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About the Cover

The cover image shows interphase FISH of paraffin sections of the breast carcinoma, showing one green/CEP17 and one to three orange/HER2 signals in the cells.

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The HER2 oncogene encodes for a receptor tyrosine kinase, which is amplified in approximately 15–20% of breast cancers. Patients with a breast cancer that over-expresses and/or is amplified for HER2 have a worse prognosis than those without such change and are eligible to receive the therapeutic agent trastuzumab, which specifically targets the gene. Given the prognostic importance and predictive utility of HER2, it is routinely evaluated on all newly diagnosed breast cancers and is recommended for evaluation in the metastatic setting and in specimens post neo-adjuvant therapy (NAT). Given the importance of this assay, the College of American Pathologists (CAP) and the American Society of Clinical Oncology (ASCO) have jointly issued guidelines for the performance and interpretation of this assay. Central to these guidelines is an algorithmic approach to the evaluation of HER2, commencing with an immunohistochemical (IHC) evaluation of the HER2 protein with an additional assay for the evaluation of the HER2 gene by in situ hybridization (ISH) for those cases with equivocal results (2+) by IHC. Canadian guidelines have also been published that mirror in large part the CAP/ASCO guidelines, with emphasis on the pre-analytical, analytical, and quality assurance (QA) procedures recommended for Canadian laboratories assessing HER2. In this issue of the journal, Dr. Hanna presents the findings of a national survey conducted to evaluate the compliance of Canadian laboratories with the above guidelines, the results of which are both interesting and informative.

Thirty-six pathologists from four regions of Canada with a special interest in breast pathology and self-identified to have a leading role in HER2 testing in their respective laboratories were invited to participate in an online survey developed jointly by Dr. Hanna, F. Hoffmann–La Roche and Summit Strategy. Approximately two thirds of the invitees, representing 22 different laboratories, participated in the survey. Many of the results are very encouraging: 82% of centres reported testing all newly diagnosed invasive breast cancers, all centres used neutral buffered formalin as the fixative of choice, 82% of centres met the recommended cold ischemic time (defined as the time from specimen excision to the initiation of tissue fixation) of ≤1 hour, and a similar number of centres reported fixing large excision specimens for the optimal time period (24–48 hours). The majority of the testing was reported to be performed on definitive surgical specimens, with core-needle biopsy specimens used in cases of locally advanced disease, patients being considered for NAT and for metastatic biopsy specimens or when the definitive surgical specimen was too small or considered too poorly fixed for optimal IHC evaluation. All laboratories were accredited to perform the tests, with 20 meeting the recommended volume of cases. In addition, the average rates of reporting of negative, equivocal, and positive cases by IHC were broadly in accordance with those published in the literature. Equivocal (2+) cases were re-evaluated with an alternative ISH method in the majority of laboratories. Encouragingly, all centres reported participation in a QA program (provincial, national, or international) for HER2 testing, and the concordance rates between local and reference laboratories for all sites were ≥95%.

However, the results were not uniformly positive and highlight specific needs that should be addressed. The fact that 50% of the respondents were from one province suggests that large swaths of the country are under-represented or not represented in this survey, introducing a potential regional reporting bias. In almost one fifth (18%) of laboratories polled, not all newly diagnosed invasive breast cancers were tested for HER2, despite its proven prognostic and predictive utility. In two centres, cases identified as being 2+ or indeterminate by IHC were not subject to any further evaluation and were apparently left unresolved; clearly this is suboptimal. While the average proportion of cases reported as negative, indeterminate, and positive are in line with the published guidelines alluded to above, the actual variance is

Competing interests: None declared
huge, with at least one centre reporting that 52% of cases evaluated by IHC fell into the 2+ or indeterminate category, thus requiring ISH for assessment of the HER2 gene status. Such reportedly high rates of indeterminate results suggest either analytical issues with the IHC assay in use or observer uncertainty. The former may result in part from the lack of a single standard antibody in use across all centres or as a result of the potential confusion and discrepancy added when more than one antibody is employed (64% of responding centres reported using more than one antibody in their initial evaluation of HER2).\(^6\) Observer uncertainty and over-reliance on the 2+ category may speak to the need for more pathology education around the interpretation and reporting of HER2 IHC, or fewer pathologists at any one site reading and interpreting the result. Currently, there is no accepted “minimum” number of HER2 samples an individual pathologist must report annually to be considered proficient. These high rates of 2+ IHC are not without consequences, leading to reflex evaluation of a case by ISH and, hence, added expense and increase in the turnaround time, which in 45% of on-site and 68% of off-site centres was greater than 10 working days. A delay in turnaround time such as this may hinder clinical management or patient eligibility for clinical trials.\(^9\) Other factors hindering or limiting the delivery of a quality service for both IHC and ISH HER2 testing are fiscal constraints and manpower restrictions.

In summary, a select review of HER2 testing across Canada suggests good compliance with national and international guidelines, while simultaneously pointing to areas that require improvement and increased resource allocation.

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References
L’oncoprotéine HER2 est un récepteur membranaire appartenant à la famille des facteurs de croissance épidermique de type tyrosine kinase; le gène qui code pour cette protéine est amplifié dans 15 % à 20 % des cancers du sein. Cette amplification qui conduit à la surexpression de la protéine HER2 est associée à un pronostic clinique défavorable; la patiente est admissible au traitement par le trastuzumab, dirigé précisément contre le gène en question. Au vu de l’importance pronostique et de l’utilité prévisionnelle de la protéine HER2, il est désormais d’usage d’évaluer son expression dans le cas de cancer du sein nouvellement diagnostiqué, et cette évaluation est recommandée en présence de métastases et après la chimiothérapie néoadjuvante. Étant donné l’importance de l’analyse de l’expression de l’oncoprotéine, le College of American Pathologists (CAP) et l’American Society of Clinical Oncology (ASCO) ont publié ensemble des lignes directrices sur l’exécution et l’interprétation de ce test. La démarche préconisée par les lignes directrices repose sur un algorithme; elle commence par l’analyse en immunohistochimie de la protéine HER2 et se poursuit par l’hybridation in situ quand le résultat de l’analyse immunohistochimique est équivoque (2+). Les lignes directrices canadiennes reprennent l’essentiel des lignes directrices américaines tout en insistant sur les normes et méthodes s’appliquant à tous les aspects de l’analyse de l’expression de l’oncogène HER2 et à l’assurance de la qualité de la procédure dans les laboratoires canadiens.

Dans le présent numéro, le Dr Hanna présente les résultats d’un sondage pancanadien sur la conformité des laboratoires au résultat équivoque (2+) selon la méthode d’hybridation in situ. De plus, il est réjouissant de constater que tous les laboratoires adhèrent à un programme d’assurance de la qualité de l’analyse de l’expression de l’oncogène HER2, provincial, canadien ou étranger, et que le taux de concordance entre le laboratoire et la norme de référence est de 95 % à tout le moins. Toutefois, les résultats ne sont pas positifs sur tous les plans, ils font ressortir des lacunes qui devraient être comblées. Comme 50 % des répondants sont de la même province, une grande partie du pays est sous-représentée ou non représentée dans le sondage, d’où le risque de distorsion régionale des résultats. Près du cinquième (18 %) des laboratoires interrogés ne procède pas à l’analyse de l’expression de la protéine HER2 dans tous les cas de cancer du sein invasif nouvellement diagnostiqués, en dépit de son utilité pronostique et prévisionnelle avérée. Deux

Aucun conflit d’intérêts à declarer
laboratoires n’approfondissent pas l’évaluation du cas dont le résultat immunohistochimique est 2+ ou de nature incertaine; manifestement, cette façon de faire laisse à désirer. Bien que les taux de résultat négatif, indéterminé et positif concordent en moyenne avec les taux dont il est question dans les lignes directrices mentionnées ici, en réalité, l’écart est énorme parfois comme l’illustre ce centre où 52 % des cas évalués à l’immunohistochimie se rangent dans la catégorie du résultat 2+ ou indéterminé et font l’objet de l’hybridation in situ. Un si grand taux de résultat indéterminé laisse entrevoir des problèmes d’ordre technique dans l’analyse immunohistochimique ou l’incertitude de l’examinateur. Les premiers s’expliquent peut-être par le fait que les laboratoires n’ont pas recours au même anticorps standard ou par la confusion ou la divergence potentielle découlant de l’utilisation de différents anticorps (64 % des répondants s’en remettent à plus d’un anticorps dans l’analyse initiale de l’expression de la protéine HER2). L’incertitude de l’examinateur et la nette propension à classer les résultats dans la catégorie 2+ soulignent sans doute la nécessité d’approfondir la formation à propos de l’interprétation et de la présentation des résultats de l’analyse immunohistochimique de l’expression de l’oncogène HER2 ou de réserver l’interprétation de ce test à un nombre restreint de pathologistes du laboratoire. À l’heure actuelle, il n’y a pas de norme quant au nombre « minimal » annuel convenu d’analyses établissant la compétence professionnelle en la matière. Les taux élevés de résultats 2+ ne vont pas sans conséquence, puisqu’ils entraînent habituellement une deuxième évaluation par hybridation in situ et de ce fait, des dépenses supplémentaires et l’allongement du délai d’exécution de l’analyse de la protéine, lequel excède les 10 jours ouvrables pour 45 % des tests effectués sur place et 68 % des tests effectués à l’extérieur. Le délai de cette longueur peut avoir des répercussions sur la prise en charge clinique ou l’admissibilité de la patiente à un essai clinique. Enfin, il faut savoir que les compressions budgétaires et la réduction de l’effectif sont d’autres facteurs susceptibles d’entraver la prestation de services de qualité dans l’analyse immunohistochimique ou par hybridation in situ de l’expression de la protéine HER2.

En bref, le sondage sur l’analyse de l’expression de l’oncogène HER2 au Canada révèle que la procédure se conforme dans une bonne mesure aux lignes directrices canadiennes et étrangères, mais il met en relief des aspects à améliorer et pour lesquels il y aurait lieu d’accroître les ressources.

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Références
Evolution of HER2 Testing in Canada

Wedad M. Hanna, MD

ABSTRACT

Purpose: The accuracy of HER2 testing plays a critical role in identifying patients who may benefit from HER2-directed therapy. Canadian Consensus for HER2/neu Testing Guidelines in Breast Cancer was published in 2007 with the aim of improving the accuracy of HER2 testing across Canada.

Method: The authors surveyed Canadian pathologists with responsibility for HER2 testing of breast cancer samples to assess the impact of the Canadian consensus guidelines on current HER2 testing procedures. Thirty-six expert pathologists were invited to participate in an online survey between April 19 and May 28, 2010.

Results: Responses were received from 22 pathologists (61%). All but one of the respondents performed HER2 testing in accordance with American Society of Clinical Oncology/CAP/Canadian consensus guidelines and used the same HER2 testing algorithm in the adjuvant and metastatic settings. All respondents participated in a quality assurance program, and concordance between HER2 testing results at local and central testing centres was high (≥95%). In addition, the survey highlighted several practical considerations – including sample collection and fixation issues – that may help to preserve, and improve upon, current good practice.

Conclusions: Overall, survey responses indicate nationwide adherence to recommended practice guidelines for HER2 testing in breast cancer, resulting in excellent inter-laboratory concordance.

RÉSUMÉ

But : L’exactitude dans la détermination du statut HER2 revêt une importance primordiale dans la sélection des patientes à qui offrir le traitement conçu pour s’opposer à l’oncogène. Les groupes qui se sont alliés dans la publication en 2007 de lignes directrices consensuelles sur la détermination du statut HER2 dans le cancer du sein avaient ainsi à cœur d’améliorer la précision de l’analyse de l’expression de la protéine HER2 au Canada.


Résultats : Vingt-deux pathologistes (61 %) ont participé au sondage. Tous, à l’exception d’un seul, exécutent l’analyse de l’expression de la protéine HER2 conformément aux lignes directrices de l’American Society of Clinical Oncology, de l’Association canadienne des pathologistes et du consensus canadien, et ils appliquent le même algorithme d’analyse au
Human epidermal growth factor 2 (HER2) is an important prognostic and predictive biomarker in breast cancer.\textsuperscript{1,2} Determination of HER2 status is recommended for all patients with invasive breast cancer at diagnosis.\textsuperscript{3} Consequently, the accuracy of HER2 testing is of great importance in treatment decision making.\textsuperscript{4}

The Canadian Consensus for HER2/neu Testing Guidelines in Breast Cancer was published in 2007,\textsuperscript{5} based on the experience of a group of Canadian pathologists and on the American Society of Clinical Oncology/College of American Pathologists Guideline Recommendations for HER2/neu Testing in Breast Cancer.\textsuperscript{6} The Canadian guidelines cover all aspects of HER2 testing, including pre-analytical steps (tissue handling and fixation), assay validation, post-analytical interpretation criteria, and training and quality assurance (QA) requirements.\textsuperscript{5} It was anticipated that widespread adoption of the Canadian guidelines would further improve the accuracy of HER2 testing in Canada. It is important to note, however, that HER2 testing policies and funding/reimbursement criteria differ between Canadian provinces.\textsuperscript{7}

The Canadian National Breast Cancer Pathology Survey was conducted to assess the current status of HER2 testing practices and procedures across Canada as a follow-up to publication of the Canadian HER2 testing guidelines. A description of the survey and a summary of the results are presented here.

**Methods**

We identified approximately 1,200 pathologists currently practising in Canada. From these we selected 36 pathologists, representative of all four regions of Canada (Ontario, Western, Quebec, and Atlantic), known to be involved in breast cancer pathology. Selected pathologists were required to hold responsibility for HER2 testing procedures, decisions, and quality assurance (QA) at a specific centre, or have a lead role in regional HER2 testing. The selected pathologists received an invitation to participate in an online survey between April 19 and May 28, 2010.

The content of the survey was developed by the author, with survey questions drafted by F. Hoffmann-La Roche Ltd. and Summit Strategy (a market research company). Prior to distribution, the survey was reviewed by the author in order to confirm the appropriateness and validity of the questions.

**Results**

**Survey Respondents**

The response rate for the survey was 61% (22/36 pathologists). These respondents represented centres in the Ontario \((n = 11)\), Western \((n = 7)\), Quebec \((n = 3)\), and Atlantic \((n = 1)\) regions of Canada. All but two respondents represented centres that provided a testing service for other hospitals.

In accordance with the Canadian guidelines, the majority of responding centres (91%) tested the required minimum of 250 samples in the 12 months preceding the survey (range: 250 to \(\geq 2,500\) samples per year).\textsuperscript{3} All other centres tested at least 150 samples per year and fulfilled QA requirements.

**Criteria for HER2 Testing at Diagnosis**

With the exception of patients diagnosed with ductal carcinoma in situ – who would not normally be tested for
EVOLUTION OF HER2 TESTING IN CANADA

HER2 status – most respondents (82%) tested the HER2 status of all breast tumours at the time of diagnosis. Samples were excluded only if microinvasive carcinoma was present, the patient was not eligible for adjuvant therapy, the clinician did not request HER2 testing, or the specimen was poorly fixed.

Are Core Needle Biopsies Used for HER2 Testing?
Most centres (82%; 18/22) carried out HER2 testing using core needle biopsies (CNBs) in addition to surgical excision samples. The most commonly cited reasons for testing CNBs included the patient being considered for neoadjuvant therapy (56%; 10/18), the presence of metastases (22%; 4/18), and the diagnosis of locally advanced breast cancer (17%; 3/18). Surgical specimens of insufficient size, inferior quality, or with poor fixation also led to CNB testing. In the absence of a CNB, 23% of respondents used a fine-needle aspirate (FNA) for testing. For patients with metastatic disease, 59% of respondents often or always used an archived tumour block for testing in cases where a HER2 test result was not available for the primary tumour. However, where an archived block was unavailable, most respondents (59%) obtained a biopsy, providing that the metastases were accessible. Other major factors limiting HER2 testing of metastases were small size of the biopsy, a compromised biopsy, and requirement for decalcification of bone tissue.

Sample Collection and Fixation
Following surgical excision, 68% of respondents reported that the breast specimens were sliced and fixed within 1 hour, while 23% took between 1 and 2 hours (Figure 1). All respondents used 10% neutral buffered formalin as a fixative in the laboratory. Most respondents (82%) fixed large excision specimens for 24–48 hours, with only 9% fixing excision specimens for less than 24 hours prior to tissue processing (Figure 2). As expected, the average fixation time for CNBs was shorter: most respondents (74%) reported a fixation time of at least 8 hours for CNBs, while 14% specified a minimum fixation time of 24 hours.

Preferred Primary HER2 Testing Methodologies
Immunohistochemistry (IHC) was used as the initial HER2 testing methodology by 91% (20/22) of respondents. Others
used silver-enhanced in situ hybridization (SISH) (5%; 1/22) or another, unspecified method (5%; 1/22). Most IHC testing was automated (86%; 19/22), with only 14% (3/22) favouring manual or mixed techniques. Half of all respondents used the rabbit monoclonal 4B5 HER2 antibody as their primary antibody (Figure 3). A secondary antibody was used by 64% (14/22) of respondents; the most widely preferred were the SP3 monoclonal antibody (NeoMarkers) and the AO485 polyclonal antibody (DAKO), each used by 43% (6/14) of respondents.

Confirmatory (Reflex) Testing Methodologies
Following an IHC 2+ Test Result
In the 20 centres that used IHC as their primary testing methodology, samples scored as IHC 2+ were retested using fluorescence in situ hybridization (FISH) (75%), SISH (20%), or chromogenic in situ hybridization (CISH) (5%). Automated ISH testing was used by 45% of respondents, with others using a range of manual and semi-automated procedures. Respondents who did not perform confirmatory testing for IHC 2+ samples cited sample availability and funding restrictions as limiting factors (Figure 4).

After FISH testing, 55% (12/22) of respondents retested samples with an equivocal FISH ratio (1.8–2.2), and 23% of respondents (5/22) performed confirmatory testing for samples with a HER2/chromosome 17 ratio of 2.2 exactly.

HER2 Status in Canadian Patients with Breast Cancer
Overall, the average proportion of IHC 0/1+, IHC 2+, and IHC 3+ test results was 65% (range 38–80%), 23% (range: 2–52%), and 12% (range: 3–20%), respectively (Figure 5). When ISH retesting of the IHC 2+ samples was taken into account, the average overall HER2-positivity rate (IHC 3+ and IHC 2+/ISH-positive) was 17.6%.

Figure 3. Preferred HER2 primary antibody used for immunohistochemistry testing. (Note: Some respondents selected more than one antibody.)

Figure 4. Identified restrictions to performing confirmatory HER2 tests. (Note: Respondents were asked to indicate all that apply.)

Figure 5. Stratification of initial HER2 test results.
Application of HER2 Testing Algorithm in Patients with Early and Metastatic Breast Cancers

All but one of the respondents reported performing HER2 testing in accordance with American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP)/Canadian guidelines and used the same HER2 testing algorithm in the adjuvant and metastatic settings.

Subsequent Retesting of HER2 Status

Approximately two thirds of respondents retested HER2 status upon completion of neoadjuvant treatment. The majority of those who did not retest specified that this was not requested by the clinician/oncologist. All centres retested the HER2 status of patients who developed metastases following adjuvant therapy for HER2-negative disease. The rate of change in HER2 status between the adjuvant and metastatic settings was reported to be 1–4% by most pathologists (Figure 6).

Turnaround Time

IHC test results were provided within 3–5 working days in 55% of on-site testing centres, with results available within 24 hours at one centre. In a further 27% of on-site centres, IHC results were available within 6–10 working days, with 14% reporting an average turnaround time of more than 10 working days. The minimum turnaround time for IHC HER2 tests performed at off-site testing facilities (32% of respondents) was 5–7 working days. Most (59%) off-site testing centres provided results within 5–10 working days of surgery, but 23% reported an average turnaround of more than 10 working days.

Most centres (68%) performed only one batch of ISH tests per week. Consequently, turnaround times for ISH-based HER2 testing were comparatively slower than for IHC testing: only 9% of on-site testing facilities and 5% of off-site testing centres provided ISH test results within 5–7 working days of surgery. An average turnaround time of more than 10 working days was reported for 45% of on-site centres and 68% of off-site centres.

Internal and External Monitoring of Testing Quality

A variety of QA programs are in place across Canada, and all respondents participated in a QA program. While national and international programs were most popular in Western Canada, Quebec, and Atlantic Canada, pathologists in Ontario favoured the provincial program (Table 1).

Reported concordance rates for HER2-positivity and negativity were ≥95% for all centres (local versus central testing). The most commonly reported challenges to the improvement of HER2 testing quality were a lack of funding/resources and a lack of time/manpower (Table 2).
Our survey of pathologists responsible for HER2 testing in breast cancer indicates a high level of compliance with recommended Canadian practice guidelines for HER2 testing. HER2 testing was performed in accordance with ASCO/CAP/Canadian consensus guidelines by all but one of the 22 respondents, and a consistent HER2 testing algorithm was applied in the adjuvant and metastatic settings. In addition, high concordance between local and central HER2 testing centres in external QA programs indicate that, overall, HER2 testing performed by Canadian centres appears accurate and reliable.

In accordance with Canadian guidelines, most respondents processed more than the recommended minimum of 250 samples during the preceding 12 months. However, some centres report performing in excess of 2,500 tests per year, and it is important that such centres have sufficient resources to manage this demand for HER2 testing in order to preserve test quality. In the majority of Canadian centres, HER2 status is routinely tested at the time of diagnosis.

Our results suggest that pathologists are concerned about the potential impact of delayed and inadequate tissue fixation on the accuracy of HER2 testing. Respondents commented that surgeons should be educated regarding the importance of prompt receipt of large specimens in the histopathology laboratory, following excision from patients, to allow for appropriate handling and immediate fixation. In addition, while a fixation time of 6–48 hours is recommended for both excisions and CNBs, there appears to be no clear consensus regarding the optimum fixation time for CNBs.

Automated IHC testing is the initial methodology in the majority of Canadian centres. Conversely, about half of ISH testing is performed manually. Confirmatory testing is generally performed in accordance with HER2 testing guidelines (i.e., samples with equivocal HER2 status either by IHC or FISH). However, a small proportion of pathologists identified restrictions, other than sample availability, to confirmatory retesting in their laboratory. Confirmatory retesting for equivocal cases is essential, and use of reference centres is a clear choice where confirmatory retesting cannot be performed on-site.

The accuracy and sensitivity of IHC testing can be affected by pre-analytical and analytical procedures, as well as by

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**Table 1. Percentage of Respondents Participating in Various QA Programs**

<table>
<thead>
<tr>
<th>QA Program*</th>
<th>All (n = 22)</th>
<th>West (n = 7)</th>
<th>Ontario (n = 11)</th>
<th>Quebec (n = 3)</th>
<th>Atlantic (n = 1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>National</td>
<td>68</td>
<td>100</td>
<td>36</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Provincial</td>
<td>59</td>
<td>29</td>
<td>91</td>
<td>33</td>
<td>–</td>
</tr>
<tr>
<td>College of American Pathologists</td>
<td>59</td>
<td>71</td>
<td>36</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Local</td>
<td>45</td>
<td>43</td>
<td>55</td>
<td>33</td>
<td>–</td>
</tr>
<tr>
<td>Nordic QC</td>
<td>27</td>
<td>43</td>
<td>18</td>
<td>–</td>
<td>100</td>
</tr>
<tr>
<td>UKNEQAS</td>
<td>14</td>
<td>29</td>
<td>9</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Others</td>
<td>13</td>
<td>–</td>
<td>18</td>
<td>33</td>
<td>–</td>
</tr>
</tbody>
</table>

*Multiple responses accepted.

QA = quality assurance.

**Table 2. Top Challenges Faced by Pathologists in Terms of QA Progress in HER2 Testing**

<table>
<thead>
<tr>
<th>Most Frequently Mentioned Challenges</th>
<th>Percentage (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of funding/budgets/resources</td>
<td>48</td>
</tr>
<tr>
<td>Lack of time/manpower/HR</td>
<td>45</td>
</tr>
<tr>
<td>Consistency of pre-analytical issues</td>
<td>25</td>
</tr>
<tr>
<td>Pathology subjectivity/opinions/interpretation</td>
<td>20</td>
</tr>
<tr>
<td>Quality/reliability of antibodies</td>
<td>19</td>
</tr>
<tr>
<td>Time/limited time to develop QA monitoring</td>
<td>14</td>
</tr>
<tr>
<td>Ongoing assessment and competency</td>
<td>14</td>
</tr>
<tr>
<td>Tumour heterogeneity</td>
<td>10</td>
</tr>
<tr>
<td>Amplification of HER2 and CEP17</td>
<td>10</td>
</tr>
<tr>
<td>External QC/ensuring external testing has QC in place</td>
<td>10</td>
</tr>
<tr>
<td>Variable fixation rates</td>
<td>10</td>
</tr>
<tr>
<td>No authority/support to implement changes</td>
<td>10</td>
</tr>
<tr>
<td>Others (miscellaneous mentions)</td>
<td>30</td>
</tr>
</tbody>
</table>

HR = human resources; QA = quality assurance; QC = quality control.
antibody sensitivity. Results should always be issued based on an approved antibody. In the event of discordance between antibodies, reflex testing should be performed using ISH. One possible area of concern is the variability in specimen-handling procedures and testing methodologies, which could account for inter-laboratory discrepancies.

Stratification of initial IHC HER2 testing results revealed substantial variation between centres in the number of samples graded as IHC 0/1+ (38–80%), IHC 2+ (2–52%), and IHC 3+ (3–20%). It seems unlikely that this variability reflects true regional variations in HER2 status. Therefore, we may assume that these results highlight technical difficulties in some testing centres. Certainly, some centres reported relying heavily on reflex testing for result confirmation. Improvements in training and standardization of testing methodologies may help to reduce the need for reflex testing.

All respondents to the survey retested HER2 status in patients with metastases, an observation that reflects the importance of high-quality HER2 testing in treatment decision making. While it is unusual for HER2 status to change during disease progression, this possibility should not be discounted. The majority of respondents retested HER2 status in patients who had undergone neoadjuvant therapy, although this is not a requirement of the Canadian or ASCO/CAP guidelines. Such retesting is important to further our understanding of the clinical impact of HER2-directed therapy.

Over half of on-site testing centres were able to obtain HER2 test results within 3–5 working days. However, around a quarter of IHC tests conducted at off-site centres took more than 10 working days. Clearly, improvements in turnaround time are desirable, particularly for off-site testing centres, as longer turnaround times may have a clinical impact for patients awaiting treatment.

In conclusion, accurate and standardized assessment of HER2 status is essential to identify patients who may benefit from HER2-directed therapy. Overall, survey responses indicate nationwide adherence to recommended practice guidelines for HER2 testing in breast cancer, resulting in excellent inter-laboratory concordance.

Acknowledgements
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References
False-Positive FISH for HER2 Amplification in a Breast Cancer Owing to Loss of CEP17: A Case Report and Literature Review

Jie Xu, PhD, FCCMG, Helen Ettler, MD, FRCP(C), Peter Engbers, MD, FRCP(C)

ABSTRACT
The authors present a case of breast cancer with equivocal status for HER2 protein overexpression by initial immunohistochemistry (IHC). Dual-colour fluorescence in situ hybridization (FISH) of paraffin sections of the tumour showed a HER2/CEP17 ratio of 2.6:1, indicative of HER2 gene amplification. However, this was interpreted as a false-positive test due to a loss of CEP17 in 92% of the cells; this was supported by a negative result on repeat IHC testing. Monosomy 17, as indicated by single CEP17, has been reported in 1–6% of patients with breast cancer and can result in false-positive result on FISH. There have been no national guidelines on how to manage and interpret such findings. The literature suggests that when a possible false-positive result on FISH for HER2 amplification due to a loss of CEP17 is indicated, a confirmation by repeat IHC testing, single-colour FISH, or array comparative genomic hybridization (CGH) can help one to make an accurate interpretation and a final decision on whether the patient is eligible for treatment with trastuzumab. More case reports and large clinical studies would help to correlate FISH with clinicopathological findings and to determine how patients with breast cancer with single CEP17, with or without HER2 gene amplification, respond to treatment with trastuzumab or other drugs.

RÉSUMÉ
Les auteurs présentent un cas de cancer du sein pour lequel l’analyse immunohistochimique initiale ne parvient pas à se prononcer quant à la surexpression de la protéine HER2. L’hybridation in situ en fluorescence à deux couleurs de la tumeur en coupes sur paraffine illustre un rapport HER2 par centromère 17 (CEP17) de 2,6 pour 1, qui laisse entrevoir l’amplification du gène HER2. Cependant, le résultat est interprété comme étant faussement positif en raison de la perte de CEP17 dans 92 % des cellules. Cette interprétation se trouve corroborée par le résultat négatif de l’analyse immunohistochimique subséquente. La monosomie 17, soit la présence d’un seul CEP17, est le lot de 1 % à 6 % des cas de cancer du sein; cet état peut entraîner un résultat faux positif à l’hybridation. Il n’y a pas de lignes directrices applicables à l’échelle du pays qui balisent l’interprétation de cette constatation, ni la conduite à tenir à la suite. La documentation propose de faire suivre l’hybridation in situ
Amplification and overexpression of human epidermal growth factor receptor 2 gene (HER2) occur in approximately 18–20% of human breast cancers and indicate a poor prognosis. Accurate assessment of HER2 status in breast cancer patients is critical for decision making in targeted therapy with trastuzumab (Herceptin, Genentech, Inc., South San Francisco, California; Hoffmann-La Roche Ltd., Basel, Switzerland) and lapatinib (Tykerb, GlaxoSmithKline, Philadelphia, Pennsylvania). Dual-colour fluorescence in situ hybridization (FISH) using a probe for HER2 (located at 17p11.2) and a control probe for centromere 17 (D17Z1/CEP17) is now a standard test, and HER2 amplification is indicated if the HER2/CEP17 signal ratio is $\geq 2.00$, as recommended by the US Food and Drug Administration (FDA), or $> 2.2$, according to the Guidelines of the American Society of Clinical Oncology and College of American Pathologists, (ASCO/CAP). Among the factors affecting interpretation of HER2 status is the finding of a single CEP17 signal. This is commonly referred to as monosomy 17 and can lead to artificial skewing of the HER2/CEP17 ratio and, thus, a false-positive result for HER2 gene amplification. There have been no national guidelines on how to manage such cases. More case studies would help to increase the understanding of mechanisms for loss of CEP17, establish appropriate test algorithms, and correlate FISH results with clinicopathological findings. Here we present a case with a false-positive FISH result, and a review of the literature.

Case Report
A 57-year-old woman was diagnosed with invasive mammary carcinoma and underwent mastectomy with sentinel lymph node biopsy. Grossly there was a firm 1.4 $\times$ 1.3 cm grey-white subareolar mass with no connection to the nipple or skin. No other abnormality was noted, and there were a total of six lymph nodes, including two separately submitted sentinel nodes. Sections were submitted after 24 hours’ fixation in neutral buffered formalin. Microscopically the tumour was an invasive mammary carcinoma with lobular features. The combined histologic grade (Scarff-Bloom-Richardson) was II/III (score 6/9: nuclear pleomorphism 2, tubule formation 3, and mitotic score 1 with a mitotic count of <5/10 high-power fields [HPFs]).

Immunohistochemical analysis for HER2/neu protein overexpression was performed on slides prepared from paraffin-embedded sections of the tumour. In our laboratory, two antibodies are used routinely to ensure appropriate sensitivity and specificity. Initial tests showed no reaction with antibody A0485 (rabbit polyclonal antibody, Dako, at a dilution of 1:500) and equivocal (2+) reactivity for antibody CB11 (mouse monoclonal antibody, Vector Laboratories, at a dilution of 1:500) with complete membrane staining of weak to moderate intensity in 20% of the invasive carcinoma. Overall, HER2 protein overexpression was interpreted to be equivocal. Interphase FISH using Abbott Path HER2 kit with dual probes for HER2 and CEP17 was also conducted on paraffin sections with the target area marked by the pathologist. A total of 90 cells were scored by three cytogenetics technologists and the results were interpreted by a certified cytogeneticist (J.X.) and included in the final pathology report.

Results
Only one CEP17 signal was seen in 92% (83/90) of the cells (Figure 1A), and 60% of the cells had 1 CEP17 and 3 HER2 signals. A small number of cells (6%) had 4 CEP17 signals, but none had $> 4$ CEP17 signals.
The FISH score showed a HER2/CEP17 ratio of 2.6, indicative of HER2 gene amplification according to the criteria of the FDA ($\geq 2.00$)\textsuperscript{1,2} or ASCO/CAP ($>2.2$).\textsuperscript{3} However, this was interpreted as likely a false-positive caused by loss of the centromeric signal in the vast majority of the cells and skewing of the HER2/CEP17 ratio.

Routine G-banding and FISH of 10 metaphase cells and 200 interphase cells of peripheral blood of the patient showed a normal constitutional female karyotype (46,XX) and, more specifically, a normal HER2 and CEP17 pattern with the 2 CEP17 signals being of approximately the same size (see Figure 1B–D). This confirmed that the loss of the CEP 17 seen in the breast carcinoma was an acquired aberration.

In light of the FISH finding in the breast cancer, repeat IHC was performed using antibody A0485 and a third antibody SP3 (rabbit monoclonal antibody, Thermo Scientific, dilution 1:100) because the performance of antibody CB11 was considered suboptimal. The result was clearly negative for overall HER2 protein overexpression: 1+ for both SP3 and A0485 with complete membrane staining of weak to moderate intensity in $<5\%$ of the invasive carcinoma. This negative result was consistent with the interpretation of the FISH test as a false-positive.

**Discussion**

It is difficult to obtain an accurate estimate of the prevalence of monosomy 17 (indicated by single CEP17) in breast cancer patients because reports vary in patient selection criteria, number of cells scored, testing methods, sample size, and even definition of monosomy 17. For example, in one study,\textsuperscript{7} monosomy 17 was based on 1.11 CEP17 signals per cell, but 1 copy of CEP17 in $>60\%$ of nuclei of invasive cells was required in another.\textsuperscript{7} Nevertheless, reported rates of monosomy 17 from large studies range from $\sim 1\%$ (2/189)\textsuperscript{4} to $\sim 5\%$ (56/1,170)\textsuperscript{8} to $\sim 6\%$ (102/1,783).\textsuperscript{7} Another term, “hypodisomy 17,” is widely used for cases with $\leq 2$ centromere 17s, including monosomy 17, and is defined as $\leq 1.75$\textsuperscript{4,8} or $<1.5$ CEP17 signals per cell. Hypodisomy is reported in 6\% (5/89)\textsuperscript{4} to 22.7\% (265/1,170)\textsuperscript{9} of breast cancer patients. Monosomy and/or hypodisomy 17 can result in skewing of a HER2/CEP17 ratio and a false-positive FISH result for HER2 amplification.

How does one determine a FISH test to be a false-positive? Table 1 shows several reported cases that had HER2/CEP17 ratios of $\geq 2.00$ or $>2.2$ but were interpreted as false-positive based on negative findings by IHC and/or array comparative genomic hybridization (CGH), or $\leq 2$ HER2 signals/cell by dual-colour FISH, or $<4$ HER2 signals/cell by single-colour FISH (INFORM HER2/neu probe; Ventana). For example, Wang et al.\textsuperscript{4} reported 2/189 cases with monosomy 17 (with 1.11 and 1.03 CEP17 signals per cell) but no HER2 protein
overexpression with scores of 1.8 and 1.9. Both cases had HER2 amplification by dual-colour FISH (with HER2/CEP17 ratios of 2.98 and 2.02) but no amplification by single-colour FISH (HER2 copy number 3.29 and 2.08 signals per cell). Corzo et al.\(^4\) reported a case with a HER2/CEP17 ratio of 2 resulting from 2 copies of HER2 and 1 copy of CEP17. This was not considered as amplification because of monosomy 17; the interpretation was supported by negative IHC. Recently Gunn et al.\(^6\) reported two false-positive cases: one case had a HER2/CEP17 of 2.86 but IHC of 1+, and another with HER2/CEP17 ratio of 2.7 and monosomy 17. In light of the questionable HER2 status by FISH and/or IHC, array CGH using probes for HER2 and the pericentric regions and 123 additional loci covering chromosome 17 were performed. Array CGH identified that both cases had loss of the complete p arm but no loss of 17q.

Studies by Gunn et al.\(^6\) demonstrate that array CGH has advantages over other methods in determining if a finding of a single CEP 17 signal is the result of loss of the centromeric probe, a chromosome segment with the probe, or the entire chromosome (monosomy 17). Therefore, CGH can be potentially used to classify so-called monosomy 17 patients into at least 3 cytogenomic subgroups, which will permit study of the clinical implications of each subgroup. It is possible, although rare, that breast cancer patients may have a constitutional reduction of centromeric copy number for D17Z1. In addition, the presence of constitutional mosaic markers involving centromere 17 has been well documented.\(^11\) Such constitutional cytogenomic rearrangements could contribute to a finding of aberrant interphase FISH patterns, including single CEP 17, in breast cancer cases. Therefore, for cases with all or the vast majority of interphase cells with a single CEP17, karyotyping and FISH of peripheral blood can be used to rule out the possibility of a constitutional basis. Some monosomy 17 cases are interpreted as HER2-positive. For example, Perez et al.\(^7\) reported that 100 of the 1,783 (6%) cases with monosomy 17 had FISH ≥2 or IHC 3+ and were interpreted as HER2-positive. Risio et al.\(^12\) suggested that HER2 amplified breast cancers with monosomy 17 were poorly responsive to trastuzumab-based treatment. The response rate was 53% in the patients with monosomy 17 in comparison with 92% in those with >2 centromeric signals (chromosome 17 eusomy/polysomy). This finding raises an interesting question as to whether monosomy 17 can serve as a biomarker for a subclassification of patients for diagnosis and treatment.

In conclusion, when dual-colour FISH indicates a possible false-positive result for HER2 amplification owing to loss of CEP17, confirmation by repeat IHC, array CGH, or single-colour FISH and consultation with the pathologists should be considered. This would help the interpretation of the HER2 status and a final decision on whether or not the patient is eligible for treatment with trastuzumab and lapatinib. Large clinical studies would help correlate the FISH results and clinicopathological findings and establish how breast cancer patients with single CEP17, with or without HER2 amplification, respond to treatment with trastuzumab or other drugs.

**Acknowledgements**

We thank the staff of the Cytogenetics Laboratory, London Health Sciences Centre, for technical assistance. The

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### Table 1. Reported Cases of False-Positive FISH Results from Loss of CEP17

<table>
<thead>
<tr>
<th>Reference</th>
<th>Dual-Colour, HER2/CEP17</th>
<th>Single-Colour, HER2/cell</th>
<th>IHC</th>
<th>aCGH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang et al.(^4)</td>
<td>2.98 (1.11 CEP17/cell)</td>
<td>3.29 HER2/cell</td>
<td>ACIS 1.8</td>
<td></td>
</tr>
<tr>
<td>Case 1</td>
<td>2.2 (1.03 CEP17/cell)</td>
<td>2.08 HER2/cell</td>
<td>ACIS 1.9</td>
<td></td>
</tr>
<tr>
<td>Corzo et al.(^5)</td>
<td>2.00</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gunn et al.(^6)</td>
<td>2.86</td>
<td>1+</td>
<td>Neg (FIR 0.97)</td>
<td></td>
</tr>
<tr>
<td>Case EQ16</td>
<td>2.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case EQ17</td>
<td></td>
<td>1+</td>
<td>Neg (FIR 1.15)</td>
<td></td>
</tr>
<tr>
<td>Present case</td>
<td>2.6 (1CEP17 in 93% cells)</td>
<td></td>
<td>1+</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) aCGH = array comparative genome hybridization; ACIS = an image analyzer (ChromaVision Medical System, Inc. San Juan Capistrano, California) – assisted IHC quantitation; CEP17 = centromere 17 sequence D17Z1; FIR = fluorescence intensity ratio; IHC = immunohistochemistry; Neg = negative.
molecular cytogenetics infrastructure used in this study was funded by the Canadian Foundation for Innovation through the Canadian Molecular Cytogenetics Platform.

References

Changes to the Editorial Board

On December 31, 2012, two members of the Editorial Board, Dr. Louis Wadsworth, section editor for hematopathology, and Dr. Eleftherios Diamandis, section editor for medical biochemistry, stepped down. I would like to thank them for their support over the past 4 years as we have worked to establish the Canadian Journal of Pathology. This is an opportunity to welcome their replacements on the Editorial Board.

Dr. Lawrence Haley, MD, FRCPC, is head of pathology and laboratory medicine at the Royal Columbian Hospital and head of hematopathology for the Fraser Health Authority in British Columbia, where he is also a clinical associate professor in the Department of Pathology at the University of British Columbia. His principal professional interests are malignant hematopathology, transfusion medicine, and teaching.

Dr. Pierre Douville, MD, FRCPC, is a senior medical biochemist at the Centre hospitalier universitaire de Quebec and a professor at Laval University. He serves on the Executive of the Canadian Association of Medical Biochemists and on the Education Committee of the Canadian Society of Atherosclerosis, Thrombosis and Vascular Biology. His professional interests lie in renal disease, dyslipidemias, and cancer biomarkers.

I look forward to working with them to promote the Canadian Journal of Pathology as a vehicle for the dissemination of advances in pathology, laboratory medicine, and science in Canada.

J. Godfrey Heathcote
Editor-in-Chief
Pathology in the 1930s and 1940s: A Poetic Critique by “the PEI–Dalhousie–Mayo Clinic Connection”: Drs. Malcolm B. Dockerty and Lewis B. Woolner

James R. Wright Jr., MD, PhD

ABSTRACT
Malcolm B. Dockerty and Lewis B. Woolner were Prince Edward Islanders who were raised on potato farms during the Great Depression. From humble beginnings, they worked their way through Dalhousie Medical School; both became world-renowned surgical pathologists, spending their entire careers at the Mayo Clinic. Both were raised loving poetry. One also wrote his own verse throughout his life and used his poetry as his memoir. Dockerty and Woolner, based upon their experiences in medical school compared with those while in practice at the Mayo Clinic, were highly critical of the state of pathology at Dalhousie in the 1930s and 1940s. Comparing pathology practice at Dalhousie and the Mayo Clinic during that time perhaps seems unfair. However, times have changed, and all pathology laboratories are now expected to meet uniform, high standards. It is useful to reflect upon how far we have come toward closing the quality gap with the very best laboratories in the world.

RÉSUMÉ
Natis de l’Île-du-Prince-Édouard, Malcolm B. Dockerty et Lewis B. Woolner ont grandi dans une ferme de pommes de terre durant la Crise de 1929. Des origines modestes qui ne les ont pas empêchés de poursuivre des études à la faculté de médecine de l’Université Dalhousie et de devenir des pathologistes qui allaient acquérir une renommée mondiale en pathologie chirurgicale. Toute leur carrière s’est déroulée à la clinique Mayo. La poésie les berce depuis leur enfance. L’un d’eux en a même écrit tout au long de sa vie, des poèmes qui forment un recueil de mémoires. Comparant leur formation médicale et celle de leurs collègues à la clinique Mayo, l’un comme l’autre ont eu des mots très durs à propos de la discipline de la pathologie à l’Université Dalhousie dans les années 1930 et 1940. Qui sait, peut-être était-il injuste de comparer l’exercice de la pathologie dans ces deux institutions à cette époque? Toujours est-il que les temps ont changé et que tous les laboratoires de pathologie doivent désormais se conformer aux mêmes normes de qualité strictes. L’histoire de ces deux éminents pathologistes nous permet de constater le chemin parcouru par les laboratoires de pathologie du pays qui se mesurent favorablement aux meilleurs du monde.
Two of the pre-eminent “American” pathologists of the 20th century, both spending their entire careers at the Mayo Clinic, were Prince Edward Islanders who were raised in poverty on potato farms. They both attended one-room country schools followed by Prince of Wales College in Charlottetown and Dalhousie Medical School (Figure 1). Both became world-renowned surgical pathologists, publishing over 750 peer-reviewed papers and book chapters between them. Each trained literally hundreds of Mayo residents and fellows in pathology and surgery. Both had strong interests in poetry and could quote the classics at length from memory. One also wrote his own verse throughout his entire life and even used his poetry as his memoir; the other loved the classics and called his colleague “a (minor) poet.” In their retirement years, both received honorary doctorates from Dalhousie. In a future paper, I will present historical vignettes of Malcolm B. Dockerty and Lewis B. Woolner, since, except for a brief mention in a 1964 CMAJ article titled “The Mayo Clinic and the Canadians,” little has been written about them. However, here I report their independent analyses of the state of pathology practice at Dalhousie in the 1930s and 1940s.

I first met Lew Woolner after I nominated him for an honorary doctorate in 2001 while I was a faculty member at Dalhousie. We met again at his home in Rochester, Minnesota, in 2010 while I was conducting research on Mayo pathologist Albert C. Broders. Lew, at 96 years old, was the only living Mayo pathologist whose practice overlapped with that of Broders. At one point in the interview, he spontaneously volunteered his thoughts on the state of the practice of pathology when he was at Dalhousie. I did not have the same opportunity to know Dockerty, as he died in 1987. To become better acquainted, I spent a day in the Mayo Historical Unit reviewing his file and reading all of his poetry; then I interviewed his friends, colleagues, and son. In Dockerty’s Rhymed Reminiscences of a Pathologist: His Life’s Story, there is a section called “Medical School Days.” Interestingly, his descriptions of pathology at Dalhousie confirmed everything Woolner had told me.

Suffice it to say, neither Maritimer seriously considered a career in pathology at Dalhousie. Both agreed that pathology at the time each had graduated from medical school was very weak. Ralph Paterson Smith (MD, Glasgow, and Diploma of Public Health, Edinburgh), was the chair of pathology from 1927 to 1949 (see Figure 1) and his pathology laboratory at the Victoria General Hospital was hugely problematic, as Dockerty documented in the poem “Our Lab (V.G. Hospital – 1934),” written while a medical student:

Our lab is in a sorry mess.
Mahaney is to blame, I guess;
Each day he’s doing less and less
To keep the place supplied.
The centrifuge is out of true,
The Bunsen burner needs a screw,
The Benedict’s is nearly through,
There’s not one single slide!
The desk is slithered o’er with grime,
The floor is slippery with slime,
Test tubes are hard as H to find,
The litmus paper’s done.
Bottles in wild confusion thrown
Assume an order all their own.
Dockerty’s poem was prefaced in his book by, “Had I not known in 1934 that Mayo’s Laboratories were a far cry from the Victoria General’s counterpart, operated by interns, I never would have considered pathology as a possible future field.”

Not only were the laboratory facilities substandard, apparently so was the chief of service. According to Woolner, Ralph Smith was “a second rate microscopist” but did concede that he “might have been OK at autopsies” (verbal communication, June 2, 2010). Dockerty is a little more charitable in his medical school poem “Ode to Pathology (V.G. Hospital – 1934),” but possibly he was swayed by the observation that “R.P. Smith gave me the highest marks ever awarded by him in pathology!” However, that did not stop Dockerty from many years later composing the following lines about Smith’s alleged tendency to write lengthy descriptive reports without actual definitive diagnoses:

The tissue section sent to me
Revealed diffuse hypertrophy,
With here and there a giant cell.
Some epithelial pearls as well.
In one small corner of the slide
Some coarse mitoses I have spied.
But on a whole, I’m not content.
I’d like to have more tissue sent.

Clearly, during Dockerty’s and Woolner’s time at Dalhousie, the clinical pathology laboratories were substandard, under-resourced, and run by unsupervised interns. Facilities were in a constant state of disrepair, and the practice of surgical pathology was characterized by equivocation rather than definitive diagnosis as required for definitive treatment. Comparing pathology practice at Dalhousie and the Mayo Clinic in the 1930s and 1940s perhaps seems unfair. However, times have changed and all pathology laboratories now are expected to meet uniform, high standards. Having trained at a top American pathology laboratory and then immediately having worked as a pathologist at Dalhousie from 1988 to 2005, it is apparent to me that the quality gap between the practice of laboratory medicine at the “Mayo Clinics of the world” versus that in “the Dalhousies of the World” has substantially narrowed. In a time period in which the practice of pathology in Canadian laboratories has been repeatedly scrutinized in the news, it is important to take an historical perspective and reflect upon how far we have come toward closing the quality gap with the very best laboratories in the world. Although “lab errors” still occur, standardized residency training followed by board certification of pathologists, standardized training of laboratory technologists, and formalized laboratory accreditation programs have made the diagnostic world much better and safer for Canadian patients today.

Acknowledgements
The following individuals and institutions are thanked for help with obtaining articles, historical materials, or historical insights: Lewis B. Woolner, MD (interview 6/2/2010, and phone interview 3/7/2012); Elizabeth Anne Woolner; Dottie Hawthorne, Mayo Clinic Libraries; Renee E. Ziemer and Kristen Van Hoven, Mayo Historical Unit; Sherry Mount; Docs for Docs.

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2. Dockerty MB. Rhymed Reminiscences of a Pathologist: His Life’s Story. Privately printed 1980:54. (Dockerty papers, Mayo Historical Unit, Rochester, MN)
**Case Report: Congenital Cervical Cartilaginous Rest**

Lamiaa Rouas, MD, Rajae Tahri, MD, Najat Lamalmi, MD, Zaitouna Alhamany, MD, Nadia Cherradi, MD

**ABSTRACT**
Congenital cartilaginous rests are branchial arch remnants. They are anatomical curiosities and are of interest to the pediatric pathologist. Histopathological examination is required to confirm the diagnosis. The authors report a case of a cervical chondrocutaneous remnant in a 13-month-old girl without other apparent abnormalities. This article reviews the clinical features, histopathology, and differential diagnosis of this extremely rare condition.

**RÉSUMÉ**
La formation cartilagineuse congénitale est un vestige embryonnaire de l’arc branchial. Essentiellement une curiosité anatomique, elle revêt de l’intérêt pour le pathologiste en pédiatrie. L’examen histopathologique est nécessaire pour confirmer le diagnostic. Les auteurs se penchent sur le cas d’une fillette de 13 mois présentant un vestige chondrocutané cervical sans autres anomalies. Ils passent en revue les caractéristiques cliniques, les aspects histopathologiques et le diagnostic différentiel de ce trouble extrêmement rare.

Congenital cartilaginous rests (CCRs), first described by Berry in 1890, are branchial arch remnants. They may be single or multiple, unilateral or bilateral, polypoid elevations, and soft or firm on palpation because of their underlying core of cartilage. Histopathological examination is required for diagnosis because of the rarity of this condition and the typically non-specific clinical picture.

**Case Report**
A 13-month-old girl presented with a solitary lesion of the lateral neck noticed since birth. Physical examination revealed a firm, sessile, mobile mass in the lower third of the neck. The overlying skin was normal. No fistula, tenderness, or other sign of inflammation was seen. No other abnormalities were noted. The lesion was surgically excised. Histopathological examination revealed skin with cutaneous...
adnexae (hair follicles), dilated blood vessels, and a central core of mature cartilage (Figure 1) that was attached to some muscle fibres on the deeper side of the specimen. These findings led to a diagnosis of cervical chondrocutaneous branchial remnants.

**Discussion**

Congenital developmental and inflammatory lesions represent the majority of the masses in the neck of the pediatric population. Among congenital lesions, cysts and sinuses are frequently seen, whereas CCRs are extremely rare. These lesions are known by a number of names: skin tags, cervical auricles, branchial appendages, branchiomas, papillomata, and chondromata. They also appear in the literature as cervical accessory tragi and wattles. The term “cervical chondrocutaneous branchial arch remnants,” proposed by Atlan et al. in 1997, is most appropriate since it describes the origin of the lesion. During embryonic development, the branchial apparatus is reshaped and structures may disappear or form vestigial remnants by the end of the embryonic period. Errors in the development of the second arch are thought to give rise to chondrocutaneous branchial remnants, although some authors believe that the cartilage arises through metaplasia of undifferentiated mesenchymal cells.

CCRs may present as dome-shaped papules or nodules on the lateral neck along the lower half or one third of the anterior border of the sternocleidomastoid muscle. These lesions are always present at birth and more commonly seen in males. Associated anomalies may be found in the auditory, respiratory, genitourinary, and cardiovascular systems. Thus, a complete physical examination of the patient and ultrasound examination of the genitourinary tract are recommended. In our case, the lesion formed a cutaneous nodule with a subcutaneous core of cartilage that extended into the deeper tissue of the neck. Muscle tissue, possibly arising from the platysma or the sternocleidomastoid muscle, was seen attached to the cartilage. The overlying dermis

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**Table 1. Differential Diagnosis of Cervical Congenital Cartilaginous Rests**

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Location</th>
<th>Age</th>
<th>Pathological Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Branchial cleft cyst</td>
<td>Lateral neck Midline</td>
<td>Infants, children, adolescents, adults</td>
<td>Cystic mass containing fluid, up to 6 cm Lined by stratified squamous epithelium, respiratory epithelium, or both; lymphoid tissue; keratinous debris</td>
</tr>
<tr>
<td>Bronchogenic cyst</td>
<td>Suprasternal notch or manubrium stern; rarely in the anterior neck or shoulder</td>
<td>At, or soon after, birth</td>
<td>Asymptomatic nodules or sinuses exuding mucoid material Cysts range from 0.3 to 6 cm Lined by ciliated, pseudostratified columnar epithelium Cyst wall contains smooth muscle, elastic fibres, and seromucous glands</td>
</tr>
<tr>
<td>Dermoid cyst</td>
<td>Midline or near-midline, upper neck, near the thyroid cartilage, and near the suprasternal notch</td>
<td>Infants, children, adolescents</td>
<td>Cystic – solid or multilocular lobulated mass up to 12 cm Lined by stratified squamous epithelium supported by a fibrous tissue wall Ectodermal derivatives present, such as hair follicles, sebaceous glands, and sweat glands</td>
</tr>
<tr>
<td>Cutaneous inclusion cyst</td>
<td>Presternal</td>
<td>Early embryonic life</td>
<td>Cystic, echolucent, filiform capsule</td>
</tr>
<tr>
<td>Chondrocutaneous vestige</td>
<td>Always at birth</td>
<td>Lower half or one third of the anterior aspect of sternocleidomastoid muscle</td>
<td>Never cystic or fistulous Polyoid lesions Fibrovascular tissue with hair follicles and small sebaceous glands and underlying fat and cartilage in the core Some follicles are vellus type May be associated with malformations</td>
</tr>
</tbody>
</table>
contained skin appendages.
The clinical differential diagnosis includes lymphadenopathy and other polypoid lesions such as fibroepithelial polyps, polypoid melanocytic nevi, and pedunculated hemangiomas. Histopathological evaluation is necessary for definitive diagnosis, but the location on the anterolateral neck of a congenital nodule or polypoid lesion is a clue. A summary of the differential diagnosis is given in Table 1. The management of CCRs involves complete surgical excision.

References

CAP-ACP NEWS
Message from the Nominating Committee

Dear CAP-ACP Members,

The Nominating Committee proposes the following Slate of Officers for June 2013; the only new nominations are for vice-president and members-at-large:

Executive:

• President – Martin Trotter
• Vice-president – Victor Tron
• Past president – Vina Alexopoulou
• Secretary treasurer – Brian Cummings
• Continuing professional development chair – Jason Ford
• Annual meeting chair – Avrum Gotlieb
• Resource development chair – Alan Spatz
• Website editor – Tadaki Hiruki
• Journal editor-in-chief (ex-officio) – Godfrey Heathcote
• Patient safety and quality assurance chair (ex-officio) – Laurette Geldenhuys
• Member-at-large – Marciano Reis
• Member-at-large – Julianne Klein

Other:

• Membership chair – Bernard Tetu

If you would like to make an alternative nomination for the positions of vice-president or member-at-large, please submit your nomination to laurette.geldenhuys@cdha.nshealth.ca. The nomination

• must be signed by five qualified ordinary members, with membership dues paid;
• must be agreed to by the proposed nominee, who must be a qualified ordinary member; and
• must be received at least 30 days prior to the Annual General Meeting.

Laurette Geldenhuys
Past President
Chair, Nominating Committee
A 25-year-old male with cystic fibrosis (CF) developed acute abdominal pain with peritoneal signs over 24 hours. On examination, there was a 3 cm tender, mobile mass in the right lower quadrant. Ultrasonography demonstrated a prominent appendix, but the radiologist was uncertain whether this represented appendicitis or mucous impaction. An appendectomy was performed (Figure 1).

On macroscopic examination, the appendix had a diameter of 2.7 cm and was distended by mucoid material. A 1.0 cm mural defect was noted, and there was purulent exudate on the external surface. Histologically, there was luminal mucus with partial compression of the mucosa and acute and chronic inflammation. The final diagnosis was perforated appendicitis in the context of CF.

Appendiceal disease in CF ranges from sterile mucous impaction to abscess. Notably, acute appendicitis has a lower prevalence (1–2%) in CF patients than in the general population (7%). The reason for this is unknown. One suggestion is that mucous distension helps to maintain luminal patency and prevents acute inflammation. The slow sterile distension may lead to higher frequencies of asymptomatic perforations that escape detection. The frequent use of prophylactic antibiotics may also decrease bacterial load or mask inflammatory symptoms. Regardless, with increasing life expectancies in the CF population, pathologists will likely encounter more cases of appendiceal disease in these patients.

References

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Understanding Antibody-Mediated Rejection of Organ Transplants: Mechanisms, Morphology, Molecular Patterns, and Personalized Precision Diagnosis

Banu Sis, MD, FRCPC

ABSTRACT
Transplantation is a life-saving treatment for patients with end-stage organ failure. While modern drugs have significantly improved 1-year transplant survival, the rate of organ transplant failure after the first year remains substantial. The lack of improvement in long-term transplant survival can be explained, at least partially, by the production of antibodies against donor antigens, which causes organ transplant damage (rejection) and failure. Clinical studies show that antibody-mediated rejection is a major problem in organ transplantation because of its negative impact on transplant outcomes and function, with no effective treatments. Microvascular endothelium is the main target of injury in antibody-mediated rejection of organ transplants. This group previously observed upregulation of several endothelial genes in kidney transplant biopsies from patients with alloantibody, indicating active antibody-mediated rejection and poor graft survival. Furthermore, endothelial molecular signals discovered a previously unknown clinical phenotype: C4d-negative antibody-mediated rejection. This article reviews the current understanding of effector mechanisms of antibody-mediated rejection, its morphological and molecular patterns in allograft tissues, and current insensitive diagnostic criteria, and discusses a new diagnostic approach that will bring pathologists closer to precision diagnosis.

RÉSUMÉ
En cas d’insuffisance organique terminale, la transplantation sauve la vie. Bien que les médicaments modernes aient amélioré grandement le taux de survie la première année de l’intervention, le taux de rejet de la greffe par la suite demeure notable. La stagnation du taux de survie au long cours s’explique, du moins en partie, par la production d’anticorps dirigés contre les antigènes du donneur, qui provoque à terme la défaillance et le rejet de l’organe transplanté. Des études cliniques démontrent que le rejet par ce mécanisme immunologique représente un problème majeur en transplantation en raison de ses répercussions sur l’issue de la transplantation et le fonctionnement de l’organe transplanté en l’absence de traitements efficaces.

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Transplantation is a life-saving treatment for patients with end-stage organ failure. In the past decade, more than 25,000 Canadians with end-stage organ failure received organ transplants, including approximately 11,000 kidney transplants. Each year in the United States, more than 28,000 solid organ transplants are performed, with 116,000 patients on waiting lists, including >91,000 needing kidney transplantation. While modern immunosuppression therapies significantly lowered the incidence of acute T-cell-mediated rejection and improved 1-year graft survival, the rate of late graft loss after the first year remains substantial. The lack of improvement in long-term graft survival is primarily due to B-cell responses and alloantibodies, and recurrent diseases.

Antibody-Mediated Rejection Is a Major Problem in Kidney Transplantation

Antibody-mediated organ rejection (ABMR) is a major problem in transplantation because it has a huge negative impact on transplant outcomes and function, and unfortunately there are no effective treatments. ABMR is caused by donor-specific antibodies (DSA) against human leukocyte antigens (HLA), ABO blood group antigens, and (possibly) non-HLA antigens. The cross-match testing (a test that determines if the recipient has antibody to the potential donor) and blood group matching pre-transplantation virtually eliminated the hyperacute ABMR phenotype from the clinic by avoiding transplantation across positive cross-matches or by use of desensitization protocols. However, transplanting cross-match negative organs cannot prevent delayed ABMR phenotypes in non-pre-sensitized or not highly pre-sensitized patients at later time points after transplantation. This is because many patients develop clinical problems due to de novo DSA or increased activity of pre-existing low-titre DSA after transplantation (22% and 15%, respectively, of kidney transplant recipients who underwent indicated biopsy). After the historical recognition of the destructive potential of alloantibodies causing hyperacute rejection in the early 1960s, the deleterious effects of alloantibodies after transplantation remained unrecognized for three decades. This ignorance ended in the early 1990s with a couple of concomitant clinical observations that recognized delayed acute rejection of kidney allografts associated with anti-HLA DSA. These clinical observations coincided with careful studies of graft pathology, which led to the discovery of C4d (a breakdown product of activated complement protein C4) staining in allograft tissues as a diagnostic biomarker for active ABMR. Acute C4d+ ABMR has since been reported to occur in about 7% of conventional (cross-match negative) kidney transplant recipients, 20–30% of biopsies with acute rejection, and 20–48% of positive cross-match patients after desensitization treatments. A decade after the discovery of C4d as a biomarker for ABMR, late graft losses due to antibody and morphological features of chronic ABMR were recognized. ABMR has been demonstrated in kidney, heart, pancreas, lung, and, less often, liver allografts and is not restricted to pre-sensitized patients. In fact, the transplant community now recognizes that de novo alloantibody production (sensitization) after transplantation is a common etiology underlying chronic graft injury. ABMR is a major cause of late kidney transplant loss. Data from two large transplant centres (Mayo Clinic and
Edmonton) and the multicentre Long-Term Deterioration of Kidney Allograft Function (DEKAF) study independently indicated that most late kidney transplant losses have a specific etiology, with ABMR being the leading cause, while idiopathic fibrosis or drug toxicity were rarely responsible for renal allograft loss.\(^5,8,36\)

**Morphological Phenotypes of ABMR**

The following are the two major phenotypes of ABMR in human kidney transplants:

1. Early/acute ABMR developing in pre-sensitized patients early after transplantation (first weeks or months)
2. Late/chronic ABMR with de novo DSA or low titres of preformed DSA developing late post-transplantation (usually after 1 year)\(^37\)

According to the international Banff classification of allograft pathology, diagnosis of ABMR requires the simultaneous presence of C4d staining along allograft capillaries, detection of DSA, and histopathological evidence of allograft tissue injury.\(^38,39\)

In acute ABMR, microvascular endothelial injury and inflammation are the predominant morphological changes observed in organ allografts. Microvascular inflammation is seen as aggregates of organ allografts (monocytes/macrophages, natural killer [NK] cells, neutrophils, and, less often, T lymphocytes) in the lumina of capillaries,\(^40–43\) and this feature is common to all organ transplants experiencing ABMR: peritubular capillaritis and glomerulitis in kidney transplants, myocardial capillaritis in heart transplants, interacinar capillaritis in pancreas transplants, and alveolar capillaritis in lung transplants.\(^25,37,38,42–46\) Active antibody-mediated injury, recognizable as C4d staining and/or microvascular inflammation, is a harbinger of subsequent chronic graft damage, especially if left untreated.\(^21,47,48\) Other pathological features of acute ABMR in kidney transplants include acute tubular injury,\(^38\) vascular thrombi, capillary dilatation and congestion, interstitial inflammation,\(^49\) and rarely intimal arteritis.\(^37\)

Smouldering or episodic alloantibody-mediated injury causes chronic structural changes in kidney allograft microvessels and arteries, interstitial fibrosis (often with inflammation in scarred areas), and tubular atrophy. Chronic ABMR of kidney

![Figure 1. Pathogenesis of antibody-mediated transplant rejection. Ig = immunoglobulin; NK = natural killer.](image-url)
Understanding Antibody-Mediated Rejection of Organ Transplants

Allografts is thus dominated by microvascular structural remodelling, which is seen as duplication and/or multilamination of basement membranes of glomerular and peritubular capillaries: transplant glomerulopathy and peritubular capillary basement membrane multilayering, respectively. Chronic ABMR in other organ allografts (non-renal) is not well defined.

Pathogenesis of ABMR

Although alloantibodies play major roles in transplant rejection, the precise mechanisms by which alloantibodies cause allograft injury, dysfunction, and loss are not well understood. However, it is well recognized that donor endothelium is the principal target of ABMR. Microvascular endothelial cells are at the interface between the allograft parenchyma and recipient blood and thus suffer as the main targets of DSA.

Effector mechanisms of ABMR (Figure 1) include downstream effects of (1) complement activation (endothelial cell lysis, chemotaxis, leukocyte recruitment, and endothelial activation); and (2) complement-independent immunoglobulin (Ig) Fc-Fc receptor-mediated tissue injury (apoptotic cell death, degranulation, phagocytosis); (3) endothelial activation that facilitates recruitment of leukocytes, platelet activation, and vascular thrombosis; and (4) influx of leukocytes promoting further tissue injury. Sustained or episodic antibody-endothelial interactions and associated intravascular inflammation result in active endothelial injury, which over time induces structural remodelling with loss of fenestrations between the endothelial cells and increased capillary basement membrane deposition, and probable downstream chronic ischemic effects of capillary remodelling on parenchymal cells of the allografts.

Complement-Dependent Mechanisms of ABMR

Antibodies activate the classical pathway of complement initiated by binding of C1q to a pair of Fc domains of IgG or IgM ligated to tissues. While IgM, IgG1, and IgG3 antibodies are effective in activating complement, IgG2 and IgG4 antibodies are less effective; thus, not all antibodies activate complement. Complement-dependent mechanisms underlying microcirculation injury involve the following:

- Split products mediate chemotaxis (C3a and C5a) and activate monocytes/macrophages and neutrophils through complement receptors (CR1, CR3), leading to microcirculation inflammation (capillaritis).
- Binding of receptors on endothelium to C3a and C5a induces endothelial activation, leading to expression/secretion of proinflammatory molecules (adhesion molecules, cytokines, chemokines), which further increase leukocyte recruitment, platelet adherence, vascular permeability, and coagulability.
- Terminal complement components (C5b-9, the membrane attack complex) trigger endothelial cell lysis by forming pores in the target cell membrane.
- Sub-lytic concentrations of C5b-C9 can also induce endothelial activation with increased expression of adhesion molecules and tissue factor, leading to microcirculation inflammation and pro-coagulant state.

Complement-Independent Mechanisms of ABMR

Although complement activation is crucial for ABMR, several effector mechanisms triggered by tissue-bound alloantibody, that is, endothelial activation and Fc receptor-mediated leukocyte recruitment and activation, do not require complement (see Figure 1). In support of this view, there is growing evidence suggesting that complement-independent mechanisms predominate particularly in chronic ABMR, causing ongoing endothelial injury and remodelling:

- Alloantibody can cause endothelial activation in the absence of complement with increased release of von Willebrand factor (VWF) and externalization of P-selectin on endothelial cells upon stimulation by anti-HLA class I.
- Activated endothelial cells express/secrete proinflammatory molecules (i.e., E-selectin, P-selectin, ICAM-1, VCAM-1, CX3CL1) that increase leukocyte recruitment. Recruited and activated leukocytes secrete cytokines such as TNF-α and IL-1β, which further increase endothelial activation by inducing expression of tissue factor, adhesion molecules, and chemokines.
Leukocytes can kill/damage endothelium via antibody-dependent cellular cytotoxicity by NK cells or monocytes/macrophages through IgG Fc/Fc receptors.

Recent clinical trial data suggest that complement-independent mechanisms may predominate in chronic ABMR. Terminal complement inhibition by eculizumab (humanized anti-C5 antibody) has been found to decrease the frequency of biopsy-proven acute ABMR in highly pre-sensitized kidney transplant recipients. However, the initial results of an eculizumab trial to prevent chronic kidney transplant damage are discouraging: anti-C5 blockade did not decrease the prevalence of chronic ABMR after 1 year in highly pre-sensitized kidney transplant recipients. In a series of positive cross-match kidney transplant recipients who were treated with eculizumab for 3–12 months, the prevalence of transplant glomerulopathy and peritubular capillaritis at 1 year was high in both eculizumab-treated and control patients (transplant glomerulopathy: 30% versus 36%, respectively; peritubular capillaritis: 56% versus 57%, respectively).

Endothelial Molecules Have Taught Us a Frightening Lesson: About 50% of Patients with Active Late ABMR Are Misdiagnosed in the Clinic

Detecting which renal transplants are at risk for antibody-mediated deterioration is an unmet need. Anti-HLA antibodies develop in 25% of renal transplant recipients and are associated with increased graft failure, but have low predictive value for rejection in individual patients. In a retrospective study of biopsies from 1,320 patients, we observed that approximately 50% of patients with HLA alloantibodies and transplant glomerulopathy were C4d negative. This earlier observation suggested that C4d may be an insensitive biomarker for chronic ABMR.

We then pursued this finding by new studies using gene microarray assessment of renal allograft biopsy tissues. We identified a literature-based “endothelial cell-associated transcript” (ENDAT) set (323 probe sets; 119 unique genes), in which genes were identified based on their selective expression in cultured human endothelial cells when compared with non-endothelial cells or by serial analysis gene expression (SAGE) libraries comparing tag frequencies in endothelial versus non-endothelial libraries. In a large number of kidney transplants biopsied for clinical indications, the biopsies with high expression of ENDATs and HLA antibodies showed histopathological lesions of ABMR (transplant glomerulopathy, capillaritis, glomerulitis, peritubular capillary basement membrane multilayering, and fibrosis/atrophy) and poor outcomes in comparison to biopsies with HLA antibodies and no ENDAT upregulation. Surprisingly, many of these active ABMR cases were missed. Only 40% of kidneys with high ENDAT expression and chronic ABMR morphology or graft loss were diagnosed by C4d positivity, an observation that was validated in an independent set of 82 kidneys. In this way, we recognized the cases of C4d-negative active ABMR and the importance of a full phenotype of patients incorporating all clinical, pathological, and outcome data.

The key message is that endothelial molecular stress plus the presence of alloantibody is a high-risk state for ABMR and graft failure. Importantly, the presence of DSA without upregulation of intragraft ENDATs is not associated with graft damage or worse outcomes compared to patients with no DSA. It should also be noted that high ENDAT expression per se is not an indicator of graft damage or eventual graft loss in patients who lack HLA antibodies. Therefore, ENDAT changes in renal transplants occur in rejection and in other forms of renal injury (infections, ischemic injury), but their impact on progressive graft deterioration and loss is principally in patients with HLA antibodies. In an extended cohort of 329 biopsies with complete DSA testing, including cases of T-cell-mediated rejection, borderline changes, glomerulonephritis, polyoma virus nephropathy, and acute tubular necrosis, some biopsies showed ENDAT expression in the absence of DSA. However, high ENDAT scores plus DSA positivity increased the specificity for ABMR (97%), capturing both C4d-positive and C4d-negative active ABMR cases. Therefore, the specificity of ENDAT scores is high in patients with DSA, detecting both active injury and ABMR histology. We recommend use of ENDAT scores together with DSA testing results.

High ENDAT expression with the presence of antibodies predicts graft loss with higher sensitivity (77% versus 31%) and slightly lower specificity (71% versus 94%) than the
presence of C4d. Thus, high intragraft endothelial transcript expression in patients with circulating alloantibody is a proven indicator of active antibody-mediated allograft damage and poor graft outcome.\textsuperscript{72} Graft survival did not differ significantly between patients with antibodies and high ENDAT with C4d or without C4d staining; thus, C4d positivity did not have an additional adverse prognostic impact over high ENDAT with HLA antibodies. We also analyzed the diagnostic value of individual endothelial transcripts. In a training set (n = 201 biopsies), we selected a cut-off signal for each gene for detecting ABMR (histology/serology) with at least 80% sensitivity by receiver operating characteristic (ROC) curve analysis. Applying the same cut-offs in an independent validation set (n = 202 biopsies), Duffy antigen receptor for chemokines (DARC) upregulation plus DSA showed 81% sensitivity and 93% specificity for ABMR, whereas C4d plus DSA showed 48% sensitivity and 97% specificity.\textsuperscript{73} Figure 2 shows the poor prognosis of kidney allografts with molecularly diagnosed ABMR (DARC plus DSA, C4d positive or negative) when compared to allografts without molecular ABMR (DSA present or absent). Thus, measuring a few endothelial transcripts in biopsies from kidneys with alloantibody is a highly sensitive and specific method to detect ABMR, and is more sensitive than C4d staining. Endothelial molecular parameters should be incorporated into the histopathology-based Banff classification to improve diagnosis of ABMR.

In our experience, most C4d-negative ABMR biopsies demonstrated chronic ABMR morphology (transplant glomerulopathy, capillaritis, peritubular capillary basement membrane multilayering, fibrosis/atrophy). Whether there is a form of acute ABMR independent of complement effects is an open question. If so, the answer will likely come from data from highly sensitized cross-match positive transplant populations. There is still much debate on whether or not C4d-negative acute ABMR truly exists in patients presenting with clinical problems. In fact, C4d staining may have a higher sensitivity for acute ABMR than it has for chronic ABMR.

**Microarrays of Kidney Allograft Biopsies Dissected: The Molecular Phenotype of ABMR**

The biopsies analyzed by gene expression microarrays displayed three intragraft molecular phenotypes during ABMR: upregulation of endothelial genes,\textsuperscript{72} NK cell–associated genes,\textsuperscript{43} and effects of interferon-gamma (IFN-\(\gamma\)).\textsuperscript{74}

**Endothelial Molecular Signals**

Many individual ENDATs were increased in ABMR and predicted future graft loss. The expression of VWF, EDN1 (endothelin 1), CAV1 (caveolin 1), CDH5 (cadherin 5), CDH13 (cadherin 13), PALMD (palmdelphin), PECAM1 (platelet/endothelial cell adhesion molecule 1), SELE (selectin E), CD34, DARC, RHOJ (ras homolog family member J), SOX7 (SRY-box containing gene 7), SOX18 (SRY-box containing gene 18), THBD (thrombomodulin), and MALL (BENE; interacts with CAV1) were higher in ABMR than in T-cell-mediated rejection biopsies.\textsuperscript{72} The
increased expression of endothelial genes in ABMR by microarrays was confirmed by reverse transcription polymerase chain reaction (RT-PCR). However, immunohistochemical staining of endothelial proteins such as VWF and PECAM1 could not be quantified for upregulation because of their high baseline staining in renal endothelium. A functional annotation analysis on endothelial transcripts that were increased in biopsies with ABMR compared to normal renal cortex showed a number of significantly enriched functional pathways operating in human ABMR biopsy tissues.

**Endothelial Cells Gain Adhesive Properties and Thereby Facilitate Leukocyte Trafficking**

Functional annotation cluster analysis using the Gene Ontology database on a list of endothelial genes that were upregulated in ABMR biopsies showed a number of significantly enriched categories: regulation of cell migration and motility, facilitation of leukocyte rolling, adhesion, and transmigration via endothelial selectins (E-selectin, P-selection), CD34, integrin ligands (VCAM1, ICAM2), PECAM1, and interaction with basement membrane components, and an increased endothelial procoagulant state (VWF) that enables platelet activation. We observed upregulation of DARC as one of the top genes increased in human ABMR samples. DARC mediates incomplete chemokine transcytosis, leading to a retention of intact chemokines on the endothelial apical surface and more leukocyte migration. Mice overexpressing DARC on vascular endothelium were reported to have enhanced leukocyte recruitment and extravasation. Thus, upregulated DARC expression on endothelium seems to be contributing to sustained microvascular inflammation and injury during ABMR in humans.

**Endothelial Cells Gain Pro-coagulant Properties and Thereby Facilitate Platelet Aggregation and Coagulation**

Functional annotation analysis on ENDATs increased in ABMR biopsies also showed significant enrichment of clusters related to coagulation and platelet activation. Interestingly, we observed VWF as the top transcript increased in biopsy tissues with ABMR. The VWF protein plays a major role in hemostasis by carrying the blood coagulation factor VIII and by linking endothelial activation to platelet adhesion and aggregation at sites of vascular injury. Endothelial cells store VWF protein as large multimers together with P-selectin in cytoplasmic Weibel-Palade bodies. Upon endothelial cell activation, immediate endothelial exocytosis facilitates the release of preformed VWF and externalization of P-selectin from the Weibel-Palade bodies to the endothelial surface. The large multimeric bundles of VWF released onto endothelial surface binds to glycoprotein Ib on platelets, leading to effective platelet activation and aggregation. Analogous to our findings in human ABMR biopsy samples, Morrell et al. observed in a mouse skin transplantation model that repeated injections of alloantibodies caused VWF release and sustained endothelial-platelet interactions mediating platelet activation and rolling in vivo. The researchers also showed that alloantibody-endothelial-platelet interactions in this model were associated with vascular inflammation, thrombi, and complement component C4d deposition in vessels.

**Increased IFN-γ Effects**

IFN-γ, a soluble cytokine and a type II interferon, which is predominantly released by antigen-triggered effector T cells and NK cells, plays major roles in allograft rejection. Both ABMR and T-cell-mediated rejection biopsies show high expression of IFN-γ induced transcripts such as CXCL11, CXCL9, and CCL5 (RANTES). IFN-γ induces expression of many genes in the donor tissue and in host infiltrating cells, inducing chemokines such as CXCL9, CXCL10, CXCL11 and major histocompatibility complex class Ia (HLA-A, HLA-B), class Ib (e.g., HLA-E), and class II (HLA-DR, -DP, -DQ, and -DQ). IFN-γ secreted by T cells and/or NK cells is essential for induction of class I and class II antigens on endothelium. IFN-γ increases antibody ligation to endothelium by increasing the density of major histocompatibility complex antigens on the endothelium and thus making antibodies more effective in inducing graft inflammation and injury.

**Innate Leukocytes, Especially NK Cells, Emerge as Effector Cells in ABMR**

Microvascular inflammation is a typical histopathological
feature of ABMR. Leukocyte recruitment to the allograft capillaries can be mediated by complement split products (C3a, C5a), activated endothelial cells, and activated platelets. There are also in vitro data that suggest alloantibody alone, independent of the complement, may trigger endothelial activation and thereby may induce microvascular inflammation.

Antibody-dependent cellular cytotoxicity (ADCC) is a powerful way to damage/kill endothelial cells coated by alloantibodies. NK cells, monocytes/macrophages, and other leukocytes have surface receptors for the Fc portion of IgG. In particular, Fc-γ receptor 3 (CD16) binding to IgG causes release of cytotoxic granules (perforin, granzymes) from NK cells or macrophages, which in turn triggers apoptosis of the target cell. Supporting the contribution of this mechanism to ABMR, we identified increased NK cell transcripts and CD56+ NK cells in biopsies with ABMR. Recently, Hirohashi et al. reported that NK cells were increased in DSA-induced obliterative arteriopathy lesions in heart allografts transplanted into immunodeficient mice that lack T and B cells and complement protein C3. They also showed that obliterative arteriopathy lesions were decreased upon depletion of NK cells with anti-NK1.1 antibodies, suggesting that NK cells in the presence of DSA mediate chronic ABMR, independently of complement activation.

Conclusion

Effector mechanisms of ABMR include downstream effects of complement activation and complement-independent Ig Fc-Fc receptor-mediated tissue injury, the involvement of activated endothelial cells that facilitates recruitment of leukocytes, platelets, and vascular thrombosis, and an influx of leukocytes promoting further tissue injury. Sustained or episodic antibody-endothelial interactions and associated intravascular inflammation result in active endothelial injury, which over time induces structural remodelling with loss of fenestrations between the endothelial cells and increased capillary basement membrane deposition, as well as their probable downstream chronic ischemic effects on the parenchymal cells of the allografts. Many of these effector mechanisms do not require complement. There is growing evidence that complement-independent mechanisms predominate in chronic ABMR causing endothelial injury and remodelling. To improve diagnosis and quantify the degree of antibody-mediated tissue injury in clinical transplants, endothelial cells provide a useful readout.

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DISPLAY CLASSIFIED
The state is charged with advancing the welfare of its citizens; a company’s purpose is to increase its owners’ wealth.

– Mindell et al., “The Corporate Capture of Public Health”

In *False Positive*, Ross Sutherland presents a series of arguments as to why “the practice of using for-profit laboratory corporations is not acceptable in the provision of an essential service.” He begins with a description of how pathologists promoted the establishment of private laboratories at a time when universal medical insurance was being introduced in the 1960s. As might be expected, this is a complicated story, which might well not be familiar to younger pathologists.

Ontario is at the centre of the author’s story, and in subsequent chapters he covers the growth of for-profit laboratory corporations despite half-hearted attempts by government to control their activities through licensing, quality management, and the limitation of overuse. It is not an edifying story, and there is little to suggest that pathologists, private corporations, or government itself really acted in the public interest.

Across the country, there is widespread belief in government in the advantages of greater integration of laboratory services. In chapter four, the author dissects the fiasco of laboratory reorganization in Ontario in the 1990s, a move resisted by private laboratories, and how government policies actually undermined its own initiatives. Even for someone who worked as a laboratory medical director during that turbulent decade in Ontario, this chapter provides new insight into the seemingly endless stream of futile attempts at reorganization and integration. The book continues with a review of the current participation of laboratory corporations in the laboratory services of each province.

In the closing chapters, the author examines the belief that “the private sector does it better” as it applies to laboratory services and comes to the conclusion that there is little or no evidence to support this. The book appears well researched, although it would have been of interest to hear more from laboratory physicians who are currently engaged with for-profit laboratory corporations.

Any discussion of laboratory corporations is likely to provoke an ideological reaction among pathologists and laboratory physicians and, after reading this book, some may still believe that the private sector is more efficient in the provision of laboratory services. These physicians presumably also believe that their hospital laboratories of today are much cleaner than they were before janitorial services were contracted out to private companies.

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Sultan Qaboos University Hospital is a government institution and a teaching hospital situated within the campus of the Sultan Qaboos University and has 550 beds capacity.

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**Kelowna** – These opportunities are situated in a breathtaking “four seasons” playground with a wide range of activities to enjoy. Kelowna is the largest city in British Columbia’s Okanagan Valley and is known for its hot summer and temperate winters. Ranked as one of the most livable cities in Canada, Kelowna attracts visitors and residents from around the world to explore what the city has to offer.

**Kelowna General Hospital (KGH)** – KGH is the major hub of 32 smaller laboratories and collection sites. It is a tertiary referral center and is co-located with a cancer center and medical school. Major programs include: renal dialysis, thoracic, neurological and cardiovascular surgery. There would be faculty privileges with KGH, Penticton Regional Hospital, Vernon Jubilee Hospital, and Kootenay Boundary Hospital. The Pathology department is expanding to include. 3 new Pathologist Opportunities – Kelowna General Hospital

**Hematopathologist** – The successful candidate will work with four General Pathologists and report to the Central Okanagan Medical Director. Highlights of the duties would include overseeing the high volume areas of the Hematology Laboratory at KGH, determining test menus offered in coordination with clinical departments and other sites through the regional Hematology Working Group, overseeing transfusion medicine and taking responsibility for quality assurance, ranking capital equipment needs, and teaching medical and laboratory students in both transfusion and hematology. Position details: 40 hours/week, on call to be determined, remuneration commensurate with provincial negotiated upper grid level of $320,958 that is dependent on experience, and relocation assistance is available. An FRCPC combined with at least 5 years of related experience would be ideal, however a FRCPC General pathologist with adequate experience would be considered as an alternative. (I-KGH-INT-198)

**Anatomic and/or General Pathologists X2** – Reporting to the Central Okanagan Medical Director while working with eight other Anatomic/General Pathologists, highlights of the opportunity include: participation in surgical pathology, cytopathology and autopsy pathology, quality assurance activities, and teaching of medical and laboratory students. Those with expertise have an opportunity to oversee sub-specialty areas: thoracic, gastrointestinal, cardiac, and/or forensic experience an asset. Position details: 40 hours/week, on call to be determined, remuneration commensurate with provincial negotiated upper grid level of $320,958 that is dependent on experience and teaching responsibilities. An FRCPC combined with at least 5 years of related experience would be ideal. (I-KGH-INT-1798)

For more information contact: physicianrecruitment@interiorhealth.ca or view us online at our website www.betterhere.ca

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**Pathologist Opportunity**

**Life style matters. . .**

Northern Health is situated in the heart of British Columbia.

Our main referral centre, University Hospital of Northern BC, includes a catchment population of 350,000 and is a teaching center affiliated with the University of British Columbia.

By joining our team of progressive Pathologists to provide clinical and anatomic pathology services, you will have the opportunity to work in a facility that boasts:

- A wide variety of active surgical programs including general, gynecologic, urologic, orthopedic and otolaryngologic specialties
- An active autopsy service in conjunction with the BC Coroner’s service
- Participation in medical student and resident teaching through the Northern Medical Program, University of Northern British Columbia, and University of British Columbia
- Generous recruitment incentives including relocation package and rural and retention fees, to name a few

If you are looking for a lifestyle change that includes a slower pace and higher standard of living, take advantage of this exciting opportunity.

For more information contact:
Sheilagh Wilson, Physician Recruitment Coordinator
Phone: 250-649-7117 Toll Free: 1-877-905-1155 Ext. 1
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