid being too specific, thus creating an endless and impractical list of actions that represent only parts of a meaningful activity, and at the same time not be so broad as to create a sum of activities that reflect an overarching domain or stage of training.

Scenario 1 – Defining an EPA

ECA – After observing a five-year-old boy riding a bike, you say:
1. From now on, you can make turns on your own.
2. From now on, you can ride your bike on your own.
3. From now on, you can perform any physical activity on your own.

EPA – You observe a resident grossing surgical specimens and conclude:
1. From now on, you can measure tumours without supervision.
2. From now on, you can gross surgical specimens without supervision.
3. From now on, you can perform any procedure without supervision.

The second activity in both examples is meaningful and can be individually measured and entrusted. Although important, Activity 1 is just part of the meaningful task, while Activity 3 encompasses many distinct activities that belong to a common domain.

Establishing the EPAs of a given specialty is an important step in the development of a competency-based program. It gives the roadmap and defines what every professional should know by the end of their
training program, regardless of where they have been trained or where they are willing to practice. It goes without saying that a training program in a world-class academic institution should and will differ from the program in a community hospital. However, both of them have to cover the professional activities considered to be the core of that profession.

A good start is to look at the clinical schedule of your division and identify the different daily activities that require staff coverage. Once the EPAs are identified, it is important to determine how you will measure competency in each EPA and the level of expertise expected from a trainee in order to transfer the responsibility to them. As supervisors, we know this transfer is gradual and not time fixed. It depends highly on the context, which includes the individual background and pace, cognitive and motor skills, environment, and stakes of the activity, to name a few; and these variables apply to both the trainee and the supervisor.

It is not infrequent to work with second-year residents who only require your assistance for specialty-specific tasks, and you allow them to do the rest without supervision. On the other hand, there are fifth-year residents who you are recurrently going to verify the clinical information in the medical record yourself before hitting the sign-out button. If we all know that, why are our residency programs time fixed? Why should we prevent the fast learner from moving on and hurry the slow learner to the next phase, generating intellectual boredom and emotional stress, respectively? A way of dealing with this situation is to determine progressive levels of competency for each EPA and formally transfer the responsibility to the trainees, or as proposed by ten Cate and Scheele in 2007: “In designing a competency-based curriculum, the entrustment of an EPA may be acknowledged more formally in a statement that implies that a trainee has demonstrated enough competence to carry out the activity in question independently from now on. A statement of awarded responsibility (STAR).”

Assessing EPAs
Assessment for Learning

Time can now be used as a resource. As residents become autonomous in different activities, they can contribute to clinical service, dedicate their time to research and education, do elective rotations, or prepare their transition to unsupervised clinical practice. But how can one determine if a resident is competent enough to bear professional responsibility? When can we remove the safety gate from the stairs, allow our children to ride their bike to school by themselves, stay home unattended, or give them the car key?

Scenario 2 – Assessing an EPA
ECA – Assessing the ability of a five-year-old boy to ride his bike:
1. Describe the parts of a bike.
2. Define inertia and describe how it relates to the braking process.
3. Describe the different steps required for riding a bike.

EPA – Assessing a Resident’s Ability to Gross:
1. Describe the anatomy of the colon.
2. Define cobblestone appearance and describe how it relates to the microscopic changes.
3. Explain how to gross a bowel segment from a patient with inflammatory bowel disease.

In both situations, answering Question 1 will demonstrate whether they memorized basic information that might not be essential for performing the task. Question 2 requires not only memorization, but also the ability to apply knowledge. Question 3 is a higher-level question in educational hierarchy, because it requires memorization as well as analysis, synthesis, integration, and application of concepts. However, it is yet knowledge-based. Even if your son knew all the answers to those questions, he won’t ride his bike to school if he cannot maintain his balance or obey traffic rules. A resident might excel in all three exams and yet perform poorly when grossing a colonic segment with inflammatory bowel disease; that is, they don’t check the specimen (although they said they would in the written exam), are unable to recognize morphological features (that they know in theory), do not submit appropriate representative sections (because of lack of procedural training), and so on. Competency also requires skills and attitudes.

The only way to determine trainee competency is by performing frequent direct observations. This is both the strength and weakness of competency-based medical education. It is a huge strength in the way that assessment is directly linked to practice, it is an effective teaching tool, and supervisors become “coaches” rather than “judges.” On the other hand, it is time and resource consuming because it requires faculty development and frequent face-to-face interaction. The education literature uses the
term “assessment-for-learning” to indicate that observation and feedback should be used to regularly verify or correct the trainee direction. The emphasis on the supervisor as a “coach” is pivotal to the learning process, and the essential role of effective feedback cannot be underestimated. Feedback is a technique that not only can, but must be learned and mastered by clinician teachers. Identification of strengths and weaknesses should ensue in reinforcement and a shared improvement plan, respectively.6,10

Scenario 3 – Giving Feedback
ECA – Giving feedback to a five-year-old boy to ride a bike who does not know how to brake:
1. You’re not riding your bike properly. You have to ride it properly.
2. You’re almost there, but you’re not using the brakes. You have to turn your pedals backwards to slow down or stop the bike.

EPA – Giving feedback to a junior resident who is grossing a gallbladder and taking inadequate samples:
1. You’re not grossing it properly. You have to gross it properly.
2. You’re almost there, but you’re not sampling appropriately. In a grossly unremarkable gallbladder, the guidelines recommend you submit three representative sections, including the cystic duct margin and fundus (and refer the resident to the appropriate guidelines).

Feedback 1 is not specific and does not tell the learner how to improve (“judging”). Conversely, Feedback 2 specifically addresses the element that needs improvement and indicates the right direction (“coaching”). It is specific, timely, non-judgemental, and has an improvement plan. Although reviewing all the elements of effective feedback is beyond the scope of this article, you would probably agree the resident from Feedback 2 would be better off.

An assessment-for-learning program should include a series of formative evaluations with feedback and followed by a low-stakes summative evaluation. After a repetition of a few “low-stakes” cycles, higher-stake summative evaluations should take place.10 For instance, in a four-week rotation, a resident could be observed grossing once a week for the first three weeks, receiving feedback after each observation, and a summative evaluation could occur in the final week. By the end of the year, the resident could have a summative evaluation to determine his level of competency and, depending on the results, they could start to gross specimens independently or even supervise junior residents (Table 1).

It seems easy enough: after determining the EPAs, we establish the levels of competency for each of them with special attention to level four, in which residents can practice without supervision. Subsequently, we develop an assessment grid for EPAs and levels of competency, and when our residents achieve level four in every EPA, they are ready for practice! (See Table 2).

Unfortunately, competency-based medical education is not a series of checkboxes.8 Deconstructing a high-complexity profession into individual activities or competency frameworks is an abstract educational exercise to better understand and align human behaviours and learning processes. The end result or outcomes are difficult to measure in a reliable and valid way, and it would be too simplistic to imagine an array of ranking scales could dependably represent the person in front of you.8

Table 1. Example of Levels of Competency for Gross Examination (adapted from ten Cate et al. 2010):

<table>
<thead>
<tr>
<th>Level of Competency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
</tr>
<tr>
<td>may gross simple specimens with full supervision</td>
</tr>
<tr>
<td>may gross complex specimens with full supervision</td>
</tr>
<tr>
<td>may gross complex specimens with reactive supervision (ad hoc)</td>
</tr>
<tr>
<td>may gross complex specimens independently</td>
</tr>
<tr>
<td>may supervise the grossing of simple and complex specimens</td>
</tr>
</tbody>
</table>

Table 2. Example of a Resident Assessment Grid for Various EPAs (adapted from ten Cate & Scheele 2007):

<table>
<thead>
<tr>
<th>EPA</th>
<th>Level of Competency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chart Review</td>
<td>X</td>
</tr>
<tr>
<td>Gross Examination</td>
<td>X</td>
</tr>
<tr>
<td>Microscopic Examination</td>
<td>X</td>
</tr>
<tr>
<td>Ancillary Tests</td>
<td>X</td>
</tr>
<tr>
<td>Clinical-Pathological Correlation</td>
<td>X</td>
</tr>
<tr>
<td>Final Diagnosis</td>
<td>X</td>
</tr>
<tr>
<td>Final Written Report</td>
<td>X</td>
</tr>
<tr>
<td>Quality Assurance</td>
<td>X</td>
</tr>
</tbody>
</table>
As Schuwirth and Ash said,10 “If you deconstruct a bicycle and reconstruct it properly, the result will again be a bicycle. But things are quite different if you try this with a frog. This, then, begs the question whether competence is more like the bicycle or like the frog.”

CBME Challenges and Directions
As we saw above, CBME relies heavily on assessment. A well-planned assessment program should create a matrix relation between general competencies and EPAs to bridge the gap between theory and clinical practice. Since EPAs reflect daily work, general competence could be inferred as trainees progress through different levels of competence.7,8 But, in order to be valid and reliable, assessment in competency-based medical education requires multifaceted assessment and multiple assessors and frequent formative sessions with effective feedback; it also has to be work-based, criterion-based, and developmental.9,10

High-quality assessment tools and assessors are prerequisites, and qualitative approaches are necessary to give a context to the abstract numbers generated or, in other words, to re integrate the concrete persons and actions under our scrutiny in a meaningful way to reflect the art and science of medical practice.9,11,12,13 A promising qualitative tool to achieve such reconciliation and to make the life of front-line clinician teachers easier (and save a lot of time on faculty development), is the use of assessment rubrics. A competent clinician should not need to (and will not) know this entire educational alphabet to properly teach and evaluate their resident, but they can easily match behavioural descriptions to their performance.9,12,13

This work in progress has recently been applied on a larger scale, and time will tell whether the sound educational theory translates into better health outcomes for our society. The RCPSC has recently launched Competence by Design (CBD), which is a multi-year initiative to implement a competency-based medical education approach to residency education and specialty practice in Canada.14 This approach will be progressively adopted by groups of disciplines and the anticipated roll-out date for anatomical and general pathology is 2016. Therefore, resistance is futile and, wherever this journey is taking us, pathology must hop on the bus.

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Egg Donor Pregnancy: A Potential Pitfall in the Diagnosis of Placental Molar Disease

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Abstract
Optimal pathologic diagnosis of hydatidiform mole (HM) is achieved using histopathology, p57 immunohistochemistry, and selective molecular genotype testing and can be rendered with formalin-fixed paraffin embedded tissues from evacuated pregnancy losses. We present a case in which the absence of relatedness between conceptus and maternal tissues in an egg donor pregnancy loss could have been misinterpreted as a dispermic complete hydatidiform mole (CHM) and conclude that laboratory diagnosis of placental molar disease may require an accurate reproductive history.

Hydatidiform molar disease (HM) is an abnormal placental growth that can lead to invasive mole and choriocarcinoma in some cases. Three cytogenetic origins are now recognized.1,2 A diandric diploid conceptus results in a CHM. Partial hydatidiform mole typically results from a diandric triploid conceptus. Recently, a biparental origin of HM due to maternal mutations in NLRP7 and KHDC3L genes has been recognized as the genetic basis for some familial moles.3

When the morphology of HM is fully developed, histopathologic diagnosis is straightforward. In early gestation, however, there is considerable overlap in the histologic appearance of CHM, PM, and hydropic abortus (HA). Through the use of obstetrical ultrasound examination, pregnancy loss is now often detected early in gestation. The subsequent evacuation of these pregnancy losses can produce specimens that are challenging to classify as CHM, PM, or HA.4

The adjunctive use of p57 immunohistochemistry greatly enhances the recognition of CHM, but cannot distinguish PM from HA.5–7 Optimal diagnosis of HM can be obtained through molecular microsatellite genotyping analysis, which can be performed on formalin-fixed paraffin embedded (FFPE) specimens.2, 5, 6, 8–18 Morphologic diagnosis of HM based only on histopathologic appearance and p57 immunohistochemistry is both imprecise and inaccurate in a substantial proportion of cases, compared to a molecular genotyping “gold standard.” A diagnostic algorithm that incorporates histopathologic examination, p57 immunohistochemistry, and molecular genotyping has been developed and implemented in some laboratories.

In this case report we describe a potential pitfall in the laboratory diagnosis of HM and conclude that an accurate clinical and reproductive history may be essential in the interpretation of molecular genotyping findings in a placental molar disease laboratory diagnostic service.

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This article has been peer reviewed.
Résumé
Pour un diagnostic pathologique optimal de la môle hydatiforme (MH), on applique à des échantillons tissulaires tirés des résidus d’une fausse couche, enrobés de paraffine et fixés au formol, des techniques d’histopathologie, d’immunohistochimie de p57 et de génotypage moléculaire. Les auteurs présentent un cas de grossesse résultant d’un don d’ovocyte où l’absence de parenté entre le conceptus et les tissus maternels pourrait avoir été confondue avec une môle hydatiforme complète issue d’une fécondation dispermique, et concluent que le diagnostic en laboratoire d’une maladie trophoblastique peut nécessiter la collecte des antécédents génésiques détaillés.

Case Report
A 36-year-old woman underwent evacuation of her uterine contents following a clinical diagnosis of “pregnancy loss.” The surgical pathology requisition noted a clinical history of “multiple missed abortions.” The specimen consisted of 8 ml. of spongy and membranous fragments and blood clot. No vesicles were noted. The entire specimen was submitted for histologic examination.

Microscopic examination revealed numerous chorionic villi and gestational endometrium. Some of the villi were irregular in outline and hydropic, but villous cisternal formation and trophoblastic inclusions were absent. Embryonic tissue was present. The pathologist was suspicious that HM was present and referred the case to the Mount Sinai consultation service. Both the immunohistochemical (IHC) and genotyping methodologies used by the service have been described in detail previously. In brief, IHC analysis for p57 protein expression was performed on slides prepared from paraffin-embedded sections, using a concentrated mouse anti-human antibody p57kip2 clone 25B2 (Leica/Novocastra, UK). Molecular genotyping was performed with genomic DNA recovered from both conceptus and maternal tissues, using a Pinpoint Slide DNA Isolation System (Zymo Research, D3001), and was subsequently purified with a Zymo Spin I Column. Following measurement of the DNA quality and quantity with a NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE), molecular genotyping analysis of both conceptus and maternal DNA samples was performed with an Aneufast QF-PCR kit (Molgentix, Barcelona, Spain). This assay contains 19 highly polymorphic short tandem repeat (STR) markers on chromosomes 13, 18, 21, X, and Y and two nonpolymorphic markers for sex determination, combined in two multiplex reactions. In this analysis, the conceptus ploidy status of these chromosomes and parental contribution of each STR can be determined by comparing the conceptus allelic pattern to the maternal allelic pattern as per the best practice guideline of the Clinical Molecular Genetics Society.

Results
Microscopic review confirmed the initial histopathologic findings (Figure 1). The p57 IHC stain showed retained staining within the chorionic villi, indicating a maternal genomic component. Based on the histopathologic and IHC findings, a diagnosis of hydropic abortus was favoured. However, since there is significant morphologic overlap between HA and PM, microdissection and genotyping were carried out. QF-PCR of the conceptus tissue detected no numerical abnormalities of chromosomes 13, 18, or 21 and was consistent with a female chromosome constitution (two X chromosomes). Only presumed paternal alleles were present at 6 loci, while the remaining 14 loci were uninformative with regards to parental contribution. Fifteen of 21 STRs examined showed a biallelic pattern. Given the available clinical history and pathologic context, the molecular genotyping result was suggestive of a dispermic CHM. Because the microscopic and IHC findings did not favour this interpretation, additional clinical history was obtained and revealed that the gestation was an egg donor conceptus.

Discussion
The Mount Sinai Department of Pathology and Laboratory Medicine in Toronto established a Placental Molar Disease Lab Diagnostic (PMD) service in 2012. Cases with a preliminary diagnosis or suspicion of HM are assessed using histopathology, p57 IHC, and selective use of molecular genotyping. In the first 16 months, the PMD service analyzed 117 cases. About two-thirds of these cases were referred from 22 outside laboratories from five provinces. A final diagnosis of HM was made in 73 cases (37 CMs and 36 PMs). The remaining 44 cases (37%) were hydropic abortuses. Our experience suggests the major use of the PMD service is to increase the specificity of a diagnosis of
HM and avoid unnecessary clinical follow-up in a substantial proportion of cases with a preliminary diagnosis or suspicion of HM. Similar studies from other consultative services have also concluded that a substantial proportion of cases with an initial morphologic suspicion or diagnosis of HM are actually non-molar gestations.

Although the selective use of molecular genotyping does permit a more definitive diagnosis of HM, and particularly PM, implementation of this diagnostic advance has been limited. Some have proposed that human chorionic gonadotropin surveillance is a less expensive and more pragmatic management. This proposal, however, commits many women with a suspicion of HM to inappropriate management and subsequent delay in reproductive activity. Such inappropriate management may also lead to significant “downstream” costs incurred by multiple clinical and laboratory visits. A comprehensive analysis of the use of molecular genotyping in the context of Canadian laboratory diagnosis of HM is needed.

In this case, the cytogenetic analysis of micro-dissected FFPE tissue from a hydropic abortus and gestational endometrium demonstrated that no maternal genome was present in the conceptus. In the context of a histopathologic suspicion of HM, this finding could have led to a mistaken interpretation of CHM. The reproductive history of egg donor pregnancy was essential in confirming the histopathologic assessment of hydropic abortus. Most molecular genotyping studies of suspected molar disease have not mentioned this potential pitfall. This case highlights that the absence of relatedness between conceptus and maternal tissues—as would be observed in an egg donor and surrogate pregnancies—may be misinterpreted as dispermic CHM in cases of suspected placental molar disease. Accurate laboratory diagnosis of placental molar disease relies not only on histopathology, immunohistochemistry, and molecular genotyping as previously described, but might also require an accurate reproductive history.

In conclusion, selective molecular genotype testing of suspected HM cases does increase the specificity of laboratory diagnosis, but egg donor and surrogate gestation is a potential pitfall. Clinical/reproductive history may be essential in the correct interpretation of molecular genotyping findings in cases of suspected HM. Optimal laboratory diagnosis of suspected placental molar disease should incorporate histopathology, immunohistochemistry, molecular genotyping, and reproductive history.

Acknowledgements: Thanks to Ms. Raquel Salamanca for her assistance.

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We acknowledge the following publication, which was published after the completion of our manuscript: Buzna N, Hui P. Egg donor pregnancy: a potential pitfall in DNA genotyping diagnosis of hydatidiform moles. Int J Gynecol Pathol 2014;33:507–10.
The Pathology Tissue and Archive Committee: Its Role in Human Tissue Research

Samantha Crombie MCISc, Karen Mackie ALT, Madeleine Moussa MBBC FRCPC, Meg McLachlin MD FRCPC and Robert Hammond MD FRCPC

Abstract
Human tissue is an indispensable resource in medical research and its nature demands consideration of a number of perspectives and regulations. Pathology departments are charged with balancing these interests and responsibilities to ensure optimal patient care while facilitating research and discovery. In response to the increasing volume, complexity, and expense of requests for tissue for research, the Tissue and Archive Committee (TAC) was established to manage all applications and disbursements according to best practices and relevant legislation.

Since its inception in 1999, all TAC applications have been tracked in a database. In its first 15 years, the TAC reviewed 895 applications. Oncology (44%) was the highest user, London Health Sciences Centre (LHSC) (32%) the most common institutional affiliation and the National Cancer Institute of Canada (NCIC) (11%) the most frequent funding source. The most recent year saw 75 new studies involving over 2,500 tissue blocks. Estimates revealed an average cost recovery of $1,333 per study; less than 1.2% of respective grant budgets. Researchers report TAC operations to be consistently in the satisfactory to excellent range.

The TAC supports research involving human tissue by means of secure and efficient access and a standardized process for vetting requests. The TAC has come to play a central role in coordination, communication and education while applying best practices towards tissue handling and patient privacy. Tracked data provides valuable insight into a number of important factors for an academic department: optimization of processes, prediction of expenditures, usage patterns, funding sources and cost-recovery. The data further underscores the magnitude of research facilitated by the Pathology Department and the importance of human tissue stewardship.

Résumé
Les tissus humains sont une ressource indispensable pour la recherche médicale, et leur nature même exige de prendre en compte un certain nombre de points de vue et de règlements. Pour assurer les meilleurs soins possibles tout en facilitant la recherche et l’avancement de la science, les services de pathologie doivent trouver un équilibre entre les divers intérêts et responsabilités. Étant donné l’augmentation de la quantité, de la complexité et du coût des demandes en tissus pour la recherche, nous avons constitué un comité sur les tissus et les archives (Tissue and Archives Committee, TAC) pour gérer toutes les demandes et les dépenses selon les pratiques exemplaires et la législation en vigueur.
Depuis son lancement en 1999, le TAC a consigné toutes les demandes dans une base de données. Pendant
les 15 premières années, le TAC a examiné 895 demandes. Les départements d’oncologie sont les plus grands utilisateurs, avec 44 % des demandes; le London Health Sciences Centre (LHSC) est l’établissement d’affiliation le plus fréquent (32 %), et l’Institut national du cancer du Canada (INCC) est la source de financement la plus citée (11 %). Dans la dernière année, 75 nouvelles études ont porté sur plus de 2 500 blocs de tissus. Selon les estimations, le recouvrement des coûts s’élève en moyenne à 1333 $ par étude, c’est à dire moins de 1,2 % des budgets de subventions. D’année en année, les chercheurs attribuent aux activités du TAC une cote allant de « satisfaisant » à « excellent ».

Le TAC favorise la recherche portant sur les tissus humains grâce à un accès sécurisé et efficace et à un processus normalisé de validation des demandes. Il joue aujourd’hui un rôle essentiel de coordination, de communication et d’éducation, tout en veillant à l’application des pratiques exemplaires de manipulation des tissus et de confidentialité des dossiers. Le suivi des données fournit de précieux renseignements sur de nombreux facteurs importants pour un département universitaire : optimisation des processus, prévisions de dépenses, schémas d’utilisation, sources de financement et recouvrement des coûts. Les données révèlent aussi l’ampleur des recherches permises par le Département de pathologie, ainsi que l’importance d’une bonne gestion des tissus humains.

Introduction

Importance of Human Tissue

Medical research in the context of human biology is unsurpassed in relevance and validity. The variety of formats (fresh, frozen, fixed, cultured and other derivatives) and matching clinical data continually fuel discovery, underscore its value and increase demand.¹²

In Canada, the greatest implications for this rising demand are for those who safeguard and provide technical and diagnostic expertise on patients’ tissues in our public health care system; our Departments of Pathology.

Issues with Human Tissue Research

Human tissues are not only a valued research tool, but an exquisitely personal element of a patient’s medical record, particularly in the era of molecular genetics. Pathology Departments find themselves at a crossroads where the vested interests of patients, physicians, hospitals, researchers, ethics boards and legal experts are in play. Pathology Departments must balance these interests and responsibilities in an ethical and secure fashion that ensures optimal patient care while facilitating valid medical research.

There are many essential and sensitive considerations to bear in mind when human tissue is to be used in research, several of which borrow expertise from outside of clinical medicine. Chief among these are issues of ownership, consent and legislation.

a) Ownership

The matter of ownership of excised human tissue is a key and at times contentious issue that remains largely undefined in Canadian law. It is not surprising then that the concept of ownership may be controversial to individuals (patients, physicians, researchers, administrators, etc.) with goals that may not be congruent. Another important role for Pathology arises then in providing guidance based on sound legal, social and philosophical footings.

b) Informed Consent

Proper consent procedures in human tissue research honour the patient’s rights by providing ample opportunity to deny or withdraw their participation without duress or prejudice. This is partly based on a person’s right to autonomy over their body and the right to privacy over linkable health information.⁴⁷ Further informed consent is required whenever there is removal of tissue that is not part of prescribed and standard medical treatment, or if the tissue can be linked to the patient.⁴⁷

At issue is when a patient’s autonomy is not respected and samples are taken without fully informed consent. This can be a particularly sensitive issue for select customs and beliefs. Furthermore, the release of personal information in association with a diagnosis or genetic trait could lead to significant disadvantage.⁷⁸ Consent processes that are mindful of such implications are essential to avoid unnecessary duress and to maintain public trust and support.
Most, if not all, Canadian universities and health care institutions have the good fortune of ready access to expertise in research ethics where standards and guidance on matters of consent are available.

c) Legislation and Privacy Laws
In issues involving human tissue for diagnostics and research, a number of acts, policies and pieces of legislation provide considerable guidance and legal obligation to health care providers including Departments of Pathology. These are international, national and provincial in scope and they share a great deal in common in their philosophy towards ethical and secure processes in handling human tissue.

Privacy legislation in Canada has evolved under the influence of a number of important documents including the Declaration of Geneva, the Declaration of Helsinki, the Tri-Council Policy Statement, Public Hospitals Act and Privacy Act. These, in turn, have influenced further legislation that should also be in the vernacular of all Canadian Departments of Pathology: the Personal Information Protection Electronic Documents Act (PIPEDA), the Personal Health Information Protection Act (PHIPA) and analogous acts in other jurisdictions.

These important documents elaborate the minimum expectations in safeguarding patient data and tissue in Canada. Those that are legislation dictate the principles and regulations for tissue committees and researchers to follow by law.

Pathology Department’s Role
The need for education and clear communication is paramount, to encourage common understanding and respect across constituencies and to assist in the evolution of policy and best practices. As the “doctor’s doctor,” diagnostician and caretaker of this resource, the Pathologist has a pivotal role to play alongside the Pathologist Assistant, Medical Laboratory Technologist and other Pathology Department staff.

One of the largest and best categorized resources of human tissue is that which is archived within Pathology Departments, retained after diagnosis in accordance with statute, lab licensing, and accreditation requirements.

The tissue can exist in various states. Although it is most readily available as formalin fixed paraffin embedded blocks, it can also be fresh, frozen and fixed without embedding. More recent years have seen digital image data and molecular profiles added to this selection. Archival tissue, merged with treatment and outcomes data, is invaluable to educators and researchers by providing the opportunity to study rare and common entities gathered under such protocols.

Pathology Tissue and Archive Committee (TAC)
To manage the knowledge-base, processes, communications, requests and disbursements, Pathology Departments are vested with a large and varied set of responsibilities. In response to the increasing volume, complexity, and expense of requests for tissue for research, the TAC was established in 1999. Its goals were and remain to safeguard patient tissue and privacy, promote best legal and ethical practices and to seek cost recovery while supporting medical education and research.

Since its inception, all requests for human tissue for research have been recorded and tracked in a database. The TAC database facilitates administrative aspects of the Committee’s work and analyses of utilization to inform best practices and standard operating procedures.

Methods
Committee Structure and Function
The TAC is comprised of a Chair, Committee members (Pathologists) and a Tissue Research Liaison. The TAC meets periodically to review policy and to optimize the application and review processes in collaboration with the REB and LHSC Privacy and Risk Management.

A request for tissue for research requires the completion of an on-line application form. The Liaison maintains a database of all applications and forwards screened applications to committee members for review. The liaison also acts to link researchers to hospital laboratory staff and provides guidance on policies and legislation as needed. Researchers are provided with a cost-estimate prior to approval. Materials and work units are tracked to provide timely and accurate billing.

The role of the Chair is to provide final adjudication for applications approved by a Committee member. Additionally, the Chair
will communicate policies, regulations and any supplementary requirements to research personnel where necessary.

**Application Form**

In addition to baseline demographics and contact information, the nature of the request is ascertained including tissue type, sample parameters, ethics approval, letters of information, consent forms and funding sources (Table 1). Select sample types may impose restrictions. For example, at our institution, fresh surgical tissue must be examined by a Pathologist (in part to satisfy the Public Hospitals Act), a portion sampled, microscopically examined (for quality assurance and patient advocacy) and retained. It is LHSC policy to retain original (diagnostic) glass slides and paraffin blocks, and protect blocks from being fully exhausted. Surgical and post-mortem tissues (5 to 10% of requests) are governed by the same regulations with the additional provision that any tissue requested from coroner’s cases must have written permission from the Chief Coroner of Ontario.

**Database**

The TAC database was constructed to afford a variety of relevant analyses including the frequency of applications from an individual, department, institution and funding source. It provides an accounting of the number of research projects supported, the value of supporting grants and costs recovered. It also provides the opportunity to analyze and predict the number of patients involved, the technical and professional workload and the volume of tissue types and formats requested.

**Survey**

User satisfaction was assessed by a voluntary and anonymized survey, gaining feedback from researchers on 10 questions regarding various administrative and technical aspects of their experience with the TAC. The survey was sent to all researchers for whom active contact information was available (n=103).

**Results**

During the TAC’s first 15 years, 895 research applications were reviewed.

Awareness of the TAC and application process was by direct inquiry (65%), word of mouth (31%) and the Internet (4%).

Figures 1-5 describe the most frequent applicants, funding sources, tissue types, and tissue formats requested. The vast majority of researchers (Figure 1) were affiliated with local institutions, although the data underrepresents extramural collaborations. Departments most heavily engaged in the use of human tissue in research were Oncology, Pathology, Urology, Transplantation Medicine and Gastroenterology respectively, collectively accounting for 74% of all requests (Figure 2). Funding sources were highly variable (Figure 3) as were tissue type (Figure 4). More than half of all requests were for archival glass slide specimens (Figure 5).

**Table 1. Variables Tracked in the TAC Database**

- Principle investigator (PI)
- PI's department
- PI's institution
- Institutional review number
- Funding source
- Decision/date of first contact and approval
- Pathology collaborator (if present)
- Tissue source (autopsy or surgical)
- Study name
- Status of request (approved or pending)
- Tissue type (organ/system) requested
- Cost estimate and final cost invoiced
- Tissue format requested
- Application update or addendum
- cost per study (process and materials)
Figure 1. Departmental affiliation of researchers

Figure 2. Institutional affiliation of researchers

*Not available* was listed for 11% of the 895 cases, due mainly to the researcher not providing that information in their application for reasons such as, the funding had not been applied for at the time of application.

*The "other" category encompasses a wide range of both surgical and autopsy specimens.

*Most of the “not available” responses occurred in the early stages of the Pathology Tissue and Archive Committee, when the records were not kept in the format of more recent times.
Table 2. Annual Administrative and Technical Workload (for 2013).

<table>
<thead>
<tr>
<th>Workload Item</th>
<th>Associated Numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>New research studies accessioned</td>
<td>75</td>
</tr>
<tr>
<td>Total blocks cut</td>
<td>2511</td>
</tr>
<tr>
<td>Tissue blanks</td>
<td>6406 at 5μm, 582 at 6μm, and 26 at 10μm</td>
</tr>
<tr>
<td>H&amp;E’s</td>
<td>1317</td>
</tr>
<tr>
<td>Curls at 20μm</td>
<td>252</td>
</tr>
<tr>
<td>Immunohistochemical stains</td>
<td>197</td>
</tr>
<tr>
<td>Immunohistochemical controls</td>
<td>134</td>
</tr>
<tr>
<td>Paraffin-dipped slides</td>
<td>125</td>
</tr>
<tr>
<td>Special stains</td>
<td>116</td>
</tr>
<tr>
<td>Large block processing</td>
<td>17</td>
</tr>
<tr>
<td>Cores from blocks (2–6 mm)</td>
<td>15</td>
</tr>
<tr>
<td>DNA preparation</td>
<td>7</td>
</tr>
<tr>
<td>Processing/embedding</td>
<td>3</td>
</tr>
<tr>
<td>Sub-block preparation</td>
<td>4</td>
</tr>
<tr>
<td>Tissue micro-array preparation</td>
<td>3</td>
</tr>
<tr>
<td>Fresh tissue collection</td>
<td>15</td>
</tr>
<tr>
<td>Whole-slide scanning</td>
<td>22</td>
</tr>
</tbody>
</table>

Table 3. Summary of Survey Results (n = 31)

<table>
<thead>
<tr>
<th>Survey Question</th>
<th>Average Rating (Or Answer)</th>
</tr>
</thead>
<tbody>
<tr>
<td>How would you rate the Tissue Committee with respect to guiding the researcher</td>
<td>3.48</td>
</tr>
<tr>
<td>How would you rate the turnaround time of the Pathology Tissue and Archives</td>
<td>3.76</td>
</tr>
<tr>
<td>Committee?</td>
<td></td>
</tr>
<tr>
<td>How would you rate the ease of access and use of the application forms?</td>
<td>3.48</td>
</tr>
<tr>
<td>How would you rate the availability of the tissues requested?</td>
<td>3.41</td>
</tr>
<tr>
<td>How would you rate the quality of the tissues received?</td>
<td>4.10</td>
</tr>
<tr>
<td>How well were issues resolved?</td>
<td>3.76</td>
</tr>
<tr>
<td>How was your overall experience with the Pathology Tissue and Archives</td>
<td>3.55</td>
</tr>
<tr>
<td>Committee?</td>
<td></td>
</tr>
<tr>
<td>How would you describe the costs associated with your application (including technical charges)?</td>
<td>3.69</td>
</tr>
<tr>
<td>How did you become aware of the Pathology Tissue and Archives Committee?</td>
<td>65% pathology, 31% word of mouth, 4% Internet</td>
</tr>
<tr>
<td>What was the approximate total value of grant(s) that supported your research?</td>
<td>(average = $114,000)</td>
</tr>
</tbody>
</table>

*Survey answers were based on a rating scale where 1 = poor, 3 = satisfactory, and 5 = excellent.
Based on a subsample of 552 studies where comparable data was available, the average number of patient samples per study was 53.88. Over 15 years, and 895 studies, this number extrapolates to 48,200 patient samples in total, underscoring the magnitude of the research facilitated by the Department of Pathology and the TAC. The data in Table 2 describes typical activity levels from one recent year (2013).

Cost recovery from researchers for technical work was also analyzed. The total cost recovery over the last three years was $239,934 representing an average annual cost recovery of $79,978 or $1,333 per study.

Thirty-one of 103 researchers (30%) responded to the survey, rating various aspects of the TAC as satisfactory to excellent (Table 3). Eleven respondents provided information on the value of the grant(s) that this work supported. The total for these studies was $11,031,500 suggesting an average grant value in excess of $1 million, from a sample skewed by one grant worth $10 million. A more realistic measure of central tendency was derived by removing the highest and lowest valued grants from this sample, leading to a more conservative average of $114,000. Based on this revised sample, it is estimated that the Pathology Department through the TAC has supported research valued at $102 million ($11,031,500) over the period under study.

**Discussion**

**Cross-roads of Patients, Pathology, Research and Legislation**

Human tissue is invaluable to medical research and proper safeguarding of this material is vital due to its sensitive nature for the individual and its value to society. Pathology Departments, and TACs where they exist, help to uphold relevant legislation (Privacy Laws, Tri-Council Policy Statement, etc.) and ensure proper stewardship of a safe and secure tissue repository that serves the best interests of patients, colleagues, researchers and society. The TAC also ensures that tissue requests are vetted in a professional and standardized manner, promoting patient advocacy as well as a fair and efficient process for researchers. Survey results confirm that researchers felt adequately informed and supported in these regards.

Key considerations related to human tissue include handling, ownership, stewardship, consent and privacy legislation. One common thread, at times the weakest, is communication. With proper knowledge, education and communication on this topic, not only do we create a more efficient and uniform approach but we better engage an important ally in medical research - our patients. Medical research hinges on this partnership and our ability to engage patients in research is fostered by clear and candid communications.

**Ownership**

Patients may believe that excised tissue continues to belong to them. The tissue originated in their body, contains their unique molecular profile, etc. Indeed, at the moment prior to excision, it does wholly belong to them and the fact that it has been removed may seem utterly insignificant to the patient. However, while the patient retains rights with regard to the usage of their tissue, the concept of ‘ownership’ legally changes at the time of excision.3 Even clinicians, in transient ‘possession’ of a patient’s tissue or cells at the moment of removal, may mistakenly imagine that they have implied rights of access or determination. It is important for Pathology Department members to have an understanding of these rights and relationships and play a role in educating the public and our colleagues.4

The common concept of ‘ownership’, that is, the notion of being in possession of something and fully in control of its disposition, is poorly suited to describe anyone’s association with excised human tissue. Once excised, described, dissected, sampled, processed, preserved, etc., the tissue is no longer materially the same and its continued presence is due largely to such rendering and caretaking.5 While ‘ownership’ is not formally transferred in the usual sense, Pathology Departments take on a legislated responsibility of guardianship in a manner analogous to that for other elements of a patient’s medical record. In the public hospital setting in Canada, this includes ensuring its availability for diagnostic purposes for the patient and for valid and ethical medical research.6,4

In Canada, legal opinion has traditionally focussed its attention on matters of patient rights, consent and custodial obligations towards human tissue, rather than wading into the murky waters of ownership.4 However, a recent case garnering headlines in the Province of Ontario concluded that excised tissue was the personal property of the hospital in question.5 While the case may not be precedent-setting with respect to tissue ownership for a variety of reasons, it confirmed that issues of rights, responsibilities,
communications, possession and disposition are critical in the proper handling of human tissue and to create a transparent and non-adversarial atmosphere for patients, hospitals and health care team members.³

In the United States, the courts have observed similar logic through several landmark decisions such as Moore v. Regents of the University of California, 793 P.2d479 (Cal. 1990) where it has been judged that patients do not retain ‘ownership’ of tissue once excised.⁷ Greater patient oversight has been granted at times, one possible common denominator being the presence of retained in-vivo functionality (embryos, ova, sperm or organs for transplantation).³

The issue is likely to be raised again as the use of human tissue in research and therapeutics continues to increase, often generating patentable and profitable products. For the time being, legal experts in Canada and the US have stopped short of creating legislation that would routinely award definitive ownership to institutions or individuals.³

Database metrics and Cost-recovery

The TAC quickly became a new vantage point for tracking workload, expenditures, and reimbursements for research involving human tissues. The database afforded an accounting of utilization at the level of the individual, department, institution, tissue type, format, and funding source. This in turn permitted analyses and predictions of workload and resource utilization that were novel, objective and accurate.

Historically, Pathology Departments bore significant costs associated with tissue requests for research. Over the last three years, the TAC has recovered approximately $239,934 (or $1,333 per study). This level of cost-recovery appeared to be reasonable from the perspective of research budgets, given the average estimated grant value of $114,000. The average estimated cost recovery of $1,333 per study, represented less than 1.2% of supporting grants. This was corroborated by researchers’ favorable rating of the availability, quality and cost of tissue preparation.

In summary, our experience in managing human tissue resources for research has provided insight into a number of related activities in a large academic centre. The TAC has evolved to play a central role in coordination, communication and education. It has applied best practices and legislation towards tissue handling and patient privacy, earning favorable ratings from users. The TAC and its database have proven to be invaluable resources for vetting requests for tissue while tracking expenditures, workload and the scope of research activities facilitated by a Pathology Department and its parent institutions.

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14. Personal Information Protection and Electronic Documents Act, SC 2000, c 5
15. Personal Health Information Protection Act, 2004, SO 2004, c3, Schedule A

The authors wish to acknowledge current and past Chairs/Chiefs of Pathology at Western University and the London Health Sciences Centre: Dr. Subrata Chakrabarti, Dr. Bertha Garcia and Dr. Godfrey Heathcote. The authors also wish to thank all past and present members of the Tissue and Archives Committee, Research Ethics Board (Western University) Privacy and Risk Management Offices (LHSC) for their time, diligence and expertise.
ARID1A Loss of Expression in Complex Atypical Hyperplasia and Grade 1 Endometrioid Carcinoma of the Endometrium

Nouf Hijazi MD, Noorah Almadani MD, C. Blake Gilks BSc, MD, David G. Huntsman MD

Abstract

ARID1A is a tumor suppressor gene that encodes the BAF250a protein, implicated in various chromatin remodeling processes, and is lost, through mutation, in many gynecological cancers. ARID1A mutation typically results in loss of expression of BAF250a protein encoded by the ARID1A gene. We studied BAF250a expression in 68 cases, consisting of 18 cases of complex atypical hyperplasia (CAH), 13 cases of grade 1 endometrioid adenocarcinoma of the endometrium (Endo Ca), 31 cases of CAH coexisting with Endo Ca, 3 cases of proliferative endometrium and 3 cases of secretory endometrium in an attempt to determine the timing of ARID1A mutation/loss of BAF250a expression during endometrial carcinoma development. Immunostaining for BAF250a protein was performed on whole tissue sections and assessed based on the presence or absence of nuclear staining. In the CAH group, ARID1A staining was retained in 14 cases (78%) with focal loss in 3 cases (17%) and complete loss in 1 case (5%). Of the 13 Endo Ca cases, 6 retained ARID1A expression (46%), five showed focal loss (38%) and two exhibited complete loss of expression (15%). In the group of 31 cases with coexisting CAH and Endo Ca, 6 CAH (19%) and 5 Endo Ca (16%) demonstrated loss of expression, manifesting as either focal or complete loss. Focal loss of expression was manifested as areas within the CAH or Endo Ca showing loss of expression in all epithelial cells within the contiguous area of loss (so-called clonal pattern of loss). Loss of ARID1A expression occurs in endometrial premalignant lesions, i.e. complex atypical hyperplasia, albeit at a low frequency (10/49 cases, 20%) with more frequent loss seen in low-grade Endo Ca (23/44 cases, 52%, p=0.002). ARID1A expression can be lost as an early event in endometrial carcinogenesis, at the premalignant phase (CAH) or later, during progression to endometrial carcinoma.

(Keywords: ARID1A, atypical hyperplasia, endometrial carcinoma)
Résumé

ARID1A est un gène suppresseur de tumeurs qui code pour la protéine BAF250a, impliquée dans divers processus de remodelage de la chromatine; dans de nombreux cas de cancer gynécologique, on observe une perte d’expression de ce gène à cause d’une mutation. Habituellement, la mutation d’ARID1A entraîne une perte d’expression du gène et l’absence de la protéine BAF250a.

Nous avons étudié la présence de BAF250a dans 68 cas, dont 18 cas d’hyperplasie atypique complexe (CAH), 13 cas d’adénocarcinome endométrioidal de l’endomètre (Endo Ca) de grade 1, 31 cas d’hyperplasie atypique complexe coexistant avec un adénocarcinome endométrioidal, 3 cas d’endomètre prolifératif et 3 cas d’endomètre sécrétoire, afin de tenter de déterminer à quel moment se produisent la mutation d’ARID1A et la perte de production de BAF250a dans l’apparition d’un carcinome de l’endomètre.

Nous avons réalisé une immunocoloration de la protéine BAF250a sur des coupes de tissu complètes et évalué les résultats en fonction de la présence ou de l’absence d’une coloration nucléaire. La coloration d’ARID1A est conservée dans 14 des cas de CAH (78 %); on observe une perte focale dans 3 cas (17 %), et une perte complète dans un cas (5 %). ARID1A s’exprime dans 6 des 13 cas d’adénocarcinomes endométrioides (46 %); 5 cas montrent une perte d’expression focale (38 %) et 2 cas montrent une perte d’expression complète (15 %). Parmi les 31 cas de CAH coexistant avec Endo Ca, 6 CAH (19 %) et 16 Endo Ca (16 %) montrent une perte d’expression (focale ou complète).

La perte d’expression focale se manifeste sous la forme de régions de l’hyperplasie atypique complexe ou de l’adénocarcinome endométrioidal de l’endomètre montrant une perte d’expression dans toutes les cellules épithéliales de la zone voisine (schéma « clonal »). La perte d’expression du gène ARID1A se produit dans les lésions précancéreuses de l’endomètre, c’est-à-dire les cas d’hyperplasie atypique complexe, mais à une fréquence peu élevée (10 cas sur 49, ou 20 %); elle est plus élevée dans les cas d’adénocarcinome endométrioidal de bas grade (23 cas sur 44, ou 52 %, p=0,002). La perte d’expression du gène ARID1A peut se produire tôt pendant la carcinogenèse endométriale, au stade des lésions précancéreuses (CAH), ou plus tard, pendant l’évolution vers un carcinome de l’endomètre.

(mots-clés : ARID1A, hyperplasie atypique, carcinome de l’endomètre)
Introduction
Endometrioid adenocarcinoma (Endo Ca) of the endometrium accounts for 7% of all invasive cancers in women, making it the most common cancer arising in the female genital tract. It typically arises in a background of complex atypical hyperplasia (CAH). Excess estrogen plays a pivotal role in the development of CAH and its progression to low grade Endo Ca and its presence is integral to most risk factors implicated in endometrioid carcinogenesis, namely obesity, anovulatory disorders, estrogen secreting tumors, etc. CAH has been established as a premalignant lesion, and will progress to Endo Ca in a significant percentage of cases if left untreated. An interplay of somatic genetic mutations in a background of unopposed estrogen is instrumental in endometrioid carcinogenesis. PTEN, a tumor suppressor gene, is lost in 20% of CAH and 80% of Endo Ca. ARID1A, a 6857-bp tumor suppressor gene, encodes the BAF 250a protein (BRG-associated factor 250a) with a specific binding motif in the human SWI/SNF complex referred to as the AT-rich interactive domain 1a (ARID1A) also known as the SWI-like gene. The SWI/SNF complex is an ATP-dependent conglomerate of proteins that regulates cell growth and development by modulating transcriptional regulation of promoter regions.

ARID1A loss of expression is a recurrent event in low grade Endo Ca of the Endometrium, and we aimed to explore the level of its BAF250a expression in CAH alone and in cases where there is CAH with contiguous low grade Endo Ca, in an attempt to determine whether ARID1A/BAF250a loss of expression is an early event in endometrial carcinogenesis.

Materials and Methods
Hysterectomy cases from the archives of Vancouver General Hospital were electronically searched to identify suitable cases, and 5 micron sections were immunostained for BAF250, using a semiautomated Ventana Discovery XT instrument (Ventana Medical Systems, Tucson, AZ, USA) and the Ventana ChromoMap DAB detection kit. Heat antigen retrieval was standard CC1 with a 2 h primary incubation. Anti-BAF250a mouse monoclonal antibody, clone 3H2 (Abgent, San Diego, CA, USA) was applied at 1:50 followed by a 16 min secondary incubation of prediluted UltraMap anti-mouse HRP (Ventana). Normal stromal cells as well as non-neoplastic cells, including endothelial cells, fibroblasts and lymphocytes, normally show BAF250a nuclear staining and were used as positive internal controls. Positive nuclear staining in CAH or Endo Ca, regardless of intensity, was considered positive. Complete absence of nuclear staining within the cells of CAH or Endo Ca (together with positive staining of normal cells) was considered negative. Partial loss of expression refers to a discrete focus within the lesion with complete absence of nuclear staining surrounded by an internal positive control and positively staining lesional cells. The terms partial and focal loss of nuclear staining are used interchangeably to describe a clonal pattern of loss. Absence of immunostaining correlates with ARID1A mutational status, albeit not perfectly.

Statistical comparison between ARID1A loss in CAH and Endo Ca was done using the Fisher’s exact test (two tailed).

Results
We studied a total of 68 cases, consisting of 18 cases of CAH, 13 cases of grade 1 Endo Ca, 31 cases of CAH coexisting with Endo Ca, 3 cases of proliferative endometrium and 3 cases of secretory endometrium. In the CAH group, ARID1A staining was retained in 14 cases (78%) (Figure 1) with focal loss in 3 cases (17%) (Figure 2) and complete loss in 1 case (5%). Of the 13 Endo Ca cases, 6 retained ARID1A expression (46%), five showed focal loss (38%) (Figure 3) and two exhibited complete loss of expression (15%) (Figure 4). In the group of cases with coexisting CAH and Endo Ca, loss of expression was seen in CAH in 6 cases (19%) and in the Endo Ca in 5 cases (16%), manifesting as focal or complete loss. Immunostaining results are summarized in Table 1. Focal loss of expression consisted of discrete areas within the CAH or Endo Ca showing loss of expression in all epithelial cells within a contiguous region, so-called clonal pattern of loss, a finding previously reported. Combining results from all cases, 10 of 49 cases of CAH showed ARID1A loss (20%), compared to 23 of 44 Endo Ca cases (52%) (p=0.002). The staining pattern of CAH and Endo Ca showed a 65% concordance rate (20/31) in cases where both components were present. Of those, 16 cases showed no loss, 3 showed clonal loss and 1 showed complete loss of staining in both components. All 6 cases of proliferative and secretory endometrium showed no loss of ARID1A staining.
TABLE 1. BAF250a Expression

<table>
<thead>
<tr>
<th>Cases</th>
<th>No loss</th>
<th>Focal loss</th>
<th>Complete loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAH (n=18)</td>
<td>14 (78%)</td>
<td>3 (17%)</td>
<td>1 (5%)</td>
</tr>
<tr>
<td>CAH component in group with co-existing Endo Ca (n=31)</td>
<td>25 (81%)</td>
<td>5 (16%)</td>
<td>1 (3%)</td>
</tr>
<tr>
<td>Endo Ca component in group with co-existing CAH (n=31)</td>
<td>15 (48%)</td>
<td>8 (26%)</td>
<td>8 (26%)</td>
</tr>
<tr>
<td>Endo Ca only (n=13)</td>
<td>6 (46%)</td>
<td>5 (38%)</td>
<td>2 (15%)</td>
</tr>
</tbody>
</table>

CAH: complex atypical hyperplasia, Endo Ca: Endometrial Carcinoma
Discussion

ARID1A, implicated in chromatin-remodeling processes,13-16 is found on the short arm of chromosome 1 (1p36.11).20 ARID1A mutations appear to be important in the development of gynecologic cancers, including ovarian endometriosis-related tumors of clear cell and endometrioid subtypes13 and cervical adenocarcinoma.21 Of ovarian carcinomas, 46% of the clear cell subtype and 30% of the endometrioid subtype showed evidence of ARID1A mutation.13 Mutations were mostly of the truncating type, evenly dispersed along the coding sequence, and strongly correlated with loss of BAF250a expression on immunohistochemical analysis. The lack of absolute concordance between mutational findings and protein expression was proposed to be secondary to the presence of a non-functional truncated protein binding to the antibody, leading to false positive protein expression by immunohistochemistry in some cases.13 A similar percentage of ARID1A loss of expression (57%) was simultaneously reported in clear cell carcinoma of the ovary.22 PIK3CA mutations have been found to coexist with foci of ARID1A loss of expression in clear cell ovarian carcinomas23,24 and ARID1A loss has been identified as an early event in oncogenesis in clear cell carcinoma.13,23,24

Two independent large scale analyses of various tumor types from different anatomic sites also demonstrated that ARID1A mutations are relatively frequent (26%-40%)25,17 in low grade endometrioid carcinoma of the endometrium, but are almost never seen in high grade serous carcinomas of the ovary and endometrium.17 A minority of typical ovarian endometriosis, in the absence of carcinoma, has also shown loss of ARID1A expression.26 ARID1A was recently found to be lost in borderline (atypical proliferative) seromucinous tumors (also known as borderline mucinous tumors of endocervical subtype) of the ovary, further implicating this gene and pathway in endometriosis-associated and hormonally driven neoplasia of both eutopic and ectopic endometrial epithelium.19

The relationship between ARID1A mutational status and clinical outcome in both ovarian clear cell and endometrioid subtypes were examined, yielding inconsistent results, with some studies demonstrating a less favorable outcome27,28 while others showed no relationship to overall survival.29,18 The prognostic significance of ARID1A mutation remains unclear at this time.

Our findings indicate that while loss of ARID1A expression can occur in endometrial preneoplastic lesions, i.e. CAH, it is at a relatively low frequency (22%) with more frequent loss seen in fully developed carcinoma (53%), a difference that is statistically significant (p=0.002). Similar results have recently been reported in a study showing loss of ARID1A staining in 16% of hyperplasia with atypia and up to 28% of endometrioid adenocarcinoma.20 Notably, we also observed cases with ARID1A loss in the Endo Ca, while the co-existent CAH lacked loss. Thus, unlike PTEN loss, which is consistently an early event in endometrial carcinogenesis,3,31 ARID1A can be lost as either an early event, where it is seen at the premalignant phase (CAH), or later during progression to carcinoma.

References:


Erratum
In the Canadian Journal of Pathology (7-2, P.13) the article entitled “CAP-ACP Workload Model - 2014 Update” did not include the names of two contributors: Dr. Carol Cheung, Department of Pathology, University Health Network, Toronto and Dr. Michael Allard, Department of Pathology and Laboratory Medicine, University of British Columbia, BC. We regret the error and apologize to the co-authors.
Managing and Leading for Science Professionals

This slim volume, subtitled *What I Wish I’d Known while Moving Up the Management Ladder*, is written by a physician scientist who moved into business administration and became a CEO (the name of the company is not revealed). The main targets of the book are physicians and scientists who are taking up management roles in medical and business organizations; the author gives much practical advice, based on his experience, as well as a simple introduction to many of the concepts of human resource management. Some chapters are more relevant to the industrial setting, while others are generally applicable to university and hospital administration. I particularly found the chapters on delegation and how to reach an appropriate and prompt decision helpful, and there is excellent advice on how to be an effective mentor. Physicians who are promoted to leadership positions often struggle with the best way to deal with former peers. This problem is discussed, as is the management of discontent among such colleagues.

I have always been somewhat sceptical of the value of general leadership courses, but, for a physician to be a successful medical administrator and leader, he or she has to learn from the wisdom of others. Some of this can be gained from the insights of novelists (Anthony Trollope’s *The Prime Minister* is an excellent example) and biographers, but books on leadership can also be a valuable resource. Some of the writing in this book is far from concise—I found myself reading some sentences two or three times before the point became clear—but each chapter contained valuable nuggets of information that I will certainly find opportunities to employ. I would recommend this book to aspiring medical administrators.

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Professor and Head, Department of Pathology,
Dalhousie University
District Chief, Pathology and Laboratory Medicine,
Capital District Health Authority
Halifax, Nova Scotia

Bertrand C. Liang
158 pages
List price: $55.81
Eighty-two percent of patients included in the study had stage M1c disease and 73% had received two or more prior therapies including ipilimumab for metastatic disease.\(^1,2\)

For more information on KEYTRUDA, please contact your Merck Oncology representative.

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**KEYTRUDA® DEMONSTRATED EFFICACY**

In an uncontrolled, open-label study of patients with unresectable or metastatic melanoma, previously treated with ipilimumab, KEYTRUDA demonstrated:\(^1,2\)‡§

### Overall response rate (n=89)

**24%**

(Primary endpoint; 95% CI: 15, 34)\(^1\)

1 complete response and 20 partial responses

### Response duration in patients who responded to therapy (n=21)

**86%**

(Secondary endpoint)\(^1\)

Of responses were ongoing at the time of analysis and the median duration of response was not reached (median follow-up of 8 months; minimum of 6 months)

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**KEYTRUDA** (pembrolizumab) is indicated for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab therapy and, if BRAF V600 mutation-positive, following a BRAF or MEK inhibitor. An improvement in survival or disease-related symptoms has not yet been established. KEYTRUDA has been issued marketing authorization with conditions, pending the results of studies to verify its clinical benefit. Patients should be advised of the nature of the authorization.

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**For more information on KEYTRUDA, please contact your Merck Oncology representative.**

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\(\text{CI=confidence interval. PD-1=programmed cell death receptor-1.} \)

\(\text{† Comparative clinical significance unknown.} \)

\(\text{‡ The efficacy of KEYTRUDA for this indication was investigated in a phase 1, multicentre, uncontrolled, open-label, dose-comparative cohort of Trial 1. To be eligible, patients needed to be refractory to ipilimumab (defined as confirmed progression following at least 2 doses of ipilimumab and within 6 months of the last dose of ipilimumab). Patients were randomized to receive 2 mg/kg (n=89) or 10 mg/kg (n=84) of KEYTRUDA every 3 weeks until unacceptable toxicity or disease progression that was symptomatic, was rapidly progressive, required urgent intervention, occurred with a decline in performance status, or was confirmed at 4 to 6 weeks with repeat imaging. Note that the 10 mg/kg dosing is not a recommended dose. Assessment of tumour status was performed every 12 weeks.} \)

\(\text{§ Based on patients with a confirmed response by independent review, including independent radiology and oncology reviews, using confirmed responses and Response Evaluation Criteria in Solid Tumors (RECIST 1.1).} \)

\(\text{¶ See the product monograph for complete dosing, dosing adjustments and administration recommendations.} \)

**References:**

Eighty-two percent of patients included in the study had stage M1c disease and 73% had received two or more prior therapies including ipilimumab for metastatic disease.1,2

For more information on KEYTRUDA, please contact your Merck Oncology representative.

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### Dosing

<table>
<thead>
<tr>
<th>2 mg/kg administered as an intravenous infusion</th>
<th>over 30 min</th>
<th>every 3 weeks</th>
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... until disease progression or unacceptable toxicity.†

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**Clinical use:**
Safety and efficacy of KEYTRUDA have not yet been established in children <18 years of age.

**Relevant warnings and precautions:**
- Immune-mediated adverse reactions including:
  - Pneumonitis
  - Colitis
  - Hepatitis
  - Nephritis
  - Endocrinopathies including hypophysitis, type 1 diabetes and thyroid disorders
  - Other immune-mediated adverse events including uveitis, myositis and severe skin reactions (reported in less than 1% of patients)
- Infusion-related reactions
- Not recommended in pregnant women
- In nursing women, a decision should be made whether to discontinue breast-feeding or KEYTRUDA taking into account the benefit of breast-feeding for the child and the benefit of KEYTRUDA therapy for the woman
- Has not been studied in patients with moderate or severe hepatic impairment
- Has not been studied in patients with severe renal impairment
- Monitor for thyroid and liver function during treatment

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The product monograph is also available by calling us at 1-800-567-2594 or by email at medinfocanada@merck.com.