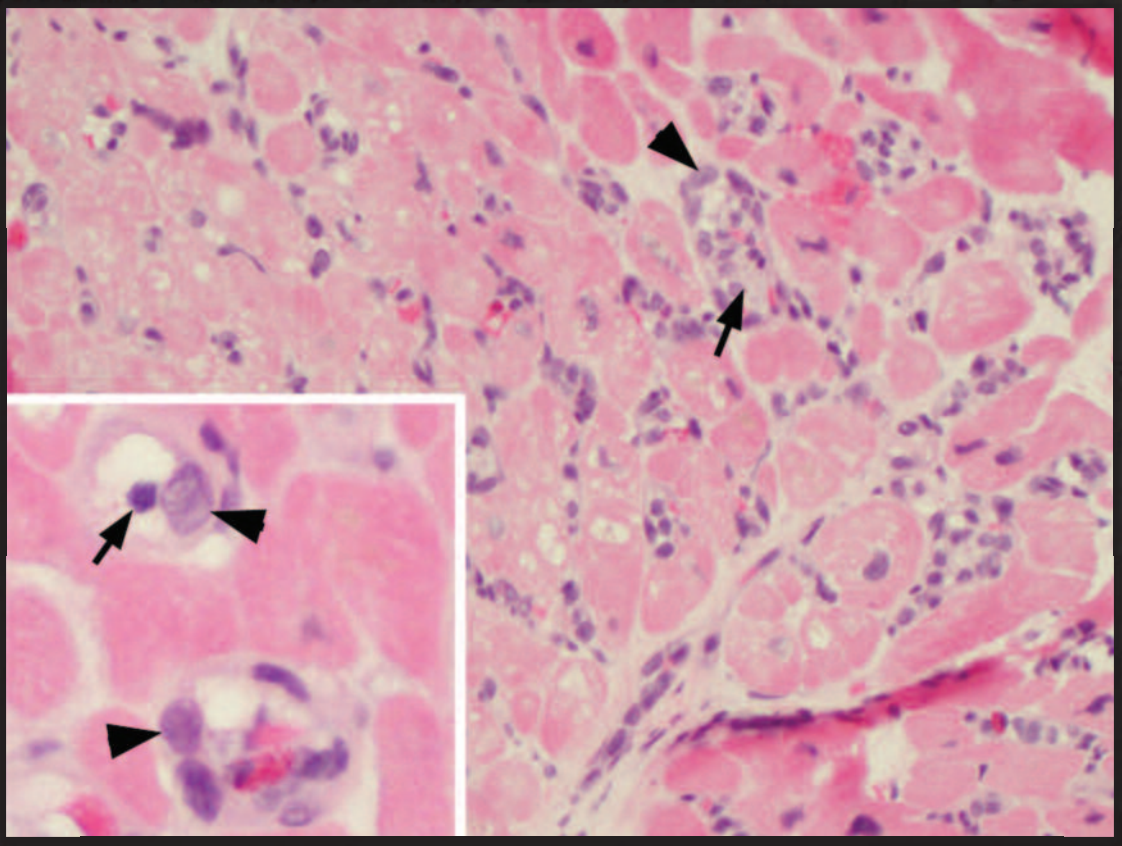
Canadian Journal of
Pathology
Volume 6, Issue 1 ▸ Spring 2014
Official Publication of the Canadian Association of Pathologists



WT-1 and ER Expression in Serous Carcinoma
Forensic Science in Canada

Publications Agreement Number 40025049 ▸ ISSN 1918-915X

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The deadline for receipt of the completed application including references is July 25, 2014. Interested candidates should submit their curriculum vitae, and arrange for three letters of recommendation to be mailed or emailed directly to:

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Canadian Journal of Pathology
Official Publication of the Canadian Association of Pathologists

Canadian Journal of Pathology • Volume 6, Issue 1, 2014

Canadian Journal of Pathology is published four timeso annually by Andrew John Publishing Inc., with offices at 115 King Street West, Dundas, ON, Canada L9H 1V1.

Publications Agreement Number 40025049 • ISSN 1918-915X

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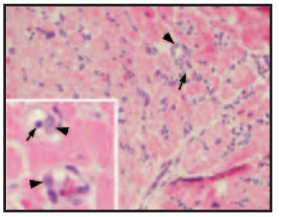

The cover image shows antibody mediated rejection of cardiac allografts: endothelial swelling *(arrowheads)* and macrophages/ inflammatory cells within capillaries *(arrows)*.

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The Patient's Record

The announcement in early February from the US Department of Health and Human Services (HHS) that patients will have direct access to completed laboratory test reports upon request set me thinking again about the many issues surrounding the communication of laboratory results and reports to physicians and patients.

For over a hundred years, at least since the time of Virchow, pathologists and, more latterly, laboratory physicians and scientists, have served as consultants to other physicians. By and large we are consulted by our colleagues but not by patients; in turn, we provide results and reports to referring physicians but not to the patients themselves. Historically, it has always been the responsibility of the referring physician to interpret the report for the patient and to decide how much detail needs to be communicated. If a patient wishes to speak directly with a pathologist, we would often obtain the agreement of the clinician first.

This approach, which has been described as "tossing the result over the wall," has served patients and physicians well, although we all recognize that there are potential problems with it. As the amount of teaching of pathology in undergraduate medical school has diminished, clinical practitioners are less comfortable in interpreting surgical pathology reports. Clinical laboratory medicine is hardly taught at all, and this has broad implications, not least unnecessary utilization of laboratory testing. Given that there is often little, or misleading, clinical information on requisitions, it is sometimes surprising that our reports make any sense at all.

Although we inhabit a changing world where "patient voice" and "patient choice" are catchphrases, it remains important for clinicians to give their patients clear advice, while avoiding any hint of pressure to accept the recommendations. Sharing of accurate information forms the basis of this joint decision making. Since many of these decisions will depend on laboratory reports, pathologists and laboratory physicians need to ensure that the tests requested are appropriate and the information provided is clear, easy to understand, and accompanied by a clinical interpretation where appropriate.

A key element in the movement away from the traditional model of doctor-patient communication is the personal health record (PHR). This may be defined as a partial or complete electronic record of relevant health information accrued during a lifetime. The health information, no matter what its source, is stored in one location and is under the control of the patient or an individual, usually a close family member, designated by the patient. Advocates of PHRs believe that better-informed patients will seek fewer costly consultations and take more responsibility for the management of their own health. Better integration of care will lead to less duplication of laboratory tests. Where PHRs have been introduced, they seem popular with patients, especially those with chronic diseases, and the ability to view laboratory reports seems to be particularly valued. Pathologists and laboratory physicians have been wary of PHRs, fearful that patients will be confused by the technical detail contained in reports and will inundate the laboratory with requests for clarification. For this reason, there has generally been a disclaimer with test reports that clarification should be sought from the referring physician, often the family doctor, and not the laboratory. Some reports are clearly "sensitive," for example, a diagnosis of cancer or a positive HIV test, and PHR systems have the potential to delay the release of results to the patient until the physician has had the opportunity to talk to him or her. This delay can, at least to some extent, be customized according to the type of test. In a demonstration project undertaken in Nova Scotia, the existence of guidelines for the release of results went a long way to alleviating the concerns of laboratory practitioners.

The new HHS rule, which can be accessed at http://www.federalregister.gov, takes the concept of the PHR a step further. Under this rule, laboratories subject to the Clinical Laboratory Improvement Amendments of 1988 (CLIA) regulations will be required to provide patients (or their designated personal representatives) with access to laboratory reports within 30 days of a request. Only reports that are finalized and ready for release need be provided, and laboratories are not required to interpret test results for patients. In a typically transparent way, the HHS document presents the rationale for this new rule and addresses the concerns raised by stakeholders. Although patients in Canada do have the right to see their own results, few seem to ask for them directly from the laboratory. It will be interesting to see if and when a similar option of direct access to laboratory results becomes the norm here.

J. Godfrey Heathcote, MA, MB BChir, PhD, FRCPC
Editor-in-Chief

Le dossier patient

Lorsque le Department of Health and Human Services aux États-Unis a annoncé au début de février que les patients pourront avoir accès eux-mêmes aux résultats de leurs analyses de laboratoire sur demande, il m'est revenu en tête les nombreux problèmes liés à la communication des rapports et des résultats d'analyse de laboratoire aux médecins et aux patients.

Voilà plus d'un siècle, depuis l'époque de Rudolf Virchow certainement, que le pathologiste, et le médecin et le scientifique spécialisés en biologie médicale par la suite, exercent à titre de consultants. Ce sont avant tout nos collègues qui nous consultent, pas les patients; c'est ainsi que nous transmettons résultats et rapports à ces médecins qui nous ont adressé des patients, mais pas aux patients. L'usage veut qu'il revienne au médecin traitant d'interpréter le rapport et de déterminer l'information qu'il communiquera à son patient. Dans l'éventualité où un patient souhaiterait parler au pathologiste, nous le ferions seulement avec l'accord de son médecin.

Les médecins et les patients n'ont rien à redire en général à propos de cette compartimentation des tâches, quoique nous sachions tous qu'elle peut être problématique. La pathologie n'est plus enseignée au premier cycle des études de médecine comme elle l'était auparavant et les cliniciens ont parfois du mal à interpréter les rapports de pathologie chirurgicale. Que dire de la biologie médicale, pratiquement absente de l'éducation médicale de premier cycle? La situation a des ramifications multiples, la prescription d'analyses de laboratoire inutiles n'étant pas la moindre. De surcroît, comme il y arrive souvent que la demande renferme peu de renseignements cliniques, ou des renseignements non pertinents, l'on s'étonne parfois de constater que le rapport se tienne un tant soit peu.

Dans la société d'aujourd'hui qui veut que le patient puisse non seulement se faire entendre mais également exercer des choix, il demeure important que le clinicien conseille son patient de manière compréhensible et précise sans pour autant l'influencer indûment dans sa décision. L'échange d'information exacte constitue l'assise de cette prise de décisions en commun. Étant donné que les analyses de laboratoire entrent en jeu dans nombre de ces décisions, le pathologiste comme le médecin spécialiste en biologie médicale doivent veiller à ce que les analyses demandées soient indiquées dans le cas en question et que l'information transmise soit claire, compréhensible et étayée d'une interprétation clinique le cas échéant.

Le dossier de santé personnel est certes un moyen de s'écarter du modèle classique de communication entre le médecin et le patient. Ce dossier électronique en tout ou en partie renferme tous les renseignements pertinents sur la santé de la personne tout au long de sa vie. L'information sur la santé, quelle que soit sa source, est stockée dans un fichier qui demeure la propriété du patient ou d'une personne désignée par le patient, un proche habituellement. Les partisans du dossier de santé personnel sont convaincus que le patient mieux informé aura de moins en moins recours à des consultations onéreuses et prendra en charge sa propre santé. En outre, le dossier favorisera l'intégration des soins qui, elle, aura pour effet de réduire le dédoublement d'analyses de laboratoire. Là où il est emplanté, il est bien vu des patients apparemment, surtout de ceux qui sont aux prises avec une maladie chronique, et les patients semblent trouver particulièrement utile de pouvoir consulter les rapports de laboratoire. Les pathologistes et les médecins spécialistes en biologie médicale sont quelque peu méfiants à l'égard du dossier de santé personnel, inquiets qu'ils sont à l'idée que les patients ne s'y retrouvent pas dans le jargon technique du rapport et qu'ils bombardent le laboratoire de questions pour obtenir des précisions. Voilà pourquoi le rapport contient généralement un avertissement indiquant que le patient doit s'adresser au médecin traitant, le médecin de famille dans la plupart des cas, pour clarifier tout aspect du rapport. Il est vrai que certains rapports sont de nature plus délicate que d'autres, notamment celui qui renferme un diagnostic de cancer ou un résultat positif au test de dépistage du VIH, mais les systèmes de dossiers de santé personnels ont prévu un mécanisme qui retarde la divulgation des résultats au patient jusqu'à ce que le médecin ait eu la possibilité d'en parler d'abord lui-même au patient. Ce report dans la divulgation des résultats peut être adapté selon la nature de l'information. Ainsi, un projet de démonstration en Nouvelle-Écosse a mis en place des lignes directrices sur la divulgation des résultats à la grande satisfaction des praticiens en laboratoire.

La nouvelle règle américaine, qui paraît à http://www.federalregister.gov, va plus loin que le dossier de santé personnel. En effet, elle stipule que les laboratoires assujettis aux Clinical Laboratory Improvement Amendments (CLIA) de 1988 seront tenus d'offrir l'accès aux rapports de

laboratoire dans les 30 jours de la demande du patient ou de son représentant désigné. Seul le rapport terminé, prêt à être consulté, doit être offert et le laboratoire n'est pas tenu d'interpréter les résultats. Par souci de transparence, le Department of Health and Human Services justifie sa nouvelle règle et prend en compte les préoccupations soulevées par les intervenants. Bien que, au Canada, les patients aient le droit de prendre connaissance des résultats de leurs analyses, ils sont peu nombreux à s'adresser au laboratoire pour les connaître. Reste à voir si cette possibilité d'accès direct aux résultats de laboratoire deviendra chose courante ici.

J. Godfrey Heathcote, MA, MB BChir, Ph. D., FRCPC
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Comparison of WT-1 and ER Expression in Serous Carcinoma Cells from Ascitic Fluid/ Peritoneal Lavage Cytological Preparations and Matched Surgical Resection Specimens

Ananta Gurung, MD, C. Blake Gilks, MD

ABSTRACT

Purpose: Immunostaining for Wilms' tumour-1 (WT-1) and estrogen receptor (ER) protein expression can be used as an aid in the diagnosis of serous carcinomas of the ovary, fallopian tube, and peritoneum. With the increasing use of neoadjuvant chemotherapy for high-grade serous carcinoma, accurate diagnosis based on cytological specimens is paramount. The aim of this study was to compare WT-1 and ER expression in CytoLyt-fixed cytological preparations and the corresponding formalin-fixed surgical specimen.

Method: Serous carcinoma cases with positive cytology and surgical specimens, taken at the same operation, were identified, and sections from surgical resection and cell block cytological preparations were immunostained for WT-1 and ER.

Results: WT-1 and ER expression was absent in the cytological specimen in 4/25 (16%) and 7/24 (29%) of cases, respectively, in which the corresponding surgical specimens showed positive staining. The differences in sensitivity in staining, comparing the cytological and surgical specimens, were significant for ER ($p = .023$) but not WT-1 ($p = .35$). Staining was unaffected by neoadjuvant chemotherapy (6 cases).

Conclusions: While WT-1 and ER will stain CytoLyt-fixed cells of serous carcinomas in most cases, occasional false-negative results occur if the same immunostaining protocol is used for both specimen types.

RÉSUMÉ

But : La coloration immunohistochimique afin de détecter une tumeur de Wilms et l'expression de récepteurs d'œstrogènes (protéines) est une épreuve utile dans le diagnostic d'un carcinome séreux de l'ovaire, de la trompe de Fallope ou du péritoine. La chimiothérapie néoadjuvante étant de plus en plus répandue dans le traitement du carcinome séreux peu différencié, l'exactitude du diagnostic établi d'après l'examen cytologique s'avère primordiale. L'étude a pour but de comparer la préparation cytologique dans CytoLyt et le prélèvement chirurgical dans le formaldéhyde dans la détection d'une tumeur de Wilms et de l'expression de récepteurs d'œstrogènes.

Méthode : Nous avons relevé des cas de carcinome séreux pour lesquels les prélèvements cytologique et chirurgical, effectués lors de la même opération, sont positifs. Dans chaque cas,

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This article has been peer reviewed.
Competing interests: None declared

nous avons analysé un fragment de la résection chirurgicale et un bloc de la préparation cytologique par l'épreuve d'immunohistochimie.

Résultats : Nous n'avons pas détecté de tumeur de Wilms dans le prélèvement cytologique de 4 cas sur 25 (16 %) ni l'expression de récepteurs d'œstrogènes de 7 cas sur 24 (29 %) pour lesquels le prélèvement chirurgical se révèle positif à la coloration immunohistochimique. La différence de sensibilité de la coloration, dans la comparaison entre le prélèvement cytologique et le prélèvement chirurgical, franchit le seuil de la signification statistique en ce qui concerne les récepteurs d'œstrogènes (p = 0,023), mais pas en ce qui concerne la tumeur de Wilms (p = 0,35). La chimiothérapie néoadjuvante (6 cas) n'altère en rien la coloration.

Conclusion : Bien que l'épreuve immunohistochimique de détection de la tumeur de Wilms et de l'expression de récepteurs d'œstrogènes agisse sur les cellules d'un carcinome séreux préparées dans CytoLyt dans la plupart des cas, le résultat faux négatif se produit à l'occasion lorsque le même protocole de coloration immunohistochimique est utilisé pour les deux types de prélèvements.

The incidence of ovarian cancer in Canada is approximately 11 cases per 100,000, with Canadian Cancer Society statistics estimating 2,600 new cases and 1,750 deaths in 2012. Though ovarian cancer is the ninth most common malignancy in women, it ranks fifth in mortality, responsible for 4.8% of cancer deaths in women.[1] The histological and molecular classification of ovarian carcinoma, with subtype-specific therapy, has evolved substantially since the most recent 2003 World Health Organization "Blue Book."[2] High-grade serous carcinoma, low-grade serous carcinomas, and other subtypes are now accepted as being distinct tumour types, clinically and pathologically, with different approaches to management. The ovarian carcinoma subtypes also show subtype-specific molecular abnormalities.[3] Low-grade and high-grade serous carcinomas account for approximately 2% and 70% of all cases of ovarian carcinomas, respectively. Low-grade serous carcinomas are rare and only recently have been accepted as a separate diagnostic entity; currently there is no universally accepted treatment approach for this tumour subtype. As this subtype responds relatively poorly to treatment with traditional high-grade serous carcinoma chemotherapeutic regimens,[4] specific targeted therapies have been proposed; however, data from clinical trials are pending.

High-grade serous carcinomas of the ovary, fallopian tube, or peritoneum usually present with extensive intraperitoneal spread and ascites.[5] A large randomized clinical trial comparing surgery followed by chemotherapy to neoadjuvant chemotherapy demonstrated that the latter approach, of treating with chemotherapy first, was associated with identical patient survival and less morbidity.[6] As such, neoadjuvant treatment for high-grade serous carcinoma is becoming increasingly common. Before starting neoadjuvant therapy, a diagnosis must first be established, and this is typically based on cytological examination of the ascitic fluid or a core biopsy of omentum.[6]

Other ovarian tumours, such as clear cell carcinoma, which is typically resistant to platinum-based chemotherapy, and metastases from the gastrointestinal tract, lung, or other non-gynecological sites, can also present with ascites and must be excluded prior to initiating therapy. Immunohistochemistry (IHC) can be invaluable in establishing the primary site in a case of metastatic adenocarcinoma. Wilms' tumour-1 (WT-1) protein expression is used as a diagnostic marker for serous tumours as it is typically not expressed in other ovarian carcinoma subtypes, gastrointestinal carcinomas, lung carcinomas, or non-micropapillary breast carcinomas.[7] As 25% of micropapillary breast carcinomas may be positive for WT-1 (albeit weak to moderate intensity in only 1–10% of nuclei), immunohistochemical expression must be interpreted cautiously, particularly if there is a history of breast carcinoma.[8] Estrogen receptor (ER) protein expression has also been shown to be positive in most serous ovarian carcinomas and is useful to distinguish them from clear cell

carcinomas of ovary, gastrointestinal carcinomas, lung carcinomas, and peritoneal mesotheliomas.[9] While WT-1 and ER positivity are useful in supporting a diagnosis of serous carcinoma, they do not allow distinction between high-grade and low-grade serous carcinoma or serous borderline tumour, and rare metastatic breast carcinomas may also show WT-1 and ER positivity, so consideration of the clinical and cytological findings must also be taken into account in reaching a correct diagnosis.

Almost all studies on the use of IHC to determine tumour cell type have been done on formalin-fixed, paraffin-embedded tissue. Ascitic fluid is often fixed in alcohol-based fixatives, such as CytoLyt, as part of liquid-based cytological preparation protocols. Although there is a need to make accurate diagnoses based on ascitic fluid in patients for whom neoadjuvant therapy is being considered, there is a lack of validation of immunomarkers in alcohol-fixed material. We therefore compared immunostaining results in CytoLyt-fixed cytological preparations with matched formalin-fixed, paraffin embedded surgical specimens for two positive immunomarkers of high-grade serous carcinoma, WT-1 and ER.

Materials and Methods

The archives of the Department of Pathology at Vancouver General Hospital were searched for histologically confirmed cases of serous carcinoma arising in the ovary, fallopian tube, or peritoneum, where there were both tissue samples and ascitic fluid/peritoneal washing cell blocks that were positive for serous carcinoma. Retrospective data collection over a 1-year period yielded 26 patients.

Specimens for cell block were received fresh and centrifuged for 15 minutes at 1,200 *g*. Supernatant was removed, and the cell pellet was re-suspended in 30 mL of ThinPrep CytoLyt Solution (Hologic Inc, Marlborough, MA). Following centrifugation for 15 minutes at 1,200 *g*, the supernatant was removed, 1–2 drops of the cell pellet were resuspended in 20 mL of ThinPrep PreservCyt Solution (Hologic Inc), and cells were transferred onto a glass slide using an automated ThinPrep 2000 Processor slide preparation unit. The remaining cell pellet was centrifuged for 15 minutes at 1,200 g, supernatant was removed, and 1–3 drops of Histogel (Thermo Scientific, Kalamazoo, MI) were added, incubated at 4°C for 5 minutes, and submitted for formalin fixation for a minimum of 24 hours. Surgical specimens were received fresh and fixed in 10% phosphate/neutral buffered formalin for a minimum of 16 hours. Due to the retrospective nature of this study and the method in which surgical and cytological specimens were received and prepared, we were unable to record or control for ischemic and fixation times.

IHC for ER and WT-1 was performed on both surgical and cytology specimens using antibodies against WT-1 (DAKO, 6F-H2 clone, 1:100 dilution) and ER (Lab Vision, SP1 clone, 1:50 dilution). Serial 3 μM sections were cut for IHC analysis and run through an automated protocol, including thermal antigen retrieval, using OptiView (Ventana Systems). For both surgical and cytology cases, controls for immunohistochemical staining included surgical specimens of an invasive malignant mesothelioma for WT-1 (positive in the tumour cells, negative in adjacent fibroadipose tissue) and a normal fallopian tube for ER (positive in fallopian tube epithelial cells, negative in surrounding supporting stroma). Internal negative controls for each sample consisted of cells that would be expected to be negative for WT-1 and ER expression (e.g., macrophages or mesothelial cells).

In both histological and cytological specimens, expression intensity (0 = minimal to absent, +1 = mild to moderate, +2 = intense) and percentage of positive cells (0 = 0–0.1%, 1 = 0.1–33%, 2 = 34–66%, 3 = 67–100%) were recorded. Statistical analysis was performed to compare the frequency of expression of WT-1 and ER in cytological preparations and the matched surgical specimens (using Fisher's exact test).

Results

In the 26 cases identified, the average patient age at time of surgery was 68 years (range 44–92 years). Of the 26 cases, three were low-grade serous carcinomas and 23 were high-grade serous carcinomas. When interpreting immunohistochemical expression, only nuclear staining of WT-1 and ER was considered positive in both surgical specimens and cytological preparations. Mesothelial cells (normally identified by the presence of "windows" between adjacent cells, "lacy skirt" cytoplasm, a low nucleus-to-cytoplasm ratio, central round to oval nuclei with prominent nucleoli, delicate chromatin pattern, and thin nuclear membranes), particularly when reactive in nature, have many overlapping features with

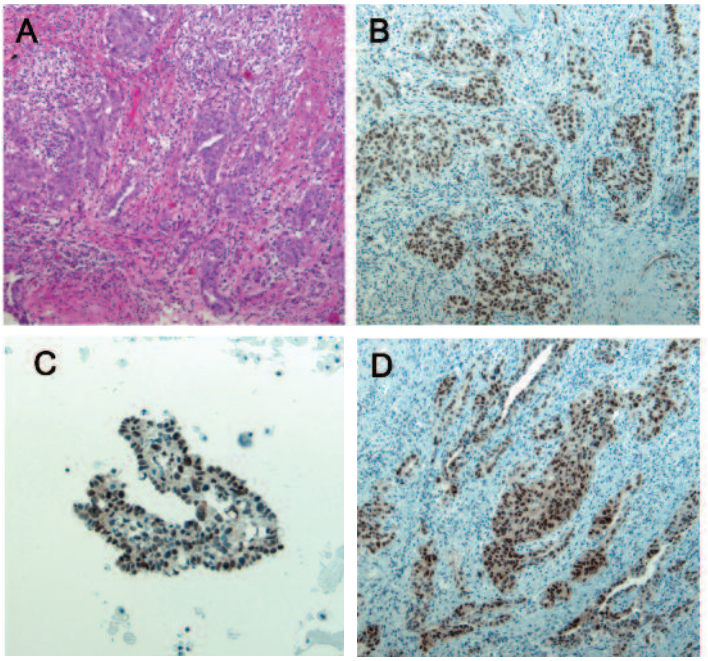

Figure 1. WT-1 and ER expression in high-grade serous carcinoma. This high-grade serous carcinoma *(A)* showed intense (2+) staining for WT-1 diffusely (percentage score 3) in both the surgical *(B)* and cytological preparations *(C)*. ER in this particular case showed intense and diffuse staining (2+, 3) staining in the surgical specimen *(D)* but mild/moderate staining diffusely (1+, 3) in the cytological preparation *(E)*. (*A*, Hematoxylin and eosin; *B–E*, immunoperoxidase)

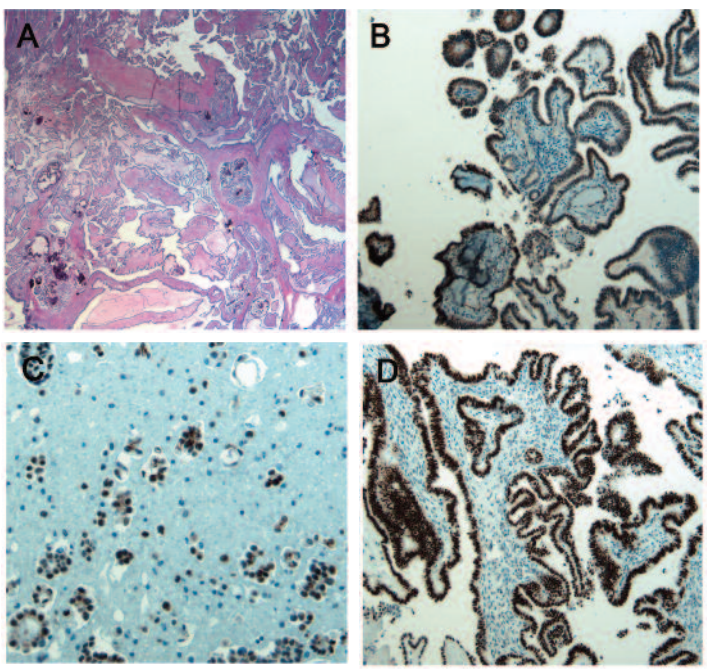

Figure 2. WT-1 and ER expression in low-grade serous carcinoma. This low-grade serous carcinoma *(A)* shows intense and diffuse (2+, 3) staining in surgical specimens of both WT-1 *(B)* and ER *(D)*. Intense and diffuse staining is seen in the matched cytological preparations for WT-1 *(C)* and mild/moderate diffusing staining for ER *(E)*. (*A*, Hematoxylin and eosin; *B–E*, immunoperoxidase)

adenocarcinoma and constitute a major diagnostic pitfall. Reactive mesothelial cells were excluded from intensity and percentage scores based on cytomorphological features; epithelial cell clusters, some branching and in papillary/micropapillary configuration, psammoma bodies, and, in the case of high-grade serous carcinoma, high-grade nuclear atypia favoured serous carcinoma. Figures 1 and 2 show representative high-grade (Figure 1) and low-grade serous carcinomas (Figure 2) stained for WT-1 and ER.

For each patient the staining intensity and the proportion of cells positive for each marker are shown in Table 1. In total, 26 cases were retrieved from the Vancouver General Hospital archives, but tumour cells were not identified in recut sections of one surgical specimen (patient 20) and an insufficient

Table 1. Comparison of WT-1 and ER Staining Intensity and Proportion of Positive Cells in Surgical Specimens and Cytological Preparations

Patient	WT-1*		ER*	
	Surgical	Cytology	Surgical	Cytology
1	2+, 1	1+, 1	2+, 2	1+, 1
2	2+, 2	2+, 3	2+. 2	Insufficient
3	2+, 3	2+, 3	2+, 3	1+, 3
4	2+, 3	1+, 3	2+, 3	1+, 2
5§	2+, 2	0, 0	2+, 3	0, 0
6§	2+, 1	2+, 3	2+, 2	1+, 1
7†	1+, 1	0, 0	2+, 2	0, 0
8§	1+, 1	2+, 2	2+, 2	2+, 2
9	2+, 3	1+, 1	2+, 2	0, 0
10	1+, 2	1+, 1	2+, 2	0, 0
11§	2+, 3	2+, 2	2+, 3	0, 0
12§	2+, 3	2+, 3	2+, 3	2+, 3
13	2+, 3	2+, 2	2+, 3	2+, 2
14	2+, 3	2+, 2	2+, 3	2+, 3
15	2+, 2	2+, 2	2+, 1	1+, 1
16	1+, 3	2+, 3	0, 0	0, 0
17	2+, 2	0, 0	2+, 2	1+, 2
18	2+, 1	2+, 3	2+, 1	2+, 2
19	2+, 3	2+, 3	2+, 2	2+, 2
20§	Insufficient	2+, 2	Insufficient	2+, 1
21	2+, 3	2+, 2	2+, 3	1+, 2
22	2+, 3	2+, 2	2+, 2	1+, 1
23†	2+, 3	2+, 3	2+, 3	2+, 3
24§	2+, 1	2+, 3	2+, 3	1+, 1
25	0, 0	2+, 2	2+, 2	0, 0
26†	2+, 3	0, 0	2+, 3	1+, 1

*Staining intensity score (0 = minimal to absent, +1 = mild to moderate, +2 = intense) and percentage of cells positive (0 = 0–0.1%, 1 = 0.1–33%, 2 =34–66%, 3 = 67–100%).
†Low-grade serous cases.
§Patients received three or four cycles of preoperative carboplatin and paclitaxel.

Table 2. Summary of WT-1* and ER† Expression in Cytological Preparations and Surgical Specimens

	Surgical Specimen +ve	Surgical Specimen –ve
WT-1 Expression		
Cytological preparation +ve	20	1
Cytological preparation −ve	4	0
ER Expression		
Cytological preparation +ve	16	0
Cytological preparation −ve	7	1

*n = 25 cases.
†n = 24 cases.

number of tumour cells was present in one cytology case for assessment of ER expression (patient 2). Statistical analysis was performed on the remaining 25 (WT-1) and 24 (ER) cases. Of the four negative WT-1 cytology cases, two were also for negative for ER (patients 5 and 7). Two cases in which ER was positive showed negative WT-1 staining (patients 17 and 26). Finally, there were five cases in which WT-1 was positive but ER was negative (patients 9, 10, 11, 16, 25). If WT-1 and ER were used alone, two and five cases would have been falsely negative, respectively, suggesting that the combination of stains increases overall sensitivity.

WT-1 expression was positive in 96% (24/25) of surgical specimens and 84% (21/25) of matched cytological preparations. This difference was not statistically significant (p =.35). In the case of ER, there was a statistically significant difference (p = .02) between ER expression in surgical specimens (96%, 23/24) and cytological preparations (67%, 16/24) (Table 2). The staining intensity and percentage of cells positive showed a wide range, with no differences in staining between low- and high-grade carcinoma cases. A single case was identified in which WT-1 expression was positive in the cytological preparation but negative on the surgical specimen. This could represent either false-positive staining in the cytological preparation or false-negative staining in the surgical specimen. False-positive staining in the cytological preparation is unlikely since the background inflammatory cells in the preparation were appropriately negative. Intriguingly, ER expression in the surgical specimen of this patient was positive when the cytological preparation was negative. As we did not control for ischemic or fixation times, one possibility for a false-negative result in the surgical specimen could be that the portion of the tumour selected for immunostaining was inadequately fixed; however, as ER expression was preserved, this explanation is also unlikely. Another possibility is that expression of WT-1 within the tumour could be patchy, and the block selected for immunostaining happened to be an area of tumour in which there was no expression.

Occasionally, WT-1 and ER immunohistochemical stains on tissue specimens showed a heterogeneous staining pattern, with peripheral areas staining more intensely and central areas staining poorly (Figure 3). This may have been secondary to zonality of staining due to poor/improper fixation. In such cases, the assessment of expression intensity and percentage

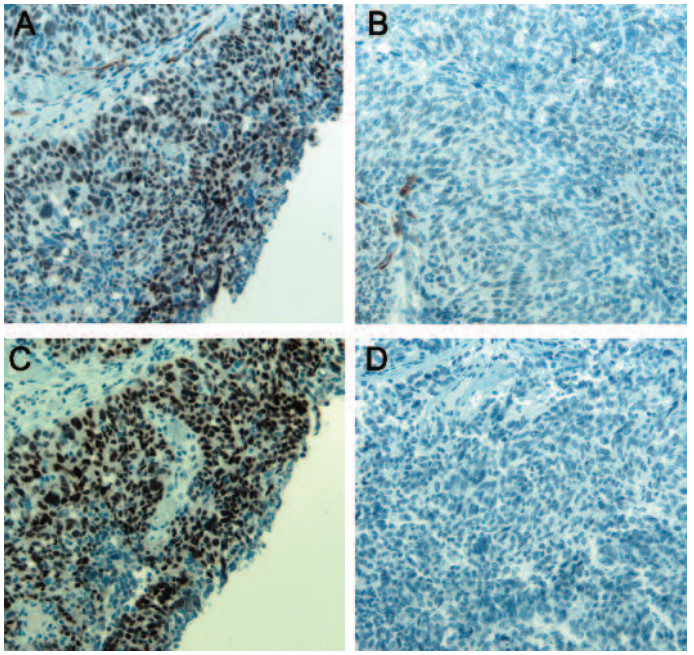

Figure 3. WT-1 and ER staining pattern. Staining at well-fixed/peripheral portions of the specimen is strong and diffuse, whereas in poorly fixed/central portions it is absent. *A* and *B*, WT-1; *C* and *D*, ER. (Immunoperoxidase)

of positive cells was performed cautiously on the well-fixed/peripheral portions, at least 1–2 low-power fields away from the tissue edge. This approach prevented false-negative (negative staining in central/unfixed portions) and false-positive (positive staining from edge effect) interpretations. In seven cases (denoted with "§" in Table 1), patients had previously undergone neoadjuvant chemotherapy (three or four cycles of carboplatin and paclitaxel).

Discussion

The subtypes of ovarian surface epithelial carcinoma are now recognized to be distinct diseases, with different risk factors, precursor lesions, genetic abnormalities, patterns of spread, and outcomes.[10,11] This has led to recommendations for subtype-specific treatment for ovarian carcinoma. For example, the consistent abnormalities in homologous recombination/double-strand break repair that characterize high-grade serous carcinomas have led to clinical trials of PARP inhibitors, with promising preliminary results.[12,13] Clear cell carcinomas show abnormal activation of pathways related to neovascularization, and the antiangiogenesis treatment sunitinib has shown activity in this ovarian carcinoma subtype in both preclinical models and patients.[14] Radiotherapy has also been associated with an improved outcome in patients with clear cell carcinoma (but not serous carcinoma).[15,16] In the case of mucinous carcinoma, HER2 amplification is present in 18% of cases and is a potential therapeutic target, while HER2 amplification is not seen in other ovarian carcinoma subtypes.[17,18] Both mucinous and clear cell carcinomas are relatively resistant to platinum-based chemotherapy,[19,20] which remains the cornerstone of treatment for ovarian cancer, as high-grade serous carcinomas respond in approximately 80% of cases.

At the same time as the importance of subtype in ovarian carcinoma management has come to be appreciated, there has been a move away from the traditional approach of surgical debulking followed by platinum/taxane combination chemotherapy as primary treatment. Neoadjuvant chemotherapy, in which three or four cycles of chemotherapy are given before surgical debulking, first entered practice for patients with bulky upper abdominal disease in whom optimal, primary surgical debulking was not possible. The apparent success of this approach in these cases led to a randomized clinical trial comparing neoadjuvant chemotherapy followed by surgery to conventional treatment of surgical debulking followed by chemotherapy. The outcomes were the same in both arms of this trial, with less morbidity in patients who received neoadjuvant chemotherapy.[6] This has led to an increasing use of neoadjuvant chemotherapy for ovarian carcinoma. This management approach is aimed at high-grade serous carcinomas, which account for the large majority of advanced-stage ovarian carcinomas and characteristically show a high rate of response to platinum-based chemotherapy. It will not be successful in chemo-insensitive subtypes such as clear cell and mucinous carcinomas.

Ovarian carcinoma subtypes can be diagnosed with a high degree of reproducibility, based on examination of hematoxylin and eosin–stained sections of resection specimens, with use of immunostaining in problematic cases.[21–23] The immunomarkers WT-1 and ER are useful positive markers of high-grade serous carcinoma as they are typically completely negative in clear cell and mucinous carcinomas.[10,21,23] In patients for whom neoadjuvant chemotherapy is being considered, the diagnosis is typically

made on a cytological specimen, as most patients with advanced-stage ovarian carcinoma have ascites. Although ovarian carcinoma subtypes can be accurately diagnosed on multiple tissue sections taken from a resection specimen, there is no evidence that accurate subtype diagnosis is possible on a cytological specimen. We recently investigated the use of a panel of immunomarkers in ovarian carcinoma subtype diagnosis, anticipating that with the emergence of neoadjuvant chemotherapy, subtype diagnosis would have to be made on small biopsy specimens, in which case immunomarkers would more often be needed to allow accurate diagnosis.[21] This approach shows significant promise but is based on the use of formalin-fixed, paraffin-embedded tissue samples.

Liquid-based cytology has become routine in many laboratories. While immunostaining has become highly reproducible over the past decade,[24] this has been achieved by paying attention to all aspects of immunostaining, including pre-analytical variables such as fixation. The fixative (10% neutral buffered formalin) and fixation times are now specified for breast cancer biomarker testing.[25,26] There have been direct comparisons of alcohol-fixed liquid-based cytology specimens to surgical specimens, with respect to ER, progesterone receptor (PR), and HER2 immunostaining. Comparison of ER expression in 41 cases of breast carcinoma cases between fine-needle aspiration cell blocks (initially fixed in 50% ethanol followed by 10% neutral buffered formalin fixation) and needle core biopsies (fixed in 10% neutral buffered formalin) showed good correlation (82% positive agreement, 100% negative agreement).[27] In the same study PR and HER2 were shown to have poor (43.7% positive agreement, 92% negative agreement) and fair (87.5% positive, 66.6% negative agreement) correlation, respectively. The conclusion was that cell block samples could be used to determine which patients may benefit from tamoxifen therapy, based on ER expression, but that the results of IHC assessment of PR and HER2 are unreliable. ER, PR, and HER2 expression have been compared in 34 cases of invasive ductal carcinoma between cell blocks (fixed in 50% ethanol followed by 10% neutral buffered formalin) and tissue blocks (fixed in formalin).[28] The conclusion from this study was that patients could be triaged to receive hormonal treatment, as good agreement was observed for expression of ER (90.4% positive agreement, 86.7% negative agreement) and PR (93.9% positive agreement, 94.7% negative agreement), but only moderate agreement was seen for HER2 expression (73.3% positive agreement, 81% negative agreement).[28]

It is currently recommended that there be revalidation of all immunostains when fixatives different from those used for the initial validation studies in the laboratory are to be used. Revalidation of nuclear immunostains such as ER and WT-1 has been recommended since, when compared with formalin-fixed tissue, suboptimal nuclear staining is observed in ethanol-fixed cytology preparations.[29] Accordingly, we undertook this study to test the sensitivity of ER and WT-1 immunostaining, comparing cell blocks fixed in an alcohol-containing medium with the corresponding formalin-fixed, paraffin-embedded specimens.

WT-1 is a tumour suppressor gene located on 11p13. It is predominantly located in the nucleus and acts as a deoxyribonucleic acid (DNA)-binding protein in the development of the genitourinary tract and tissues from the inner layer of the intermediate mesoderm. WT-1 is normally expressed in ovarian granulosa cells and surface epithelium, the fallopian tube, mesothelial cells, Sertoli cells of the testis, and mesangial cells of the kidney and spleen. It is also expressed in a variety of diseases including tumours of the ovarian surface epithelium (particularly serous carcinomas), malignant mesotheliomas, Wilms' tumour, rhabdoid tumours of the kidney, acute myeloid leukemia, and desmoplastic small round cell tumour. ER is expressed in various tumours including ovarian serous and endometrioid carcinomas, breast carcinoma, and endometrial carcinoma. Importantly, mucinous and clear cell carcinomas are typically ER and WT-1 negative.

We found that WT-1 was expressed in 96% (24/25) of surgical specimens and 84% (21/25) of matched cytological preparations. ER expression was present in 96% (23/24) of surgical specimens and 66% (16/24) of cytological preparations. These are higher rates of positivity in the surgical specimens than we previously found in studies using tissue microarrays[10] and presumably reflect improved detection in whole sections of antigens that can show some variability in expression. We did not see any differences in staining between cases in which patients had received prior neoadjuvant chemotherapy, a finding that has been reported

previously.[30] Discordant staining (absent staining in cytological preparations where there was positivity in the matched surgical specimens) was observed for WT-1 in 17% (4/24) and for ER in 30% (7/23) of cases.

There was an overall decrease in sensitivity for WT-1 and ER immunostaining. The question is how to act upon these results. One possibility would be to accept that there is a modest reduction in sensitivity when cell blocks fixed in alcohol are used, knowing that in most cases there will be appropriate staining. Sensitivity in detecting serous carcinoma could potentially be improved with additional immunohistochemical stains such as PAX-2, which is positive in approximately 67% of serous carcinomas of the ovary.[31] Another option would be to adjust the staining protocol in the hope of achieving identical results in the cytological and surgical specimens; it has been suggested that elimination of the thermal antigen retrieval step improves staining in alcohol-fixed specimens,[32] although in our experience an increase in antibody concentration may also be required. It is possible that equivalence is not possible as these are intrinsically different samples, since exfoliated cells may have detached days earlier, with resulting changes in expression levels. Interestingly, Kinsella et al. found that even formalin-fixed cell blocks were suboptimal for HER2 assessment, indicating that fixation is not the only variable related to assessment of expression in cell blocks.[33]

This study does serve to highlight one of the problems with revalidation of staining protocols in non-standard fixatives; it is difficult or impossible to obtain the large numbers of specimens with varied tissue diagnoses that were used for the original validation. In this case, for example, we did not test specificity of WT-1 and ER staining as we do not have a wide range of non-serous carcinomas fixed in alcohol and prepared as cell blocks for assessment. Based on our results, it is possible to obtain useful information based on alcohol-fixed, liquid-based cytology specimen, but such results must be interpreted with caution, as immunostaining in this setting is not as well validated and false-negative results can occur.

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Prospective Assessment of C4d Immunohistochemistry in Cardiac Transplant Biopsies: Cost-Effectiveness Analysis

Bin Xu, MDCM, PhD, René P. Michel, MDCM, FRCPC, A. Kevin Watters, MDCM, Richard S. Fraser, MDCM

ABSTRACT

Background: Antibody-mediated rejection (AMR) may lead to cardiac allograft dysfunction and decreased patient survival. The International Society for Heart and Lung Transplantation (ISHLT) 2011 working formulation recommended that AMR be diagnosed on the basis of histopathological or immunopathological findings and that immunohistochemical study for C4d be performed routinely. The aim of the current study was to evaluate the incidence of pathological and immunopathological AMR using the scoring system proposed by ISHLT in 2011 and to estimate the laboratory cost and the diagnostic gain of routine versus selective C4d immunohistochemistry (IHC) in endomyocardial biopsies (EMBs).

Methods: We prospectively evaluated 146 EMBs from 58 patients with histology and C4d IHC. The cost-effectiveness of selective C4d IHC based on 2005 ISHLT guidelines and the cost-effectiveness of routine C4d IHC as suggested by the 2011 ISHLT guidelines were calculated and compared.

Results: Of the 146 EMBs analyzed, 9 (6%) from 7 patients (12%) were classified as pAMR 1 (H+) (histological AMR alone); 1 biopsy (1%) from 1 patient (2%) was diagnosed as pAMR 1 (I+) (immunopathological AMR alone); and 2 (1%) from 2 patients (3%) were defined as pAMR 2 (histological and immunopathological AMR). No patient was diagnosed with pAMR 3. The average cost-effectiveness ratio increased eightfold with routine as compared with selective C4d IHC. The additional cost to detect one case of pAMR 1 (I+) was estimated at $5,427.00.

Conclusion: The tentative scoring system for AMR and C4d positivity proposed by the ISHLT 2011 guidelines is a useful tool with which to evaluate AMR and to clarify the existing controversy regarding the incidence of AMR in patients with cardiac transplants. We found that routine C4d IHC yields a minimal diagnostic gain at an additional cost of $5,427.00 per case compared with selective C4d IHC. Given that a consideration of cost should be involved in implementing any laboratory test, the routine use of C4d IHC in assessing AMR can be questioned.

RÉSUMÉ

Contexte : Le rejet de greffe cardiaque médié par les anticorps peut entraîner la détérioration du greffon et abréger la survie du patient. L'International Society for Heart and Lung

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This article has been peer reviewed.

Competing interests: None declared

Transplantation (ISHLT) a recommandé en 2011 le diagnostic du rejet humoral sur la foi de l'examen histopathologique ou immunopathologique et l'exécution de l'épreuve d'immunohistochimie de détection de C4d. La présente étude a pour but d'évaluer l'incidence du rejet humoral confirmé à la pathologie et à l'immunopathologie selon le système de notation proposé par l'ISHLT en 2011 et d'estimer les coûts de laboratoire et le gain diagnostique liés à l'épreuve immunohistochimique exécutée à la biopsie endomyocardique dans tous les cas par opposition à son exécution dans certains cas seulement.

Méthode : Nous étudions de façon prospective 146 biopsies effectuées chez 58 patients, accompagnées d'analyses histologiques et de la détection immunohistochimique de C4d. Nous avons évalué la rentabilité relative de la détection immunohistochimique sélective de C4d comme le préconisent les lignes directrices de 2005 de l'ISHLT et de la détection immunohistochimique générale de C4d ainsi que le recommandent les lignes directrices de 2011.

Résultats : Du lot des biopsies analysées, 9 (6 %) effectuées chez 7 patients (12 %) sont classées dans la catégorie pAMR 1 (H+) (rejet humoral sur la foi de l'histologie), 1 (1 %) (1 patient, 2 %) se range dans la catégorie pAMR 1 (I+) (rejet humoral sur la foi de l'immunopathologie) et 2 (1 %) effectuées chez 2 patients (3 %) sont classées comme étant pAMR 2 (diagnostic histologique et immunopathologique). Il n'y a pas de diagnostic pAMR 3. Le rapport coût-efficacité moyen augmente d'un facteur de huit quand on passe de la détection immunochimique sélective de C4d à la détection générale. Le coût supplémentaire de la détection d'un cas pAMR 1 (I+) est estimé à 5 427 $.

Conclusion : Le système de notation de la détection du rejet humoral et de la présence de C4d proposé dans les lignes directrices de l'ISHLT en 2011 est un outil utile pour évaluer le rejet humoral et clarifier la controverse à propos de l'incidence du rejet humoral de greffe cardiaque. Nous constatons que la détection immunohistochimique générale de C4d se traduit par un minime gain sur le plan diagnostique au coût supplémentaire de 5 427 $ le cas comparativement à la détection immunohistochimique sélective. Étant donné que l'aspect des coûts devrait être pris en considération avant d'adopter toute analyse de laboratoire, il y aurait lieu de remettre en question l'utilité de la détection immunohistochimique courante de C4d dans l'évaluation du rejet humoral.

Antibody-mediated rejection (AMR), mediated by donor-specific antibodies, is associated with an increased incidence of allograft dysfunction, cardiac allograft vasculopathy, and reduced survival.[1,2] In 2005, the International Society for Heart and Lung Transplantation (ISHLT) recognized AMR as a distinct entity.[3,4] According to this group, a diagnosis of AMR can be suspected on the basis of histological findings (such as capillary endothelial swelling, the presence of macrophages or neutrophils within capillaries, and interstitial edema/hemorrhage) and can be confirmed by immunophenotypic evidence of capillary deposition of immunoglobulin and complement as provided by immunofluorescence or immunohistochemistry (IHC).[1–3,5,6] Subsequent efforts to assess the incidence and clinical significance of AMR have yielded inconsistent results. For example, the reported incidence of immunoreactivity for complement component 4d (C4d) varies from 4.1% to 55.5%, partially due to the lack of a consensus scoring scheme.[7–23] Moreover, although recent evidence has shown that asymptomatic AMR is associated with an increased incidence of cardiac allograft vasculopathy and long-term mortality,[24–26] the clinical significance of just the presence of C4d within the capillaries remains controversial.[7,10,11,14,19,22,23,27–29]

In 2011, ISHLT revised the diagnostic criteria for AMR and

Table 1. Scoring Scheme for C4d Immunoreactivity

Distribution	Intensity of Staining
Negative	0: negative
Focal: 1–<10% of capillaries positive for C4d	1+: weak
Multifocal: 10–50% of capillaries positive for C4d	2+: moderate
Diffuse: >50% of capillaries positive for C4d	3+: strong

proposed a pathological diagnosis of AMR solely based on histopathological findings or immunopathological findings or both in endomyocardial biopsy (EMB) specimens.[1,2] In this proposal, IHC or immunofluorescent assessment of C4d is considered mandatory for evaluating AMR. The current study was undertaken to (1) evaluate the incidence of AMR in our institution according to 2011 ISHLT guidelines and (2) estimate the cost and diagnostic gain of routine C4d IHC as compared with selective C4d IHC.

Materials and Methods

We prospectively evaluated 146 consecutive EMBs in 58 patients performed between January 1, 2011, and June 30, 2012, at McGill University Health Centre. The number of biopsies per patient ranged from 1 to 12. We documented the presence or absence of the following histological parameters: (1) capillary endothelial swelling, (2) macrophages or neutrophils within capillaries, and (3) interstitial edema and/or hemorrhage. The grade of acute cellular rejection was assigned on the basis of the 2005 ISHLT classification system.[4] For immunohistochemistry, polyclonal anti-human C4d antibody from ALPCO (Windham, New Hampshire, US) at a dilution of 1:30 was used. The distribution and intensity of the C4d immunoreactivity within the capillaries were evaluated according to a scoring scheme modified from the Banff system for renal transplant biopsies (Table 1).[30] A positive result for C4d was defined as multifocal or diffuse staining of any intensity, as defined by the 2011 ISHLT guidelines.[1,2] Nonspecific C4d staining was documented whenever noted. Additional immunohistochemical testing for the presence of capillary-associated macrophages (CD68) and/or T lymphocytes (CD3) was performed when considered necessary. All statistical analyses were carried out with *STATISTICA* software (StatSoft Inc., Tulsa, Oklahoma, US), and p values of less than 0.05 were considered statistically significant. The number and percentage of cases with features of histological AMR, immunopathological AMR, or both were calculated with the Fisher exact test.

The estimated laboratory costs for evaluating an EMB specimen with or without C4d IHC were calculated in Canadian dollars, based on manufacturers' pricing information for reagents and antibodies, the expense of tissue processing and handling at the institutional level, and the reimbursement rate of Régie de l'assurance maladie du Québec. Professional fees related to the testing were not included in the analysis.

Results

Incidence of Histological and Immunopathological AMR

Of the 146 EMB specimens analyzed, 135 (92%) from 54 patients (93%) showed no evidence of histological AMR whereas 11 (8%) from 9 patients (16%) demonstrated features suggestive of histological AMR (Table 2). Of these 11 biopsy specimens, 2 (1%) from 2 patients (3%) showed multifocal ($n = 1$) or diffuse ($n = 1$) C4d immunoreactivity and can be classified as pAMR 2 (pathological AMR with both histological and immunopathological findings) based on the 2011 ISHLT guidelines (Figure 1).[1,2] The other nine biopsy specimens were classified as pAMR 1 (H+) (histological AMR alone). One of the 135 biopsy specimens (1% of biopsies and 2% of patients) without histological evidence of AMR revealed multifocal weak C4d labelling within capillaries and was thus classified as pAMR 1 (I+) (immunopathological AMR alone). The other 134 biopsy specimens (92%) from 54 patients (93%) were categorized as pAMR 0. No biopsy specimen was classed as pAMR 3. Among these 134 biopsy specimens, 9 (6%) from 9 patients (16%) were focally (1–10% of capillaries) positive for C4d with weak ($n = 5$) or moderate ($n = 4$) intensity. Overall, 12 biopsy specimens (8%) were classified as pAMR 1 and above by the 2011 ISHLT classification, including 9 cases of pAMR 1 (H+), 1 case of pAMR 1 (I+), and 2 cases of pAMR 2. The incidence of C4d positivity was significantly higher in biopsy specimens that were suggestive of histological AMR (2 of 11

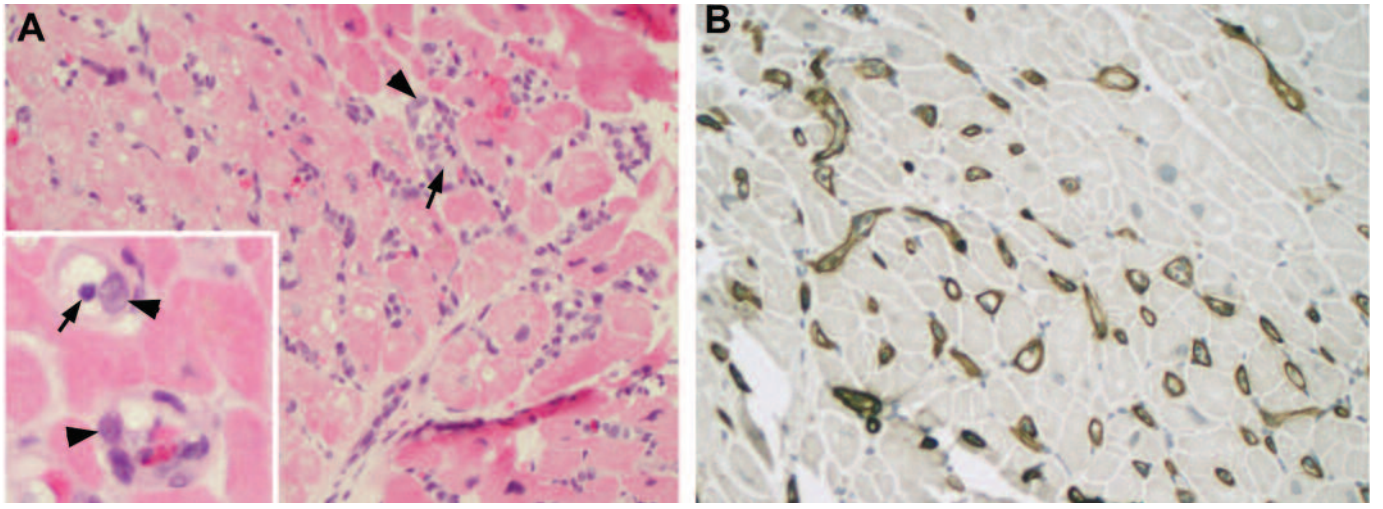

Figure 1. Histological and immunopathological antibody-mediated rejection (AMR). *A*, Histological AMR: presence of endothelial swelling *(arrowheads)* and macrophages and inflammatory cells within the capillaries *(arrows)*. *B*, Immunopathological AMR: diffuse and strong C4d labelling within the capillaries. (*A*, hematoxylin and eosin; *B*, immunoperoxidase)

Table 2. Incidence of Histological and Immunopathological Antibody-Mediated Rejection

	Number of Biopsies		
	C4d (-)	C4d (+)	Total
Histological AMR (-)	134 (92%)	1 (1%)	135 (92%)
Histological AMR (+)	9 (6%)	2 (1%)	11 (8%)
Total	143 (98%)	3 (2%)	146 (100%)

AMR = antibody-mediated rejection.

Table 3. Cost-Effectiveness of Selective versus Routine C4d Immunohistochemistry in Cardiac Transplant Biopsies*

C4d IHC	Number of Biopsies	Estimated Total Cost†	Number of Positive C4d	Average Cost-Effectiveness
Selective	11	$442.20	2	$221.10/biopsy
Routine	146	$5,869.20	3	$1,956.40/biopsy
Marginal cost-effectiveness ratio‡:				$5,427.00/biopsy

IHC = immunohistochemistry.
*All costs and calculations are in Canadian dollars. Professional fees related to the testing were not included in analysis.
†Estimated cost for one C4d immunohistochemical analysis is $40.20.
‡Defined as the additional cost of detecting one additional case of positive C4d by routine C4d IHC as compared with selective C4d IHC.

[18%]) than in those without histological AMR (1 of 135 [1%]) (Fisher exact test, $p = 0.015$). Coexisting acute cellular rejection (ACR) was commonly observed in biopsy specimens with pAMR 1 and above (9 of 12 [75%]). Seven of 9 biopsy specimens classified as pAMR 1 (H+) and 1 of 2 cases of pAMR 2 demonstrated ACR grade 1R whereas the biopsy specimen with pAMR 1 (I+) showed an ACR of grade 2R (ISHLT 2005 classification).[4]

Background C4d staining was found in 90 biopsy specimens (62%), notably in the serum ($n = 21$, 14%), myocytes ($n = 18$, 12%), the internal elastic membrane and smooth muscle of arterioles and venules ($n = 17$, 12%), the intima of small arterioles or venules ($n = 11$, 8%), the cytoplasm of endothelial cells ($n = 10$, 7%), and the interstitial tissue ($n = 9$, 6%). Nonspecific background artifact was evident in 43 cases (30%). This background staining was not considered to significantly interfere with the interpretation of rejection.

Cost-Effectiveness Analysis of Selective versus Routine C4d IHC in EMB Specimens

The established testing protocol for EMB specimens at our institution prior to 2011 included three slides stained with hematoxylin and eosin and one stained with Masson trichrome; the estimated cost was $29.30 per biopsy. Routine C4d IHC was introduced in January 2011 at an additional cost of $40.20 per case. The cost of the primary antibody alone was $14.16 per slide. The average cost-effective ratios, as calculated by dividing the total cost of a particular protocol (selective versus routine C4d IHC) by the number of positive C4d cases detected by the program, were $221.10 per case using selective C4d IHC and $1,956.40 per case using routine C4d IHC (Table 3). The marginal cost-effective ratio, defined as the additional cost of implementing routine C4d IHC (as opposed to selective C4d IHC) divided by the number of additional cases detected by routine C4d IHC, was $5,427.00 per case (see Table 3).

Discussion

AMR as defined by the 2005 ISHLT guidelines required clinicopathological correlation whereby it first had to be suspected by graft dysfunction and histological features and subsequently had to be confirmed by immunopathological studies (using antibodies against immunoglobulin, complement, or CD68) or by detection of serum donor-specific antibodies.[4] The lack of a clear definition for immunopathological and histological AMR in the 2005 ISHLT guidelines resulted in variability in its interpretation and reported incidence. In the interpretation of immunopathological AMR for example, the scoring scheme for C4d varied considerably among studies, leading to a wide range of incidence of positivity (4.1–55.5%).[7–23] In 2011, ISHLT published updated guidelines for AMR in an effort to clarify and standardize its diagnostic criteria.[1,2] One major change in these guidelines is a scoring scheme for C4d in which only multifocal or diffuse capillary staining is considered positive. When revisiting the literature that had this more stringent definition for C4d positivity, we found that the positive rate in published reports ranged from 4.1% to 11.5%.[7–23] In our study, only 3 biopsy specimens (2.1%) from 3 patients (5.4%) fulfilled the 2011 ISHLT diagnostic criteria for immunopathological AMR whereas 9 biopsy specimens (6.2%) from 9 patients (15.5%) with focal C4d immunopositivity were classified as negative for immunopathological AMR.

C4d IHC or immunofluorescence is now considered mandatory for evaluating AMR by the 2011 ISHLT guidelines.[1,2,4] This change is based on the notion that C4d deposits can be observed in cases lacking histological evidence of AMR and may predict graft dysfunction or poor long-term outcomes.[16,20,29] Given that cost should be considered when decisions are made to implement any laboratory test, the routine use of IHC to detect AMR can be questioned. Our analysis shows that the laboratory cost of C4d IHC increased approximately eightfold – from $221.10 to detect one positive case based on 2005 ISHLT guidelines to $1,956 to detect one positive case based on 2011 ISHLT guidelines. In the present group of biopsies, only 1 of 146 specimens was classified as pAMR 1 (I+) (i.e., immunopathological AMR without histological AMR). The extra cost required to detect one additional case of immunopathological AMR by 2011 ISHLT guidelines as compared with the 2005 guidelines is estimated to be $5,427.00. In our experience, routine C4d IHC for cardiac transplant biopsy specimens appears to have only a small diagnostic gain over C4d IHC based on histological suspicion.

A decision to include or exclude a test in a laboratory menu is based on a number of factors in addition to cost, such as test sensitivity and specificity, contribution to diagnosis, benefit to patient care, prognostic value, and expert panel recommendations or guidelines. How much weight each of these factors should have in such a decision is difficult to indicate precisely and will vary depending on the nature of the abnormality the test addresses. However, as the issues of resource limitation and allocation become increasingly significant in our society, it is important that pathologists give cost-appropriate consideration when deciding to include a test as part of routine diagnosis.

In conclusion, we found that the presence of immunopathological AMR alone is an uncommon finding that has an estimated laboratory cost of $5,427 per case. Until sufficient evidence is gathered in larger series or meta-analyses employing the same diagnostic criteria proposed by the 2011 ISHLT guidelines, our findings suggest that

routine C4d IHC for cardiac transplant biopsy specimens might not be a cost-effective way to assess AMR and that implementing ISHLT guidelines into our daily practice may not be appropriate.

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Forensic Science in Canada: Report of a Multidisciplinary Discussion

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ABSTRACT

Reliable forensic science is the cornerstone of many court cases in Canada, yet the system that delivers our forensic science is at a critical juncture. Public confidence and judicial confidence in our practices have been eroded by several high-profile inquiries into the damage wrought by faulty forensic evidence. In the wake of these failures, Canadian forensic scientists have responded with a number of important reforms, much to the benefit of the public. In the larger article on which this summary is based, forensic experts from across Canada describe the current state in forensic science and make recommendations to improve services. The unanimous conclusion is that forensic sciences in Canada must grow and develop to enhance public health, public safety, and justice. Continued and sustainable improvements in all the disciplines of forensic science will require the coordinated efforts of academic institutions, government, stakeholders in the justice sector, and forensic scientists.

RÉSUMÉ

Les sciences judiciaires exercent une influence déterminante dans nombre de causes au Canada, mais le système qui chapeaute les sciences judiciaires est à une croisée des chemins critique. Plusieurs enquêtes largement médiatisées sur les conséquences de preuves scientifiques erronées ont mis à mal la confiance du public et celle de l'appareil judiciaire envers notre pratique d'expertise scientifique. Devant ces erreurs professionnelles, les scientifiques experts canadiens ont entrepris d'importantes réformes pour le plus grand bien du public. Dans l'article, des experts en sciences judiciaires du pays offrent un aperçu de l'état des lieux en sciences judiciaires et formulent des recommandations dans le but d'améliorer les services. Ils sont unanimes à conclure que les sciences judiciaires doivent connaître un essor et se perfectionner pour améliorer la santé et la sécurité du public et l'administration de la justice. L'amélioration continue et durable de toutes les disciplines des sciences judiciaires nécessitera l'intervention coordonnée des établissements universitaires, des administrations publiques, de l'appareil judiciaire et des scientifiques experts.

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This article has been peer reviewed.
Competing interests: Dr. Pollanen is the director of the Centre for Forensic Science and Medicine and director of the Forensic Pathology Residency Training Program at the University of Toronto, and is chief forensic pathologist of the Ontario Forensic Pathology Service, in Toronto.

In May 2012, the Centre for Forensic Science and Medicine at the University of Toronto convened a multidisciplinary workshop to examine the reliability and sustainability of Canada's forensic sciences. This event was organized in response to both public and judicial concerns about the accuracy of forensic evidence being presented in Canada's courts – a situation thrown into the spotlight by several high-profile cases of wrongful conviction and subsequent public inquiries uncovering faulty forensic evidence.

The workshop brought together key scientists working across the country in the following disciplines: forensic pathology, forensic anthropology, forensic odontology, forensic nursing, forensic entomology, forensic physical sciences, forensic toxicology, forensic biology, and forensic psychiatry. The resulting report includes a comprehensive discussion of each area and concludes with detailed recommendations for critical improvements needed to ensure that Canada's justice system is adequately served by its forensic science community. The full text of the report is available in electronic format from the website of the University of Toronto Centre for Forensic Science and Medicine (http://www.forensics.utoronto.ca/Page969.aspx). This article is a summary of that report.

Scientists taking part in this multidisciplinary discussion were asked to provide descriptions and analyses of the states of their disciplines. They were further asked to do so through the lens of the three main components of healthy intellectual inquiry: service, education, and research.

Each chapter of the report is prefaced by a summary and overview and is then divided into the following sections:

- **Service** (covering legal framework, facilities, professional standards, and workforce)
- **Education** (covering undergraduate, graduate, post-graduate, and continuing education, as well as professional certification and specialized training where applicable)
- **Research** (covering publications, institutions, professional organizations and groups, and national and international committees and networks)

Each chapter concludes with detailed recommendations for improvements to the relevant discipline.

Legal Framework and Professional Standards

Canada's forensic scientists work under a wide variety of legal frameworks. This is because the country's medical, judicial, and death investigative services are overseen by a combination of provincial, territorial, and federal agencies. Thus, standards and practices differ from province to province, sometimes markedly. This situation is further complicated by the fact that forensic scientists may work in two separate legislative contexts: death investigations (overseen by coroners and medical examiners) and criminal investigations (overseen by police but with input from workers from child welfare and other agencies).

Many forensic disciplines operate smoothly despite these differences. Forensic psychiatry, for instance, works within a mostly uniform legal framework across the country. Forensic biology operates within a robust and well-defined legal framework, largely due to the uniform acceptance of deoxyribonucleic acid (DNA) evidence by Canadian courts. In contrast, forensic nursing is governed by twelve provincial associations and a professional body (the Canadian Nurses Association) that has awarded the field "emerging group" status but has yet to develop credentials and standards for practitioners across Canada. Forensic dentists struggle with issues of professional and personal liability, particularly in bite-mark-opinion cases, due to the blurring of jurisdictional lines from province to province. Practitioners in the forensic physical sciences may be bound by federal legislation (such as the Criminal Code of Canada and the Canada Evidence Act) and internal regulations (such as a Police Services Act) as well as standard operating procedures and the International Standards Organization standards of the laboratories where they perform their work. Forensic pathologists must contend with a patchwork of systems whereby they have the statutory responsibility to order an autopsy in some provinces whereas that responsibility rests with a coroner in other provinces.

The authors of the report conclude that forensic science has suffered from this patchwork of provincial and federal agencies and recognize that a united strategy has been difficult to forge when so many forensic activities fall between municipal, provincial, and federal mandates. They also suggest that the solution to some of these challenges resulting from this may lie in the area of professional standards and credentialing. Credentialing of Canada's forensic scientists is absent in some disciplines, fragmentary in others, and neither universally accepted as necessary nor even considered desirable by others. Some disciplines (such as forensic anthropology, odontology, and entomology) may seek professional certification through

United States or international credentialing organizations. There is, however, no legal requirement that they do so. The authors propose that professional credentialing, along with the development of Canada-wide standards (or adaptation of existing international standards), would help ensure uniform quality in the delivery of forensic services across the country.

Facilities

The quality and availability of facilities for forensic work also vary across the country. Many forensic pathologists work in provincial morgue facilities either in hospitals or in dedicated forensics units; these facilities vary widely in their quality. Other practitioners work in the country's three major government laboratories: the Centre of Forensic Sciences, with locations in Toronto and Sault Ste. Marie; the Royal Canadian Mounted Police forensic laboratories, located in Vancouver, Winnipeg, and Halifax; and Quebec's Laboratoire de sciences judiciaires et de médecine légale, in Montréal.

Some disciplines, such as forensic entomology and forensic anthropology, are connected to academic institutions, so while practitioners of such disciplines may work from universities, they often rely on cooperation with other agencies to secure facilities for dedicated forensic work. Disciplines requiring specialized equipment, such as forensic dentistry, suffer from a lack of dedicated facilities. Despite these challenges, the authors do not identify any insurmountable facility-related problems with Canada's forensic sciences.

Workforce

Two major issues arise from the report's discussion of Canada's existing forensic science workforce. The first is the low number and geographical separation of forensic scientists across the country, due in part to Canada's small and geographically scattered population. In practice, this translates to critical shortages in some areas of the country, practitioners burdened with heavy caseloads in others, and many investigations that could benefit from forensic science expertise going without such expertise. The second issue is the fee-for-service model used to pay for many forensic science services. This structure effectively restricts the involvement of forensic specialists in many cases in which they could be of great help. The authors recommend that funding models emphasizing full-time personnel rather than fee-for-service providers be developed within existing budget frameworks, and they identify forensic nursing and forensic pathology as disciplines that stand to benefit greatly from this reform.

Education

One of the biggest challenges facing Canada's forensic science community is the lack of graduate and postgraduate training available in its academic institutions. At this time, a number of forensic disciplines have no mechanism for educating the next generation of practitioners; these include forensic anthropology, forensic odontology, forensic toxicology, and forensic biology. Forensic pathology, forensic nursing, and forensic psychiatry are supported by some graduate and postgraduate training programs and should continue to be so. Students interested in forensic entomology may study in Canada under the supervision of one of the two American Board of Forensic Entomology–certified practitioners.

The field of forensic toxicology has perhaps the least-developed educational infrastructure of all Canada's forensic sciences. Not only is there no formal training available in Canada at either the undergraduate or graduate levels, there are also no formal requirements for continuing education or professional development within the field. Currently, those practising in Canada may hold anything from a college diploma to a graduate degree. At the same time, Canada's forensic toxicologists are coming under increasing scrutiny by the courts. The situation is exacerbated by the lack of formal education and a credentialing mechanism for these practitioners.

Canadian forensic physical sciences analysts are largely educated through in-house police training followed by internship or understudy programs and by professional certification. These analysts specialize in forensic identification (FI), which includes fingerprint, footprint, tire-track, and friction-ridge analysis; firearms and tool identification; and blood pattern analysis. Although the educational background of Canada's forensic physical sciences personnel can vary widely (for some Royal Canadian Mounted Police officers, it is a high school diploma, followed by in-house training), the Canadian model of in-house training followed by internship or understudy, by certification, and by mandatory re-certification every few years has been recognized internationally and duplicated by agencies outside the country. One of the challenges faced by the forensic physical sciences community is that trained FI experts are often transferred

from unit to unit on the basis of the host police agency's operational needs. The authors note that these policies were developed at a time when FI duties were much less complex, as were the expectations of both the judicial system and society in general. This is a situation that warrants discussion and a policy response.

The in-house and on-the-job training model can also be found within the forensic nursing discipline, in which nurses wishing to practise as sexual assault nurse examiners, legal nurse consultants, or forensic nurse death investigators undertake a combination of clinical training and online learning modules. Basic undergraduate training in forensic sciences is now available at several Canadian universities, but the authors caution that these programs are somewhat generalized and that, in any case, they must still be augmented by graduate and postgraduate programs. The overwhelming conclusion is that Canada must develop masters- and doctoral-level research-focused degree programs for all forensic disciplines. This development would help to address the need for multidisciplinary cross-training among scientists, police, lawyers, and judges.

Research

In chapter after chapter, one reads the same story about research: Canadian research in the forensic sciences is almost non-existent. This is due largely to the fact that no national grant-funding agency has a mandate to advance the forensic sciences. Forensic science generates novel questions and issues quite distinct from the usual mainstream scientific problems, which means it does not fit well into the mandate of existing agencies such as the Canadian Institutes of Health Research, the National Science and Engineering Research Council, the Social Sciences and Humanities Research Council, and the Canadian Police Research Centre. This lack of research funding is a pivotal concern for Canada's forensic sciences because it leads logically to an impoverished research culture. Unsustainable workloads and diminished travel budgets are also part of this equation.

Establishing a robust culture of research and development in Canada's forensic science community is a vital first step to expanding Canada's educational offerings in these disciplines. At the moment, Canada faces grave challenges in recruiting and retaining high-quality faculty and students, many of whom can find fully funded opportunities to work and study in Europe or the United States. Research is important in preparing Canada to respond to new technologies, advance its disciplines, and train new generations of practitioners.

Recommendations

Forensic science has evolved from parent core-scientific disciplines, but the overarching principles of these disciplines still apply – that is, creating a healthy intellectual climate means embracing the virtuous cycle of service, teaching, and research. There is no shortage of casework to be done in Canada at the moment, although there are certainly challenges in meeting these needs with our small and geographically scattered workforce. There are also many scientific discoveries to be made and young minds to nurture. As much attention should be paid to teaching and research as to case work, because they are equally important. To that end, the authors make the following recommendations:

- Canada's forensic science community should work with governments to establish a sustainable culture of research.
- Canada should establish its own training programs for forensic scientists. These programs must educate the next generation of practitioners but should also include continuing education opportunities and multidisciplinary cross-training for scientists, police, lawyers, and judges.
- Canada's forensic scientists should establish and continuously renew best practices in the following areas:
  - Clinical and practical guidelines and standards
  - Professional certification
  - Accreditation standards and programs
  - Culture of scientific neutrality
  - Systemic response to error (when error occurs)
- Canada should address pressing administrative and regulatory issues, including the following:
  - Development of memoranda of understanding (MOUs) between fee-for-service forensic scientists and service end-users
  - Creation of funding models that emphasize full-time personnel over fee-for-service providers
  - Alignment of best practices in forensic pathology with death investigation policies and procedures
  - Establishment of workload standards
  - Implementation of peer-review and other quality-management systems

- Development of career paths for FI officers that will allow long-term professional commitment to the important activity of FI

Conclusion

The science that underlies so many court cases in Canada requires scrutiny. The volunteerism, good intentions and ad hoc organizational efforts of Canada's forensic scientists are no substitute for a thoughtfully designed system of service delivery. Other jurisdictions, including the United States, have begun the process of critically evaluating these systems in their respective countries, and Canada cannot afford to lag behind in this respect. Why is this so important? Because Canadians hold that peace, order, and good government are their most fundamental values. Establishing and maintaining a just peace is thus *the* core mission of government. And a just peace cannot rest on a foundation of bad science.

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Contributors to the larger publication, *Forensic Science in Canada: A Report of Multidisciplinary Discussion*, include those listed below.

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I have read with interest the article "Who Owns Diagnostic Specimens" by Ogbogu and Mengel in your issue of Fall 2013. It appears that their concern is to control and limit the use of surgically excised tissue specimens and protect their exploitation by unidentifiable person or organizations in undefinable circumstances, in a legal situation which is itself undefined. This is an impossible task; no one can predict what the law may do in such a situation, or protect against its acts. The best we can do in such circumstances is firmly to define what we considered to be the most desirable situation and keep a careful watch on any legal proceedings that may arise in our own or related jurisdictions; the most likely source by far is that fertile field of medicolegal litigation, the United States. At present it is necessary to trust someone or something, and this could be done in the consent form of the institution, signed by the patient or surrogate. Ownership is defined in law; I am not aware of any legal definition of *stewardship*. In my *Shorter Oxford English Dictionary*, *steward* occupies a half-column of small print ranging over a number of interesting situations but nowhere touching on the one in question.

The situation would be clarified by the inclusion in the institutional consent form of a section such as this: "Any material removed from my body, including tissue, aspirate, or other bodily component or derivative shall become the property of [the institution] and may be used for diagnosis, teaching, research, or quality control provided that I am not personally identifiable in any publicly accessible report or publication."

This seems to me the best we can do in the present very undefined situation.

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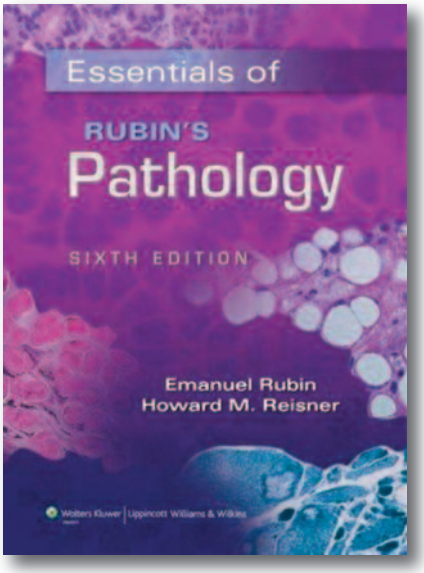
Essentials of Rubin's Pathology, 6th Edition



Emanuel Rubin and Howard M. Reisner
Lippincott Williams & Wilkins,
Philadelphia, 2014
ISBN: 978-1-4511-1023-4
704 pages
List price: $114.50

Essentials of Rubin's Pathology, Sixth Edition, serves as a companion to *Rubin's Pathology: Clinicopathologic Foundations of Medicine* and is aimed at students of the health professions.

As stated in the preface of this text, literacy in pathology is the bedrock of practice and research for the student of medical science. This assertion has special significance in the modern era of medical education, in which the teaching of pathology has slowly receded. Consequently, textbooks often provide most of the pathology education to interested medical trainees.

This text is structured similarly to its perhaps-better-known competitor *Pathologic Basis of Disease* by Robbins and Cotran; the first nine chapters are dedicated to general principles of pathology, and the following 20 chapters are assigned to individual organs or organ systems. Do not be misled by the size of this companion text – it is an easy read, packed with useful information. Most of the text is arranged as paragraphs, with scattered bulleted lists where appropriate. Each chapter is authored by well-known experts in the field. The depth of pathology details provided is surprising, given that this serves as an abridged version of a larger text. Each chapter is logically organized and makes effective use of icons and coloured subtitles to guide the reader. Gross photos and photomicrographs are superb and plentiful. Coloured illustrations (many are full page) helpfully illustrate several general concepts and key pathobiological processes.

Some more recent developments in pathology are not included in this general text. For example, there is no mention of the recently proposed changes to the classification of pulmonary adenocarcinoma, and more could be said on the topic of serrated polyps of the colorectum. Furthermore, the statement that there is no identifiable precursor to high-grade serous carcinoma of the ovary is perhaps inaccurate.

Overall, I would recommend this text to medical students and pathology residents. In my opinion, it is an impressively detailed pathology compendium and provides a solid foundation upon which to build one's pathology knowledge. For those especially interested, the larger text might be even more rewarding.

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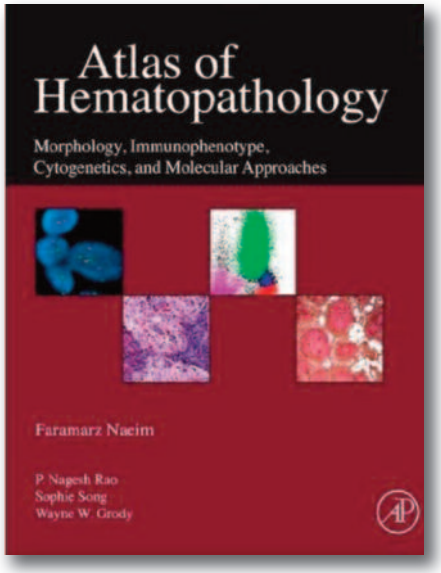
Atlas of Hematopathology: Morphology, Immunophenotype, Cytogenetics, and Molecular Approaches



Faramarz Naeim, P. Nagesh Rao, Sophie Song, and Wayne W. Grody
Elsevier Academic Press, Boston, 2013
ISBN: 978-0-12-385183-3
743 pages

Atlas of Hematopathology is a well-organized text focusing on the typical features demonstrated by hematological disorders. Information is presented in logical sequence: morphology, immunophenotype, genetic studies, and differential diagnosis. There are abundant representative illustrations of blood and marrow findings, and these are often supplemented by high-quality images of cytogenetic and molecular tests. Immunophenotypic findings are mainly based on flow cytometry with supplemental immunohistochemistry where appropriate. A limited number of images of cytochemical findings are provided, in keeping with current practice. The text is concise, focusing on the most important diagnostic features, allowing the reader to quickly review a disease entity.

The text begins by providing a brief review of the structure and function of hematopoietic tissues. The principles underlying the ancillary tools utilized by hematopathologists (flow cytometry, immunohistochemistry, genetic studies) are also reviewed in this section. The remainder of the text primarily addresses neoplastic hematopathology, both bone marrow diseases and lymphomas. Benign disorders are not emphasized.

This text utilizes the *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues*, Fourth Edition (2008). Although not numerous, many useful tables are provided, outlining the World Health Organization diagnostic criteria or key features in differential diagnosis. Prognostic features are included for many disorders. Appropriately, there is little information about treatment.

This book is easy to read, and oncologists and clinical hematologists, as well as trainees in these programs, will find this a useful resource. The text can provide practising pathologists and trainees in pathology a quick review of the important diagnostic considerations. In addition, *Atlas of Hematopathology* will be a useful addition to the library of physician educators since it provides a concise logical approach that is an excellent framework for teaching.

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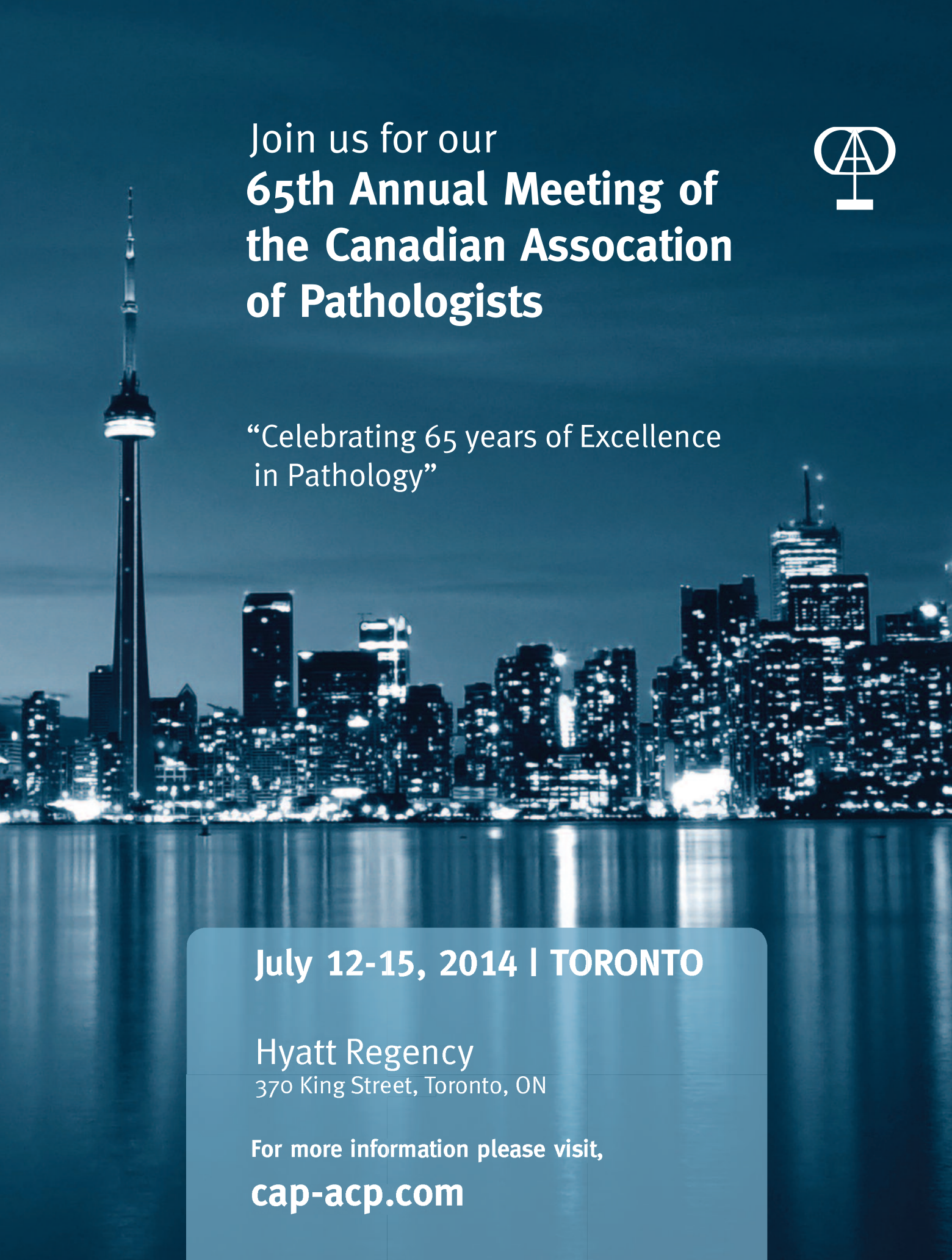

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