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About the Cover

This image shows the spindle cell component with bland nuclei and scant mitotic figures of a SETTLE.

For Instructions to Authors, please visit http://www.andrewjohnpublishing.com/CJP/instructionstoauthors.html
The classification of hematolymphoid neoplasms has been a battlefield for the lumpers and splitters for the past half-century, and every year the long-suffering trainees in pathological disciplines are expected to learn the latest disease designation. Little do they know that in another 5 years, all will change and they will have to remember a new stratification system. The ebb and flow of these classification systems is a testament to our lack of understanding of the precise pathobiology of many of these diseases. Little change has occurred over a period of 60 years in the classification of the group of disorders known as myeloproliferative syndromes, now known as myeloproliferative neoplasms (MPN), which were first described by William Dameshek in the early 1950s. The myeloproliferative syndromes are known to progress in some patients to a blast cell crisis that resembles an acute leukemia. In contradistinction, the classification and comprehension of the group of hematological disorders now known as the myelodysplastic syndromes (MDS) has evolved extensively during my professional career. During residency training, I recall presenting a case in which a diagnosis of chronic monocytic leukemia had been rendered and mentioning to the amusement of the assembled audience that perhaps this condition fell within the spectrum of a group of conditions that evolved into acute myeloblastic leukemia (AML) and should perhaps be classified as a “preleukemic syndrome.” The term preleukemic syndrome began to be applied in the 1970s to a “grab bag” of hematological disorders that could not otherwise be classified, but the term soon became passé when it became clear that, although some patients did eventually develop AML, not all of them did and many demonstrated a smouldering illness with chronic cytopenias rather than culminating in a blast cell crisis. The bone marrow in these patients was often hypercellular and showed morphologically abnormal, but very active, hematopoiesis. This led to the conclusion that the formation of mature blood elements in this group of patients was functionally ineffective. The idea evolved that the microscopic finding of dysplastic hematopoiesis – myelodysplasia – was the central morphological feature in patients with these disorders.

The umbrella term of MDS evolved, and an initial classification was published in 1982 by the French-American-British (FAB) Cooperative Group. We now understand that these disorders are clonal but only approximately 50% have an easily recognizable cytogenetic defect to prove this clonality. The ineffective hematopoiesis and chronic cytopenias are now believed to be the result of increased apoptosis. In the past 30 years, further refinements to the initial FAB classification have been made. These changes have improved management; we now delineate cases previously designated as MDS that we now classify and treat ab initio as AML. There are also criteria to identify high-grade MDS and, consequently, some patients can be given a worse prognosis. These revisions are described in the recently published WHO Classification of Tumours of the Haematopoietic and Lymphoid Tissues (2008). This same proposal has now excluded chronic myelomonocytic leukemia from the MDS family and suggests that, since this condition has characteristics of both MDS and MPN, it is best classified under myelodysplastic/myeloproliferative neoplasms (MDS/MPN). The proposal also includes the somewhat controversial “provisional entity” of refractory cytopenia of childhood (childhood MDS), in which subtle dysplastic features, affecting at least 10% of erythroid and granulopoietic precursors, are used to distinguish the condition from an emerging bone marrow failure syndrome. Risk stratifications based on the severity of the cytopenias are used to determine prognosis. We now appreciate that the risk of an individual developing MDS increases with age, to such an extent that perhaps as many as 0.75 of 1,000 persons over the age of 64 years will be affected. Despite the evolution of disease classification, the MDS remain a very challenging group of conditions to diagnose. A large component of the diagnostic formulation rests on the recognition of morphological findings, a very subjective process; these dysplastic changes may be difficult to appreciate and are always troublesome to quantify. These are problems not only for the individual pathologist but also for reference groups: in the latest proposal from the World Health Organization (WHO), although major progress has been made, there is still a lack of consensus as to whether blast cell percentages should be calculated on all marrow nucleated cells or just on the nonerythroid nucleated cell component. This is a significant problem because differentiation of one subtype from another rests upon blast cell percentages.

The invited review by Vercauteren and Karsan in this edition of the Journal describes the new directions in comprehending MDS and outlines new advances in our understanding of the molecular pathogenesis of one particular type of myelodysplastic syndrome. It also describes and emphasizes the increasing use of molecular genetic techniques, which are
beginning to be used in the diagnosis and stratification of the disorders. Much of the work that has expanded our knowledge of these conditions has been done in Canada, and it is most appropriate that this review should appear in a Canadian publication. As with many neoplastic conditions, one hopes that eventually this improved understanding of the pathobiology will lead not only to clarification of diagnostic parameters but also to improved, and possibly targeted, therapy. As pathologists, we should welcome objective advancements in diagnostic techniques that can augment and justify our more subjective morphological impressions.

Louis D. Wadsworth, MB ChB, FRCP, FRCP C
Section Editor, Hematopathology

References
Dans la classification des tumeurs hématolymphoïdes au cours du dernier demi-siècle, deux camps s’opposent : les tenants de la catégorisation selon les différences et les fervents de la catégorisation selon les similitudes. Il s’ensuit que, chaque année, les résidents des disciplines du domaine de la pathologie, surchargés déjà, doivent retenir la toute dernière désignation des maladies, sans se douter que, durant cette période de cinq ans, la classification évoluera probablement et qu’ils devront se familiariser avec un nouveau système. Ces courants qui modulent la classification de ces tumeurs témoignent de notre savoir restreint sur la biopathologie précise de nombre de ces maladies.

Notons que la classification du groupe des syndromes myéloprolifératifs, décrits pour la première fois par William Dameshek au début des années 1950 et désormais désignés par l’expression tumeurs myéloprolifératives (TMP), a peu changé en 60 ans. Dans certains cas, le syndrome myéloprolifératif évolue vers la crise blastique qui ressemble à la leucémie aiguë. À l’opposé, la classification du groupe d’hémopathies appelées syndromes myélodysplasiques (SMD) et les connaissances sur ce sujet ont grandement évolué durant ma carrière. Je me rappelle avoir présenté, durant ma résidence, un cas dont le diagnostic était une leucémie monocytaire chronique et avoir suggéré, au scepticisme manifeste de l’auditoire, de peut-être ranger cette affection dans la catégorie des troubles évoluant vers la leucémie myéloblastique aiguë (LMA) et de la classer comme une « préleucémie ». Ce terme de préleucémie a vu le jour dans les années 1970 pour désigner un méli-mélo d’hémopathies que l’on n’arrivait pas à classifier, mais il n’a pas eu la vie longue, car il est vite devenu évident que, même si l’affection se transforment effectivement en LMA dans certains cas, elle prenait la forme d’une leucémie aiguë (LMA) et de la classer comme une « préleucémie ». Ce terme de préleucémie a vu le jour dans les années 1970 pour désigner un méli-mélo d’hémopathies que l’on n’arrivait pas à classifier, mais il n’a pas eu la vie longue, car il est vite devenu évident que, même si l’affection se transforment effectivement en LMA dans certains cas, elle prenait la forme d’une leucémie aiguë (LMA) et de la classer comme une « préleucémie ». 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seulement problématiques pour le pathologiste, mais également pour ceux qui établissent les groupes de référence : la dernière édition de la classification de l’OMS, malgré ses avancées, ne fait pas l’unanimité sur la question de savoir s’il est nécessaire de calculer le pourcentage de cellules blastiques parmi toutes les cellules nucléées de la moelle ou parmi les cellules nucléées à l’exclusion de la lignée érythrocytaire\(^5\). La question est importante, car la distinction entre les sous-types repose sur le pourcentage de cellules blastiques.

L’article de fond de Vercauteren et Karsan qui paraît dans le présent numéro fait le point sur les connaissances acquises à propos des SMD et offre un aperçu de ce que nous savons maintenant de la pathogenèse moléculaire d’un syndrome myélodysplasique en particulier\(^6\). En outre, il met l’accent sur l’usage croissant des techniques de génétique moléculaire, notamment dans le diagnostic et la classification de ces affections. Comme c’est au Canada que s’est déroulée pour la majeure partie la recherche qui a étendu notre savoir sur ces tumeurs, il appert tout indiqué que cette synthèse paraisse dans une revue canadienne. Reste à espérer que cette connaissance plus approfondie de la biopathologie de ces tumeurs débouchera sur la précision des critères diagnostiques et l’amélioration du traitement. Et nous, les pathologistes, devrions saluer ces percées objectives des techniques diagnostiques qui viennent corroborer nos constatations morphologiques au premier abord subjectives.

References

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Message from the Nominating Committee

Dear CAP-ACP Members,

The Nominating Committee proposes the following Slate of Officers for July 2012; the only new nominations are for continuing professional development chair and resource development chair:

- **President** – Vina Alexopoulou
- **Vice-president** – Martin Trotter
- **Past president** – Laurette Geldenhuys
- **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)** – Diponkar Banerjee
- **Member-at-large** – Beverley Carter
- **Member-at-large** – Sylvia Asa

Other:

- **Membership chair** – Bernard Tetu

If you would like to make an alternative nomination for the position of continuing professional development chair or resource development chair, 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

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For further information please click:  [http://www.cap-acp.org/ResReview.cfm](http://www.cap-acp.org/ResReview.cfm)
We report a case of a hemodialysis-dependent, 47-year-old male with a history of hypertensive nephrosclerosis who underwent a cadaveric renal transplantation. Postoperatively, the patient was treated with oral sodium polystyrene sulfonate (Kayexalate) for hyperkalemia. Shortly thereafter, the patient required re-intubation, which was complicated by the aspiration of gastric contents. Months later, bronchoscopy was performed due to a persistent left upper lobe infiltrate on imaging. Transbronchial lung biopsies showed typical histological features of sodium polystyrene sulfonate aspiration (Figure 1).

Sodium polystyrene sulfonate is a cation exchange resin administered orally or rectally for the treatment of hyperkalemia. It is well known to cause necrosis of the lower gastrointestinal tract, where the sodium polystyrene sulfonate crystals need to be distinguished from cholestyramine, a bile acid binding resin. Aspiration pneumonitis caused by sodium polystyrene sulfonate is much rarer and is infrequently reported in the literature. Sodium polystyrene sulfonate appears microscopically as angular fragments of foreign material with parallel lamination (Figure 2). The alignment of these laminae between fragments suggests that they represent an artefact of sectioning. Sodium polystyrene sulfonate is strongly basophilic when stained.
with hematoxylin and eosin and is weakly birefringent under polarized light. The differential diagnosis includes aspirated calcific or organic material. The identity of sodium polystyrene sulfonate can be confirmed by infrared microspectrophotometry, although the microscopic appearance and clinical history are usually sufficient for the diagnosis. The clinical significance of sodium polystyrene sulfonate aspiration is unclear: it is most often a purely incidental finding, although it may present as aspiration pneumonitis.

References
Synoptic Reporting on Breast Cancer Biomarkers: A Novel Quality Improvement Module

Sharon Nofech-Mozes, MD, Tanya Jorden, MLT, Wedad M. Hanna, MD, FRCPC, Mahmoud A. Khalifa, MD, FRCPC

ABSTRACT
Currently, testing for biomarkers such as estrogen receptor, progesterone receptor, and HER2/neu is undertaken in all newly diagnosed cases of invasive breast cancer. Reports of biomarker test results in synoptic format may be linked to the initial surgical pathology report through the creation of discrete data fields. This approach facilitates data entry and promotes the efficient retrieval of data for analysis.

In the past decade, medical institutions and cancer agencies have acknowledged the need for standardization in pathology reporting. Cancer Care Ontario (CCO) endorsed the College of American Pathologists (CAP) cancer checklists for five disease sites, including the breast, and mandated their synoptic reporting in 2004–2005. The aim was to ensure efficient, uniform, and complete province-wide reporting of all scientifically validated data elements in each report. Subsequently, there has been overwhelming evidence that synoptic formatting has also led to improved quality of pathology reporting in Ontario. To date, there are more than 60 site-specific cancer protocols and checklists developed by CAP that serve as a valuable resource for pathologists to effectively provide the information necessary for their synoptic reports. Parallel to the pathology reporting project in Ontario, information technology (IT) solutions were needed to allow entry of the synoptic report tumour parameters as discrete data fields (DDFs). Information entered as DDFs can now be electronically transmitted to CCO and can be retrieved by individual pathology departments, researchers, and healthcare policy makers.

Breast cancer biomarkers such as estrogen receptor (ER), progesterone receptor (PR), and HER2/neu are currently tested in all newly diagnosed cases of invasive breast cancer and occasionally in recurrences and metastatic disease. The assessment of biomarker status in breast cancer is frequently completed after the surgical pathology report has been signed out, and results are therefore released as an addendum to the
original report. Another reason for the two-step reporting on biomarkers in breast cancer is the fact that some of the testing, particularly for HER2/neu, is centrally performed in reference laboratories. In these cases, the laboratory performing the test typically issues a report with limited diagnosis and the biomarker status of the tumour, without completing the entire CAP checklist. On the other hand, the referring laboratory typically issues a synoptic report followed by an addendum once the biomarker status is completed. Irrespective of whether testing for biomarkers is performed locally or in a reference laboratory, the final report is typically in a narrative mode, requiring text editing and multiple intermediate steps involving pathologists and transcriptionists, leading to potential reporting and typographical errors. Moreover, narrative-style reporting does not allow for easy data mining or statistical analysis. It requires the use of retrieval flags, natural language searches, and manual data searches.

Errors in testing of breast cancer biomarkers can be reduced by the adoption of several quality assurance measures. The recent errors in hormone receptor testing in Newfoundland were historical in magnitude and triggered a judicial inquiry. In its final report on the matter, the Commission of Inquiry made landmark recommendations aimed at building system safeguards against future erroneous testing in this area. Consequently, awareness of quality improvement issues around breast cancer biomarker testing was heightened in all anatomical pathology laboratories, and the attention of hospital and health care administrators was captured. This was reflected in the number of publications that followed. As with the Commission of Inquiry report, most of these publications focused on biomarker testing and reporting criteria. The recently published American Society of Clinical Oncology (ASCO)/CAP recommendations for immunohistochemical testing of ER and PR in breast cancer include a periodic trend analysis to help ensure the expected number of ER-positive cases of breast cancer in the patient population served by the laboratory. Nevertheless, limited attention has been given to date to the reporting format. In this article, we introduce a simple template-based biomarker synoptic reporting module that facilitates data entry and produces a clear tabulated final report that allows for data mining and statistical analysis. This innovative tool allows transfer of the parameters as DDFs to tumour registries and central data repositories that can then link them to surgical pathology reports.

Materials and Methods
Our local laboratory information system (LIS) is the Sunquest CoPathPlus Anatomic Pathology System (Version 4.1, Tucson, Arizona), which uses mTuitive (Centerville, Massachusetts) as its synoptic reporting module. A portion of the breast cancer 2010 CAP checklist served as the basis for an mTuitive biomarker synoptic report. Drop-down menus, number fields, and text boxes were created for users to select. Like some other LISs, Sunquest CoPathPlus allows the insertion of only one synoptic report per specimen. The functionality to add a synoptic checklist to an addendum report is also not possible. To enable the usage of a second synoptic report for surgical pathology specimens that use an “S” numbering wheel (for surgical pathology), we have created a separate “B” wheel (for biomarkers) in Sunquest CoPathPlus. The completed synoptic file is then uploaded to our institutional Sunquest CoPathPlus database.

The biomarker report option appears in the drop-down list on the initial CoPath synoptic selection window and is suggested as a default for all specimens accessioned under the B wheel. Once the biomarker report is selected, the pathologists fill in the form by entering number fields and drop-down menus. There is an option to insert notes using free-text boxes after each section. When a test result field is populated, details regarding the antibody used and vendor are automatically entered. For instant review, a summary of all completed fields is displayed on a table at the bottom half of the mTuitive window. Upon completion, the pathologist submits the form to CoPath and the report gets integrated into the Final Diagnosis field.

Results
The biomarker report is accessible from all departmental computers with an mTuitive licence. The selection of this report brings up the complete form. Specific attributes are provided in drop-down lists, number fields, or free-text fields. The attributes relate to the results of ER and PR testing using immunohistochemistry and HER2/neu testing using immunohistochemistry and/or in situ hybridization (silver-enhanced in situ hybridization [SISH] or fluorescence in situ hybridization [FISH]; Figure 1). In addition, attributes related to the adequacy of specimen fixation and external and internal controls are captured with default values. These are suppressed; that is, they are captured but not printed on the final report (Figure 2). The completed form tabulates all the entries containing only selected attributes, and “not
applicable” data do not appear on the report. This allows for a concise synoptic report (Figure 3). If changes to the synoptic report are required, users can navigate back to the mTuitive form and make any changes before signing out the final report. Once electronically transmitted to CCO, the surgical pathology and biomarker synoptic reports can become “associated” with the same cancer in an individual patient, even though reports remain independent pathology reports.

The Ontario Cancer Registry is a population-based registry that uses probabilistic linkage to identify and collect information on every newly diagnosed case of cancer in the province using multiple data sources. For in-house cases, the medical record number ties together all records pertaining to an individual, that is, both the surgical and the biomarker report, by a sequential computer linkage used by the Ontario Cancer Registry Information System. However, because medical record numbers vary among institutions, for consultation cases, the Ontario Health Insurance Plan number and name are used to connect all records pertaining to an individual.

Discussion

We introduced a new concept of reporting biomarkers through a separate synoptic report, currently created for the three most commonly tested markers in breast cancer, ER, PR, and HER2/neu. This application can be extended to include other emerging markers in breast cancer such as Ki-67, or biomarkers in other tumours such as C-KIT and KRAS. This system is more practical than commonly used narrative reports as it decreases error rates by limiting the redundant steps from the pathologist to the transcriptionist and then back to the pathologist. In addition, reports become uniformly structured with standardized terminology used consistently by pathologists. The form is easy to implement and can increase workflow efficacy. The incorporation of the biomarker report required the creation of a new specimen class and number wheel with an accessioning process; updates and modifications

Figure 1. mTuitive form with entry fields for attributes related to the results of ER, PR, and HER2/neu testing. Fields that need to be filled in are shown in the upper left corner; completed items are displayed for confirmation in the lower panel. Reproduced with permission from mTuitive.

Figure 2. mTuitive form: attributes related to adequacy of specimen fixation and external and internal controls are captured with default values. Reproduced with permission from mTuitive.

Figure 3. Example of a final concise synoptic report. Reproduced with permission from Sunquest CoPathPlus.
are however fairly easy. The number wheel specifies the type of specimen in our LIS. Using the prefix \( B \) allows our users to quickly identify the specimen as a biomarkers case. The number wheel is composed of specimen classes that help to further categorize the specimen as referred consultation cases or in-house routine cases. The creation of different specimen classes allows for different workflows and efficient data mining. In practice, a log is generated daily with all orders for biomarker studies and then cases are re-accessioned and assigned a B number (Figure 4). The designated number wheel can be created in many LIS systems. If an LIS permits a synoptic report to become part of an addendum, the biomarkers synoptic report may be incorporated without the added complexity of a new number wheel.

The data generated are kept in DDFs that can be retrieved for retrospective analysis. The current version of CoPath is limited with regards to the data that can be exported, although complex queries can be created using multiple steps, and this facilitates the collection of data. In compliance with the ASCO/CAP recommendation for periodic trend analysis, a semiannual report on the proportion of ER- and PR-positive cases, broken down by age and observer, may be created. In addition, we analyze the percentages of positive and equivocal HER2/neu cases and the proportion of amplified equivocal cases. Periodic monitoring serves as a tool for the detection of technical errors, as well as systemic analytical or pre-analytical mistakes. In addition to being a robust reporting and quality assurance tool, a synoptic format offers a significant advantage over a narrative report. The former generates a prospectively collected dataset in which each individual element can be analyzed for research purposes.

From the point of view of a health system or cancer agency, including data on biomarkers in administrative and population-based databases may add substantial information on prognostic and predictive markers in breast cancer and allow monitoring of compliance with testing recommendations throughout the province. Given the difficulty in incorporating biomarker data in the main surgical pathology report or in capturing them later, we would like to suggest a module that allows adding the biomarkers to data elements collected on breast cancer. Regardless of whether a separate biomarker synoptic checklist is submitted as an addendum or with a designated number wheel, this is the preferred method of biomarker reporting from a local quality assurance perspective and for data collection for cancer registries.
References

A family of tumours arises from either ectopic thymus or rudimentary branchial pouches that retain the potential to differentiate along the thymic line. This family has been divided into four categories: (1) ectopic cervical thymoma, (2) ectopic hamartomatous thymoma, (3) spindle epithelial tumour with thymus-like differentiation (SETTLE), and (4) carcinoma showing thymus-like differentiation (CASTLE).1 Approximately 35 cases of SETTLE have been reported, typically in children and young adults.1–9 SETTLE is considered a potentially malignant tumour and has a propensity to develop late distant metastases. The 59-year-old man with mediastinal and thyroid masses in the case of SETTLE reported here died from the tumour.

Case Report
A 59-year-old man presented with shortness of breath and paroxysmal nocturnal dyspnea of 4 months’ duration. Fatigue and a 9 kg weight loss were noted over the same period. He smoked and was addicted to alcohol, and his past medical history included a multi-nodular goitre. Clinical examination revealed jaundice and an elevated jugular venous pulse. Initial investigations demonstrated disseminated intravascular coagulation. The patient was transferred to a tertiary care centre, where further investigations revealed a mediastinal mass, bilateral pleural effusions, and ascites. Echocardiography showed a large mass in the right ventricle, filling the right atrium and originating from the superior vena cava (SVC). Because of the risk of bleeding, a biopsy was not attempted. With a presumptive diagnosis of lymphoma, the patient was given a single dose of radiation; however, he died 5 days later. At autopsy, a $10 \times 8 \times 4$ cm mass was found between the two lobes of the thyroid gland. The remainder of thyroid tissue contained scattered necrotic, friable yellow foci. The tumour extended into the mediastinum forming a hemorrhagic $12 \times 7 \times 6$ cm mass obstructing the SVC with extension into the right atrium and right ventricle (Figure 1). The
thymus was not identified grossly. Microscopic examination revealed a highly cellular neoplasm traversed by irregular thin and thick sclerotic bands that gave rise to multiple nodules (Figure 2). The tumour had a biphasic pattern, characterized by the merging of cytologically bland and mitotically inactive spindle-shaped to oval-shaped cells with tubulo-papillary epithelial structures. The former component accounted for approximately 70% of the entire tumour and formed vague rosettes. The spindle cells possessed oval nuclei with delicate chromatin and inconspicuous nucleoli (Figure 3). The glandular component showed narrow tubulo-papillary structures that were lined by bland cuboidal to columnar cells. Lymphocytes were sparse. No lymphovascular invasion by tumour cells was present. Interestingly, the thyroid mass showed both components, whereas the mass involving the mediastinum, SVC, and the heart showed only the spindle cell component. Immunohistochemical studies showed that the spindle and glandular components were positive for pan-cytokeratin, vimentin, and smooth muscle actin but negative for CD5. High-molecular-weight cytokeratin was positive in the spindle cell component and negative in the glandular one. Neuroendocrine markers, including chromogranin, synaptophysin, and calcitonin, were not expressed. Furthermore, CD99 and epithelial membrane antigen (EMA) were both negative. Thyroid transcription factor-1 (TTF-1) was positive in the glandular element but negative in the spindle cell component. Thyroglobulin was positive in both the glandular and spindle cell components. The
Discussion

Embryologically, the thymus gland is derived from the endoderm of the third branchial pouch. By the 6th week of development, the thymic primordia begin their descent caudally through the neck to the anterior mediastinum. Chan and Rosai reviewed the literature on intrathyroidal thymic tumours and postulated that a family of tumours arose from either the ectopic thymus or the rudimentary branchial pouches that retain the potential to differentiate along the thymic line. They divided the reviewed cases into four categories: (1) ectopic cervical thymoma, (2) ectopic hamartomatous thymoma, (3) SETTLE, and (4) CASTLE. The biological behaviour of the first two tumours is invariably benign, and they show the same histological findings as intrathymic thymomas. The last two, which have characteristic histological and clinicopathological features, are generally considered malignant.

Approximately 35 cases of SETTLE have been reported so far in the literature. This tumour usually presents as a thyroid or neck mass in children and adolescents. The age range is between 2 and 59 years, with median age at presentation being approximately 14 years; the gender distribution is approximately equal. Macroscopically, these tumours range in size from 1.8 to 12 cm in maximal dimension. The case reported here involved the upper end of the age range and both the thyroid and the mediastinum. The tumour measured 10 × 8 × 4 cm, and it formed a firm mass located between the two lobes of the thyroid.

The histopathological features of SETTLE, which vary relatively little from case to case, consist of an infiltrative, variably myxoid, fascicular-to-reticular proliferation of uniform spindled cells in a partially hyalinized stroma, with transition to areas showing overt epithelial differentiation in the form of small glands and glomeruloid papillary structures. The spindle cells are monotonous, with pale, elongated nuclei and subtle nucleoli. Only occasional pleomorphism or mitotic activity (>1 mitotic figure per 50 high-power fields) is present. The nodules of the cells are usually separated by thick fibrous bands. A reticular pattern caused by the accumulation of intercellular fluid or mucusubstance is usually present. The epithelial component consists of cuboidal or ciliated respiratory epithelial cells that form tubules, papillae, sheets, or mucin-secreting glands. Electron microscopy reveals thymoma-like epithelial features with numerous intercellular junctions and tonofilaments, prominent cellular processes, intracellular glycogen, abundant mitochondria, and well-developed endoplasmic reticulum. The differential diagnosis of SETTLE is important and includes synovial sarcoma; CASTLE; the spindle cell variant of medullary thyroid carcinoma; spindle cell sarcomas, including leiomyosarcomas; and malignant teratoma.

Immunohistochemistry is particularly valuable in establishing the diagnosis. In a recent study by Folpe et al., SETTLE showed extensive expression of high-molecular-weight cytokeratins in seven of eight cases (88%). Expression of low-molecular-weight cytokeratins and epithelial membrane antigen was confined to only scattered cells in seven of eight (88%) and four of eight (50%) cases, respectively. Pan-keratin and high-molecular-weight keratin were expressed in this case and, similar to the findings of Folpe et al., cytokeratin 7 was positive and cytokeratin 20 was negative. In Folpe’s series, expression of CD99 and bcl-2 were seen in six of eight (75%) and seven of eight (88%) cases, respectively, although CD99 was not expressed in this case. Expression of vimentin, muscle-specific actin, and smooth muscle actin has also been reported in the literature, which is consistent with the findings in this case. One case reported by Erickson et al. showed that fluorescent in situ hybridization and reverse transcriptase polymerase chain reaction (PCR) were negative for the X;18 translocation typical of synovial sarcoma; cytogenetic analysis revealed a normal 46, XY karyotype without any clonal abnormality. Xu et al. described SETTLE in a 6-year-old boy, which showed somatic mutations at codons 13 and 15 of the KRAS gene.

The lack of whorl formation and lymphocytic infiltrate differentiates this case from CASTLE, which is a variant of an intrathyroidal epithelial thymoma and is histologically similar to thymic carcinoma of the lymphoepithelioma or squamous cell variety. CASTLE almost always occurs in older patients (age range: 25–69 years). The spindle cell variant of medullary thyroid carcinoma does not show the
biphasic morphological pattern of SETTLE and reveals calcitonin and chromogranin immunoreactivity.\textsuperscript{15} Aggressive spindle cell sarcomas, including leiomyosarcomas, show marked atypia and do not contain epithelial components.\textsuperscript{16} Moreover, such tumours are mitotically active, with coagulative necrosis. In malignant teratoma, the presence of mesenchymal tissue or neuronal components is helpful to distinguish it from SETTLE. Clinically, most thyroid teratomas occur in neonates; malignant forms, which are rare in adults, are more frequently seen in young women.\textsuperscript{15}

Treatment of SETTLE generally consists of surgical excision with or without adjuvant therapy. Despite treatment, the prognosis is not always favourable. Among the cases previously reported, four had pulmonary metastases and died 6 to 8 years after the initial diagnosis.\textsuperscript{12,16,17} One patient had a solitary renal metastasis 22 years after diagnosis\textsuperscript{18}; one died at 14 months because of treatment complications.\textsuperscript{3} A single patient developed metastases in the mediastinum and lung 25 years after the primary tumour in the thyroid was resected.\textsuperscript{3} Only two patients were free of disease 10 and 12 years after diagnosis.\textsuperscript{1,19} Therefore, it seems SETTLE must be considered a potentially malignant tumour with a propensity to develop delayed distant metastases.

Acknowledgement
Dr. Sylvia Asa confirmed the diagnosis of SETTLE in this case.

References
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Co-expression and Cellular Localization of p16 (INK4A) and Mib-1 (Ki-67) Help in Differentiating High-Grade Squamous Intraepithelial Lesions from Immature Squamous Metaplasia of the Uterine Cervix

Md Shahrier Amin, MD, PhD, Mary Senterman, MD, FRCSC, FRCPC, Shahidul Islam, MD, PhD, FRCPC, FCAP

ABSTRACT

Purpose: High-grade squamous intraepithelial lesions (HSILs) of the cervix are a precursor to squamous cell carcinoma. However, many cases present with “borderline” morphological features that are difficult to differentiate from benign entities such as immature squamous metaplasia (ISM). The INK4A gene product p16 has been used as a marker of transforming human papillomavirus (HPV) infections, but accurate interpretation of p16 staining is very difficult. The use of a second marker such as Ki-67 (Mib-1), which is expressed in the nucleus of cells that are no longer in the G0 phase of the cell cycle, may help in differentiating these two entities. The authors hypothesized that simultaneous evaluation of p16 and Mib-1 expression profiles and cellular localization would better differentiate HSIL from ISM.

Methods: Immunohistochemistry was performed with commercially available monoclonal antibodies against p16 and Mib-1 on 15 cases of HSIL and 36 cases of ISM. Expression of p16 was scored as negative, focally positive, or diffusely positive. Mib-1 nuclear expression was assessed in the basal, parabasal, and intermediate layers of the epithelium. The assessment of immunostains was undertaken by two independent pathologists.

Results: Eighty percent (12/15) of cases of HSIL showed diffuse and strong nuclear and cytoplasmic staining of p16 and a strong nuclear pattern of Mib-1 in the basal, parabasal, and intermediate cells. This pattern of immunostaining was considered as positive for HSIL and was corroborated by the pattern of immunostaining in the equivocal cases. In 33% (12/36) of cases of ISM, p16 expression was generally strong and was associated with positive Mib-1 staining in all layers. These cases were reclassified as HSIL. In 53% (19/36) of cases of ISM, p16 and Mib-1 were found to be negative, further confirming the initial diagnosis.

Conclusion: When the morphological distinction between ISM and HSIL is difficult, the latter must be excluded by staining for both p16 and Mib-1. The interpretation of immunostaining of these two antigens is often difficult as there is variation in the pattern of staining. Our study shows that diffuse and strong nuclear and cytoplasmic p16 expression, in combination with...
strong Mib-1 nuclear expression in basal, parabasal, and intermediate cells, is most consistent with HSIL rather than ISM.

RÉSUMÉ

But : La lésion intraépithéliale malpighienne de haut degré de malignité du col de l’utérus constitue un signe précurseur du carcinome malpighien. Dans bien des cas toutefois, les anomalies morphologiques sont « à la limite » de la malignité, d’où la difficulté de les distinguer de l’entité bénigne, telle la métaplasie malpighienne immature. Il existe bien le test de détection de la protéine p16 INK4a, marqueur cytologique de la carcinogenèse induite par le papillomavirus humain, mais son interprétation exacte demeure très difficile. Un autre marqueur, Ki-67 (Mib-1), présent dans le noyau des cellules prolifératives qui n’en sont plus à la phase G0 du cycle cellulaire, pourrait s’avérer utile dans la distinction entre les deux entités. Les auteurs soutiennent que l’évaluation simultanée de l’expression de la protéine p16 et de celle de Mib-1, et leur localisation cellulaire, représente une méthode plus précise de différenciation de la lésion intraépithéliale malpighienne de haut degré de malignité de la métaplasie bénigne.

Méthode : Pour l’analyse immunohistochimique, nous avons eu recours à des tests de détection par anticorps dirigés contre p16 et Mib-1, offerts sur le marché, pour évaluer 15 cas de lésion intraépithéliale malpighienne de haut degré de malignité et 36 cas de métaplasie. L’expression de p16 est qualifiée de négative, de positive localisée ou de positive diffuse. L’expression nucléaire de Mib-1 est évaluée dans les couches basale, parabasale et intermédiaire de l’épithélium. Deux pathologistes déterminent les résultats de l’immunocoloration en toute indépendance l’un de l’autre.

Résultats : Dans 80 % des cas de lésion intraépithéliale malpighienne de haut degré de malignité (12 sur 15), les résultats indiquent l’expression diffuse de p16 et sa forte présence nucléaire et cytoplasmique ainsi que la présence marquée de Mib-1 dans le noyau des cellules des couches basale, parabasale et intermédiaire de l’épithélium. Ce schéma d’immunocoloration confirme la nature de l’entité, soit la lésion intraépithéliale malpighienne de haut degré de malignité, diagnostic corroboré par le schéma de coloration dans les cas équivoques. Dans 33 % des cas de métaplasie (12 sur 36), l’expression de p16 est forte en général et elle s’accompagne du marquage positif de Mib-1 dans toutes les couches. Nous avons donc classé ces cas comme des lésions intraépithéliales malpighiennes de haut degré de malignité. Dans 53 % des cas (19 sur 36), il n’y a pas d’expression de p16 et de Mib-1, ce qui confirme le diagnostic initial.

Conclusion : Lorsque la distinction morphologique entre la métaplasie et la lésion intraépithéliale de haut degré de malignité est difficile à établir, il convient d’écarter la possibilité qu’il s’agisse de la seconde éventualité par l’immunomarquage de p16 comme de Mib-1. L’interprétation de l’immunocoloration de ces deux antigènes est complexe dans bien des cas en raison de la variation du schéma de coloration. Notre étude démontre que l’expression de p16 diffuse et sa forte présence nucléaire et cytoplasmique, couplées à l’expression nucléaire marquée de Mib-1 dans les couches basale, parabasale et intermédiaire, sont révélatrices de la lésion intraépithéliale malpighienne de haut degré de malignité, excluant ainsi la possibilité d’une métaplasie malpighienne immature.
Cervical carcinoma continues to be a significant cause of mortality and morbidity worldwide. There are still 500,000 new cases and 274,000 cervical cancer deaths worldwide each year, with 80% occurring in underdeveloped countries. Even in developed countries such as Canada where the incidence has significantly decreased, there are still about 1,300 new cases (1.6% of all cancers) and 380 cancer deaths (1.1% of all cancer deaths) annually. Much of the decrease in incidence is attributable to organized screening and intervention programs detecting precursor high-grade squamous intraepithelial lesions (HSILs) at an early stage. Differentiating high-grade precursors from mimics such as immature squamous metaplasia (ISM), atrophy, and reactive/reactive changes is subject to inter-observer variability, leading to a high rate of false-positive (5–70% for Papanicolaou [Pap] tests) and false-negative (20–30% for Pap tests) results. Ancillary immuno-histochemistry panels using markers such as p16 have therefore been used in the diagnosis.

p16 is the product of the INK4A gene on chromosome 9. It is a cyclin-dependent kinase 4 (CDK4) inhibitor and an integral component of the retinoblastoma protein (Rb) mediated control of the G1–S phase transition of the cell cycle. p16 inactivates cyclin D1–CDK4/6 complexes and prevents expression of cyclin E, which is essential for progression through the cell cycle. Rb inhibits transcription of p16. In high-risk human papillomavirus (HPV) infections, expression of the E7 oncoproteins not only inactivates p16 but also degrades Rb, causing the release of the transcription factor E2F and an upregulation of p16. Overexpression of p16 has been used as a marker for E7 gene activity and transforming HPV infections. However, focal or even diffuse p16 immunoreactivity can be seen in a non-HPV infected cervix. In a recent meta-analysis, immunostaining for p16 was found to be reported as positive in 2% of normal biopsies, with a confidence interval (CI) ranging from 0.4 to 30%. Among dysplastic lesions, p16 positivity ranged from 38% (95% CI 23–53%) in CIN1 to 68% (95% CI 44–92%) in CIN2 and 82% (95% CI 72–92%) in CIN3. Several evaluation strategies have been proposed for establishing the threshold values above which a sample can be considered as p16 positive, with most studies using the scheme proposed by Klaes et al.

In both Pap tests and biopsy specimens, difficulty often arises in differentiating HSILs from ISM. We propose that using a complementary marker for proliferating activity with p16 will better differentiate HSILs from ISM. Ki-67 expression is extensively used as a surrogate marker for determining proliferative activity in different types of malignancies. It is expressed in the nucleus of cells that are no longer in the G0 phase of the cell cycle. Immunoreactivity to the anti-Ki-67 antibody, Mib-1, is usually limited to the basal and parabasal layers of the cervix, but Mib-1 immunoreactivity extends to the superficial layers in HSILs. In ISM, however, the expression should not extend beyond the parabasal layer. To test our hypothesis, we assessed both p16 and Mib-1 immunoreactivity in a series of HSIL and ISM cases from our institution.

Materials and Methods
We studied 38 cervical biopsies and 13 cases involving loop electrosurgical excision procedures (LEEPs) of the cervix from the surgical pathology archives of our institution between 2008 and 2010. Immunohistochemical staining was performed on 4 µm thick, formalin-fixed, paraffin-embedded tissue sections using antibodies against p16 (sc-56330, Santa Cruz Biotechnology Inc., Santa Cruz, California) and Mib-1 (M7240, Dako, Burlington, Ontario). Slides were deparaffinized in xylene and rehydrated through a graded alcohol series before being placed in a 3% hydrogen peroxide/methanol blocking solution to quench endogenous peroxidase activity, followed by subsequent antigen unmasking. Incubation with the primary antibodies was performed on 4 µm thick, formalin-fixed, paraffin-embedded tissue sections using antibodies against p16 (sc-56330, Santa Cruz Biotechnology Inc., Santa Cruz, California) and Mib-1 (M7240, Dako, Burlington, Ontario). Slides were deparaffinized in xylene and rehydrated through a graded alcohol series before being placed in a 3% hydrogen peroxide/methanol blocking solution to quench endogenous peroxidase activity, followed by subsequent antigen unmasking. Incubation with the primary antibodies was performed overnight at room temperature and at appropriate dilutions. After being washed with TBS, the slides were incubated for 30 minutes at room temperature with goat anti-mouse or anti-rabbit immunoglobulin G (IgG) conjugated to a horseradish peroxidase–labelled polymer. Reactions were developed with 3,3′-diaminobenzidine chromogen and counterstained with hematoxylin. For negative controls, incubation with the primary antibody was substituted with non-immune mouse or rabbit serum. Negative controls were included with every case.
All the slides were reviewed independently by two gynecological pathologists (M. S. and S. I.) and a resident in anatomical pathology (S. A.). In each case, localization of the lesion was confirmed with slides stained with hematoxylin and eosin (H & E). Staining in normal areas was used as an internal control. The expression of p16 was scored as negative when there was no immunoreactivity, focally positive if there was only a weak cytoplasmic or nuclear blush in only a small focus, and diffusely positive when there was a strong and generalized staining of both the nucleus and cytoplasm in a contiguous fashion, imparting a “painted” appearance. Mib-1 nuclear expression was assessed in the basal, parabasal, intermediate, and superficial layer keratinocytes. Mib-1 expression was considered negative if there was nuclear staining limited to the basal, or a few parabasal, cells and positive when nuclear staining was detected in all cell layers of the epithelium.

**Results**

There were 38 biopsies and 13 LEEPs. A preliminary diagnosis of HSIL had been made in nine of the biopsies and six of the LEEPs, whereas ISM with atypia (cannot rule out HSIL) was diagnosed in 29 cervical biopsies and seven of the LEEPs. Both gynecological pathologists had agreed on these initial diagnoses. A summary of the immunohistochemical findings is seen in Table 1. Four different patterns were recognized:

- p16 positive, Mib-1 positive
- p16 positive, Mib-1 negative
- p16 negative, Mib-1 positive
- p16 negative, Mib-1 negative

**p16 Positive, Mib-1 Positive**

Twelve of the 15 cases (80%) that had initially been diagnosed as HSIL showed strong “painted” positive staining for p16 in both the cytoplasm and nucleus (Figures 1-3).

![Figure 1](image1.png) **Figure 1.** Top, hematoxylin and eosin; middle, Mib-1; bottom, p16. High-grade squamous intraepithelial lesion in a cervical biopsy. Immunostaining shows strong “painted” positive p16 staining, as well as Mib-1-positive cells in all layers of the epithelium.

<table>
<thead>
<tr>
<th>Initial Diagnosis</th>
<th>p16 Positive, Mib-1 Positive</th>
<th>p16 Positive, Mib-1 Negative</th>
<th>p16 Negative, Mib-1 Negative</th>
<th>p16 Negative, Mib-1 Positive</th>
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<tbody>
<tr>
<td>ISM with atypia (36)</td>
<td>12 (33%)</td>
<td>1 (3%)</td>
<td>19 (53%)</td>
<td>4 (11%)</td>
</tr>
<tr>
<td>HSIL (15)</td>
<td>12 (80%)</td>
<td>0 (0%)</td>
<td>3 (20%)</td>
<td>0 (0%)</td>
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HSIL = high-grade squamous intraepithelial lesion; ISM = immature squamous metaplasia.
Figure 2. Left Panel, hematoxylin and eosin; middle panel, Mib-1; right panel, p16. Cervical biopsy showing “immature squamous metaplasia with atypia,” which was found to be positive for both p16 and Mib-1. The diagnosis was amended to “immunophenotype favouring high-grade squamous intraepithelial lesion.”

Figure 3. Left Panel, hematoxylin and eosin (H & E); middle panel, Mib-1; right panel, p16. Cervical biopsy initially diagnosed as immature squamous metaplasia with atypia by morphological assessment of the H & E-stained section. Immunostaining shows only faint blush-like staining for p16. Mib-1-positive cells are present in the basal and parabasal layers but are also noted in the superficial layers. Follow-up showed positivity for human papillomavirus DNA. The case was therefore diagnosed as a squamous intraepithelial lesion.

Figure 4. Left panel, hematoxylin and eosin; middle panel, Mib-1; right panel, p16. Cervical biopsy with features of immature squamous metaplasia with atypia in an area of active inflammation. There is increased proliferative activity, as assessed with Mib-1 in superficial layers. However, p16 staining was negative.
Immunoreactivity was diffuse and involved the entire thickness of the epithelium. These areas showed sharp contrast to the adjacent normal cervical epithelium. In all these cases, Mib-1-positive nuclei were seen in all layers of the epithelium. Distinct HPV cytopathic effects were seen and reported in some of these cases (four of 12 = 33.3%). Interestingly, positive immunostaining for p16 was also seen in 12 of the 36 cases (33%) that were initially diagnosed as “ISM with atypia, cannot exclude HSIL.” Four of these cases showed only patchy or blush staining with p16 that could otherwise be considered as weakly positive or even negative. However, immunostaining with Mib-1 showed proliferative activity in all layers of the epithelium. Based on the immunohistochemical findings, the diagnosis was amended to “immunoprofile most consistent with HSIL” in these cases (see Figure 6).

**p16 Positive, Mib-1 Negative**

None of the cases of HSIL that expressed p16 was negative for Mib-1. However, focal p16 staining was seen in one case of ISM. In this case, the staining was limited to patches of epithelium and single cells with only a faint cytoplasmic blush. Mib-1 staining was usually limited to the basal and, at most, parabasal cells (Figure 3). There was no evidence of HPV cytopathic effects and only mild atypia, but follow-up with polymerase chain reaction (PCR) for HPV infection was positive in two repeated tests. Further follow-up of this case did not show any changes to support HSIL, and the diagnosis of a low-grade squamous intraepithelial lesion was maintained.

**p16 Negative, Mib-1 Positive**

None of the cases of HSIL showed a p16-negative, Mib-1-positive pattern of staining. However, four cases of ISM (11%) showed positive Mib-1 staining in the superficial layers of the epithelium (Figure 4). Careful examination showed that these areas were at or near areas of extensive acute inflammation. Follow-up did not show any evidence of a high-grade lesion.

**p16 Negative, Mib-1 Negative**

Three of the 15 cases (20%) of HSIL did not show any staining for p16 or Mib-1 in the superficial layers of the epithelium. On the other hand, 19 of the 36 cases (53%) of ISM showed a lack of staining for p16 in the epithelium and no proliferative activity by Mib-1 staining in the superficial layers (Figure 5).

**Discussion**

The current study affirms previous findings that using morphological features as the sole criterion to differentiate HSIL from ISM with atypia is often misleading. A distinct “painted” positive p16 along with Mib-1 staining in all layers is seen in 80% cases of HSIL. p16 staining may be equivocal in ISM, in which case, Mib-1 positivity in all layers helps to push the diagnosis in favour of dysplasia. Such cases used to be followed up according to the guideline for “atypical squamous cells–cannot exclude high-grade squamous intraepithelial lesion” (ASC-H). Infection of the basal layer cells with a high-risk HPV (HR-
HPV) lies at the cornerstone of cervical carcinogenesis. Limited expression of viral oncoproteins (such as E5, E6, and E7) in the basal cells enhances proliferation and lateral expansion of the infected cells. With persistence of the infection, “late” viral gene expression occurs in the suprabasal layers, followed by the assembly and release of complete viral particles in the more superficial layers, leading to progressively significant atypia, as is seen in different types of SIL. This might explain why strong p16 staining is seen in HSIL in all the layers of the epithelium and associated with a high proliferative activity as evidenced by strong Mib-1 expression.

In the current study, strong p16 and Mib-1 immunoreactivity was noted in most cases of HSIL. However, 20% of cases did not show any p16 or Mib-1 reactivity, although there was clear evidence of atypia. This may indicate that other pathways are also involved in the pathogenesis of cervical carcinoma. Alternatively, increased p16 could override the mitotic drive caused by the expression of E6 and E7 genes. Some previous studies reported 100% positivity for p16 and Mib-1 in cases of HSIL. Others reported variable immunoreactivity to p16, with some showing a lack of p16 staining. However, these studies were based on different interpretations of the pattern of immunostaining. The neoplastic potential of the cases that are negative for p16 is not known. Further studies are required to determine the optimal management, with either more extensive follow-up or intervention with a curative intent.

An important conclusion from the current study is that morphological assessment should not be used as the sole criterion to exclude HSIL. Thirty-three percent (12/36) of our cases of “ISM with atypia” cases were reclassified as HSIL based on positive p16 and Mib-1 staining (Figure 6). In a recent similar study, the authors also noted only fair to moderate agreement between the reviewers regarding a diagnosis of HSIL based on H & E–stained sections alone. About 19% of cases of ISM in that study were reclassified as HSILs based on p16 and Mib-1 positivity. Our numbers are slightly higher, yet in a similar range. For example, in most of the cases of HSIL, p16 immunoreactivity was strongly “painted” positive. However, in some of them (approximately four of 12) the staining was found to be weak or equivocal. Mib-1 staining in such cases was found to be useful to support a diagnosis of HSIL. Considering the H & E morphology as a gold standard (before reclassification), in our cohort of 51 cases we noted a slight improvement in specificity, sensitivity, and positive predictive value (PPV) by using p16 and Mib-1 together (p16 and Mib-1 combined: sensitivity 81%, specificity 61%, PPV 52%) over Mib-1 alone (sensitivity 75%, specificity 54%, PPV 43%). However, Pinto et al. did not find a significant difference in performance between using p16 and Mib-1 alone or in combination. In contrast, in another large multicentre study evaluating the distribution of p16 and Mib-1 staining in a range of cervical lesions, the authors reported increased positive staining of p16 and Mib-1 with worsening diagnoses. Despite poor agreement between endocervical and ectocervical specimens, it was found that using both p16 and Mib-1 together increased the sensitivity and specificity by about 5% (p16: sensitivity 90%, specificity 85%; Mib-1: sensitivity 89%, specificity 87%; p16 and Mib-1 combined: sensitivity 94%, specificity 90%). The improved accuracy in delineating morphologically inseparable lesions is very important as it may change the course of patient management. In our study, we noted improvement in diagnostic accuracy when both tests were used. Therefore, using both p16 and Mib-1 staining is more useful than each alone to differentiate the difficult-to-diagnose cervical lesions. Consistent with the initial diagnosis, 53% of our cases of ISM with atypia did not show any immunoreactivity for either p16 or Mib-1. In one case, we found positive staining for p16 without positivity for Mib-1 in the superficial layers. Interestingly, this case was found by PCR to be positive for...
HPV infection. Similar cases were also encountered by Pinto et al. and are presumed to be the result of high levels of p16 due to the release of its gene from inhibition by Rb. p16 may exert inhibiting effects on residual, unsequestered Rb, overriding the mitotic drive stimulated by E7 and E6 oncoproteins. Therefore, these lesions may represent early stages of HSIL or, alternatively, regressing HSIL. One of the pitfalls of our study is that we did not have the HPV status available for most of our cases. However, persistent HPV infection, rather than a single positive test, is considered a more reliable determinant of carcinogenesis.

References
ABSTRACT
Follicular lymphoma (FL) and mantle cell lymphoma (MCL) are neoplasms of mature B lymphocytes that typically occur in older individuals. Despite certain clinical and histopathological similarities, FL and MCL differ with respect to their underlying biology and clinical course, such that their correct histopathological diagnosis is important. The authors describe a case of MCL diagnosed incidentally in a pericolic lymph node removed during a hemicolectomy. Initial histopathological findings, including an abundant expression of Bcl-2 oncoprotein within follicle centres, justified an initial impression of FL. However, additional immunostains revealed the co-expression of CD5 and cyclin D1 in neoplastic cells that is characteristic of MCL. This case is informative in illustrating the morphological spectrum of MCL. Furthermore, the authors’ findings support the practice of performing a judiciously designed panel of immunostains that includes anti-CD5 and anti-cyclin D1, even in cases in which morphological findings appear to justify an unequivocal diagnosis of FL.

RÉSUMÉ
Le lymphome folliculaire et le lymphome du manteau sont des tumeurs des lymphocytes B matures qui se manifestent en général chez la personne âgée. Même s’ils ont en commun certains aspects cliniques et histopathologiques, ces lymphomes se distinguent l’un de l’autre sur les plans du mécanisme biologique fondamental et de l’évolution clinique, d’où l’importance de l’exactitude du diagnostic histopathologique. Les auteurs décrivent un cas de lymphome du manteau diagnostiqué de façon fortuite dans un ganglion lymphatique péricolique excisé lors d’une hémicolectomie. Les premières constatations histopathologiques, dont la surexpression de l’oncoprotéine Bcl-2 dans les centres folliculaires, justifient l’impression initiale de lymphome folliculaire. Cependant, d’autres analyses d’immunocoloration révèlent la surexpression de CD5 et de la cycline D1 dans les cellules tumorales, trait caractéristique du lymphome du manteau. Le cas a ceci d’instructif qu’il illustre le spectre morphologique du lymphome du manteau. De plus, les constatations des auteurs appuient l’exécution d’un groupe judicieusement choisi d’immunolocoarations visant notamment CD5 et la cycline D1, même si les résultats morphologiques penchent pour un diagnostic de lymphome folliculaire.
Follicular lymphoma (FL) typically presents as lymphadenopathy consequent to the presence of an expanded population of neoplastic B lymphocytes arranged in lymphoid follicles. The neoplastic follicles of FL retain morphological and immunophenotypic features of non-neoplastic follicles. Accordingly, FL may be difficult to distinguish from follicular lymphoid hyperplasia, in addition to other indolent lymphomas. Like FL, mantle cell lymphoma (MCL) is a neoplasm of mature B lymphocytes that usually involves lymph nodes and frequently presents as disseminated disease. However, FL and MCL differ considerably in their underlying biology and clinical behaviour. While the median survival for FL exceeds a decade, MCL typically responds poorly to therapy, such that median survival is approximately 3 years. Moreover, many MCL patients are eligible for experimental therapies, making its pathological distinction from FL critical.

Case Report
An 80-year-old man underwent right hemicolectomy with lymph node dissection for a tubulovillous adenoma. One of the pericolic lymph nodes contained prominent lymphoid follicles, some of which transgressed the nodal capsule (Figure 1A). Follicle centres contained a dominant population of small lymphoid cells with small, dark, somewhat-angulated nuclei (Figure 1B). Large lymphoid cells with vesicular nuclei were present in smaller numbers, and mantle zones were thin or absent. These morphological findings justified an initial, provisional diagnosis of FL. Initial IHC findings supported this impression: most cells within follicle centres expressed CD20 but not CD3 (Figure 1C), staining for the Bcl-2 oncoprotein was relatively intense (Figure 1D), and anti-CD23 highlighted follicular dendritic networks. However, further immunostaining indicated that the predominant intrafollicular cells unequivocally co-expressed CD5 and cyclin D1 (Figure 1E and F, respectively) and did not express CD10 or Bcl-6, although anti-Bcl-6 stained a minor cell population within the follicles. These findings justified a final diagnosis of MCL in which the appearance of FL was largely attributable to extensive infiltration of non-neoplastic, secondary lymphoid follicles by MCL cells. Staging investigations revealed no evidence of more extensive lymph node involvement. The patient is being managed conservatively.

Discussion
MCL can mimic several indolent B-cell lymphomas, including marginal zone lymphoma and small lymphocytic lymphoma, as well as aggressive lymphomas, such as diffuse large B-cell lymphoma, especially the CD5-positive variant, and precursor lymphoid neoplasms. Abundant expression
of Bcl-2 in follicle centre cells is characteristic of FL. However, Bcl-2 is also expressed abundantly and consistently in MCL. Strong Bcl-2 staining in the follicle centres in our case, a consequence of follicle centre colonization by MCL, might have been misinterpreted as evidence of FL. Schuetz et al. recently described two cases of MCL mimicking FL.4 However, since neither of these was stained for Bcl-2, we believe that ours is the first report to underscore intense staining for Bcl-2 in follicle centres as a potential diagnostic pitfall. Although it is reasonable to question the need for IHC in lymphoma cases where the morphological features appear conclusive, the case described here would likely have been misdiagnosed as FL had “confirmatory” immunostains not been undertaken. Therefore, our experience with this case tends to support the practice of performing a panel of corroborative immunostains that includes at least CD5 and Bcl-6 and perhaps cyclin D1, even in cases where a diagnosis of FL seems justified on morphological examination alone.

Lymphomas discovered incidentally in lymph nodes resected for other reasons have not been characterized extensively but may pursue a less aggressive clinical course, suggesting that conservative clinical management may be justified.5,6

References
Myelodysplastic Syndromes: Evolving Insights
Suzanne M. Vercauteren, MD, PhD, Aly Karsan, BA, MD

ABSTRACT
The classification, pathogenesis, and diagnosis and of the entities that comprise myelodysplastic syndromes are reviewed. Factors governing prognosis are discussed, as are newer therapeutic modalities.

RÉSUMÉ
Les auteurs passent en revue la classification, la pathogenèse et le diagnostic des affections regroupées sous le vocable de syndromes myélodysplasiques. Ils examinent également les facteurs qui modulent le pronostic et les nouvelles modalités thérapeutiques.

The term myelodysplastic syndrome (MDS) incorporates a heterogeneous group of clonal stem cell disorders characterized by peripheral blood cytopenia(s) due to ineffective hematopoiesis and dysplasia in one or more of the myeloid lineages. Patients with MDS have an increased risk of developing acute myeloid leukemia (AML) or marrow failure. MDS is the most common hematological malignancy, with an overall incidence of 3–5/100,000 individuals per year, although recent evidence suggests that the incidence is significantly underestimated. The incidence increases dramatically with age, from 0.4/100,000 per year in children to approximately 100/100,000 per year in individuals over 70 years of age.

Pathogenesis
MDS can arise either de novo or secondary to benzene or agrochemical exposure, cytotoxic drug therapy, or radiation. In addition, MDS can occur as part of the evolution of an inherited bone marrow failure syndrome (Fanconi’s anemia, dyskeratosis congenita, Shwachman-Diamond syndrome), and cases of familial MDS have also been described. It is thought that MDS occurs as a result of genetic changes in a primitive CD34+ hematopoietic cell. However, standard cytogenetics on bone marrow samples detects an abnormal karyotype in only approximately 50% of patients with MDS. Some interesting new insights into the development of MDS have been made, particularly in the area of molecular pathobiology. We and others have shown that genetic abnormalities can be detected in the majority of patients with MDS with a normal karyotype using high-resolution array platforms. Although some recurring genetic changes have been seen, the vast majority of changes are sporadic and their significance in the initiation or progression of MDS remains unknown.

Mutations in tumour suppressor genes do not seem to play a major role in the pathogenesis of MDS, with two exceptions (Table 1): the neurofibromatosis type 1 (NF1) gene is frequently mutated in childhood MDS, and mutations in the p53 gene are seen in 5–10% of adult patients with MDS. However, recently, mutations in TET2, ASXL1, and EZH2 have been identified in MDS. These genes may have a tumour suppressor function, suggesting that tumour suppressor genes are more important than previously thought. Proto-oncogene mutations in MDS include NRas/K-Ras, Runx1/AML1, Jak2, Flt3, and EVI-1, although thus far none has been shown to be a crucial player (see Table 1). TET2 mutations appear to be the most common in MDS, with approximately 20% of patients with MDS harbouring this mutation. Although still controversial, preliminary studies have shown that TET2 mutations cause...
hypomethylation of deoxyribonucleic acid (DNA), but it is not well understood how this would lead to MDS.\textsuperscript{30} It is currently unclear how important these mutations are in the pathogenesis of MDS and whether testing patients with MDS for these mutations should become a routine part of diagnostic work.

Defects in ribosome biogenesis play a role in the inherited bone marrow failure syndromes. In addition, haploinsufficiency of the ribosomal protein RPS14, resulting in a block in the processing of the 18S ribosomal ribonucleic acid (rRNA), which then inhibits the formation of the 40S ribosomal subunit, has been identified as a cause for the severe macrocytic anemia in one subtype of MDS, deletion 5q syndrome.\textsuperscript{31} However, this abnormality does not explain the accompanying thrombocytosis, megakaryocytic dysplasia, and propensity to progress to AML or marrow failure. MicroRNAs (miR) are small non-coding RNAs that can bind to the 3′ untranslated region of genes, thereby suppressing or repressing the transcription of that particular gene. These small RNAs may play a role in the pathogenesis of MDS. Our group has recently demonstrated that miR-145, which is located on the commonly deleted region of 5q, is hemizygously expressed in deletion 5q syndrome, while miR-146a is lost in the majority of deletion 5q MDS.\textsuperscript{32} Depletion of miR-145 and miR-146a in mice gives rise to a syndrome very similar to deletion 5q syndrome in humans with marrow hypercellularity, thrombocytosis, and micromegakaryocytes, as well as a propensity to progress to marrow failure or AML. However, macrocytic anemia is not observed in these mice.\textsuperscript{32} Other important factors in the pathogenesis of MDS include the action of inflammatory cytokines such as tumour necrosis factor α and interleukin-6, as well as the interaction of clonal hematopoietic precursors with the bone marrow environment.\textsuperscript{33–35} It is thought that the bone marrow stroma of patients with MDS conveys pro-apoptotic signals to MDS precursor cells, resulting in ineffective hematopoiesis, a hypercellular marrow, and peripheral cytopenias.\textsuperscript{35–37}

### Diagnosis

Most patients with MDS present with cytopenias, particularly anemia, and symptoms related to these cytopenias (fatigue, shortness of breath, bruising/bleeding, infection). Organomegaly is an infrequent finding. Assessment of the complete blood count with review of the peripheral blood smear, bone marrow aspirate and biopsy, and cytogenetics are essential tools in the diagnosis of MDS. The peripheral blood smear should be examined for red blood cell anisopoikilocytosis, hypogranulated or hyposegmented neutrophils (Pelger-Hüet anomaly), large or hypogranular platelets, and the presence of blast cells. Review of the bone marrow must include an assessment of the cellularity, scanning for the presence of erythroid, granulocytic (including increased blast cells, abnormal localization of immature myeloid precursors [ALIP] in the biopsy), and megakaryocytic dysplasia, and reticulin staining to detect the presence of fibrosis in the biopsy. A Perls’ stain for the presence of ring sideroblasts should also be performed on the bone marrow aspirate.

Cytogenetic analysis has a major diagnostic and prognostic role for patients with MDS, and recurrent abnormalities are used in recent classification schemes (see below). Unbalanced abnormalities are more commonly seen than balanced

<table>
<thead>
<tr>
<th>Genetic Abnormality</th>
<th>Frequency in MDS</th>
<th>Clinical Correlation</th>
<th>Prognosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1</td>
<td>10%</td>
<td>JMML</td>
<td>Poor</td>
</tr>
<tr>
<td>p53</td>
<td>5–10%</td>
<td>Complex cytogenetics</td>
<td>Poor</td>
</tr>
<tr>
<td>N-Ras/K-Ras</td>
<td>10%</td>
<td>Progression to AML</td>
<td>Poor</td>
</tr>
<tr>
<td>Runx1/AML1</td>
<td>Rare</td>
<td>Familial MDS, therapy-related MDS, progression to AML</td>
<td>Poor</td>
</tr>
<tr>
<td>Jak2</td>
<td>5% MDS, 50% RARS-T</td>
<td>RARS-T</td>
<td>Unknown</td>
</tr>
<tr>
<td>Flt3</td>
<td>Rare</td>
<td>Progression to AML</td>
<td>Poor</td>
</tr>
<tr>
<td>Tet2</td>
<td>20%</td>
<td>N/A</td>
<td>Unknown</td>
</tr>
<tr>
<td>EVI-1</td>
<td>Rare</td>
<td>N/A</td>
<td>Poor</td>
</tr>
</tbody>
</table>

AML = acute myeloid leukemia; JMML = juvenile myelomonocytic leukemia; MDS = myelodysplastic syndrome; N/A = not applicable; NF1 = neurofibromatosis type 1; RARS-T = refractory anemia with ring sideroblasts and thrombocytosis.
abnormalities. Deletion of the long arms of chromosome 5 and 7 as well as deletion of the whole chromosome 5 or 7 are frequent findings. Trisomy 8, deletion Y, and deletion 20q are recurrent cytogenetic changes seen in MDS but are not considered diagnostic except in the presence of morphological changes consistent with MDS.

A diagnosis of MDS can be entertained when one or more of the following features is seen: (1) blast cells forming between 5 and 20% of nucleated cells in the blood or bone marrow; (2) dysplasia in at least 10% of cells in one or more hematopoietic lineage; or (3) abnormal cytogenetics. Given the heterogeneous nature of MDS, the subjective assessment of dysplasia, and the broad differential diagnosis for cytopenias, dysplasia, or increased blast cells, an accurate diagnosis of MDS can be very challenging. Included in the differential diagnosis are benign causes such as cobalamin or folate deficiency, liver disease, excessive alcohol intake, medication-induced effects, AML, paroxysmal nocturnal hemoglobinuria, aplastic anemia, hairy cell leukemia, and myeloproliferative neoplasms (MPN).

In 2007 consensus statements, developed by an International Working Conference in Vienna, outlining the minimal diagnostic criteria for MDS were published. In addition to the above-mentioned criteria for MDS, three co-criteria were described: (1) an abnormal immunophenotype as seen by flow cytometric analysis; (2) clear molecular signs in human androgen receptor gene assay (HUMARA; this assay detects X chromosome lyonization and therefore possible clonality, but this assay can only be done in females), gene chip profiling, or point mutation analysis; and (3) a reduction in colony formation (colony-forming unit [CFU] assay). Flow cytometry is capable of identifying aberrations on immature and mature myelomonocytic cells and has been shown to be of diagnostic and prognostic value in MDS. For example, expression of the lymphoid marker CD7 on myeloblasts correlates with a poor clinical outcome in patients with MDS. A significant number of molecular abnormalities can be detected in patients with MDS who have normal karyotypes by the use of array comparative genomic hybridization (CGH) platforms, and these molecular changes carry diagnostic and prognostic significance. Reduced colony formation, as measured by the CFU assay, is seen in the majority of patients with MDS, but this test lacks sensitivity or specificity to aid in the diagnosis of MDS.

In summary, although the diagnosis of MDS can be challenging due to disease heterogeneity and a broad differential diagnosis, newer diagnostic tools are emerging that may assist the hematopathologist in making this diagnosis.

**Classification**

In the early 1980s, a morphology-based classification system was developed. This French-American-British (FAB) classification scheme proposed five major subtypes of MDS:

1. Refractory anemia (RA)
2. Refractory anemia with ring sideroblasts (RARS)
3. Refractory anemia with excess of blasts (RAEB)
4. Refractory anemia with excess of blasts in transformation (RAEB-T)
5. Chronic myelomonocytic leukemia (CMML)

In 2001, the World Health Organization (WHO) refined the FAB system by decreasing the blast count for MDS to a maximum of 19%, acknowledging the existence of other cytopenias beside anemia and adding cytogenetic findings to classify MDS. In addition, a category of MDS/MPN was included, which described clonal hematopoietic diseases with overlapping clinical, laboratory, or morphological findings of MDS and MPN. This WHO classification was revised in 2008 and recognized childhood myelodysplastic syndrome as a separate entity (Table 2). The provisional entity, MDS/MPN unclassifiable – RARS associated with marked thrombocytosis, has been added to the MDS/MPN classification (see Table 2).

**Prognosis**

Morphologically, MDS can be divided into three risk groups based on the duration of survival and the progression to AML. The low-risk group includes refractory cytopenia with unilineage dysplasia (RCUD) and RARS, the intermediate-risk group includes refractory cytopenia with multilineage dysplasia (RCMD) and RAEB-1, and the high-risk category is RAEB-2. The International Prognostic Scoring System (IPSS) was developed to include cytogenetics as a prognostic marker. This scoring system is based on the percentage of
### Table 2. Classification of MDS According to WHO 2008

<table>
<thead>
<tr>
<th>WHO Type</th>
<th>Blood Findings</th>
<th>Bone Marrow Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MDS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refractory cytopenia with unilineage dysplasia</td>
<td>• Cytopenia</td>
<td>• Unilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td>• No or rare blasts</td>
<td>• &lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td>• &lt;1 × 10^9/L monocytes</td>
<td>• &lt;15% ring sideroblasts</td>
</tr>
<tr>
<td>Refractory anemia with ring sideroblasts</td>
<td>• Anemia</td>
<td>• Erythroid dysplasia only</td>
</tr>
<tr>
<td></td>
<td>• No blasts</td>
<td>• ≥15% ring sideroblasts, &lt;5% blasts</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia</td>
<td>• Cytopenias (bicytopenia or pancytopenia)</td>
<td>• Dysplasia in ≥10% of cells in two or more myeloid cell lines</td>
</tr>
<tr>
<td></td>
<td>• No or rare blasts, no Auer rods</td>
<td>• &lt;5% blasts in marrow, no Auer rods</td>
</tr>
<tr>
<td></td>
<td>• &lt;1 × 10^9/L monocytes</td>
<td>• &lt;15% ring sideroblasts</td>
</tr>
<tr>
<td>Refractory cytopenia with multilineage dysplasia</td>
<td>• Cytopenias (bicytopenia or pancytopenia)</td>
<td>• Dysplasia in ≥10% of cells in two or more myeloid cell lines</td>
</tr>
<tr>
<td>and ringed sideroblasts</td>
<td>• No or rare blasts, no Auer rods</td>
<td>• ≥15% ring sideroblasts, &lt;5% blasts</td>
</tr>
<tr>
<td>Refractory anemia with excess of blasts 1</td>
<td>• Cytopenias</td>
<td>• Unilineage or multilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td>• &lt;5% blasts, no Auer rods</td>
<td>• 5–9% blasts, no Auer rods</td>
</tr>
<tr>
<td>Refractory anemia with excess of blasts 2</td>
<td>• Cytopenias</td>
<td>• Unilineage or multilineage dysplasia</td>
</tr>
<tr>
<td></td>
<td>• 5–19% blasts, Auer rods ±</td>
<td>• 10–19% blasts, Auer rods ±</td>
</tr>
<tr>
<td>MDS associated with isolated del(5q)</td>
<td>• Anemia</td>
<td>• Normal to increased megakaryocytes</td>
</tr>
<tr>
<td></td>
<td>• &lt;5% blasts</td>
<td>• with hypolobulated nuclei</td>
</tr>
<tr>
<td></td>
<td>• Platelets normal or increased</td>
<td>• &lt;5% blasts, no Auer rods</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Isolated del(5q)</td>
</tr>
<tr>
<td>Myelodysplastic syndrome – unclassifiable</td>
<td>• Cytopenias</td>
<td>• Does not fit other categories of dysplasia</td>
</tr>
<tr>
<td></td>
<td>• ≤1% blasts</td>
<td>• &lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• If no dysplasia, MDS-associated karyotype</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Marrow usually hypocellular</td>
</tr>
<tr>
<td>Childhood myelodysplastic syndrome (provisional)</td>
<td>• Pancytopenia</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• &lt;5% blasts</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Marrow usually hypocellular</td>
</tr>
<tr>
<td><strong>MDS/MPN</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic myelomonocytic leukemia</td>
<td>• &gt;1 × 10^9/L monocytes</td>
<td>• &lt;20% blasts</td>
</tr>
<tr>
<td></td>
<td>• No BCR/ABL1 fusion gene</td>
<td>• Dysplasia in ≥1 myeloid lineage</td>
</tr>
<tr>
<td></td>
<td>• No PDGFR A or B</td>
<td>• If no dysplasia, genetic abnormality or monocytosis &gt; 3 months and all other causes monocytosis excluded</td>
</tr>
<tr>
<td></td>
<td>• &lt;20% blasts</td>
<td>• Hypercellularity with granulocytic proliferation and dysplasia</td>
</tr>
<tr>
<td>Atypical chronic myeloid leukemia, BCR-ABL1 negative</td>
<td>• ≥13 × 10^9/L WBCs with prominent dysgranulopoiesis</td>
<td>• &lt;20% blasts</td>
</tr>
<tr>
<td></td>
<td>• No BCR/ABL1 fusion gene</td>
<td>• Hypercellularity with granulocytic proliferation and dysplasia</td>
</tr>
<tr>
<td></td>
<td>• No PDGFR A or B</td>
<td>• If no dysplasia, genetic abnormality or monocytosis &gt; 3 months and all other causes monocytosis excluded</td>
</tr>
<tr>
<td></td>
<td>• Neutrophil precursors ≥10% of WBCs</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• No/minimal basophilia or monocytosis</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• &lt;20% blasts</td>
<td>• Hypercellularity with granulocytic proliferation and dysplasia</td>
</tr>
<tr>
<td>Juvenile myelomonocytic leukemia</td>
<td>• &gt;1 × 10^9/L monocytes</td>
<td>• &lt;20% blasts</td>
</tr>
<tr>
<td></td>
<td>• No BCR/ABL1 fusion gene</td>
<td>• Hypercellularity with granulocytic proliferation and dysplasia</td>
</tr>
<tr>
<td></td>
<td>• &lt;20% blasts</td>
<td>• If no dysplasia, genetic abnormality or monocytosis &gt; 3 months and all other causes monocytosis excluded</td>
</tr>
<tr>
<td></td>
<td>• ≥2 of following: HbF ↑, immature granulocytes in PB, &gt;10 × 10^9/L WBCs, chromosomal abnormality or GM-CSF hypersensitivity in vitro</td>
<td></td>
</tr>
<tr>
<td>Myelodysplastic/myeloproliferative neoplasm – unclassifiable</td>
<td>• Features of MDS and MPN</td>
<td>• &lt;20% blasts</td>
</tr>
<tr>
<td></td>
<td>• Does not fit in other categories</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• &lt;20% blasts</td>
<td>• Hypercellularity with granulocytic proliferation and dysplasia</td>
</tr>
</tbody>
</table>

GM-CSF = granulocyte-macrophage colony-stimulating factor; HbF = fetal hemoglobin; MDS = myelodysplastic syndrome; MPN = myeloproliferative neoplasms; PB = peripheral blood; PDGFR = platelet-derived growth factor receptor; WBC = white blood cell; WHO = World Health Organization.

Adapted from WHO. 49
blast cells, karyotype, and the number of cytopenias and divides MDS patients into four risk groups (low, intermediate-1, intermediate-2, and high). Germing et al. have added lactate dehydrogenase (LDH) levels to the IPSS score. An elevated LDH is associated with a poorer outcome, and this correlation is more pronounced in patients in the low- or intermediate-1-risk group. In 2005, a WHO Prognostic Scoring System (WPSS) was proposed that relies on the WHO subtype, transfusion requirements, and cytogenetics. Transfusion requirements have also been shown to be an independent prognostic factor in MDS and are thought to be a reliable factor for classifying the severity of MDS and comorbidities. Using this newer parameter, the prognosis can be evaluated at any time during the course of the disease, unlike with the IPSS, which can only predict the prognosis at time of diagnosis. A modified WPSS substituting transfusion dependence for hemoglobin level has just been published. Bone marrow fibrosis and molecular abnormalities are also independent risk factors but are currently not included in prognostic systems. Since transfusion dependence is strongly correlated with poorer survival, the hypothesis has been put forward that the poorer survival is partly due to transfusion-induced iron overload as high serum ferritin levels can also be correlated with poorer survival. Iron chelation appears to improve outcome for MDS patients, suggesting that iron overload is a predictor for poor survival, but prospective studies are still ongoing.

Treatment
The only potentially curative treatment for MDS to date is hematopoietic stem cell transplantation. However, most patients with MDS are ineligible for this treatment modality because of older age and comorbidities. Supportive care is one of the mainstays of treatment and consists of transfusions of red blood cells (RBCs) or platelets as well as the administration of hematopoietic growth factors. As anemia impacts the quality of life significantly, RBC transfusions are a common treatment modality in MDS. Although its use is widespread, RBC transfusion is by no means an inexpensive option. The cost of transfusing one unit of RBC is estimated at C$420, excluding the cost of drug administration, professional fees, laboratory testing, disposable equipment, and treatment of complications. Patient-related loss of productivity and expenses are also excluded and may cost an additional C$450 per unit. Platelet transfusions are given at a lower frequency but costs are comparable or slightly higher per unit. Although not approved by the US Food and Drug Administration (FDA), erythropoietin stimulating agents (ESAs) are still considered a standard of care and are the most commonly prescribed agents for patients with MDS in the United States. ESAs remain front-line therapy for anemia in low-risk or intermediate-1-risk MDS patients according to recent National Comprehensive Cancer Network Guidelines. The percentage of patients with MDS responding to ESAs varies depending on the study. A large meta-analysis showed that approximately 50% of patients with MDS have an erythroid response to standard-dose erythropoietin, with 65% of patients responding to high-dose erythropoietin. It has been shown that serum erythropoietin levels and transfusion history can also predict the ESA response. Flow cytometric assessment of the phenotype of myeloid blasts in patients with MDS in combination with serum erythropoietin level also seems to predict the response to ESA. Concerns exist that ESA may promote tumour growth, cause heart disease and strokes, and hasten death. However, two recent studies did not find a difference in AML transformation between MDS patients that did or did not receive ESAs. Romiplostim is a fusion protein that mimics the action of thrombopoietin, the growth factor for megakaryocytes. It has been approved by the FDA for the treatment of idiopathic thrombocytopenic purpura and is currently being investigated for the treatment of thrombocytopenia in patients with MDS. A recent study with a small number of low- and intermediate-1-risk MDS patients receiving romiplostim showed a trend in decreased, clinically significant thrombocytopenia and a decreased need for platelet transfusions. Due to the relatively low number of patients, these results were not statistically significant, and larger studies will be required.
Over the past decade, new drugs have emerged for the treatment of MDS. Lenalidomide is a derivative of thalidomide that is used in the treatment of multiple myeloma. The mechanism of action of lenalidomide is complex and includes a direct antitumour effect, inhibition
of the microenvironment support for tumour cells, and an immunomodulatory effect. Lenalidomide has shown promising results in the treatment of the MDS subtype deletion 5q syndrome. A landmark phase I/II study showed that 76% of MDS patients with a deletion 5q as an isolated change, or accompanied by other cytogenetic abnormalities, had a response to lenalidomide, with 45% of patients achieving a complete molecular response. Sixty-seven percent of patients became transfusion independent, with more than half remaining transfusion independent for at least a year. There was no clear association between karyotype complexity and the achievement of a cytogenetic response. Hypomethylating agents including decitabine and azacitidine are also under investigation for the treatment of MDS. Although decitabine has not been shown to increase survival, 5-azacitidine has been shown in two independent studies to prolong survival modestly. The results with these new, but expensive, medications are promising and, although they are not likely to be curative, these drugs may improve life expectancy and quality of life.

Conclusions

Over the past decade, significant progress has been made in many aspects of MDS. In particular, a number of mechanisms have been described that may play a role in the pathogenesis of MDS and may open the door for more targeted therapies. In addition, diagnostic standardization and categorization and the use of new diagnostic tools will allow more accurate diagnosis of MDS and facilitate therapeutic trials. With the emergence of prognostic classification systems, patients with MDS can be evaluated for appropriate therapeutic strategies and effectiveness of these treatments. With the arrival of several new treatment agents, patients with MDS can hope for a longer life expectancy and increased quality of life.

References


Molecular Tools and Infectious Disease Epidemiology

As a professor of epidemiology and a director of the Center for Molecular and Clinical Epidemiology of Infectious Diseases in Michigan, Betsy Foxman uses her expertise to clearly summarize the interplay between epidemiology and molecular biology. The incorporation of molecular tools in epidemiological studies provides new insight into the understanding of disease transmission, pathogenesis, and disease evolution: “Molecular tools let us see the world anew.” With a comprehensive review of the applications and caveats of currently used molecular methods, this book is well suited for any student or resident with interests in epidemiology, microbiology, or infectious diseases. The chapters progress to explain the evolution of molecular tools in epidemiology, the implications and appropriateness of their use, ethical concerns that may arise, and finally future opportunities in research and development. Overall, this book is an excellent resource that provides the foundation for practical experiences in molecular epidemiology.

Jason J. LeBlanc, PhD
Division of Medical Microbiology
Department of Pathology and Laboratory Medicine
Queen Elizabeth II Health Sciences Centre
Halifax, Nova Scotia

Legacy of Excellence: The Armed Forces Institute of Pathology, 1862–2011

This book’s title, its coffee table format, and its profuse illustrations all point to Legacy of Excellence as a tribute to the now defunct Armed Forces Institute of Pathology (AFIP). What began as the Army Medical Museum in 1862 during the American Civil War became reinvented as the tri-service Armed Forces Institute of Pathology in 1949; a few years later this institution relocated to the Walter Reed Army Medical Center campus. For the next 60 years, it developed an international reputation for its diagnostic referral and consulting services (over 3 million tumour cases), its countless publications, and its military and civilian medical education courses (approximately 1.6 million CME hours). Many readers of Legacy of Excellence will likely be AFIP alumni and will recognize in its pages colleagues who made this pathology centre what it was; those who are not will learn what they will now miss owing to a 2005 US Presidential decree to close AFIP. This book captures wonderfully the amazing range of AFIP’s routine pathological activities, as well as its extraordinary forensic achievements (e.g., in the aftermath of the 9/11 attacks).

J. T. H. Connor, PhD
John Clinch Professor of Medical Humanities and History of Medicine
Faculty of Medicine, Memorial University
St John’s, Newfoundland
MOLECULAR ONCOLOGIC PATHOLOGY FELLOWSHIP PROGRAM in CANADA

Toronto, Kingston, Vancouver, Victoria, Calgary

“TFF STIHR* in Molecular Pathology of Cancer at CIHR” is funded jointly by the Terry Fox Foundation (TFF) and the Canadian Institutes of Health Research (CIHR). This is a specialized research training program for “Clinician-Scientists in Molecular Oncologic Pathology”, available at any of the four training centres:

**Toronto:** Princess Margaret Hospital/Ontario Cancer Institute  
**Kingston:** Queen’s University  
**Vancouver/Victoria:** BC Cancer Agency, Vancouver and Vancouver Island Centres  
**Calgary:** Alberta Cancer Research Institute and Tom Baker’s Cancer Centre

Accepted fellows are funded by the program for 2 years to receive research training in the pathobiology and molecular pathology of human cancer. Trainees will be exposed to a comprehensive range of leading edge laboratory techniques and their applications to molecular pathology research. In addition to formal and self-directed learning, each fellow undertakes an in-depth research project that should lead to publication in high impact journals. Fellows may elect to combine or continue this training program in post-graduate studies that lead to a M.Sc. or Ph.D. degree.

This Training Program is designed for MD/MBChB pathologists who will have completed their residency or clinical fellowship and wish to develop additional research expertise for an academic career in molecular pathology.

For further information and application details please contact:

Dr. Ming-Sound Tsao  
Tel. (416) 340-4737; e-mail: Ming.Tsao@uhn.on.ca

or

Margaret Jusczczak  
Tel. (416) 340-4800 ext. 5938; E-mail: Margaret.Jusczczak@uhn.on.ca

Website: [http://molecularpathology.ca](http://molecularpathology.ca)

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