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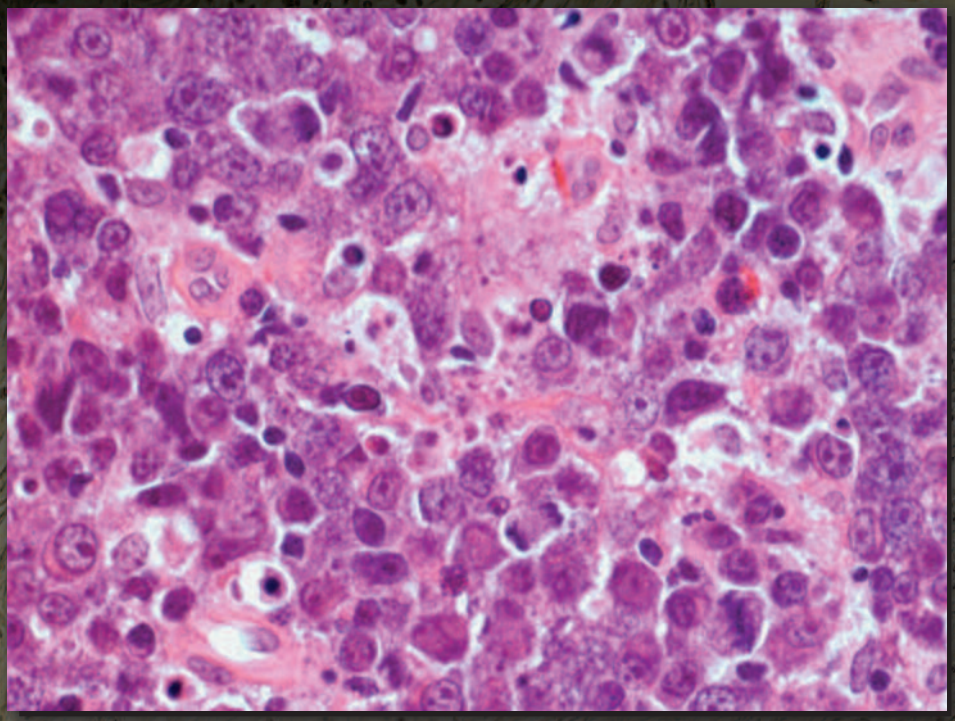

Nucleolous as a Biomarker in Cancer

Measurement of Pathologists' Workload

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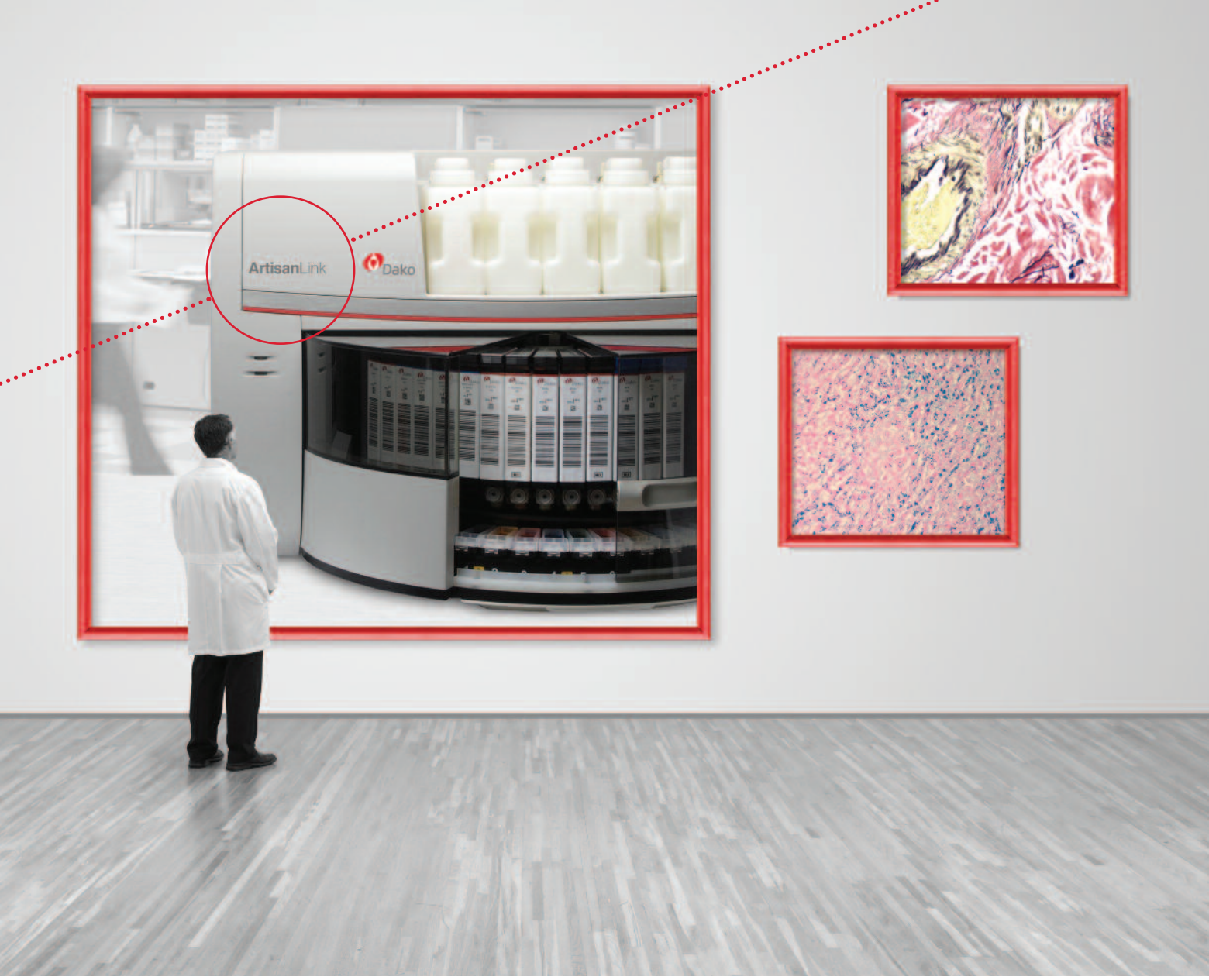

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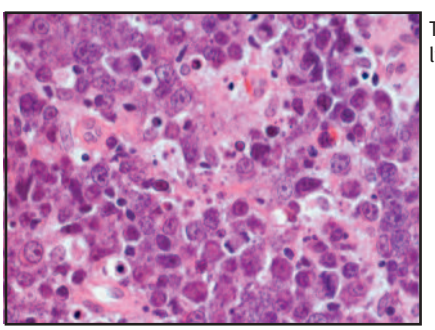

This image shows plasmablastic lymphoma of the maxilla.

EDITORIAL

Pathology Informatics

There is no doubt that the entire health care industry is going through the early stages of an information revolution. Look anywhere and you hear buzzwords such as *electronic health records (EHRs), electronic medical records (EMRs), standards,* and *telemedicine*. In a parallel fashion, the field of laboratory medicine is undergoing its own information revolution, whether internally motivated (e.g., digital microscopy, whole slide scanning) or driven by the momentum of the evolving health care complex (e.g., terminology standards for laboratory data to EHRs/EMRs). As laboratory professionals, it is imperative that we embrace this change and not be left out. Most of all, we need to position our training programs and departments with regard to pathology informatics in such a way that we can take a leadership role in both the laboratory and health care system.

The concept of pathology informatics is very broad and refers to the science of the capture, storage, and processing of information in the laboratory setting. Functionally, this includes areas such as security and privacy, terminology standards, imaging, decision support, interface development, modelling, and bioinformatics. On a day-to-day level, all of us are "informaticians" as we interpret findings in clinical samples, capture those data in a laboratory information system, and disseminate data to the clinical users. Historically, this type of informatics has been taught very well in our pathology training programs and continues to be. Programs must now integrate the technological side of pathology informatics into their residency training through lectures, courses, or exposure at conferences. As an example, programs such as hematological pathology have made it explicit in their specialty training requirements that the resident may take selectives in informatics. No matter how it is done, it is crucial that informatics teaching not only focuses on laboratory-centric informatics, such as the nuances of laboratory information systems or imaging, but also involves larger problems that the health care community wrestles with. Doing this will help to diversify our residents' knowledge, allow them to guide the diagnostic laboratory through its own changes, and help them be conversant with the rest of the evolving health informatics universe.

The role of the informatician in the pathology laboratory must also be recognized, valued, and functionally integrated into the laboratory. As an example, at the Capital District Health Authority in Halifax, we have created within the Department of Pathology and Laboratory Medicine a Pathology Informatics Group (PInG), where there is centralized administration of the laboratory information system and the provision of other value-added services. Our laboratory has leveraged this expertise in transfusion services to improve the management of human and blood resources. Since 2006, we have saved over $800,000 per annum in discarded red cell costs and reduced the average age of red cell units at transfusion by 9 days by using informatics to examine our inventory and supplier and by creating a maximum surgical blood ordering schedule derived from a high-resolution database. We have also examined red cell trafficking at an institutional level to determine areas where we could improve the supply chain and reduce both wastage and aging of red cells. These efforts have benefited our laboratory, the patients, and the institution, and have also reduced the financial burden on the taxpayer in a resource-constrained environment. While other departments may choose to do it differently, all need to harness the interest in informatics and utilize it.

It is also important that national specialty societies recognize the value of pathology informatics. The Canadian Association of Pathologists is creating an Informatics Interest Group, which will have its inaugural meeting at the 2010 CAP-ACP meeting in Montreal later this year. Perhaps in the near future, the CAP-ACP meeting could include presentations on pathology informatics-related topics from Canadian and international speakers as part of its regular conference program. *The Canadian Journal of Pathology* is looking into the future of pathology practice by incorporating a section for pathology informatics and quality management at its outset, and I encourage laboratory specialists with an interest in informatics to consider publishing their work in the journal.

We are in the early days of an information revolution in health care in general and, likewise, in the diagnostic pathology laboratory. If we as laboratory professionals are to remain relevant and contribute to future developments in health care – and, most importantly, lead those changes – we need to position ourselves appropriately. Through efforts to include and value informatics in the CAP-ACP, our own diagnostic laboratories, and our training programs, we will be able to shape the change that will inevitably come.

Calvino Cheng
Section Editor, Pathology Informatics
and Quality Management

L'informatique en pathologie

À l'évidence, toute l'industrie des services de santé est à l'aube d'une révolution informationnelle. Partout, tous n'ont à la bouche que les mots *dossier de santé électronique (DES), dossier médical électronique (DME), normes* et *télémédecine*. En parallèle, le domaine de la médecine de laboratoire traverse sa propre révolution informationnelle sous l'impulsion du milieu lui-même (p. ex., microscopie numérique, balayage d'une coupe entière) ou du système de santé en pleine évolution (p. ex., normes terminologiques des données de laboratoire en prévision du DES ou du DME). Il est impératif que nous, les professionnels de laboratoire, soyons à bord de ce train en marche. Qui plus est, nous devons déterminer la place de l'informatique dans nos programmes de formation et au travail de telle manière que nous exercions un rôle de chef de file en médecine de laboratoire comme dans le système de santé.

L'informatique en pathologie est une notion large qui désigne la saisie, le stockage et le traitement de l'information au laboratoire. Sur le plan fonctionnel, elle comporte de multiples aspects, dont la sécurité et la confidentialité, les normes terminologiques, l'imagerie, le soutien décisionnel, le développement d'interfaces, la modélisation et la bio-informatique. Dans l'exercice de notre profession, nous pouvons nous considérer comme des « informaticiens » en ce sens que nous interprétons les résultats d'analyse des prélèvements, que nous saisissons ces données dans le système d'information du laboratoire et que nous les transmettons aux utilisateurs en pratique clinique. Nos programmes de formation en pathologie ont toujours été conçus pour enseigner l'informatique telle que nous l'appliquons. Cependant, ils devront désormais prévoir l'enseignement des aspects techniques de l'informatique en pathologie au cours de la résidence par des exposés, des cours ou la participation à des conférences sur le sujet. Citons, à titre d'exemple, le programme de formation en pathologie hématologique qui offre un choix de cours obligatoires en informatique durant la résidence. Quelle que soit la formule, l'enseignement de l'informatique ne doit pas couvrir seulement l'informatique de laboratoire en étudiant les différences entre des systèmes d'information ou modes d'imagerie en laboratoire par exemple, mais s'attarder également aux grandes questions auxquelles s'attaque la vaste communauté des services de santé. Une telle orientation favorisera la diversification des connaissances de nos résidents, préparera ceux-ci à guider le laboratoire diagnostique au cours de sa propre phase de changement et fera en sorte qu'ils seront au courant de ce qui se passe dans l'univers de l'informatique de la santé en pleine mutation.

Il nous faut en outre prendre en compte la place de l'informaticien dans le laboratoire de pathologie et valoriser le rôle de cet intervenant au regard du fonctionnement du laboratoire. Dans cette optique, nous avons mis sur pied le Groupe d'informatique en pathologie au sein du Service de pathologie et de médecine de laboratoire à la Régie de la santé Capital District à Halifax, qui assure l'administration centralisée du système d'information du laboratoire et offre d'autres services à valeur ajoutée. Notre laboratoire a mis à contribution cette expertise dans les services transfusionnels afin d'améliorer la gestion des ressources humaines et du stock de produits sanguins. Depuis 2006, nous avons engendré des économies de plus de 800 000 $ par an en ce qui a trait au rejet de concentrés de globules rouges et abaissé de neuf jours l'âge moyen du concentré au moment de la transfusion grâce au recours à l'informatique pour examiner l'inventaire et la fourniture de produits sanguins et pour planifier l'ordonnance de sang chirurgical maximale à l'aide d'une base de données de haute précision. Nous avons également étudié le commerce de globules rouges à l'échelle de l'organisme pour déterminer les points de la chaîne d'approvisionnement à améliorer et réduire tant le gaspillage que le vieillissement des concentrés de globules rouges. Ces efforts se sont révélés fructueux pour le laboratoire, pour les patients et pour l'organisme, ainsi que pour les contribuables dont le fardeau financier a été allégé dans un contexte de ressources limitées pour tout le monde. D'autres services s'y prendront autrement, mais l'essentiel consiste à adopter l'informatique au laboratoire.

Il est tout aussi important que les associations nationales de spécialistes soient conscientes de l'utilité de l'informatique en pathologie. L'Association canadienne des pathologistes forme un groupe d'intérêt en informatique qui tiendra sa première réunion au Congrès de 2010 à Montréal. Peut-être y aurait-il lieu que le congrès de l'Association prévoie dans son programme des exposés sur des sujets ayant trait à l'informatique présentés par des conférenciers du pays ou de l'étranger. La *Revue canadienne de pathologie* quant à elle se penche sur l'avenir de l'exercice de la pathologie en créant une rubrique sur l'informatique et la gestion de la qualité en pathologie; d'ailleurs, j'en profite pour inviter les spécialistes de laboratoire s'intéressant au domaine de l'informatique à

publier leurs communications dans la revue.

Nous sommes à l'aube d'une révolution informationnelle à la fois dans le domaine de la santé en général et dans le domaine de la pathologie diagnostique. Si nous, les professionnels de laboratoire, avons à cœur d'offrir des services pertinents aujourd'hui comme demain et de participer à l'évolution du système de santé – et, plus important encore, de diriger ces changements –, nous devons agir dès maintenant. En nous efforçant de promouvoir l'informatique à l'Association, dans nos laboratoires diagnostiques et nos programmes de formation, nous serons bien placés pour façonner ce changement inéluctable.

Calvino Cheng
Éditeur de la rubrique Informatique
et gestion de la qualité en pathologie

ORIGINAL ARTICLE

Canadian Association of Pathologists Guidelines for Measurement of Pathologist Workload

Raymond Maung, MD, MBA, FRCPC

ABSTRACT

The level 4 equivalent (L4E) system of workload measurement in anatomical pathology has been endorsed by the Canadian Association of Pathologists. This system is applicable to general anatomical pathology practice and may be adapted to include some aspects of clinical pathology. The L4E system is primarily based on specimen complexity and clinical significance. This article details the general rules for application of the system.

RÉSUMÉ

L'Association canadienne des pathologistes a adopté la méthode L4E (level 4 equivalent) de détermination de la charge de travail en anatomopathologie. Cette méthode, qui s'applique en anatomopathologie générale, peut être adaptée à l'anatomopathologie clinique. La méthode L4E tient compte principalement de la complexité des cas et de leur importance clinique. L'article précise les règles générales d'application de la méthode.

The Honorable Mr. Justice Paul S. Creaghan, in his report on the Commission of Inquiry into Pathology Services at the Miramichi Regional Health Authority, commented that "medicine has for too long perhaps been a rather hierarchical profession and I have some suspicion that pathologists have been near the bottom of the 'pecking order.'"[1] This attitude toward laboratory services and its needs has resulted in chronic underfunding in terms of equipment and technical and professional resources in Canada and in other countries.[2–4] The fact that most pathologists are on salary or contract payments may also have contributed to the chronic understaffing of pathology departments. Only imprecise information on how to properly staff a pathology department has been available,[5–7] and most of this was obtained before current guidelines for the detailed reporting of cancer specimens[8,9] and quality assurance were established.[1,10–12]

There have been recent attempts to capture anatomical pathology workload by various authors and institutions,[2,13–15] and the Canadian Association of Pathologists (CAP-ACP) is committed to developing guidelines that are fair to pathologists, institutions, health authorities, and provincial governments, as well as the public. These guidelines will allow pathologist workforce planning and benchmarks to be established for a reasonable, practical, and safe workload. Only in this way will the pathology information needed for proper patient care be provided.

Previous studies have shown the following:

- Population-based benchmarks have a role in pathologist workforce planning but do not constitute a workload measurement system.[2]
- A workload measurement system must take into account case complexity. Workload measurements based simply on case accessions or specimen counts are inadequate.[2,13–15]
- Workload measurement systems designed for

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anatomical pathology, although not ideal for clinical laboratory disciplines, can be adapted to measure a pathologist's workload related to direct patient care in the laboratory specialties of hematopathology, clinical chemistry, and medical microbiology (unpublished studies Provincial Workload Advisory Committee of British Columbia).[16]

- Pathologist workload measurement should encompass the direct clinical care involved in generating a pathology report, and include other patient care–related activities, for example, consultation, clinical rounds, and quality assurance.[2,13]
- Pathologists with university academic appointments and an expectation of academic productivity should have a portion of their time contractually assigned to these activities. Their service commitment should be reduced to reflect these academic commitments.[13]
- Workload benchmarks must consider that pathologists require additional time for administrative functions, system management, and continuing professional development.[13]

CAP-ACP recommends the use of complexity-weighted workload measurement and endorses a modified level 4 equivalent (L4E) system to measure workload in anatomical pathology, with modifications for clinical laboratory disciplines. The L4E system is designed for general anatomical pathology practice and is not directly applicable to specialized practice such as neuropathology or pediatric pathology.

The Level 4 Equivalent System

The L4E system assigns consensus-based relative workload units to diagnostic pathology, taking into account time required, medical value to clinicians and patients, clinical urgency, and medicolegal responsibility. Recommended annual L4E workload is applicable to an average pathologist performing direct patient care duties, including quality assurance activities and professional development. The system does not include academic (research and teaching) or administrative activities.

The key component of the L4E system is weighting of different specimen types and pathologist activities relative

Table 1. L4E Relative Weighting of Pathologist Workload Activities

	Work Activity		Relative Value (L4E)
Surgical pathology*	Level 1		0.15
	Level 2		0.33
	Level 3	Any special stains (≤3)	0.5
	Level 4	are considered part of the case	1
	Level 5		5
	Level 6		15
	Special stains and IHC (≥4 per case); if ≤3, considered part of the case workup		+1 L4E to the case
	Immunofluorescence		+ 0.5 L4E to the case
	Intraoperative consultation		3
	Each additional intraoperative consultation on same case		2
Cytopathology	Exfoliative cytology (urine and sputum) including Papanicolaou's smears that are reviewed and reported by pathologists		1
	All other non-gynecological cytology		2
	Performed FNA biopsy		3
	Performed FNA biopsy with immediate review		5
Autopsy pathology	Routine full autopsy (adult and pediatrics)		24
	Complex full autopsy (medicolegal and hospital)		48
	Limited autopsy		18
	External only autopsy		10
	Brain and/or spinal cord, full neuropathology		18
Consultations (second opinion)	Internal consultation (for each case although multiple pathologists may have seen the case)		1 L4E
	Complex case consultation		Original level × 1.5
	Case review (e.g., cancer centre, external request)		Original level × 0.75

FNA = fine-needle aspiration; IHC = immunohistochemistry; L4E = level 4 equivalent.
*Details of surgical pathology complexity levels are shown in Table 3.

to level 4 surgical pathology specimens (reviewed in Maung[2]). This approach allows for a flexible system adaptable to changes in pathology practice and work complexity.

The recent application of the original L4E system to workload measurement in British Columbia, Alberta,[14] and Manitoba[15] demonstrated that although relative weighting assigned to less complicated specimens (levels 1–3) is appropriate, complex specimens warrant higher relative workload values, as do autopsies, frozen sections (intraoperative consultations), and cytopathology cases. The recommended weighting system is a consensus of the different systems (Table 1).

Despite the potential advantages of using more than six complexity levels, six levels are currently used in British Columbia, Alberta, and Ontario, and in current procedural terminology (CPT) coding in the United States. The retention of six complexity levels for the L4E system is advisable.

Using the L4E system, in most instances each specimen (but not each case) is assigned a complexity level based on the final pathological diagnosis. However, for more complicated cases, including all level 6 and some level 5 cases, the appropriate complexity level is assigned to an entire case, not each individual specimen. The L4E system assumes that the pathologist is totally responsible for gross examination, microscopic examination, and final reporting on every case.

Workload Recommendations

Based on regression analysis of original survey data,[2] with the modifications noted above (i.e. changes in weighting of level 5 and 6 cases, autopsies, intraoperative consultations, and cytopathology cases), the recommended workload per pathologist full-time equivalent (FTE) is 5,453 L4E per year (range 5,277–5,640; approximately ± 3.5%). To account for minor adjustments in specimen categorization and for the inclusion of four or more immunohistochemical stains per case, there is a positive bias of 2.5%, giving a mean of 5,589 L4E per FTE. Internal and external consultations are an integral part of the diagnostic evaluation of many cases, but the associated workload is not adequately measured in most institutions. Since such consultations are an important part of professional quality assurance, it is appropriate that they be integrated into the L4E system.

The productivity of a pathologist depends to some extent on the pattern of practice and on factors such as adequate technical, secretarial, and information technology support. Anatomical pathology can be divided into three practice patterns with somewhat different productivity:

1. Specialized: pure anatomical pathology practice
2. Independent: general anatomical/general pathology practice with in-house immunohistochemistry and an adequate number of colleagues for intradepartmental consultation
3. Rural: group of one to three pathologists with no in-house immunohistochemistry and insufficient colleagues for intradepartmental consultation

In these different practices, it is reasonable to expect the average workloads to be slightly different within the modified L4E system (Table 2).

Categorization of Anatomical Pathology Specimens

The general rules for the application of the L4E system are set out in Table 3. The complexity associated with individual specimens (level) and procedures (L4E) is shown in Table 4.

Table 2. Recommended Annual Average Workload per Pathologist (Modified L4E)

	Rural	Independent	Specialized
Mean	5,589	6,316	7,043
Lower limit (—3.5%)*	5,393	6,095	6,797
Upper limit (+3.5%)*	5,784	6,537	7,290

L4E = level 4 equivalent.
*The regression analysis in the original study indicated that 3.5% represents one standard deviation.[2]

Table 3. General Rules for Categorization of Surgical Specimens* to Reflect the Degree of Difficulty and Effort

	Description	Level	Comment
Rule 1	Biopsies other than skin (gastrointestinal, genitourinary, etc.), e.g., screening biopsies for IBD are given level 4 or 5 depending on total number of tissue biopsy fragments irrespective of the number of containers they are submitted in	4	1–4 biopsy fragments for same diagnostic purpose
		5	5 or more biopsy fragments for same diagnostic purpose
Rule 2	Core biopsies (prostate, breast, etc.), e.g.:	4	1–4 core for same diagnostic purpose
	• 2 breast core biopsies from right upper + 2 core biopsies from right lower lesion = level 4 x 2	5	5–20 cores for same diagnostic purpose
	• 4 breast core biopsies from single lesion = level 4 x 1		
	• 5 breast core biopsies from single lesion = level 5 x 1	6	≥21 cores for same diagnostic purpose
Rule 3	Curettings and tissue fragments (uterine curettings, bladder, TURP, etc.)	4	1–4 blocks for same diagnostic purpose
		5	5 or more blocks
Rule 4	Small organs and surgical excisions, benign or malignant (e.g., lumpectomy, hysterectomy ± SO, adrenalectomy, thymectomy, thyroid resections, etc.)	4	1–4 blocks
		5	5–25 blocks
		6	26 or more blocks
Immunohistochemistry – if 3 or fewer stains considered part of the case†		0 L4E	
Immunohistochemistry – if 4 or more stains, 1 L4E added to the case		+1 L4E	

IBD = inflammatory bowel disease; L4E = level 4 equivalent; SO = salpingo-oophorectomy; TURP = transurethral resection of the prostate.
*A specimen is defined as the content of a single container received from a particular patient.
†A case includes all containers received from the same operation under one accession number.

Table 4. Assignment of Relative Complexity to Specimens and Procedures

System	Description	Complexity (Level or L4E)	Comment
Autopsy	Brain and/or spinal cord, full neuropathology	18 L4E	
Autopsy	Complex – medicolegal and hospital	48 L4E	
Autopsy	External only	10 L4E	
Autopsy	Full pediatric	24 L4E	
Autopsy	Full uncomplicated autopsy	24 L4E	
Autopsy	Partial	18 L4E	
Breast	Implant capsules, gross and micro	3	
Breast	Implant capsules, gross only	1	
Breast	Lumpectomies alone, benign or malignant, (includes gynecomastia)	4/5/6	Rule 4
Breast	Mastectomy partial/full, with/without nodes, for malignancy; sentinel nodes included	6	Sentinel nodes not categorized separately
Breast	Needle core biopsy	4/5/6	Rule 2
Breast	Reduction mammoplasty	4	
Consult	For difficult cases	150% of original level	

Table 4. Assignment of Relative Complexity to Specimens and Procedures *(cont)*

System	Description	Complexity (Level or L4E)	Comment
Consult	Internal	4	Whether examined by one or multiple pathologists
Consult	Routine review for cancer clinic	75% of original level	No gross done
CVS	Aneurysm contents – gross and micro	2	
CVS	Aneurysm contents, thrombus, hematoma, atheromatous plaque – gross only	1	
CVS	Artery – biopsy	4	
CVS	Atheromatous plaque – gross and micro	2	
CVS	Cardiac, explant	6	
CVS	Cardiac, myocardial biopsy without EM, includes transplant	5	
CVS	Heart valve – gross and micro	3	
CVS	Heart valve – gross only	1	
CVS	Hematoma – gross and micro	3	
CVS	Pericardial biopsy	4	
CVS	Ventricle heart, aneurysm, atrium partial resection	4	
CVS	Vessels, vein – varicose veins, gross and micro	2	
CVS	Vessels, vein – varicose veins, gross only	1	
Cytology	Fluids and FNA	2 L4E	
Cytology	Pap smears, urine and sputum	1 L4E	
EM	Any biopsy	6	Any specimen that includes EM is upgraded to level 6 (not an additional level 6)
Endocrine	Adrenal resection	4/5/6	Rule 4
Endocrine	Parathyroid – biopsy	4/5/6	Rule 4
Endocrine	Pituitary biopsy/resection	5	
Endocrine	Thyroid – lobectomy or total thyroidectomy	4/5/6	Rule 4
Endocrine	Thyroid – thyroidectomy with neck dissection, malignant	6	
Eye	Conjunctiva – biopsy, benign, includes pterygium	3	
Eye	Conjunctiva – biopsy, premalignant or malignant	4	
Eye	Cornea, benign	3	
Eye	Cornea, premalignant or malignant	4	
Eye	Enucleation, benign	5	
Eye	Enucleation, malignant	6	
Eye	Evisceration	4	
Eye	Orbital exenteration	6	
Eye	Orbital biopsy	4	
Frozen	For immunofluorescence	3	
GIT	Gallbladder, benign	3	
GIT	Gallbladder, malignant	4/5/6	Rule 4
GIT	Fissure/fistula in ano	3	
GIT	Mouth to anus – biopsy	4/5	Rule 1
GIT	Mouth to anus – resection with node dissection, malignant	6	
GIT	Mouth oral to anus – resection, benign	4/5/6	Rule 4
GIT	Polyps, mouth to anus	4	For each separate/discrete polyp identified

Table 4. Assignment of Relative Complexity to Specimens and Procedures *(cont)*

System	Description	Complexity (Level or L4E)	Comment
GIT	Hemorrhoids	3	If gross, only 1; if gross and micro, 3
GIT	Liver biopsy/wedge resection, for medical conditions (includes pretransplantation and transplant)	5	
GIT	Liver biopsy/wedge resection, for metastases	4	
GIT	Liver resection	4/5/6	Rule 4
GIT	Pancreas – core biopsy	4/5/6	Rule 2
GIT	Pancreas – segmental or total resection, benign	4/5/6	Rule 4
GIT	Pancreas – segmental or total resection, malignant	6	
GIT	Peritoneal biopsy	4/5	Rule 1
GIT	Pilonidal sinus/cyst	3	
GIT	Small bowel biopsy for transplant	4/5	Rule 1
GIT	Stoma – enterostomy, ileostomy, colostomy, etc., and donuts	3	
GIT	Vermiform appendix – incidental and no pathology	2	
GIT	Vermiform appendix – neoplastic	4/5/6	Rule 4
GIT	Vermiform appendix – non-neoplastic	3	
Gyne	Bartholin gland – abscess/cyst	3	
Gyne	Cervix – biopsy or curettings	4/5	Rule 1 or 3
Gyne	Cervix – cone/LEEP biopsy	5	
Gyne	Endometrial biopsy/curettings	4/5	Rules 1 or 3
Gyne	Fallopian tube – biopsy	4/5	Rule 1
Gyne	Fallopian tube resection for benign and malignant conditions	4/5/6	Rule 4
Gyne	Fallopian tubes – sterilization	2	
Gyne	Fallopian tubes or contents – ectopic pregnancy	4	
Gyne	Hydatid of Morgagni	3	
Gyne	Hysterectomy ± adnexa, benign conditions	4/5/6	Rule 4
Gyne	Hysterectomy ± adnexa, malignant condition	6	
Gyne	Hysterectomy ± adnexa, prolapse	4	
Gyne	Leiomyoma(s) – with/without uterus	4/5/6	Rule 4
Gyne	Omentum	4	
Gyne	Ovarian biopsy or wedge resection	4	
Gyne	Ovary with/without tubes, benign or malignant	4/5/6	Rule 4
Gyne	Placenta – gross and micro	4	
Gyne	Placenta, multiple gestation – gross and micro	5	
Gyne	Products of conception, missed/spontaneous	3	
Gyne	Products of conception, therapeutic (family planning)	2	
Gyne	Vagina repair	2	
Gyne	Vulva/vagina – malignant with nodal dissection	6	6
Gyne	Vulva/vagina – resection, without nodal dissection	4/5/6	Rule 4
Gyne	Vulva/vagina/perineal – biopsy	4/5	Rule 1
Head/neck	Cholesteatoma	3	
Head/neck	Larynx – biopsy	4/5	Rule 1
Head/neck	Larynx – partial or total resection with nodes, malignant	6	
Head/neck	Larynx – partial or total resection, nonmalignant	4/5/6	Rule 4
Head/neck	Lip biopsy/wedge resection	4	
Head/neck	Mucus retention cyst – salivary/oral	3	

Table 4. Assignment of Relative Complexity to Specimens and Procedures *(cont)*

System	Description	Complexity (Level or L4E)	Comment
Head/neck	Nasal/sinonasal polyps – inflammatory or allergic	3	
Head/neck	Nasal cartilage – gross only	1	
Head/neck	Odontogenic tumour resection	4/5/6	Rule 4
Head/neck	Odontogenic/dental cyst	4	
Head/neck	Oral, paranasal sinus, nose, mucosal biopsy	4/5	Rule 1
Head/neck	Paranasal sinus, biopsy/curettings	4	
Head/neck	Pharynx, biopsy	4	
Head/neck	Salivary gland biopsy	4/5	Rule 1
Head/neck	Salivary gland resection, benign or malignant	4/5/6	Rule 4
Head/neck	Teeth – gross only	1	
Head/neck	Thyroglossal duct/cyst	4	
Head/neck	Tongue biopsy	4/5	Rule 1
Head/neck	Tongue resection, benign or malignant	4/5/6	Rule 4
Hem/lymph	Adenoid/tonsils, 15 and under – gross and micro	2	
Hem/lymph	Adenoid/tonsils, 15 and under – gross only	1	
Hem/lymph	Adenoid/tonsils, 16 and over – gross and micro	3	
Hem/lymph	Adenoids/tonsils – malignant, resection with nodal dissection	6	
Hem/lymph	Bone marrow biopsy	5	
Hem/lymph	Extranodal lymphoma, biopsy	5	
Hem/lymph	Lymph node – hematolymphoid neoplasm or infection	5	
Hem/lymph	Lymph node – metastatic tumour	4	
Hem/lymph	Lymph node – regional resection, per side of body	5	
Hem/lymph	Lymph node – sentinel node(s) with tumour resection	6	
Hem/lymph	Lymph node – sentinel node(s) alone	4	For each identified numbered sentinel node
Hem/lymph	Mediastinal mass/tumour	4/5/6	Rule 4
Hem/lymph	Spleen – diagnostic or for tumour	5	
Hem/lymph	Spleen – trauma	2	
Hem/lymph	Thymus – tumour resection	4/5/6	Rule 4
Intraop consult	First specimen	3 L4E	
Intraop consult	Second and subsequent specimens on same case	2 L4E	
Male	Foreskin incidental in pediatrics 15 years and below	2	
Male	Foreskin, 15 years and over	3	Foreskin <1 year (?)
Male	Hydrocele sac	1 or 2	If gross, only 1; if gross and micro, 2
Male	Penis resection for malignant conditions	6	
Male	Prostate – needle core biopsies	4/5/6	Rule 2
Male	Prostate – prostatectomy, benign	4/5/6	Rule 4
Male	Prostate – prostatectomy, malignant	6	
Male	Prostate – TURP	4/5	Rule 3
Male	Testis, orchidectomy for carcinoma of prostate	2	
Male	Testis, orchidectomy for primary benign or malignant condition	4/5/6	Rule 4
Male	Testicular biopsy	4	
Male	Testicular biopsy for medical conditions	5	
Male	Testis – appendix	2	
Male	Testis, appendage	3	

Table 4. Assignment of Relative Complexity to Specimens and Procedures *(cont)*

System	Description	Complexity (Level or L4E)	Comment
Male	Testis, spermatocele	3	
Male	Testis, varicocele	3	
Male	Vas deferens, for sterilization	2	
Male	Vas deferens, not for sterilization	3	
Misc	Abscess	3	
Misc	Branchial cleft cyst	4	
Misc	Calculus (stone), foreign body	1	
Misc	Hernia sacs	1 or 2	If gross, only 1; if gross and micro, 2
Misc	Material passed per vaginam or other orifices	3	
Misc	Mesothelium (peritoneum/pericardium/pleural) – biopsy/tissue	4/5	Rule 1
Misc	Thrombus or embolus or blood clot	1 or 2	If gross, only 1; if gross and micro, 2
Nervous	Brain biopsy	5	
Nervous	Brain cyst	4	
Nervous	Brain/meninges – trauma – gross and micro	2	
Nervous	Brain/meninges – tumour resection	5	
Nervous	CNS, spinal cord – tumour resection	5	
Nervous	Muscle biopsy, metabolic and medical conditions	5	
Nervous	Nerve biopsy	5	
Nervous	Nerves, confirm nerve (vagus, sympathectomy, ganglia)	2	
Orthopedic	Amputation, extremities , traumatic – gross and micro	4	
Orthopedic	Amputation, extremity, benign and nontraumatic condition	5	
Orthopedic	Amputation, finger and toes, benign and nontraumatic		
Orthopedic	Amputation, finger and toes, malignant	5	
Orthopedic	Amputation, finger and toes, traumatic – gross and micro	2	
Orthopedic	Amputation, finger and toes, traumatic – gross only	1	
Orthopedic	Amputation/disarticulation, extremity, malignant condition	6	
Orthopedic	Bone – exostosis	3	
Orthopedic	Bone – metastatic tumour and pathological fracture	4	
Orthopedic	Bone biopsy for medical and metabolic disorders	5	
Orthopedic	Bone biopsy or curettings for metastatic carcinoma	4	
Orthopedic	Bone biopsy or curettings for primary bone tumour	5	
Orthopedic	Bone fragments requiring histology	3	
Orthopedic	Bone, femoral head, benign conditions – gross ± micro	3	
Orthopedic	Bone, primary bone tumour – resection	6	
Orthopedic	Intervertebral disc – gross	1	
Orthopedic	Intervertebral disc – gross and micro	2	
Orthopedic	Joint resection	4	
Orthopedic	Joint, bursa	3	
Orthopedic	Joint, cartilage and shavings – gross and micro	2	
Orthopedic	Joint, loose body – gross and micro	2	
Orthopedic	Joint, loose body – gross only	1	
Orthopedic	Joint, meniscus – gross and micro	2	
Orthopedic	Joint, meniscus – gross only	1	
Orthopedic	Joint, synovium – biopsy	4	

Table 4. Assignment of Relative Complexity to Specimens and Procedures *(cont)*

System	Description	Complexity (Level or L4E)	Comment
Orthopedic	Joint, synovium cyst	3	
Orthopedic	Rib, incidental, gross only	1	
Pediatric	Gross and micro, full examination	6	
Pediatric	Gross only	5	
Respiratory	Lung – biopsy (transbronchial or wedge)	4/5	Rule 1 or 3
Respiratory	Lung – resection (segmental, lobe, total), benign conditions	4/5/6	Rule 4
Respiratory	Lung – resection (segmental, lobe, total), malignant conditions	6	
Respiratory	Lung transplant biopsy	5	
Respiratory	Lung, explant	5	
Respiratory	Pleural biopsy	4/5	Rule 1
Respiratory	Respiratory tract (trachea to lung) – all biopsies	4/5	Rule 1 or 3
Skin	Epidermal inclusion cyst	3	
Skin	Finger and toe nail – gross only	1	
Skin	Adnexal tumours	4	
Skin	All benign tumours (includes typical nevus) except adnexal tumour	3	
Skin	All malignant tumours except basal cell carcinoma	4	
Skin	Atypical nevus and melanoma (without minimal data set)	4	All melanocytic lesions including melanoma if no checklist completed
Skin	Basal cell carcinoma	3	
Skin	Alopecia	5	
Skin	Immunofluorescence	5	
Skin	Inflammatory skin disease	4	
Skin	Large excisions	4/5/6	Rule 4
Skin	Malignant condition with nodal dissection	6	Melanoma, squamous or Merkel cell carcinoma
Skin	Melanoma with minimal data set	5	
Skin	Plastic repair – gross and micro 2		
Soft tissue	Carpal tunnel tissue	3	
Soft tissue	Fibromatosis – palmar/plantar/others	3	
Soft tissue	Ganglion cyst	3	
Soft tissue	Lipoma or traumatic neuroma	3	
Soft tissue	Muscle biopsy	5	
Soft tissue	Soft tissue, benign tumours other than lipoma and traumatic neuroma	4/5/6	Rule 4
Soft tissue	Soft tissue, débridement	3	
Soft tissue	Soft tissue, malignant – radical surgery	6	
Soft tissue	Soft tissue, malignant tumour, biopsy or excision	4/5/6	Rule 4
Urinary	Immunofluorescence – kidney, (includes transplanted kidney)	3	
Urinary	Kidney – biopsy for allograft rejection	5	
Urinary	Kidney – biopsy with EM	6	
Urinary	Kidney – biopsy without EM	5	
Urinary	Kidney – partial or total nephrectomy, malignant (includes ureteric lesions)	6	
Urinary	Kidney – partial or total nephrectomy, benign (includes ureteric lesions)	4/5/6	Rule 4

Table 4. Assignment of Relative Complexity to Specimens and Procedures *(cont)*

System	Description	Complexity (Level or L4E)	Comment
Urinary	Ureter/urethra – biopsy or resection for benign lesions	4/5/6	Rule 4
Urinary	Urinary bladder – biopsy or TUR	4/5	Rule 1 or 3
Urinary	Urinary bladder – partial or total resection, benign (includes urethral lesions)	4/5/6	Rule 4
Urinary	Urinary bladder – partial or total resection, malignant (includes urethral lesions)	6	
Urinary	Urinary tract, ureter and urethra – biopsy	4/5	Rule 1

CNS = central nervous system; consult = consultation; CVS = cardiovascular system; EM = electron microscopy; FNA = fine-needle aspiration; GIT = gastrointestinal tract; gyne = gynecological; hem/lymph = hematology and lymphatics; intraop consult = intraoperative consultation; L4E = level 4 equivalent; LEEP = loop electrosurgical excision procedure; misc = miscellaneous; Pap = Papanicolaou's; TUR = transurethral resection; TURP = transurethral resection of the prostate.

Comment

Although different models of workload analysis in anatomical pathology lead to similar conclusions about what is a reasonable and safe workload for a pathologist (Table 5),[2,17,18] the use of the L4E system of measurement derives from an earlier study that demonstrated its superiority to the other indicators.[2] This document contains the simple guidelines by which the L4E system can be applied in any pathology laboratory. After much discussion, in which pathologists representing all 10 Canadian provinces participated, the guidelines were endorsed by CAP-ACP at its Annual General Meeting in Halifax, Nova Scotia, in July 2009. Nevertheless, it was recognized at that meeting that, with continuing changes in pathology practice, the document would need to be updated at regular intervals. The L4E system was not designed to serve as a template for the equitable distribution of work between pathologists in any particular pathology department but, rather, as an indicator of the number of pathologists that would be required to handle that department's workload safely. Further evaluation may allow it to be modified so that it can be used to calculate appropriate daily caseloads for individuals.

Given that throughout Canada many smaller laboratories are staffed by general pathologists or by anatomical pathologists with clinical pathology responsibilities, it should be emphasized that there is no established model for clinical pathology (CP). There are many studies that indicate that the manpower in anatomical pathology (AP) can be used as

Table 5. Comparison of the Various Models of Workload Analysis

Model	Recommendation per FTE in L4E
L4E study	3,455 (range 3,362–3,554)
Royal College of Physicians and Surgeons of Canada*	3,278
Royal College of Pathologists (UK) – modified to Canadian working conditions†	3,570 (4 h/session) 3,123.75 (3.5 h/session)
Medical Group Management Association (US)‡	3,442 (mean) 3,702 (75th percentile)
Manitoba Model§	3,550–3,800
Mean	**3,513**
Median	**3,552**
Standard deviation	**209**

FTE = full-time equivalent; L4E = level 4 equivalent.

* The Royal College of Physicians and Surgeons of Canada recommends one tissue pathologist for a population of 24,500, and the original L4E study regression analysis indicates 1 FTE for a population of 25,819 (3,455 × 24,500/25,819 = 3,278 L4E).

†The Royal College of Pathologists (UK) recommendation: *original* – 40 wk/y × 7.5 sessions/wk × 4 h (3.5 h)/session × 10 units/h = 12,000 (10,500) units; *usual Canadian situation* – 42 wk/y × 8.5 sessions (15% PD time)/wk × 4 h/session × 10 units/h = 14,280 units. Comparative studies show that 1 L4E = 4 UK units. Therefore, annual workload per FTE = 14,280/4 = 3,570 L4E.

‡CPT code 88305 is very similar to level 4 specimens = 1.12 RVU professional component. A study by RPOptions for the British Columbia government has shown that because of different methodology in the categorization of specimens, there is 16.9% overcounting compared with L4E methodology. The Medical Group Management Association recommends on average 4,639 (75th percentile 4,989) RVU per pathologist. The equivalent L4E will be [4,639 × (100% – 16.9%)]/1.12 = 3,442 L4E (75th percentile = 3,702 L4E).

§The Manitoba Model uses 7 categories (vs. 6 categories in other models). It includes only microscopy since gross examination is performed exclusively by pathology assistants. If a 25% or 20% discount is included for gross examination, the recommendation from the Manitoba Model of 7920 PCU is equivalent to 3,550 or 3,800 L4E per FTE.

a baseline to calculate the number of FTEs needed in clinical pathology. A large survey in the United States[19] indicated the following:

- For academic institutions and institutions with residents, the appropriate AP:CP ratio is 1.5:1.
- For community institutions, the appropriate AP:CP ratio is 2:1.

In this context, clinical pathology also includes direct patient consultation and administration. Although not totally satisfactory, this guideline may, in most situations, give a reasonable estimate of the number of FTEs needed to provide adequate laboratory services in an institution. Further studies are necessary in the Canadian setting to determine the appropriate human resource needs for hematopathology, clinical chemistry, microbiology, cytogenetics, and molecular pathology. A possible scheme for the application of the L4E system to diagnostic activities in clinical pathology laboratories is shown in Table 6.

The impact of professional extenders is another area in which there is no consensus. In some institutions there are dedicated pathologists' assistants (PAs) and, in others, trained histotechnologists who perform some or all of the gross examinations. These examinations are carried out under the supervision of a pathologist, who signs out the case, reviews the gross dictation of the PAs, and re-examines and takes more blocks from the specimen if necessary. The pathologist is thus ultimately responsible for the work of the professional extender. As with other professional extenders working with, for example, lawyers, accountants, or dentists, the degree of autonomy and responsibility of the professional extender depends on the ability, training, and experience of the individual, as well as the level of comfort and trust of the professional who takes responsibility for the work. The degree of autonomy and extent of the gross examination by the PAs and trained technologists should be at the discretion of the pathologist who will be responsible for the case. How the work of professional extenders should be accounted for in the L4E system remains to be determined.

Table 6. Possible Workload Values for Examples of Diagnostic Activities in Clinical Pathology

Interpretative Reports: 1 L4E	
Serum protein electrophoresis	
Cardiac enzymes	
Routine blood culture interpretation	
Gram stain interpretation	
Peripheral blood smear	
Routine Clinical Consultations: 2.0 L4E	
Hematopathology	Flow cytometry
	Coagulation
	Fluid morphology
	Semen analysis
	Consultation for test selection
Transfusion medicine	Routine transfusion/blood products consultation
	Routine transfusion reactions
	Interpretation of antibody investigations
	Autologous blood transfusion consultations
Microbiology	Interpretation of culture results and susceptibility testing
	Review and consultation for complicated infections
	Fungal/parasite identification and interpretation
	Consultation for test selection
Clinical chemistry	Consultation in lipid clinics
	Consultation over metabolic and endocrine problems
	Consultation for test selection
Complicated Clinical Consultations*: 10 L4E	
Investigation of infection outbreak	
Consultation in and investigation of complex metabolic disorders	
Consultation in and investigation of complex coagulation disorders	

L4E = level 4 equivalent.
*May include chart and laboratory results review and recommendations.

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ORIGINAL ARTICLE

Sudden Infant Death Syndrome and the Prosecutor's Fallacy

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ABSTRACT

The evaluation of recurrent infant deaths among siblings remains a vexing problem for pathologists. It is commonly held that recurrent unexplained infant deaths within the same family are suspicious for infanticide. Statistical assessments of the probability of recurrent deaths being attributable to sudden infant death syndrome (SIDS) *versus* infanticide have suffered from two errors. The first error is that the statistical odds can be miscalculated by assuming independence of the two death events. The second and more important error is related to the so-called prosecutor's fallacy and stems from the improper framing of the initial question. Instead of asking the probability of more than one future SIDS death in a randomly chosen family, we need to ask a different question: What is the probability, given that more than one child has already died, of a family experiencing two SIDS deaths *versus* the probability that at least one of the deaths was infanticide. This article demonstrates a simple mathematical solution for weighing the likelihood of these alternative scenarios. Based on published epidemiological data, the a priori odds that multiple deaths are due to SIDS rather than infanticide is much higher than is generally believed.

RÉSUMÉ

L'évaluation de plus d'un décès infantile dans une même famille demeure une tâche difficile pour le pathologiste. Il est admis généralement que la survenue de plus d'un décès infantile inexpliqué dans une famille donne lieu à un soupçon d'infanticide. La détermination statistique de la probabilité de la cause de ces décès, soit la mort subite du nourrisson (MSN), soit l'infanticide, est entachée de deux erreurs. L'une d'elles est une erreur de calcul de la probabilité statistique provenant de l'hypothèse voulant que les deux incidents mortels soient indépendants l'un de l'autre. L'autre, la plus importante, qui s'apparente à l'argumentation fallacieuse d'un procureur, découle de la formulation inappropriée de la question initiale. Plutôt que de se demander quelle est la probabilité de plus d'une MSN dans une famille choisie au hasard, nous devons poser la question suivante : quelle est la probabilité qu'un deuxième décès infantile dans une famille soit imputable à la MSN par rapport à la probabilité qu'au moins l'un des décès soit un infanticide? L'article propose une solution mathématique simple à la pondération de la probabilité de ces deux scénarios. D'après des données épidémiologiques publiées, la probabilité a priori que les décès soient tous des MSN et non pas l'issue d'un infanticide est beaucoup plus élevée qu'on a tendance à le croire.

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The evaluation of recurrent sudden infant death syndrome (SIDS) includes an adequate postmortem examination to exclude disorders with familial recurrence, such as cardiac disorders, respiratory tract anomalies, central hypoventilation syndromes, and inborn errors of metabolism. There is, however, no universally accepted protocol for the SIDS postmortem examination. Despite the absence of accepted standards in the evaluation of SIDS, pathologists are commonly called upon to give expert court testimony in such cases. Statistical assessment as part of that testimony, however, must be approached with circumspection since it is outside the expertise of most pathologists. As one commentator has noted, "The understanding that statistics is a difficult subject is not widespread."[1] Furthermore, the need to engage in a discussion on the proper use of mathematics in the legal system is well recognized.[2] Perhaps the commonest and most dangerous mistake has been dubbed "the prosecutor's fallacy." Although it was described over 20 years ago,[3] the prosecutor's fallacy remains largely unknown to most pathologists. The purpose of this article is to describe this error and demonstrate a simple mathematical solution for weighing the likelihood of alternative scenarios.

The example of the Sally Clark murder trial in the United Kingdom graphically illustrates the prosecutor's fallacy. Ms. Clark was tried and convicted of murdering her two infant sons in 1999. The defense argument was that the children had died of SIDS. A pediatric expert witness testified at trial that the chance of one SIDS death in an affluent British family was 1 in 8,500; therefore, the chance of two SIDS deaths was this figure squared or 1 in 73,000,000. (Actually, the proper calculation would have been $1/8{,}500 \times 1/8{,}500 = 1/72{,}250{,}000$.) These are long odds indeed and enough for the jury to render a guilty verdict.

Intuitively, it seems that innocence was unlikely, but this argument suffers from two important errors. The first error was that the statistical odds were (mis)calculated by assuming the independence of the two death events. The 1 in 73,000,000 statistic was thought to be a reasonable estimate of the chance that a family chosen at random from the population would experience two SIDS deaths. The likelihood of two SIDS deaths within the same family is actually much higher since two SIDS deaths occurring in the same family may have a common genetic or environmental cause.[4] Secondly, and more importantly, what we really need is a measure of something very different. We want to know the probability of a family having two SIDS deaths *versus* the probability of a double infanticide (or the probability of two SIDS deaths *versus* the probability that at least one of the deaths was an infanticide).

To determine these relative probabilities, we first need to gather some probability estimates from the literature. For the risk of a SIDS death in an affluent British family, we may use the estimate presented at the original trial: 1 in 8,500. The chance of a second or subsequent SIDS death in the same family, given that one has already occurred, is greater than this due to common genetic or environmental factors. The probability of a second or subsequent SIDS death may be as high as 1 in 100,[4] but let us use a conservative estimate of 1 in 300. For the United Kingdom, the infant (under 1 year) murder rate is 15.2 per million per year.[5] The risk of someone committing a second murder is harder to calculate. The recidivism rate for violent crime is in the range of 30%.[6] For this demonstration, let us assume the probability of a second (and each subsequent) infanticide is 10%.

Utilizing these data, Table 1 shows the relative probabilities for every possible combination of SIDS and murder for up to three unexplained infant deaths in the same family. For one death, the a priori probability of SIDS is 88.6%. Surprisingly, the a priori probability of two SIDS deaths in the same family is 20.5% (odds of about 1 in 5). This number is clearly very different from the 1 in 73,000,000 estimate (0.0000000136%) of having two SIDS deaths in the same family cited in the above court case. It should be noted that the recidivism rate of 10% used here is probably an overestimate of the actual odds of a parent murdering a second child. If we use a risk of 1 in 50 of a second murder occurring in the same family, the relative a priori probability of two SIDS deaths then becomes 56.3%.

When we consider the situation of three infant deaths within the same family, the probability that all three were SIDS falls to 0.8%, again assuming a risk of 1 in 10 for subsequent murders in the same family. However, extending the original argument used in the Sally Clark case, the risk of three SIDS deaths could erroneously be calculated as ($1/8{,}500 \times 1/8{,}500 \times 1/8{,}500 = 1/614{,}125{,}000{,}000$).

Table 1. A Priori Probabilities of SIDS versus Murder for One, Two, and Three Unexplained Infant Deaths in the Same Family*

1 death	(SIDS, murders)			(1, 0)		(0, 1)			
	Probability formula			p_1		q_1			
	Relative probability			88.6%		11.4%			
2 deaths	(SIDS, murders)		(2, 0)		(1, 1)		(0, 2)		
	Probability formula		p_1p_2		$2p_1q_1$		q_1q_2		
	Relative probability		20.5%		0.2%		79.3%		
3 deaths	(SIDS, murders)	(3, 0)		(2, 1)		(1, 2)		(0, 3)	
	Probability formula	$p_1p_2^2$		$3p_1p_2q_1$		$3p_1q_1q_2$		$q_1q_2^2$	
	Relative probability	0.8%		0.0%		0.3%		98.8%	

SIDS = sudden infant death syndrome.
*Where p_1 = probability of a SIDS death = 1/8,500; p_2 = probability of each subsequent SIDS death = 1/300; q_1 = probability of an infanticide = 1/65,789; and q_2 = probability of each subsequent infanticide = 1/10.

Several conclusions can be drawn from this demonstration. The first is that commonly held beliefs about the relative a priori probabilities of multiple SIDS deaths are not supported by epidemiological data. In fact, the a priori odds that multiple deaths are due to SIDS rather than infanticide is much higher than generally believed. The second point is that the use of statistical inference to give the probabilities of competing explanations may not be intuitive. However, the probability method I have illustrated here is simple to use and, if properly applied, could be used to calculate probabilities for other scenarios.

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Oral Extranodal Lymphoproliferative Disorders of B-Cell and T-Cell Origin

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ABSTRACT

There have been very few reports of the relative incidence of oral lymphomas since the reclassification of non-Hodgkin's lymphomas by the World Health Organization (WHO) and the introduction of new entities, some of which present frequently or predominantly in the oral cavity. The Canadian experience has not been explored or compared with similar studies from other geographical regions.

This study reports the clinical and microscopic details of 88 cases of lymphoproliferative neoplasms of B- and T-lymphocytes, reclassified according to WHO criteria published in 2008, from 1995 to mid-2009 inclusive (a 14.5-year period), in a Canadian population. It compares these data with other populations in like publications.

Diffuse large B-cell lymphomas were seen most often, accounting for 40.9% of the total number, whereas follicular lymphomas accounted for 18.2%. Extranodal marginal zone (mucosa-associated lymphoid tissue [MALT]) lymphomas (also 18.2%) were found to be disproportionately more common and T-cell lymphomas disproportionately less common in the oral region than in other sites. Rare lymphomas, including B-cell lymphoblastic lymphoma, sporadic Burkitt's lymphoma, and plasmablastic lymphoma, which has a marked predilection for the oral mucosa, are documented in greater detail.

The widespread use of the WHO classification of B- and T-cell lymphoproliferative disorders has allowed a global comparison of the incidence of lymphomas of the oral region. Only minor geographical differences in relative incidence were found in this comparative study. A variety of lymphoproliferative disorders occur in the oral cavity, including rare entities that may cause diagnostic difficulties.

RÉSUMÉ

Seules quelques études font état de l'incidence relative des lymphomes buccaux depuis la reclassification des lymphomes malins non hodgkiniens effectuée par l'Organisation mondiale de la santé (OMS) et la recension de nouvelles entités dont certaines se manifestent fréquemment ou principalement dans la cavité buccale. Aucune n'évalue la situation au Canada, ni ne la compare à celle d'autres régions.

La présente étude décrit les aspects cliniques et microscopiques de 88 syndromes lymphoprolifératifs B ou T reclassés conformément aux critères de l'OMS publiés en 2008,

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diagnostiqués dans la période de 14,5 ans allant de 1995 au milieu de 2009 au Canada. Elle compare ces données à celles sur d'autres populations dans des publications de même envergure.

Le lymphome B diffus à grandes cellules est le plus fréquent, représentant 40,9 % des cas, tandis que le lymphome nodulaire regroupe 18,2 % des cas. Le lymphome du tissu lymphoïde associé aux muqueuses (correspondant également à 18,2 % des cas) est, toute proportion gardée, plus fréquent dans la cavité buccale qu'ailleurs, alors que c'est l'inverse pour ce qui est du lymphome T. L'article documente avec précision les lymphomes rares, dont le lymphosarcome lymphoblastique B, le lymphome de Burkitt sporadique et le lymphome plasmablastique à prédilection marquée pour la muqueuse buccale.

L'adoption à grande échelle de la classification de l'OMS des syndromes lymphoprolifératifs B ou T facilite l'évaluation mondiale de l'incidence des lymphomes de la cavité buccale. L'étude comparative ne décèle que de légères différences d'incidence entre les diverses régions géographiques. Un certain nombre de tumeurs lymphoprolifératives ont pour siège la cavité buccale, dont des entités rares qui peuvent poser des difficultés d'ordre diagnostique.

In 1994, the Revised European-American Classification of Lymphoid Neoplasms (REAL classification) was developed,[1] which was further refined in the classifications published by the World Health Organization (WHO) in 2001[2] and updated in 2008.[3] Significantly, the WHO classification has gained widespread acceptance, allowing for the first time comparative analysis of worldwide published data. Reports on lymphomas of the oral and maxillofacial region using the WHO classification[4–6] or REAL classification[7] are very few indeed. We report 88 such lymphoproliferative lesions of B-cell and T-cell origin in a mixed Canadian population consisting predominantly of Caucasian individuals of European background; the lesions are classified according to the WHO criteria of 2008.[3] In addition, we document in greater detail rare oral lymphomas that may present diagnostic difficulties.

Patients and Methods

All cases involving oropharyngeal soft tissues and jaw bones diagnosed microscopically as Hodgkin's lymphoma, non-Hodgkin's lymphoma, atypical lymphoproliferative disease, plasmacytoma, and multiple myeloma for the 14.5-year period from 1995 to mid-2009 inclusive were retrieved from the archives of the Oral Pathology Diagnostic Service at University of Western Ontario, in London. Hematoxylin and eosin–stained tissue sections and all available existing immunohistochemical stains were reviewed. Additional immunohistochemical stains were applied to representative tissue sections in cases that required further evaluation, using a Vector Impress or Ultraview technique and commercially available primary antibodies with appropriate negative and positive controls. Markers included *BCL2*, CD3, CD20, CD23, CD30, CD43, CD45RO, CD138, cyclin D1, anaplastic lymphoma kinase 1 (ALK1), immunoglobulin A (IgA), IgG, IgM, kappa, and lambda from DakoCytomation, Carpentia, California; *BCL6* from Cell Marque, Rocklin, California; CD5, CD10, and CD56 from Vector Laboratories, Burlingame, California; CD79a from Immunotech, Marseille, France; terminal deoxynucleotidyl transferase (TDT), polyclonal, from Supertechs Inc, Rockville, Maryland. Immunostains performed prior to 2006 were done with the avidin-biotin complex (ABC) system.

All authors reviewed and, when necessary, reclassified the cases according to WHO definitions.[3] In cases of diagnostic disagreement, cases were reassessed, further immunohistochemical stains were ordered, if appropriate, and a consensus diagnosis was reached. In selected cases, an in situ hybridization for Epstein-Barr virus–encoded small ribonucleic acids (EBER) study was performed. Molecular and cytogenetic studies for translocations or gene rearrangements were not routinely performed. Flow

Table 1. Clinical Data of 88 Oral B- and T-Cell Lymphomas, by Diagnosis

Lymphoma	Number (%)	Mean Age (Range)	Sex	Biopsy Site
DLBCL	36 (40.9)	65.2 (25–89)	M: 19, F: 17	Md: 3, Mx: 8, S: 25
Follicular	16 (18.2)	67.9 (44–90)	M: 5, F: 11	Md: 2, Mx: 0, S: 14
Marginal	16 (18.2)	70.6 (53–93)	M: 4, F: 12	Md: 2, Mx: 0, S: 14
Mantle	3 (3.4)	63, 78, 91	M: 3	Md: 0, Mx: 0, S: 3
SLL	1 (1.1)	52	M: 1	Md: 0, Mx: 0, S: 1
Other*	3 (3.4)	9, 29, 82	M: 2, F: 1	Md: 0, Mx: 2, S: 1
Per. T-cell	1 (1.1)	37	F	Md: 0, Mx: 0, S: 1
Nasal NK/T	1 (1.1)	46	F	Md: 0, Mx: 1, S: 0
MF	2 (2.3)	56, 78	M: 2	Md: 0, Mx: 0, S: 2
Plasma cell	9 (10.2)	67.2 (51–83)	M: 6, F: 3	Md: 8, Mx: 0, S: 1
Total	88	(9–93)	M: 42, F: 46	Md: 15, Mx: 11, S: 62

DLBCL = diffuse large B-cell lymphoma, not otherwise specified; follicular = follicular lymphoma; mantle = mantle cell lymphoma; marginal = extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma); Md = mandible; MF = mycosis fungoides; Mx = maxilla; nasal NK/T = extranodal natural killer/T-cell lymphoma, nasal type; per. T-cell = peripheral T-cell lymphoma, not otherwise specified; plasma cell = plasma cell myeloma/solitary plasmacytoma of bone; S = soft tissues; SLL = small lymphocytic lymphoma.
*Includes B-cell lymphoblastic lymphoma, sporadic Burkitt's lymphoma, and plasmablastic lymphoma.

cytometry was not performed on the cases.

Patient sex and age at the time of biopsy, the site of the oral/perioral lesion, and historical data were recorded. Since staging was usually carried out by clinicians after the biopsy, staging information was not available for most cases.

Results

There were no recorded cases of oral Hodgkin's lymphoma in the 14.5-year period. All cases presented in the oral and maxillofacial area as extranodal masses. The final diagnoses of five cases diagnosed prior to 2001 were changed from B-cell lymphoproliferative disorders, small cell type, to extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) or mantle cell lymphoma when the WHO classification[3] and additional immunohistochemical studies were considered.

There were 80 lesions submitted directly to our biopsy service, and 8 lesions were referred from other centres. Table 1 lists the relative incidence of the various types of lymphoproliferative neoplasm, data concerning patient age

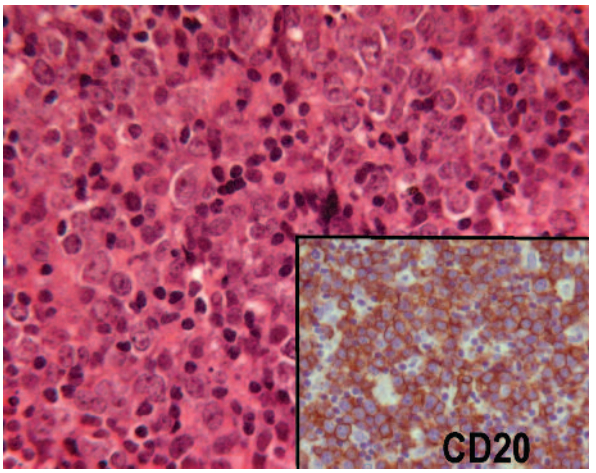

Figure 1. Diffuse large B-cell lymphoma showing cells with large vesicular nuclei, one or more peripherally located nucleoli, and abundant cytoplasm, with positive immunoreactivity for CD20. (Hematoxylin and eosin, objective lens 40×; anti-CD20/hematoxylin, objective lens 10×)

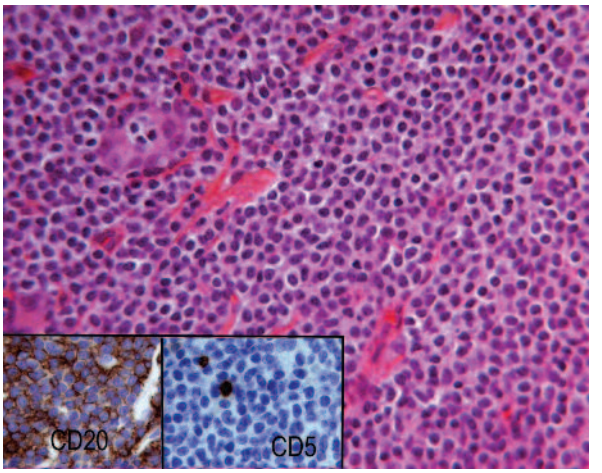

Figure 2. Extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma) consists of lymphocytes that often exhibit characteristic clear cytoplasm and small monotonous nuclei (monocytoid appearance). Positive immunoreactivity is seen for CD20 but not for CD5, which is expressed by only scattered benign T cells. (Hematoxylin and eosin, objective lens 25×; anti-CD20/hematoxylin, objective lens 25×; anti-CD5/hematoxylin, objective lens 25×)

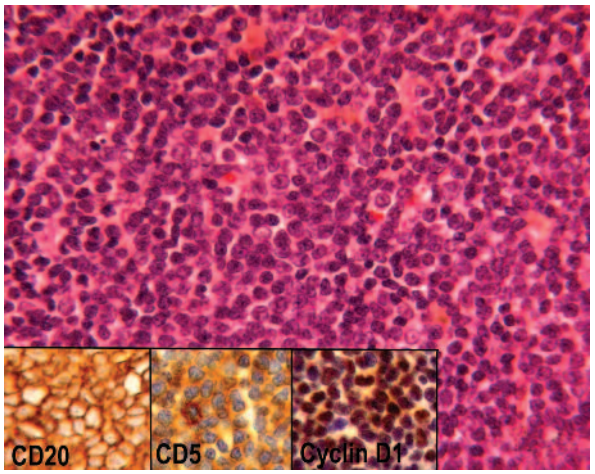

Figure 3. Mantle cell lymphoma, another tumour of relatively small cells, shows immunoreactivity for CD20, CD5, and cyclin D1 (nuclear). (Hematoxylin and eosin, objective lens 40×; anti-CD20/hematoxylin, objective lens 40×; anti-CD5/hematoxylin, objective lens 40×; anti-cyclin D1/hematoxylin, objective lens 40×)

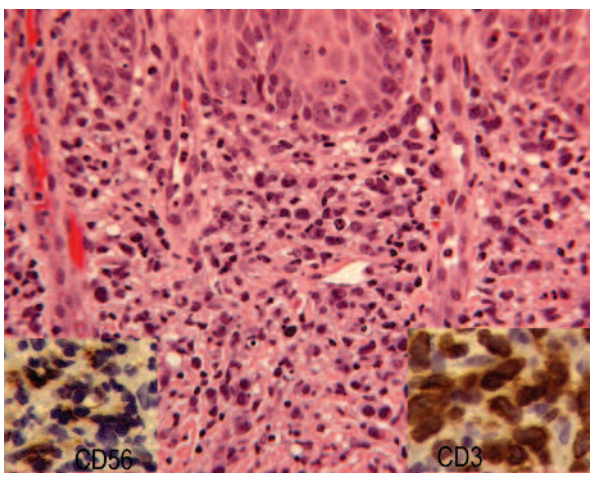

Figure 4. This lymphoma was CD20 negative and CD5 positive, indicating a probable T-cell lymphoma. T-cell lineage was confirmed with CD3 staining; CD56 staining, the microscopic morphology, the site, and clinical features were consistent with an extranodal natural killer/T-cell lymphoma, nasal type (previously termed *midline lethal granuloma*). (Hematoxylin and eosin, objective lens 25×; anti-CD3/hematoxylin, objective lens 40×; anti-CD56/hematoxylin, objective lens 40×)

and sex, and the site of occurrence.

Diffuse large B-cell lymphomas (DLBCLs) (Figure 1) were the most common type, accounting for 40.9%. All of these lesions were CD20 positive, and the majority showed positivity for CD10 and/or *BCL6*. Follicular lymphomas and marginal zone lymphomas (Figure 2) were the next most common, accounting for 18.2% each. Of the 16 follicular lymphomas, five were classified as grade 1, nine were grade 2, and two were grade 2a. None were classified as grade 3b. Nine cases (10.2%) of plasma cell neoplasm were diagnosed in our series. Mantle cell lymphomas (Figure 3), which were all cyclin D1 positive, comprised only 3.4% of the lesions. T-cell lymphomas (Figure 4) were rarely found, comprising, as a group, only 4.5% of the total number of cases.

The most common soft tissue site was the palatal mucosa, which was affected in 24 cases (27.2%), with the involvement of the hard palate mucosa, soft palate, or both. Other soft tissue sites included the buccal mucosa/buccal vestibules (17.0%), gingiva/alveolar mucosa (10.2%), upper lip mucosa (4.5%), floor of mouth (3.4%), lower lip mucosa, tonsillar fossa, tongue, floor of the maxillary sinus, and facial/neck skin. Almost 44% (7 of 16) of follicular lymphomas occurred in palatal mucosa.

Twenty-four lesions were intraosseous. The mandible was the site of predilection for plasma cell myelomas (8 of 9 cases). DLBCL was the predominant type (8 of 11) of maxillary lymphoma. The oral and perioral soft tissues and jaw bones were the primary sites of discovery in 71 of the 88 patients (80.7%) (Table 2).

Table 2. Comparison of the Incidence of Lymphomas Whose Initial Biopsy Was from Oral/Jaw Tissues to Those With Known Lymphoma/CLL Elsewhere at the Time of Oral Biopsy

Lymphoma	Apparent Oral Primary	Oral as Known Secondary
DLBCL	31	5
Follicular	12	4
Marginal zone	14	2
Mantle cell	2	1
SLL	0	1 (CLL)
Other*	3	0
Per. T-cell	1	0
Nasal NK/T	1	0
MF	0	2
Plasma cell	7	2
Total	71 (80.7%)	17 (19.3%)

CLL = chronic lymphocytic leukemia; DLBCL = diffuse large B-cell lymphoma, not otherwise specified; follicular = follicular lymphoma; mantle = mantle cell lymphoma; marginal = extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma); MF = mycosis fungoides; nasal NK/T = extranodal natural killer/T-cell lymphoma, nasal type; per. T-cell = peripheral T-cell lymphoma, not otherwise specified; plasma cell = plasma cell myeloma/solitary plasmacytoma of bone; SLL = small lymphocytic lymphoma.
*See text for details.

The three lesions listed within the "other" group in Table 2 were examples of rare, very aggressive B-cell lymphomas:

1. **Sporadic Burkitt's lymphoma.** One case of Burkitt's lymphoma caused right maxillary and mandibular gingival enlargement and loosening of the teeth in a 29-year-old Caucasian male. The classic morphology with angular and moulded, somewhat granular, nuclei, a high mitotic rate, and a "starry sky" pattern was present. Cytoplasmic immunostaining was strong for CD20 and CD10, as was nuclear staining for *BCL6*. The neoplastic cells did not stain for CD5, *BCL2*, and TDT. Fluorescence in situ hybridization studies showed that 98% of tumour cells were positive for the *IgH/MYC* gene fusion (t[8:14]). The patient refused medical treatment initially and was not compliant with a subsequent chemotherapeutic regimen. The patient died of his disease after 1 year with widespread involvement of other tissues and multiorgan failure.
2. **Plasmablastic lymphoma.** This rare type of lymphoma (Figure 5) presented in the right anterior maxilla of an 82-year-old Caucasian female, causing spontaneous loss of the right maxillary cuspid. It extended as a red mass onto the alveolar mucosa and adjacent palate. The patient was not known to have a human immunodeficiency virus infection or other immunosuppressive disorder. Microscopically, the dense infiltrate of neoplastic, mostly large lymphocytes with vaguely plasmacytoid features did not stain for CD20, CD10, *BCL6*, *BCL2*, CD7, CD43, CD45, CD79a, or TDT. Positive staining was found for CD138, CD4, CD56, and EBER. In situ hybridization for light chains was negative for lambda and positive for kappa. Other immunostains and studies were not done. The morphology, negative staining for CD20 and CD79a, and positive staining for CD4 and CD56 exclude the diagnosis of Epstein-Barr virus–positive diffuse large B-cell lymphoma of the elderly, and the diagnosis of multiple myeloma was ruled out histologically and clinically. The patient received chemotherapy but died of her disease after 8 months.
3. **B-cell lymphoblastic lymphoma.** This case of B-cell lymphoblastic lymphoma occurred in a 9-year-old Caucasian male. He had a 2-month history of a progressive, painless enlargement of his left buccal mucosa and recent onset of enlargement of left cervical lymph nodes. Computed tomography scans showed the involvement of his left maxillary sinus. Microscopically, the cells sometimes exhibited clefting of small nuclei, a fine chromatin pattern, and nuclear moulding in packed areas. Mitotic activity was high. Immunostaining was positive for CD20, CD10, *BCL2*, and TDT. The patient underwent chemotherapy and was clinically disease free at his 6-year follow-up.

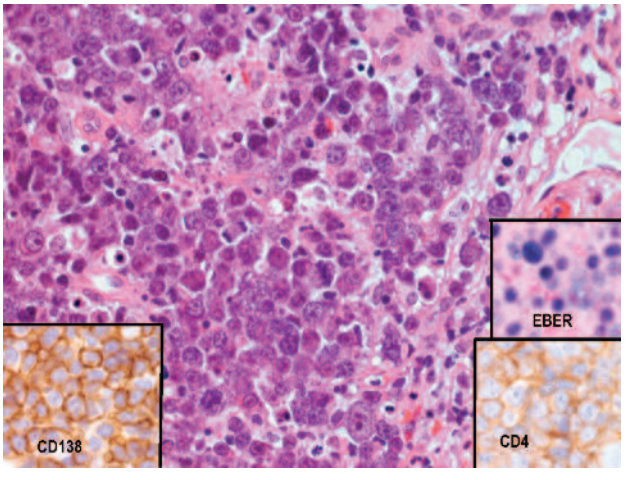

Figure 5. Plasmablastic lymphoma shows packed atypical cells with highly pleomorphic nuclei and a vaguely plasmacytoid appearance. The positive staining for CD138 and EBER, and lack of staining for CD20, CD79a, and T-cell antibodies are important clues in reaching the correct diagnosis. The aberrant expression of CD4 may lead to the misdiagnosis of a T-cell lymphoma. (Hematoxylin and eosin, objective lens 40×; anti-CD4/hematoxylin, objective lens 40×; anti-CD138/hematoxylin, objective lens 40×; Epstein-Barr virus–encoded small RNAs [EBER], objective lens 40×).

In our biopsy service, for the time period 1995 to mid-2009 inclusive, lymphoproliferative neoplasms were the third most common malignancy after squamous cell carcinoma and salivary gland malignancies (Table 3). The relative incidence was 6.5%. Lymphomas comprised over 23% of non-squamous cell oral cancers. As a group, lymphomas of B cells and T cells were more common than any individual malignant salivary gland tumour (mucoepidermoid carcinoma, 75 cases; adenoid cystic carcinoma, 46 cases; polymorphous low-grade adenocarcinoma, 33 cases). Geographical analysis of our cases showed no significant clustering within the province of Ontario.

Table 3. Incidence of Malignant Tumours Submitted to Biopsy Oral Pathology Diagnostic Service between 1995 and Mid-2009

Tumour	Cases (%)
Squamous cell carcinoma, including variants	979 (72.0)
Epithelial salivary gland tumours – malignant	199 (14.6)
Lymphoproliferative diseases	88 (6.5)
Lymphomas (non-Hodgkin's)	79
Plasma cell neoplasms	9
Hodgkin's lymphoma	0
Bone/cartilage malignancies	25 (1.8)
Leukemia	10 (0.7)
Odontogenic carcinoma/sarcoma	9 (0.7)
Soft tissue malignancies	6 (0.4)
Others (e.g., melanoma, basal cell carcinoma)	44 (3.2)

Discussion

Comparisons of the incidence of oral B- and T-cell lymphomas using the classifications preceding the REAL and WHO classifications are not valid since entities such as marginal zone lymphomas and mantle cell lymphomas were included under other diagnoses. Comparison is only valid between studies using similar diagnostic criteria, of which there are few. There is a clear need for more data from different geographical regions for epidemiological comparison and analysis. Recent publications by Kemp et al.[4] from Boston, Massachusetts, Kolokotronis et al.[5] from Greece, and van der Waal et al.[6] from the Netherlands used the WHO classification of 2001,[2] and the study by Solomides et al.[7] from Philadelphia, Pennsylvania, used the REAL classification,[1] which is sufficiently close to the WHO classification to include it in a comparison. Although totalling only 169 cases, the relative incidence of the various types of lymphomas in these articles is given in Table 4.

In the five studies, B-cell lymphomas make up 95.7% of oral lymphomas. In our study, 95.5% were derived from B cells, and 4.5 % were of T cell origin. Solomides et al. reported a 92% B-cell predilection in their series.[7] Kemp et al. had only one T-cell lesion in 40 cases (97.5% B cell),[4] while neither van der Waal et al.[6] nor Kolokotronis et al.[5] reported any T-cell lesions. Compared with the body overall,[3] there appears to be a greater relative incidence of B-cell tumours in the oral mucosa. This is somewhat unexpected since the oral mucosa has a rich T-cell population and T-cell mediated diseases are seen with high frequency in clinical practice (e.g., lichen planus and aphthous ulcers).

Similar to the other studies, we found DLBCL to be the most common type (40.9%). Interestingly, 21 of the 36 DLBCLs were *BCL2* positive, and 21 showed positive immunostaining for CD10 and/or *BCL6*, suggesting that the majority of oral DLBCLs arises from follicular centre cells.

Another surprising finding is the rarity of small lymphocytic lymphomas in the oral cavity. Kemp et al.[4] described two cases, and only one of our cases was CD20, CD5, and CD23

Table 4. Geographical Comparison of Relative Incidence of Oral Lymphoproliferative Neoplasms of B-Cell and T-Cell Origins

	London, Ont, 2009*	Boston, Mass, 2008[4]	Philadelphia, Pa, 2002[7]	The Netherlands, 2004[6]	Greece, 2005[5]
No. of cases	88	40	71	40	18
B-Cell Origin	**Relative Incidence – Percentage of Total Cases**				
DLBCL	41	58	68	50	56
Follicular	18	15	6	20	11
Marginal zone	18	13	15	5	28
Plasma cell	10	8	0	3	0
Mantle cell	3	0	3	15	6
SLL	1	5	0	0	0
Burkitt's	1	0	0	3	0
Other B-cell	3	0	0	0	0
T-Cell Origin	**Relative Incidence – Percentage of Total Cases**				
MF	2	0	0	0	0
Per. T-cell	1	0	8	0	0
Nasal NK/T	1	3	0	0	0

DLBCL = diffuse large B-cell lymphoma, not otherwise specified; follicular = follicular lymphoma; mantle = mantle cell lymphoma; marginal = extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma); MF = mycosis fungoides; nasal NK/T = extranodal natural killer/T-cell lymphoma, nasal type; per. T-cell = peripheral T-cell lymphoma, not otherwise specified; plasma cell = plasma cell myeloma/solitary plasmacytoma of bone; SLL = small lymphocytic lymphoma.
*Current study, performed at University of Western Ontario.

positive, in a patient with known chronic lymphocytic leukemia. No cases were reported by van der Waal et al.,[6] Solomides et al.,[7] or Kolokotronis et al.[5] It is possible that small lymphocytic lymphoma cells do not have a predilection to infiltrate the oral mucosa, or they may infiltrate oral tissues without producing a focal mass that would lead to diagnosis. The relative rarity of this indolent lymphoma and the predilection for DLBCL suggests a tendency for more aggressive lymphomas to occur in the oral mucosa. However, contrary to this trend is the relative abundance of extranodal marginal zone lymphomas. Although not surprising, since these cells are known to home to mucosal surfaces in an organ-specific manner, the equal relative incidence of oral marginal zone lymphomas and follicular lymphomas was unexpected, especially when given that follicular lymphomas are generally much more common than marginal zone lymphomas.[3]

This study illustrates that rare types of both T- and B-cell lymphomas occur in the oral and maxillofacial regions and may cause considerable diagnostic difficulty for pathologists who do not specialize in lymphoproliferative disorders. For example, the CD20-negative, CD4- and CD138-positive plasmablastic lymphoma, such as the one documented in this study, has an 85% predilection for the oral cavity,[8,9] yet it is still rare and may easily be misdiagnosed as a T-cell neoplasm because of its unusual immunophenotype.

We conclude from comparison of reported data that the relative incidence of oral non-Hodgkin's lymphomas is more or less similar in Ontario, the northeastern United States, the Netherlands, and Greece. Although B-cell lymphomas are generally more common than T-cell lymphomas, this preponderance is exaggerated in the oral cavity where over 95% of lymphomas are of B-cell origin. Extranodal marginal zone lymphomas of mucosa-associated lymphoid tissue (MALT lymphomas) occurred with unexpected frequency and must now be considered a major subtype of oral lymphoproliferative neoplasia. Rare oral lymphomas with unusual microscopic features and/or unexpected immunophenotypic profiles occur, sometimes with a predilection for the oral region (e.g., plasmablastic lymphoma). Non-routine immunostains are often required to avoid misdiagnoses that may affect treatment and disease outcome. Consequently, it is highly recommended that opinions be obtained from experts in lymphoproliferative diseases to help reach the correct diagnosis in these challenging cases.

Acknowledgement

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Nucleolus as a Biomarker in Cancer: Past and Future

Kendra L. Cann, PhD, Graham Dellaire, PhD

ABSTRACT

Changes in the nucleolus have long been associated with cell proliferation and tumorigenesis. However, the full extent to which the nucleolus provides a window into cellular well-being was not fully appreciated until the development of advanced imaging techniques and proteomics screens, which revealed the nucleolus as a dynamic structure composed of hundreds of proteins with astonishing functional diversity. No longer just a producer of ribosomes, the nucleolus has emerged as an important cell stress sensor and mediator of tumour suppression. Therefore, it is not surprising that changes in nucleolar composition and dynamics are associated with malignancy and are providing novel biomarkers for cancer diagnosis and prognosis.

RÉSUMÉ

Depuis longtemps, on associe l'altération du nucléole à la prolifération cellulaire et à l'oncogenèse. Toutefois, nous ne connaissions pas la pleine mesure du nucléole à refléter le bien-être cellulaire jusqu'à l'arrivée des techniques d'imagerie de pointe et de la protéomique qui ont mis au jour la structure dynamique du nucléole composée de centaines de protéines d'une diversité fonctionnelle stupéfiante. Plus qu'un simple producteur de ribosomes désormais, le nucléole est également un important capteur de stress cellulaire et médiateur de la suppression tumorale. Par conséquent, rien de surprenant à ce que l'altération de la composition et de la dynamique nucléolaires soit associée à la malignité et donne lieu à des biomarqueurs inédits utiles dans le diagnostic du cancer et à son pronostic.

Biomarkers, such as abnormally expressed proteins and genetic alterations, are used in the diagnosis and prognosis of many human diseases. Because cancer development is generally associated with uncontrolled cell proliferation, proliferation markers represent an important class of biomarkers for these diseases. Such markers include mitotic cell indices, active deoxyribonucleic acid (DNA) synthesis, the presence of cell cycle proteins such as Ki-67 and proliferating cell nuclear antigen (PCNA), and the size and number of nucleoli.[1]

The Past: AgNOR Staining, Nucleolar Size, and Malignancy

The nucleolus is the largest structure in the nucleus and is easily visualized using even light microscopy. One of the main functions of the nucleolus is the production of ribosomes (Figure 1), the protein/ribonucleic acid (RNA) complexes that translate messenger RNA (mRNA) sequences into protein.[2,3] Proliferating cells have greater protein requirements than their noncycling counterparts, and because of this growth factors and oncoproteins that promote proliferation also up-regulate ribosomal RNA

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Competing interests: None declared

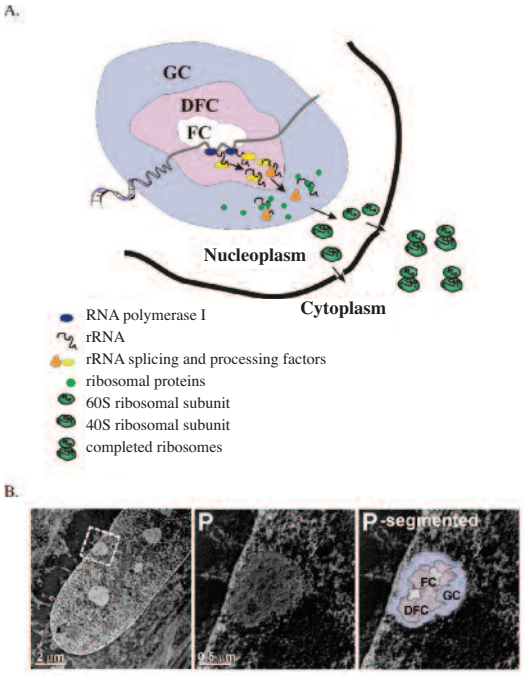

Figure 1. Nucleolar structure and function in ribosome biogenesis. *A*, Regional demarcation of ribosomal biogenesis in the nucleolus and the cell. Using electron microscopy, three morphologically distinct regions of the nucleolus can be visualized. Fibrillar centres (FCs) are surrounded by the dense fibrillar component (DFC), which is further encircled by the granular component (GC).[3] RNA polymerase I–mediated transcription of the rDNA genes occurs at the interface of the FC and the DFC. The newly made transcripts radiate out into the DFC and eventually progress into the GC. Along the way, the transcripts are cleaved, processed, and complexed with ribosomal proteins. Following further maturation steps, the 40S and 60S ribosomal subunits are exported into the cytoplasm to form the completed ribosomes (reviewed in Boisvert et al.[2] and Raska et al.[3]). *B*, Electron spectroscopic imaging analysis of the nucleolus.[46] The image on the left is a 155 eV phosphorus-enriched electron micrograph in which a region of interest containing a single nucleolus was chosen (white box) to be shown at higher magnification in the adjacent electron micrographs. The central image (P) is the corresponding phosphorus micrograph, which visualizes DNA and RNA, and the image on the right (P-segmented) has the FC, DFC, and GC regions delineated on the phosphorus micrograph.

(rRNA) transcription, increasing the size and number of nucleoli.[3,4] Therefore, rapidly proliferating cells, including malignant cells, are associated with nucleolar hypertrophy, and this connection has been known for over 100 years.[4,5] However, it was not until the identification and standardization of a silver nitrate staining technique[6,7] that the relationship could be properly investigated.[4,5] This technique relies on the argyrophilic nature of several nucleolar proteins that associate with repeats of the ribosomal DNA (rDNA) genes, which encode the sequence for the rRNAs.[8] The rDNA repeats comprise the nucleolar organizer regions (NORs), and the resulting silver-stained structures are termed the AgNORs.[3,5] The AgNORs can be quantified through counting or by a morphometric method that uses computer-assisted image analysis to measure the area of AgNOR staining in each cell.[7] Furthermore, because AgNOR size is inversely related to cell doubling time and tumour mass doubling time, it is unique among the proliferation markers in that it can be used to gauge tumour proliferation rate, not just the proportion of tumour cells that are proliferating.[9]

While AgNOR staining cannot be used to diagnose malignancy, over 60 studies in more than 20 different types of cancers have shown that the nucleolar parameter is an independent prognostic variable (reviewed in Derenzini et al.[4] and Pich et al.[10]). Cancers analyzed included breast carcinomas, leukemias, and prostatic carcinomas (see Derenzini et al.[4] and Pich et al.[10] and the references therein). Overall, tumours with high AgNOR scores are poorly differentiated with high metabolic activity, abnormal DNA content, and a high proliferation rate, all indicative of a malignant phenotype.[10] Finally, the AgNOR staining can also reflect the underlying oncogene and tumour suppressor status of the cancer cells. For example, the critical tumour suppressors Rb and p53 can both inhibit rRNA transcription, and human breast cancer tumours with mutated or deleted Rb or p53 have significantly larger nucleoli, and therefore higher AgNOR scores, than tumours with normal Rb and p53 statuses.[11,12]

The Present: Nucleolar Proteins

The advent of advanced microscopic techniques, including fluorescence microscopy and live-cell imaging,[13] and large-scale proteomic studies using mass spectrometry[14,15] has enabled much more extensive analyses of nucleolar dynamics, structure, and biochemical composition. From these data, it has become clear that the role of the nucleolus in cells extends far beyond ribosome biogenesis. For example, of the over 700 human proteins identified in the

nucleolus, only approximately 30% function in ribosome biogenesis.[2] Other nucleolar proteins have functions that include regulation of the cell cycle, DNA repair, cell senescence, and the cellular stress response.[2,3,5,16] As such, it is perhaps not surprising that changes in the levels and/or subcellular localizations of many nucleolar proteins have also been identified as biomarkers in their own right (Figure 2). First, two well-established cancer biomarkers are associated with the nucleolus: telomerase[17] and Ki-67.[18] The telomerase enzyme is a protein/RNA complex that extends the telomeres, the terminal segments of the chromosomes, which would otherwise become successively shorter with each DNA replication.[19] In the majority of human cancers, telomerase is overexpressed, which allows the cells to replicate indefinitely.[20] The proliferation marker Ki-67 is used extensively in pathology,[18] and this nucleolar protein functions in rRNA transcription.[21] In fact, overexpression of nucleolar proteins as proliferation markers is a common theme; two other such markers are Nop2/p120[22] and Mina53.[23]

Other important nucleolar biomarkers are those involved in the regulation of the p53 tumour suppressor; p53, which is mutated in over 50% of cancers,[24] is a gatekeeper that regulates the cell cycle checkpoints, apoptosis (programmed cell death), and cell senescence.[25] Normally, the p53 protein is maintained at a very low level and is only stabilized following cellular stress,[26,27] DNA damage,[27] or aberrant oncogene expression.[28] Nucleolar proteins represent one of the key mechanisms for regulating p53,[27] with the nucleolar tumour suppressor ARF (alternate reading frame) being the prime example.[29] ARF is normally found predominantly in the nucleolus; however, when nucleolar structure is disrupted, ARF relocalizes to the nucleoplasm, where it helps prevent p53 degradation.[29] Other nucleolar proteins that can regulate p53 include nucleostemin, nucleophosmin, and nucleolin,[26,30–32] and all of these proteins have been identified as biomarkers. Overexpression, loss of expression, and/or changes in localization of these proteins have been shown to correlate with either malignancy or more aggressive disease.[33–37] However, it is important to note that the functions of these proteins are not restricted to the regulation of p53 and include ribosome biogenesis and genome maintenance, indicating that altered expression or localization of these proteins has the potential to cause multiple effects.[30,32,36,38]

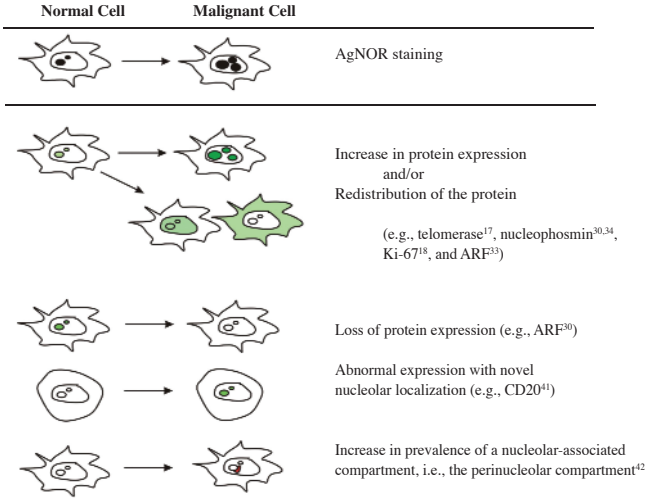

Figure 2. Using the nucleolus as a biomarker: the past and the present. The use of the nucleolus as a biomarker in cancer has expanded from AgNOR staining to include the evaluation of nucleolar proteins for diagnostic and prognostic purposes. Pathologically significant changes in nucleolar proteins include overexpression, loss of expression, and relocalization. ARF = alternate reading frame.

In addition, the nucleophosmin gene, *NPM1*, is fused to the anaplastic lymphoma kinase *(ALK)* gene in the most frequent translocation found in anaplastic large cell lymphoma, the myeloid leukemia factor 1 *(MLF1)* gene in myelodysplastic syndrome, and retinoic acid receptor alpha *(RARA)* gene in acute promyelocytic leukemia (APL).[39] Furthermore, approximately 35% of adult patients with acute myeloid leukemia have mutations in NPM that cause it to be relocalized to the cytoplasm.[30] Consequently, normal and mutated NPM have been used for cancer diagnosis and prognosis and for monitoring minimal residual disease.[40]

The abnormal localization of proteins to the nucleolus may also serve as biomarkers. For example, the nucleolar localization of CD20, which is normally expressed on the membrane of B cells, has been shown to be a marker for T-cell neoplasms.[41] Undoubtedly, more examples of aberrant nucleolar localization of proteins will be found in other malignancies.

One of the main themes that can be drawn from the

nucleolar biomarkers described above is that changes in the biochemical composition of the nucleolus, through changes in either the level of protein expression or localization, frequently occur during carcinogenesis and that this can lead to changes in the very topography of the nucleus. Perhaps one of the best examples of this is the perinucleolar compartment, which is located at the nucleolar periphery. This is not found in normal cells, and its presence has been identified as a cancer marker with prognostic value for solid tumours.[42]

The Future: Using the Nucleolus to Identify Functional Pathologies in Tumour Suppressor Pathways

Ideally, cancer treatment regimens should be based on the underlying integrity of cellular pathways, and the nucleolus provides an important window into the status of the cell stress response. For example, the nucleolus undergoes specific changes in composition and structure in response to chemotherapeutic agents that damage cellular DNA, including releasing proteins that regulate the tumour suppressor p53.[26,29–32] Therefore, deviation from the normal nucleolar "response" to chemotherapy could be used to identify problems with the p53 pathway where no overt mutations have been identified. The nucleolus thus represents a potential biomarker for functional changes in molecular pathways associated with tumour suppression. Furthermore, changes in nucleolar function could be used to monitor treatment response and, if used in an ex vivo analysis of biopsies, as a way to select the most effective cancer treatment for individual patients.

The nucleolus represents just one of the nuclear structures that undergo morphological changes under stress or DNA damage. Another example is the promyelocytic leukemia (PML) nuclear bodies, which function in tumour suppression, apoptosis, cell senescence, and DNA repair[43] and which increase in number following DNA damage and cellular stress.[43,44] Interestingly, the PML protein can also function in the nucleolus to help regulate p53.[45] In this way, the dynamics of multiple nuclear compartments, including the nucleolus and PML nuclear bodies, can be used to provide a functional "read-out" of the integrity of key pathways involved in cancer prevention and development, as well as in the response to chemotherapy.

In conclusion, nucleolar number, morphology, and biochemical composition reflect overall cellular health and the integrity of pathways involved in cell-cycle progression, the cell stress response, the maintenance of genetic integrity, and tumour suppression. The usefulness of the nucleolus in cancer pathology is no longer restricted to AgNOR status but now includes a rapidly expanding repertoire of nucleolus-associated proteins that serve as biomarkers (see Figure 2) for cancer diagnosis, prognosis, treatment selection, and treatment monitoring.

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