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ANATOMIC PATHOLOGY

Pseudosarcomas of Soft Tissue

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One of the most common and important pitfalls in soft tissue pathology are the so-called pseudosarcomas. These lesions are nonneoplastic; however, their rapid growth, hypercellularity, cytologic atypia, and mitotic activity makes them prone to be misinterpreted as sarcoma. The most common of these lesions are fibroblastic/myofibroblastic and matrix-forming proliferations, including nodular fasciitis, proliferative fasciitis and myositis, ischemic fasciitis, massive localized edema, myositis ossificans, and bizarre parosteal osteochondromatous proliferation and related entities. Most of these lesions rarely recur following simple excision; therefore, their accurate recognition helps prevent excessive therapy.

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Immunohistochemical Markers as Predictive Tools for Breast Cancer

Walker, R A

Steroid receptors have been used for predicting outcome and response to therapy of breast cancer for many years. This has been the predominant cancer where oncologists have used such markers clinically to select treatment options. Assessment of receptors and other markers was by biochemical methods but practice has changed, with immunohistochemistry now being the major assay used. It has also taken over from other techniques such as flow cytometry and immunoassay. Despite its extensive use there are still issues around the methodology, interpretation and quantification that those assessing results and those applying the results must be aware of. These problems have been highlighted in a recent perspective and in recommendations from the Ad-Hoc Committee on Immunohistochemistry Standardization, USA. This review will consider general points that relate to these issues and are applicable to all markers, and then discuss those markers that are used either routinely or in a research setting for prediction. The important issue is that the markers will be used to determine therapy, so a false negative or a false positive result could impact on patient survival.

OESTROGEN AND PROGESTERONE RECEPTORS

The oestrogen receptor was first identified in the 1960s. The analyses of oestrogen and progesterone receptor in breast cancers quickly provided the evidence that they could aid the identification of those cancers that were more likely to respond to endocrine treatment. The assays were dependent on the homogenisation of frozen tumour tissue with the preparation of a cytosol for subsequent ligand binding. The most widely used method was the dextran-coated charcoal assay (DCC), with results being expressed as fmol/mg cytosol protein, i.e. the receptors could be quantified. Response data showed that not only was the presence of ER important, but also the amount in aiding prediction. The presence of PgR, which is induced by oestrogen, is also a predictor of response. The DCC assay had the advantage of providing a quantifiable level of receptor, but it required fresh tissue and the level of receptor could be influenced by the presence of large amounts of normal breast and/or stroma. Such factors led the drive for histological based methods and the development of monoclonal antibodies to ER and PgR that could be used in fixed tissue and applied routinely. For the methods to be clinically valuable they have to have the same predictive power as the original biochemical assay.

Although there have been many publications about ER immunohistochemistry, there is still debate about quantification and what is required clinically. Fisher et al compared various methods of scoring ER and PgR, involving percentage ranges and intensity, both summated and as a product, and concluded that the “any-or-none” method was just as good at prediction, and simpler. However, Barnes et al, in a very thorough comparison of scoring methods, showed that there was a correlation between greater extent of staining and likelihood of favourable response. In neoadjuvant endocrine treatment the Allred score has been of value in identifying those cases more likely to respond. The BIG 1–98 trial of adjuvant endocrine therapy in postmenopausal women identified differences in outcome between those cases that were ER negative and had 1–9% of positive cells and those with $\geq 10\%$ positive cells, indicating the importance of detecting low levels of receptor. Schnitt, in a “Comments and Controversies”, has highlighted that variation in pre-analytical factors and assays will affect attempts to standardise quantification of ER by immunohistochemistry, but has also suggested that the highly sensitive antibodies and detection systems cannot identify differences in amounts in the higher staining tumours. From a clinical perspective, having sensitive techniques that can detect low levels of ER is important. The Allred score concentrates on this low end, is easy to use and is recommended by the author.

Those undertaking interpretation of ER and PgR should be aware of all of these many factors. [Table 1](#) gives recommendations for staining and assessment.

Critical factors	
Staining	Optimal fixation Antigen retrieval—citrate pH 6.0, but test higher pH buffers if suboptimal staining Antibody validated against biochemical assay Positive control with range of staining; choose test tissue with normal breast included if possible Quality assurance, internal and external
Assessment	Nuclear staining only; cytoplasmic staining may be due to excess antigen retrieval Only invasive cancer assessed Strong relationship with grade, so if grade I low/negative repeat Use a recognised scoring system. Allred score easy to use and identifies low positive cases

Table 1 Recommendations for staining and assessment of ER and PgR

HER2

The clinical importance of amplification of human epidermal growth factor receptor 2 (HER2) (also known as HER-2/neu/c-erb B2) in breast cancer was recognised in 1987. Numerous subsequent studies found that either HER2 gene amplification or protein expression predicted for poor prognosis. Following the development of a humanised monoclonal antibody against HER2 (trastuzumab), the reasons for establishing the HER2 status of breast cancers changed, since it is a prerequisite for trastuzumab's clinical use. Trastuzumab was originally licensed for the treatment of patients with metastatic disease who had HER2 positive cancers. More recently several prospective randomised trials have shown that adjuvant trastuzumab reduces the risk of recurrence and mortality in patients with HER2 positive early stage breast cancer. This resulted in it being licensed for adjuvant use and being endorsed by the UK National Institute for Clinical Excellence (NICE).

The principal testing methods used are immunohistochemistry and/or in situ hybridisation using either fluorescence (FISH) or a chromogen. In comparison to ER data on response, information is limited as to whether HER2 overexpression as detected by immunohistochemistry or HER2 gene amplification as detected by FISH is a better predictor. Data from the metastatic setting suggests that there is a higher overall response in patients with HER2 FISH positive than FISH negative cancers, but the overall response rate of the patients (all with HER2 positive breast cancers) to single agent trastuzumab was around 35 percent. There are insufficient data comparing immunohistochemistry and FISH in prediction of response to adjuvant trastuzumab. Comparisons of local and central testing of cases entered into two of the adjuvant trials has shown that there are discordances, although concordance was better for FISH than for immunohistochemistry. Data presented at the American Society in Clinical Oncology in 2007 raised issues about the reliability of testing. In the NSABP B-31 trial, retesting of cancers centrally resulted in 9.7% being reassessed as negative; however, some of these patients had benefited from trastuzumab. The main message from this rather confusing data is that for each testing laboratory, adequate numbers should be assessed, all tests should be standardised with good quality control, and there should be participation in external quality assessment.

Immunohistochemical analysis of HER2 is either by the use of FDA approved commercial assay systems, such as Hercep Test (Dako, Ely, UK) and Ventana Pathway (now using clone 4B5), or in-house systems using polyclonal antisera (A0485, Dako) or monoclonal antibodies (CB11, Novocastra; TAB250, Zymed).

There are a variety of factors that can modify immunoreactivity for HER2 and therefore affect interpretation, which have been referred to above. These include: poor fixation, which can be a particular problem in excision specimens; crushing of tumour cells and edge artefact staining in NCB; batch variation of assay kits; excess antigen retrieval; and excess nuclear counterstain.

Assessment

It is important that only invasive carcinoma is assessed. The scoring system used is the same whichever assay is employed and is shown in [Table 2](#).

Score to report	HER2 protein assessment	Staining pattern
0	Negative	No staining is seen or membrane staining is <10% of invasive tumour cells
1+	Negative	Faint/barely perceptible membrane staining detected in >10% of invasive tumour cells
2+	Equivocal	Weak to moderate complete membrane staining in >10% of invasive tumour cells or <30% with strong complete membrane staining
3+	Positive	Strong complete membrane staining in >30% of invasive tumour cells

2+ cases should be assessed by FISH, as should other cases where there is heterogeneity, problems with immunohistochemistry interpretation and problems relating to fixation.

Table 2 Immunohistochemical assessment of HER2

Only membrane staining of invasive cells is considered. Cancers are categorised as negative if no staining is seen or membrane staining is <10% of invasive cells; 1+ (and therefore negative) if there is faint membrane staining in >10% of cells). Equivocal or 2+ staining is weak to moderate complete membrane staining in >10% of cells or <30% with strong complete membrane staining. This requires further analysis by another system to check amplification status. A positive case is 3+ which is strong membrane staining in >30% of cells. There has been a change from 10% to 30% in the recent ASCO/CAP guidelines which is being endorsed in the updated UK HER2 testing recommendations. HER2 overexpression is more likely to be present in a grade 2 or grade 3 invasive breast cancer. Unlike ER, staining should not be present in normal breast.

The main problems in interpretation and in intra- and inter-observer variation arise with cases that are at the 1+/2+ borderline and the 2+/3+ borderline. It is these categories that can be affected by the technical issues outlined, so it is particularly important that those undertaking interpretation are aware of the impact of these issues and that regular audits are undertaken.

OTHER MARKERS

Epidermal growth factor receptor

Epidermal growth factor receptor (EGFR, also HER1) is a type 1 tyrosine kinase receptor that is expressed in normal breast. The frequency of detection by immunohistochemistry in breast cancers varies between different studies and can range from 15% to >60%. The reasons for this variation relate to differences in methodology, antibodies used, interpretation and the cancers studied. Unlike ER and HER2 there is no other recognised assay that antibodies and the immunohistochemical technique can be compared to. The EGFR PharmDx assay (Dako, UK) is licensed in the USA for testing colon cancer and is a similar assay to the HercepTest (Dako) with a scoring system of 0 to 3+. Reported studies vary from using an H score system to positive if any staining is present.

The presence of EGFR in breast cancers is associated with a lack of ER and poor prognostic features. There are other reasons why assessment of EGFR could be of value. Tyrosine kinase inhibitors of EGFR, such as gefitinib, are now available and are being tested in trials of advanced and early breast cancer. Although the results so far are not promising, if EGFR testing is to be used as a method of patient selection, there needs to be better standardisation of the assay.

Basal markers

Gene expression profiling has identified different subgroups of breast cancers, that link to patient outcome. One subgroup that was associated with poor outcome expressed genes characteristic of basal or myoepithelial cells of normal breast. Most of these are high grade and lack ER, PgR and HER2 and have a higher risk of brain and lung metastases. However, there is no accepted consensus on the immunohistochemical profile that defines these basal like cancers. Most studies include cytokeratins 5/6 and/or 14, but Nielson et al define them as lacking ER, PgR and HER2, expressing basal cytokeratins and EGFR and c-KIT. Matos et al consider them to express P-cadherin and p63 more frequently and recommend that cytokeratin 5, p63 and P-cadherin can be used to distinguish a basal like carcinoma. Rakha et al have proposed that basal cytokeratins (5/6 and 14) can be used to define basal like carcinomas irrespective of the expression of other markers.

The response of patients with basal like breast cancer to chemotherapy has been reported as both poor and good. There are similarities between basal like breast cancers and those cancers arising in women with BRCA1 mutations. Therapeutic approaches that have potential in BRCA1 deficiency, for example carboplatin and PARP inhibitors, could be of value in the management of basal like cancers, and clinical trials of the management of ER, PgR, HER2 (triple) negative cancers are being undertaken. EGFR is expressed at a high frequency in basal like cancers, so they could benefit from EGFR inhibitors. The identification of this group of cancers is going to become increasingly important as therapeutic strategies become more refined and targeted.

Proliferation markers

The Ki-67 antigen is expressed in the nucleus of cells in all phases of the cell cycle and is a useful marker of cell proliferation. The MIB1 antibody is reactive against the antigen in fixed, embedded tissue and gives comparable results to the original Ki-67 antibody, which was only reactive with frozen tissue. Several studies have shown that both Ki-67 and MIB1 staining are of prognostic value.

Changes in Ki-67 expression following preoperative endocrine treatment can predict long term outcome. The rationale is that endocrine treatments act by inhibiting tumour cell proliferation, so decreases in Ki-67 after short-term treatment indicate effective responses. Pretreatment assessment of Ki-67 has also been shown to predict response to preoperative chemotherapy. A decrease in Ki-67 was found to predict good clinical response to neoadjuvant chemotherapy in a study using cytology, but a subsequent study using NCB was less conclusive, particularly for pathological response. A problem with these studies is that the pretreatment assessment has to be done on NCBs, i.e. relatively small samples. If there is intratumoural heterogeneity of Ki-67 expression, this will affect the counts obtained from small samples. One issue with the use of Ki-67/MIB1 is the lack of an agreed scoring method and the definition of low/high, positive/negative. For assessing changes, percentage of positive (any staining) cells (counting 1000–3000 cells) has been used, whereas others estimated the percentage of positive nuclei within the area of highest positivity. Assessment of whole tumour sections has been of 10–20 random fields at $\times 400$ to give a percentage, but cut-off levels have varied from positive if $>5\%$ of cells staining with 20% as high, to $\leq 9.5\%$ low, $>9.5\% - \leq 15.5\%$ intermediate and $>15.5\%$ high, when compared to histological grade.

Other proliferation markers that have been evaluated immunohistochemically in breast cancer include cyclin E, cyclin D1, p21 and p27, but there is no strong evidence for their use as predictive markers outside of clinical research.

Apoptotic proteins and p53

As with proliferation markers, there have been many immunohistochemical studies evaluating expression of apoptotic proteins including bcl-2, bax, bcl-x and survivin, but for various reasons including availability of suitable antibodies, methods of evaluation and lack of strong evidence, these are not suitable as routine predictive markers.

p53 has been considered as a potential predictor of response of breast cancers to chemotherapy, but much of the data comes from mutation analysis. Immunohistochemistry detects stabilised p53 protein, which may reflect a mutation but will not detect protein truncation mutations; there are also problems in evaluation and defining what is positive.

Topoisomerase II alpha

Topoisomerase II alpha is a target of anthracycline action, a chemotherapeutic drug that is frequently used in the management of breast cancer. The gene encoding this is TOPO2A which maps to 17q21 and can be co-amplified with HER2. There are

conflicting reports as to whether TOP2A amplification can be used as a predictor of response to anthracycline based chemotherapy, although recent reports suggest that it could be a useful marker. This study found that there was a good correlation between amplification and immunohistochemical detection of the protein using the antibody Ki-S1 when >25% cells staining was used as the cut-off for defining overexpression.

THE FUTURE

Will immunohistochemistry remain the main method for assessing predictive markers? There has been debate for sometime about its role in HER2 testing, with some centres preferring frontline FISH, to which TOP2A could be added. Real time (quantitative) PCR is being used to assess gene expression levels for ER and HER2. Commercial assays that cover expression of a range of genes, e.g. Oncotype Dx are available. If molecular assays become automated on the scale of biochemical assays and cost per test becomes competitive, the use of immunohistochemistry, with its problems around quantification, could change.

CONCLUSIONS

Table 3 presents a summary of the various markers discussed and their potential roles in prediction.

Established and in routine clinical use	Potential for clinical use; need refinement of scoring systems or antibodies	Research interest, less likely to be used clinically
Oestrogen receptor	Epidermal growth factor receptor	P53
Progesterone receptor	Ki-67 (MIB-1)	Cyclin E, cyclin D1, p21, p27
HER2	Topoisomerase II alpha	Bcl2, bax, bcl-x, survivin

Table 3 Markers and their value in prediction in breast cancer

Despite evaluation of many markers, ER remains the most reliable and best example of a predictor of treatment response for breast cancer. HER2 is used as a marker to select patients for a specific form of treatment, trastuzumab, but there is insufficient data about response. Immunohistochemical determination of these markers is of value, but there has to be standardisation of fixation, methodology and interpretation, and the person undertaking the interpretation has to be aware of these technical pitfalls and the expected patterns of reactivity in relation to breast cancer pathology.

Take-home messages

- * Immunohistochemistry is the major assay used for determining markers in breast cancer, but there remain issues relating to tissue fixation, methodology, interpretation and quantification.
- * The oestrogen receptor is the most reliable and best example of a predictive marker.
- * Those undertaking interpretation must understand the technical pitfalls and be aware how expression relates to the nature of the breast cancer.

* Newer markers will require further evaluation and standardisation before they can be used for patient management.

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Fine Needle Aspiration Cytology of Papillary Lesions of the Breast - How Accurate is the Diagnosis?

Tse GM, Ma TK, Lui PC et al

AIM: Cytological diagnosis of mammary papillary lesions is difficult. We reviewed the previous cytology diagnosis of 23 papillomas and 11 papillary carcinomas and specific cytological features that may assist in differentiating these entities.

METHODS: The cytology preparations were reviewed for (i) overall cellularity; (ii) epithelial cell ball devoid of fibrovascular cores; (iii) single cells; (iv) papillary fragments and their morphology.

RESULTS: The overall diagnostic accuracy was 59%, atypical rate was 24%, and the error (combined false positive and negative) rate was 17%. For overall cellularity, six, 14 and three cases of papillomas and six, three and two cases of papillary carcinoma showed low, moderate to high cellularity respectively. Cell balls were present in mild to moderate number in 20 papillomas and ten papillary carcinomas. The background single cells were absent, present in low or moderate to high numbers in seven, ten and six papillomas and three, three and five papillary carcinomas respectively. Papillary fragments were absent, present in small, moderate or large quantities in nine, four, eight and two papillomas and six, three, one and one papillary carcinomas respectively. There is no demonstrable quantitative difference between papilloma and papillary carcinoma for all these parameters. Qualitatively, the cell balls and single cells showed higher degree of atypia in papillary carcinoma, and the papillary fragments were more elaborate and slender.

SUMMARY: Cytological diagnosis of papillary lesions shows significant error rate with overlapping features. Cellular atypia and fragments with long and slender papillae with ramifying edges favours papillary carcinoma.

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Flat Epithelial Atypia of the Breast

Melinda F. Lerwill

Flat epithelial atypia is a presumably neoplastic alteration of terminal duct-lobular units that is characterized by the replacement of the native luminal epithelium by ductal cells demonstrating low-grade cytologic atypia. The atypical cells maintain a “flat” pattern of growth without evidence of architectural atypicality. Morphologic,

immunohistochemical, and molecular investigations support that flat epithelial atypia represents an early step in the evolution of low-grade ductal carcinomas. It is frequently seen in association with atypical ductal hyperplasia, low-grade ductal carcinoma in situ, invasive tubular carcinoma, and lobular neoplasia. The risk for subsequent breast carcinoma remains to be defined, but flat epithelial atypia likely represents a nonobligate precursor with an extended time course to progression. Certain benign alterations may superficially mimic its appearance; careful attention to cytologic and architectural characteristics can help one distinguish these unrelated entities from flat epithelial atypia.

INTRODUCTION

Flat epithelial atypia is a modern name for an alteration of terminal duct-lobular units that was first recognized more than a century ago^{1,2} and appears to represent an early stage in the development of low-grade ductal carcinoma.³ Flat epithelial atypia is characterized by the replacement of native luminal cells by one to several layers of monomorphic epithelial cells with low-grade cytologic atypia. The atypical cells are frequently columnar but are occasionally cuboidal. As the cells increase in number, they pseudostratify but maintain a “flat” pattern of growth along the ductal or acinar wall; that is, they do not form architecturally atypical structures such as micropapillae, trabecular bars, or cribriform spaces. In essence, flat epithelial atypia can be defined as a ductal epithelial proliferation demonstrating low-grade cytologic atypia in the absence of architectural atypia.

HISTOLOGIC FEATURES

A diagnosis of flat epithelial atypia is primarily a cytologic one, requiring medium-power to high-power microscopic evaluation to recognize the presence of low-grade cytologic atypia. Architectural features do not play as large of a role in the diagnosis of flat epithelial atypia as they do in other mammary epithelial proliferations, although certain architectural alterations are helpful for identifying involved terminal duct-lobular units on scanning magnification. In particular, the involved lobules are enlarged when compared to adjacent normal lobules (Figure 1, A and B). This enlargement is due to dilatation of the terminal ductules and acini, the degree of which is variable. In some examples, the distended glands can span a millimeter or more. The terminal ductules often show the earliest evidence and greatest degree of alteration, with the changes then progressively affecting the acini. In well-developed examples of flat epithelial atypia, the dilated acini appear cystic and rounded. Somewhat branching configurations may be seen in earlier, less well-developed lesions. The intralobular stroma frequently become diminished as the glandular spaces become increasingly dilated. Although flat, the lesion demonstrates distinguishing features on scanning magnification. Most notably, one sees a uniform increase in epithelial thickness along the entire perimeter of involved glands (Figure 1, B). This is largely caused by the pseudostratification of the atypical cells as they increase in number. The atypical cells also are usually larger and taller than normal luminal cells, and this too contributes to the increased epithelial height. The evenly thick, bandlike epithelium of flat epithelial atypia is rather distinctive when seen lining dilated cysts, as it contrasts with both the thin, flattened epithelium usually encountered in nonapocrine cysts and the papillary hyperplasia of proliferative apocrine cysts.

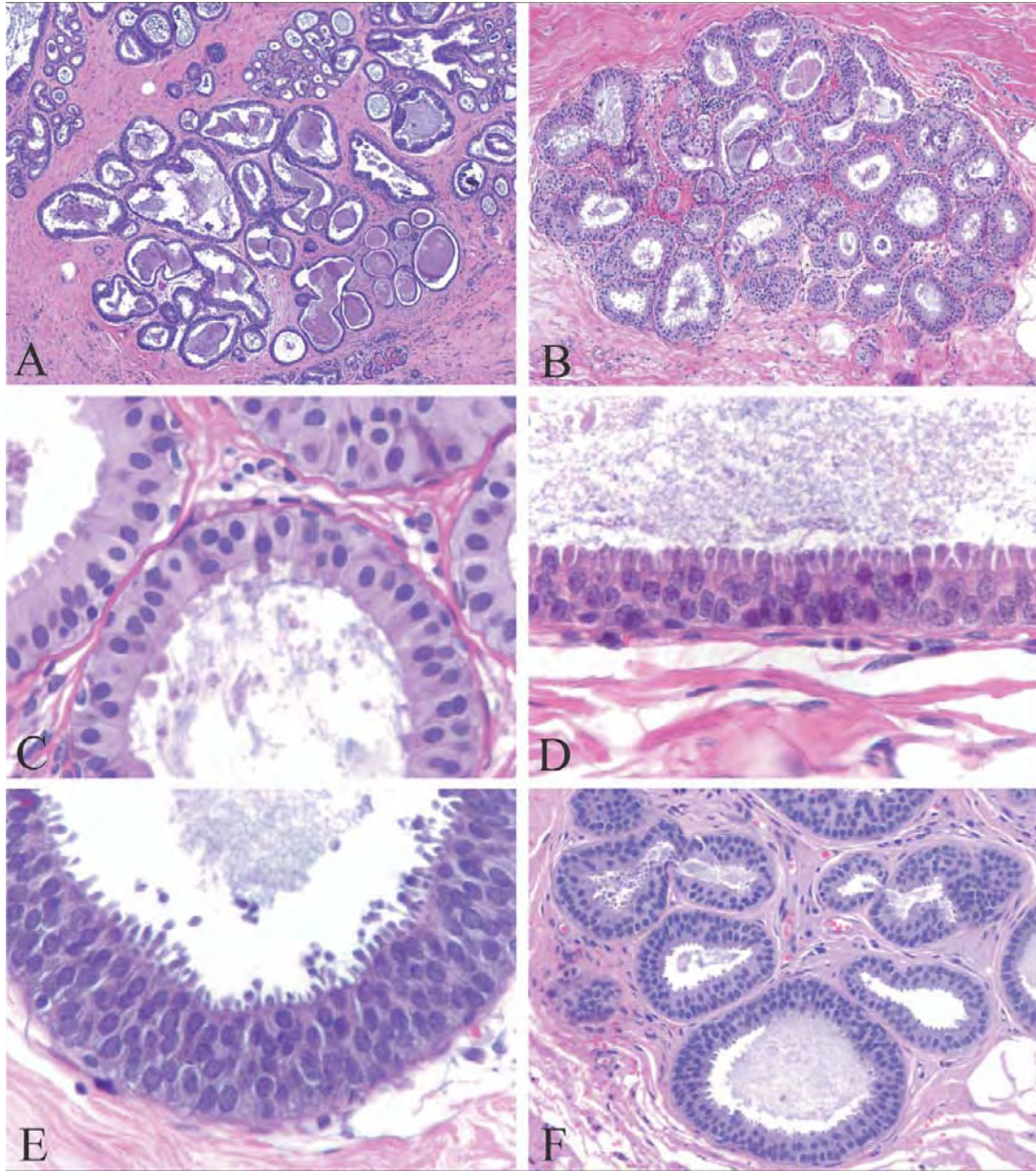


Figure 1. Histologic features of flat epithelial atypia. A, Variably dilated terminal duct-lobular units containing intraluminal secretions and associated calcifications (hematoxylin-eosin, original magnification $\times 62.5$). B, Enlarged terminal duct-lobular unit with prominently thickened epithelial layer (hematoxylin-eosin, original magnification $\times 125$). C, Classic low-grade ductal atypia (hematoxylin-eosin, original magnification $\times 800$). D, Variant nuclear morphology with more open chromatin and small nucleoli (hematoxylin-eosin, original magnification $\times 800$). E, Characteristic pseudostratification of the atypical cells; myoepithelial cells are inconspicuous (hematoxylin-eosin, original magnification $\times 800$). F, Even nuclear spacing imparts an orderly appearance (hematoxylin-eosin, original magnification $\times 250$).

CONCLUSION

Flat epithelial atypia encompasses a wide spectrum of changes, ranging from minimal examples that many would not recognize as atypical to ones that border on ductal carcinoma in situ. Increasing evidence supports that flat epithelial atypia represents an early step in the evolution of certain low-grade in situ and invasive ductal carcinomas. Its presence in a biopsy should prompt one to look for associated ductal carcinoma, atypical ductal hyperplasia, and lobular neoplasia. If identified in a core biopsy, flat epithelial atypia warrants follow-up excision. Our understanding of the biologic behavior of flat epithelial atypia remains incomplete, but it likely represents a relatively indolent, nonobligate precursor to low-grade ductal carcinoma. Additional studies that better define its clinicopathologic characteristics, the risk for subsequent breast cancer, and the time course to progression are needed in order to determine optimal clinical management.

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Cardiac Botryomycosis: An Autopsy Report

Gupta, K; Das, A; Radotra, B D; Bhalla, A

Visceral botryomycosis is rare, and documented sites are lung, brain, kidney, liver and prostate. This report describes a rare autopsy case of disseminated visceral botryomycosis, with bulky, grape-like botryomycotic vegetations in the heart, and similar abscesses in the lungs and bone marrow. This is the first such report in the literature to the best of our knowledge.

Botryomycosis or bacterial pseudomycosis is an uncommon chronic, suppurative granulomatous disease with grains of bacteria. The lesions begin at a cutaneous wound and usually invade deeper tissues including muscle and bone. The term was proposed by Rivolta and is derived from the Greek words for “bunch of grapes” (botryos) and “fungus” (mycosis), as the histological pattern was attributed to a fungal infection. Histologically, it shows presence of eosinophilic material surrounding densely packed micro-organisms associated with a suppurative focus. Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa are the frequent causative organisms. It occurs in two forms. The cutaneous form is more common and accounts for 75% of the reported cases. Visceral disease is rare, and documented sites are lung, brain, kidney, liver and prostate. We describe a rare autopsy case of disseminated visceral botryomycosis with involvement of heart, valves, lungs and bone marrow. To our knowledge, this is the first such report in the literature.

CASE SUMMARY

A 34-year-old female presented with moderate grade, intermittent fever associated with chills and dry cough. A week later she developed backache and macular, non-itching, petechial rash, which first appeared over the legs, and later coalesced and turned black with necrosis of the skin. Soon she developed breathlessness that progressed to

orthopnoea. On examination, she was afebrile with tachycardia and increased respiratory rate. There was hepatomegaly; however, the spleen was not palpable. Investigations revealed anaemia, neutrophilic leucocytosis and thrombocytopenia. Renal function tests were deranged with raised urea and creatinine. Microscopic examination of urine revealed the presence of pus cells, red blood cells and bacteria. Blood culture grew *S aureus* after 24 h. Ultrasound abdomen confirmed hepatosplenomegaly. Oliguria, metabolic acidosis and bradycardia were documented before she succumbed to her illness.

A complete autopsy was performed following an informed consent. Externally, a purpuric rash was noted over both legs. The heart weighed 310 g, with the presence of multiple vegetations (0.8–4 cm) attached to tricuspid valvular leaflets ([Fig 1A, B](#)), with the presence of ring abscesses. The underlying valve was destroyed; however, the remnants of anterior and posterior leaflets were not thickened. The chordae tendinae were ruptured. The right ventricular endocardial surface just beneath the tricuspid valve showed similar linear abscesses (1–2 cm) over the trabecular muscles. The outflow chamber of right ventricle including the pulmonary valve and trunk were within normal limits. The left atrial and the ventricular endocardium and the chambers were essentially normal except for the anterior mitral valvular leaflet, which showed small, friable 3–4 mm vegetations on its atrial surface away from the free border. The aorta and its valve were normal. On microscopy, the vegetations were chiefly composed of large colonies of Gram-positive cocci with exuberant Splendore-Hoeppli phenomenon ([Fig 1C, D](#)). Bacterial colonies were also seen completely carpeting the right atrial and ventricular endocardium, with formation of abscesses within the myocardium ([Fig 2A, B, C](#)). Ring abscesses were confirmed microscopically. The lungs weighed 870 g, with patchy areas of consolidation, mild pleuritis and small abscesses (0.8–1.0 cm) present bilaterally. Microscopically, the abscess revealed characteristic zonation with central core of eosinophilic granules displaying Splendore-Hoeppli phenomenon, along with the presence of Gram-positive cocci ([Fig 2D](#)). The peripheral zones showed the presence of neutrophils and lymphomononuclear cells. Bronchopneumonia and diffuse alveolar damage was noted. The kidneys weighed 260 g and on microscopic examination showed immune-complex mediated injury with focal mesangioproliferative pattern. The tubules showed presence of casts with foci of moderate inflammatory infiltrate with occasional abscess formation within the interstitium. Liver and spleen were enlarged weighing 1600 g and 180 g, respectively. Microscopically, liver and spleen revealed features of shock, with the presence of neutrophils and haematopoietic precursors within the dilated sinusoids. Botryomycotic abscesses and necrotic foci were noted within the marrow spaces in the section from lumbar vertebra ([Fig 2E, F](#)). Hilar lymph nodes revealed reactive follicular hyperplasia with preserved lymphoid population. Brain, gastrointestinal tract including oesophagus and stomach, and pancreas, were normal. A final diagnosis of disseminated visceral botryomycosis with involvement of heart, valves, lungs and bone marrow was made.

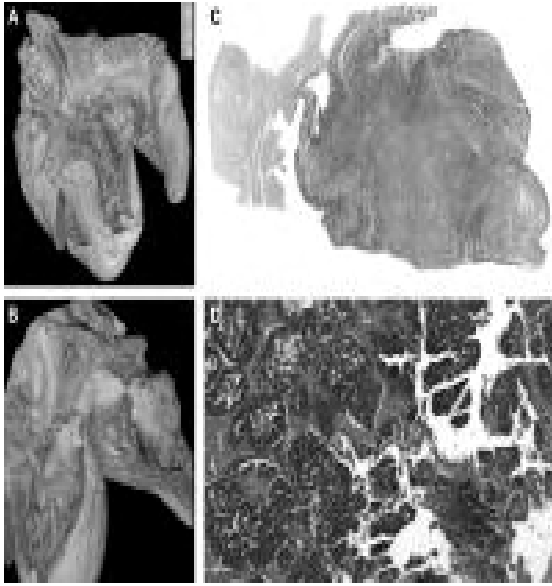


Figure 1 (A) and (B) Large, bulky vegetations attached to the tricuspid valve with grayish white cut surface (B). (C) and (D) Scanned image of the giant botryomycotic vegetation delicately attached to the tricuspid valve (periodic acid–Schiff stain). High power shows colonies of Gram-positive cocci with exuberant Splendore-Hoeppli phenomenon ($\times 100$, Gram stain).

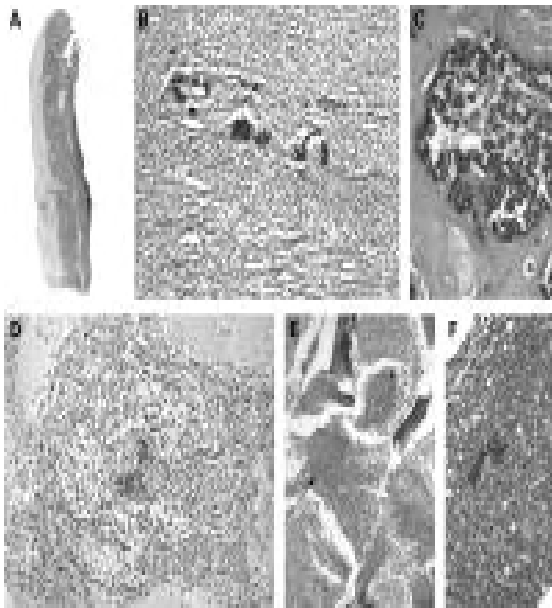


Figure 2 (A) Scanned image of the right ventricular wall carpeted by bacterial colonies (H&E stain). (B) and (C) Botryomycotic abscess within the myocardium with destruction of the myocardial fibres ($\times 100$, $\times 400$, respectively, H&E stain). (D) Botryomycotic abscess within the alveoli with characteristic zonation ($\times 100$, H&E stain). (E) and (F) Botryomycotic abscess within the marrow spaces ($\times 40$, $\times 100$, respectively, H&E stain)

DISCUSSION

Botryomycosis was first established to have a bacterial nature by Magrou, who isolated *S aureus* from a pulmonary botryomycotic lesion in a horse. It is generally considered to be a progressive pseudomycosis caused by non-filamentous bacteria, and histopathology shows a central focus of suppuration within which the grains or granules, which are the organised aggregates of bacteria.

In humans, the disease occurs in two broad categories: cutaneous and visceral forms. Visceral botryomycotic abscess predominantly occurs in lungs, but has also been reported in the liver, kidney, trachea, heart, bowel and brain. Since its initial description in humans in 1913, the disease known as botryomycosis has been difficult to distinguish from actinomycosis. Pathologically, both diseases display the Splendore-Hoeppli phenomenon, which is the deposition of periodic acid–Schiff positive, eosinophilic material around the bacterial colonies. The two are distinguished satisfactorily with the help of Gram stain and Grocott Gomori methamine-silver stain. Routine Gram staining of the granules of botryomycosis will reveal the organism responsible.

Recently, *Erysipelothrix rhusiopathiae*, a filamentous Gram-positive bacterium, has been documented to cause endocarditis in humans, with the presence of botryomycotic vegetations on mitral, aortic and tricuspid valves. An autopsy report on involvement of the myocardium with large botryomycotic *S aureus* vegetations and dissemination, with similar abscess formation in lungs and bone marrow, has not been reported in the literature to the best of our knowledge. The vegetations in the present case revealed extensive colonies of Gram-positive cocci with exuberant Splendore-Hoeppli phenomenon, and the paucity of the inflammatory response distinguishes these vegetations from those seen in a case of acute infective endocarditis. The most characteristic feature of the bacteria in botryomycotic vegetations is that, instead of spreading throughout the infected tissue, they group together to form conglomerates resembling the sulfur granules of actinomycotic infection.

The pathogenesis of these abscesses is not well understood. The immune status of the host and microbial infectivity have been implicated in the pathogenesis. An imbalance between the virulence of the organism and host resistance may result in incomplete removal of bacteria by the host, leading to formation of bacterial granulomas. A review of the literature by Brunken et al noted that many patients had immunological abnormalities, with these lesions being more often seen in patients with cystic fibrosis, diabetes mellitus, HIV infection and defects in cellular immunity. The HIV test performed by ELISA in the patient was negative, and moreover the reactive follicular hyperplasia in the lymph node indicated a preserved lymphoid population.

The cutaneous lesions on the legs served as the portal of entry for *Staphylococcus*, which lodged on the right side of the heart and produced bulky botryomycotic vegetations and later disseminated to the systemic circulation. The involvement of the tricuspid valve is more often seen in intravenous drug misusers and is usually fungal in origin. The

occurrence of such large bulky vegetations with destruction of tricuspid valve as seen in this patient is rare.

These lesions are unresponsive to medical therapy and surgical intervention is required. In a case of infective endocarditis having bulky, large grape-like vegetations with presence of extensive bacterial colonies lying embedded in exuberant eosinophilic hyaline material, a diagnosis of botryomycosis should be kept in mind.

Take-home messages

* The heart and its valves are one of the rare sites of involvement by visceral botryomycosis.

* Immune status of the host and microbial infectivity have been implicated in the pathogenesis of botryomycosis.

* Cardiac botryomycosis should always be suspected in a case of infective endocarditis showing large, bulky grape-like vegetations with the presence of extensive colonies lying embedded in exuberant eosinophilic, hyaline material.

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BOTTOM LINE

Canada's Pathology

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Critical issues in Canadian anatomic pathology have surfaced like a crashing wave. Reports from the judicial inquiry into erroneous breast cancer estrogen and progesterone receptor testing, the public inquiry into faulty forensic pathology, and investigations into pathology misdiagnoses in New Brunswick and, most recently, a pathologist's high error rate in Owen Sound, Ontario, have occupied the minds of Canadian pathologists for months and eroded public confidence. But few would dispute pathology's importance. Pathological analysis of tissues is the basis of most health care decisions regarding diagnosis and, increasingly, treatment. As well, it provides links to understanding disease processes. As we wait for the recommendations that will emerge from these inquiries, it is critical to reflect in a general sense on why, with such a fundamental and essential role in patient care, it looks as though Canadian pathology laboratories are unravelling at the seams and, perhaps more importantly, what can be done about it.

Sir William Osler wrote "As is your Pathology, so is your Medicine." His words are as true today as they were in his time. Specimens submitted to a pathology laboratory are examined individually by a pathologist — a labour intensive, nonautomated process that requires scientific acumen and interpretive skills. Canada's population has grown, our understanding of disease has evolved, and screening programs and novel targeted therapies requiring confirmation of appropriate targets in tissues have been developed.

With these advances, the pathologist's volume of work has increased and the complexity of each case has multiplied. Gone are the days when a brief note describing the type of cancer, its extent and margins sufficed. Today, extensive tissue sampling, exhaustive microscopic examinations and ancillary tests, many of which determine therapy and predict outcome (such as estrogen receptor, progesterone receptor and human epidermal growth factor receptor-2/neu tests) as well as synoptic reporting are essential. All of these factors have overwhelmed the pathology laboratory. Unlike a clinic, which may book a limited number of patients per day, the pathology laboratory cannot restrict the number of patient specimens received.

Despite this environment of increased workload and complexity, Canadian laboratories deliver high-quality pathology results, and it would be incorrect to generalize from specific incidents to the overall state of pathology in the country. Most Canadian pathologists and technologists do an excellent job within tight timelines and against numerous confounding odds, including a severe and long-standing shortage of human resources. The Canadian Association of Pathologists estimates we will need about 500 pathologists over the next 10 years to keep up with the current demands, and the Canadian Society for Medical Laboratory Science states that more than 50% of medical technologists will retire over the next 8 years.

Medical errors that may lead to adverse patient outcomes can occur anywhere, especially in an over-strained health care system. In 1999, an American Institute of Medicine report on patient safety prompted the examination of medical errors in all fields of medicine in the United States, including pathology. In response, the Joint Commission on the Accreditation of Healthcare Organizations as well as the College of American Pathologists revised and strengthened accreditation standards in laboratory medicine with respect to patient safety. A number of variables have been identified that contribute to pathology errors, including preanalytical technical issues (e.g., tissue fixation time). Surprisingly, the importance of standardization and minimization of these technical variations has only recently been appreciated and is now being incorporated into practice guidelines.

In Canada, some provincial laboratory accreditation and external proficiency testing programs are in place: Ontario has the Quality Management Program–Laboratory Services and British Columbia has the Diagnostic Accreditation Program. Unfortunately, proficiency testing varies from province to province. More important, Canada lacks an organization to deal with technical proficiency on a national scale. The Canadian Association of Pathologists recently created a National Standards Committee for Immunohistochemistry; however, this grassroots effort lacks official governmental standing or funding, and its recommendations will not be binding.

Although there is some momentum for technical quality-assurance programs, the Canadian health care system does not have a well-resourced approach to quality assurance of the analytical or professional component of anatomic pathology. It is accepted that peer review is an important method of error reduction. This can take many forms (random retrospective review, prospective targeted review or interdepartmental

conferences) and occurs to variable degrees in virtually all pathology departments, especially larger regional centres, academic hospitals and cancer treatment facilities. However, implementing and monitoring activities such as these requires a level of human resources most laboratories can ill afford, especially when already besieged with serious technical and professional staff shortages. Yet, the benefits could be far-reaching, and analyses of errors and discrepancies could uncover reasons for their occurrence that may have less to do with individual performance and far more to do with systemic problems, including human-resource issues, high workload pressures, pathologist fatigue and burnout, factors related to solo or small practices, or a lack of resources for ongoing continuing professional development.

Canadian laboratories are not unique in facing workload and human-resource issues or problems pertaining to medical error and patient safety. However, they are unique in that they lack a national quality-assurance program such as exists within the College of American Pathologists, the British Royal College of Pathologists and the Royal College of Pathologists of Australasia. These organizations oversee and administer a wide variety of quality-assurance initiatives, and there is evidence to attest to the validity of these strategies in decreasing error rates. External quality assessment or voluntary proficiency testing are components in all of these programs, and although there is considerable debate surrounding them, there is evidence to support their value in improving reporting consistency.

All of us share the same goal — the delivery of the very highest quality of laboratory services in Canada. The judicial and public inquiries occurring in different parts of the country will make specific recommendations; however, we can start improving the system now with 3 broad first steps.

First, urgent attention to the serious human-resource issues should help alleviate long-standing staffing problems and improve future laboratory performance.

Second, local hospital administrations and provincial ministries of health should immediately fund quality-assurance efforts in the laboratory system. Far too often the laboratory is asked to develop these initiatives as well as absorb the impact of new clinical programs, changes in clinical practice, new therapies and new techniques without any consideration of appropriate additional resources.

And finally, we must create an appropriately resourced national body to promote excellence in the practice of laboratory medicine in Canada. Such an organization, similar to others around the world, would link together existing provincial laboratory accreditation programs and provide quality assurance to other regions. It could also set national standards and guidelines, establish voluntary professional proficiency quality assurance, coordinate educational activities, and advise and guide human-resource planning.

These 3 actions are absolutely essential if we are to continually improve and sustain a high-quality Canadian laboratory system. Laboratories provide time-critical information

for patient care and even one error may have a devastating effect. By making quality assurance and patient safety a priority at all levels, we will be able to restore confidence in pathology to patients, clinicians and ourselves.

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