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# NEWSPath

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## ANATOMIC PATHOLOGY

### Inflammatory Myofibroblastic Tumours: Where are we now?

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#### Abstract

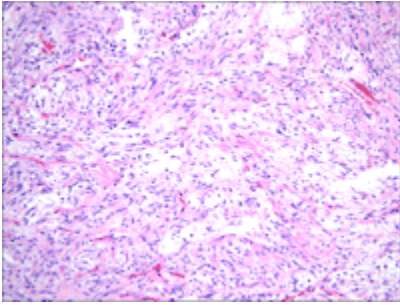
Inflammatory pseudotumour is a generic term applied to a variety of neoplastic and non-neoplastic entities that share a common histological appearance, namely a cytologically bland spindle cell proliferation with a prominent, usually chronic inflammatory infiltrate. Over the last two decades, inflammatory myofibroblastic tumour (IMT) has emerged from within the broad category of inflammatory pseudotumour, with distinctive clinical, pathological and molecular features. IMT shows a predilection for the visceral soft tissues of children and adolescents and has a tendency for local recurrence, but only a small risk of distant metastasis. Characteristic histological patterns include the fasciitis-like, compact spindle cell and hypocellular fibrous patterns, which are often seen in combination within the same tumour. Chromosomal translocations leading to activation of the ALK tyrosine kinase can be detected in approximately 50% of IMTs, particularly those arising in young patients. This review will examine the pathological, and molecular genetic features of IMT and discuss an approach to diagnosis and differential diagnosis.

The term "inflammatory pseudotumour" has been used to describe a wide range of reactive and neoplastic lesions, including inflammatory myofibroblastic tumour (IMT), pseudosarcomatous myofibroblastic proliferations of the genitourinary (GU) tract, infectious and reparative processes, and inflammatory pseudotumours of lymph node, spleen and orbit. Over the last two decades, IMT has emerged as a distinct entity with characteristic clinical, pathological and molecular features. However, confusion remains regarding the distinction of these tumours from other lesions in the "inflammatory pseudotumour" family, as well as from non-neoplastic fibrosclerosing processes and malignant neoplasms with a prominent inflammatory infiltrate. This review will examine the clinicopathological and molecular features of IMT and discuss its differential diagnosis, with emphasis on other entities included under the umbrella of inflammatory pseudotumour.

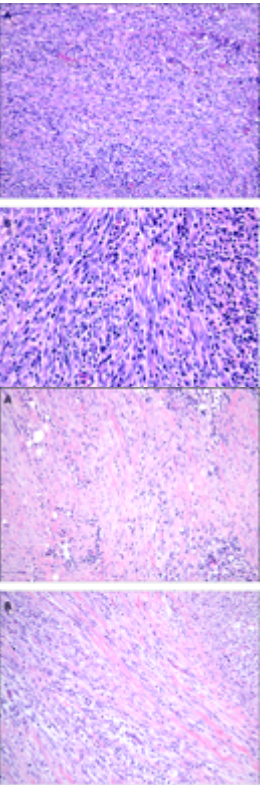
#### Pathological Features

Histologically, IMTs are characterised by a variably cellular spindle cell proliferation in a myxoid to collagenous stroma with a prominent inflammatory infiltrate composed primarily of plasma cells and lymphocytes, with occasional admixed eosinophils and neutrophils. Coffin *et al* described three basic histological patterns, which are often seen in combination within the same tumour: a myxoid/vascular pattern, a compact spindle cell pattern, and a hypocellular fibrous (fibromatosis-like) pattern. The myxoid/vascular pattern has a fasciitis-like appearance, with loosely arranged plump spindle cells in an oedematous or myxoid stroma and a prominent vasculature (Fig. 1). The inflammatory

infiltrate in these areas often contains more neutrophils and eosinophils and fewer plasma cells than in the other two patterns. The compact spindle cell pattern is characterised by a cellular proliferation of spindle cells with a fascicular or storiform architecture in a collagenous stroma (Fig. 2). These foci typically show numerous plasma cells and lymphocytes intimately admixed with the spindle cells, but discrete lymphoid follicles and aggregates of plasma cells are also common. The fibromatosis-like pattern is relatively hypocellular, with elongated rather than plump spindle cells in a densely collagenous background containing scattered lymphocytes, plasma cells and eosinophils (Fig. 3). Focal dystrophic calcification and even metaplastic ossification can be seen in hyalinised areas. Foamy histiocytes are prominent in a minority of IMTs.



**Figure 1** A gastric inflammatory myofibroblastic tumour with a fasciitis-like appearance. Note the loosely arranged spindle cells and the prominent myxoid stroma.

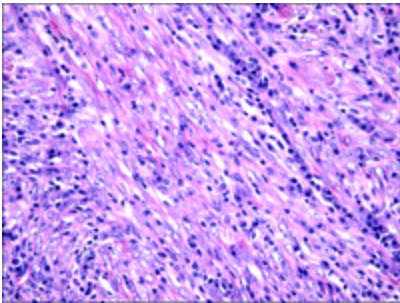


**Figure 2** A cellular inflammatory myofibroblastic tumour of the lung showing (A) a fascicular architecture and (B) numerous admixed plasma cells and lymphocytes.

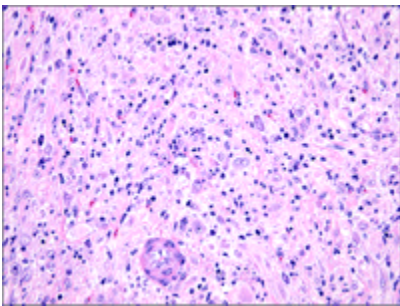
**Figure 3** A hypocellular abdominal inflammatory myofibroblastic tumour (A) resembling desmoid fibromatosis. Note the hyalinised stroma and scattered plasma cells. (B) A more cellular area composed of plump myofibroblasts.

The spindle cells of IMT are typically uniform and predominantly myofibroblastic in appearance, with pale eosinophilic cytoplasm, plump ovoid to tapering vesicular nuclei

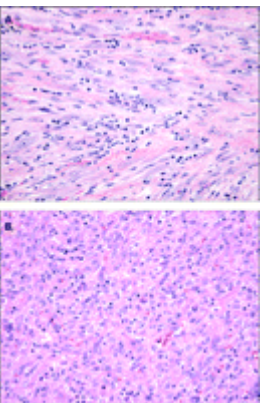
and one or two small nucleoli (Fig 4). Mild nuclear pleomorphism may be seen, but hyperchromasia is absent. Approximately one half of cases contain scattered "ganglion-like" cells (Fig 5): larger polygonal cells with abundant amphophilic to eosinophilic cytoplasm, large vesicular nuclei and prominent nucleoli, similar to those seen in proliferative fasciitis. Mitotic activity is generally low (0–2 mitoses per 10 HPF), and atypical mitoses are rare. Necrosis and vascular invasion have been reported in typical IMTs but are very infrequent. Rarely, IMTs may undergo histological evolution to a morphologically higher grade lesion (Fig. 6) with increased cellularity, marked nuclear atypia, frequent mitoses, atypical mitotic figures, and/or necrosis. The cytological features of the morphologically higher grade tumours are variable, including hypercellular spindle cell, epithelioid/histiocytoid, or round cell morphology.



**Figure 4** Typical cytomorphology in an inflammatory myofibroblastic tumour. The uniform spindle cells contain ovoid nuclei with vesicular chromatin and pale eosinophilic cytoplasm. Note the prominent plasma cells.



**Figure 5** "Ganglion-like" cells in a colonic inflammatory myofibroblastic tumour: large polygonal cells with abundant amphophilic cytoplasm, vesicular nuclei, and prominent nucleoli.



**Figure 6** Recurrent abdominal inflammatory myofibroblastic tumour showing histological progression. (A) The primary tumour was composed of plump spindled myofibroblasts in a collagenous stroma with prominent lymphocytes. (B) The recurrence was a highly cellular tumour composed of histiocytoid cells with mild nuclear atypia and a high mitotic rate.

By ultrastructural analysis, IMTs are composed predominantly of myofibroblasts with a smaller fibroblastic component; the ganglion-like cells show features of fibroblasts. As

expected given their myofibroblastic differentiation, IMTs are positive for smooth muscle actin in 80–90% of cases and express desmin and calponin in 60–70%, although reactivity for these markers is often focal. Approximately one-third of tumours show focal keratin reactivity, which is not unexpected given that myofibroblasts, similar to smooth muscle cells, may be keratin positive. The plasma cell infiltrate is polyclonal.

Due to the prominent inflammatory infiltrate and associated systemic symptoms in a minority of patients with IMT, a viral aetiology has been proposed, but the evidence in this regard is unconvincing. A small subset of so-called inflammatory pseudotumours of lymph node, liver and spleen contain detectable Epstein–Barr virus (EBV); however, it is likely that most if not all of these cases do not represent true IMTs (see below). The presence of EBV in classic IMTs of other anatomical sites is rare. One group identified human herpesvirus-8 (HHV-8) DNA in both pulmonary and extrapulmonary IMT, but expression of HHV-8-associated antigens was not investigated, and other authors have not found evidence for HHV-8 infection in IMT.

### **Diagnostic Approach And Differential Diagnosis**

In our experience, IMTs are more commonly overdiagnosed than underdiagnosed. Attention to the clinical context will often prevent this error—in particular, one should be wary of making the diagnosis of IMT in a middle-aged or elderly patient. The anatomical site also provides clues, since these tumours have a strong predilection for visceral organs and the deep soft tissues of the abdomen, pelvis, and retroperitoneum. IMT should be considered a diagnosis of exclusion in the skin and superficial somatic soft tissues, lymph node, spleen or bladder, where close histological mimics are more common (see discussion below). The histological differential diagnosis of IMT depends in part on the dominant pattern—myxoid/vascular, compact spindle cell or fibromatosis-like. In general, immunohistochemistry does not play a major role in confirming the diagnosis, due to the variable expression and lack of specificity of myofibroblastic markers. ALK positivity is helpful if present, but its absence does not exclude the diagnosis of IMT, particularly in adults. We will first consider the specific differential diagnosis of IMT based on histological pattern, and then discuss criteria for the diagnosis at several specific anatomical sites, focusing on those in which the distinction between "inflammatory pseudotumour" and IMT is poorly defined.

The compact spindle cell pattern of IMT is particularly difficult to distinguish from a variety of histological mimics. Occasional spindle cell sarcomas, spindle cell melanomas, and sarcomatoid carcinomas contain a marked inflammatory infiltrate, and may show only mild cytological atypia. However, plasma cells are generally not a prominent component of the inflammatory infiltrate in these other tumour types, and most examples show at least focally prominent nuclear hyperchromasia, atypical mitoses, necrosis or vascular invasion, all of which are very unusual in IMT. Dedifferentiated liposarcoma can be particularly challenging, especially on a biopsy specimen, as morphologically low grade lesions are often remarkably bland, and immunohistochemistry for MDM2, a sensitive marker of dedifferentiated liposarcoma, is not helpful in this distinction, given that a significant proportion of IMTs show nuclear MDM2 expression. However,

dedifferentiated liposarcoma usually presents in an older age group than IMT, and adequate sampling of the mass and adjacent soft tissue will usually reveal a higher grade component and/or areas of well-differentiated liposarcoma. Due to their predominance among mesenchymal tumours of the GI tract and mesentery, gastrointestinal stromal tumours (GISTs) may be considered in the differential diagnosis of cellular IMTs in the abdomen. However, the pale eosinophilic, syncytial cytoplasm and cytological uniformity of GISTs contrast with the plump myofibroblasts, scattered ganglion-like cells and collagenous background seen in IMT. Furthermore, although an inflammatory infiltrate may be seen in GISTs, it is generally patchy, and plasma cells are infrequent. Immunohistochemistry is helpful in this differential diagnosis, as IMTs are consistently negative for c-kit. Other entities to consider in the differential diagnosis of cellular IMTs are dendritic cell neoplasms, which characteristically show an evenly distributed chronic inflammatory infiltrate admixed with the spindle cell component. Follicular dendritic cell (FDC) and interdigitating dendritic cell (IDC) sarcomas are readily distinguished from IMT by immunohistochemistry, as the former express CD21 and/or CD35, and the latter are uniformly positive for S100 protein. Fibroblastic reticulum cell (FBRC) tumours are exceptionally rare with only few reports in the literature, most of which have arisen in lymph nodes, where IMT is very uncommon. FBRC tumours may be histologically indistinguishable from IMT and have a similar immunoprofile, including variable expression of SMA, desmin and keratin. Indeed, Rosai *et al* have recently proposed that IMTs show fibroblastic reticulum cell, rather than myofibroblastic, differentiation, but this hypothesis remains speculative. Finally, inflammatory leiomyosarcoma, although a rare histological variant, deserves mention, as it shares several features with IMT, including a predilection for young adults, a mixed storiform and fascicular spindle cell architecture, and a prominent inflammatory infiltrate, often with numerous foamy histiocytes. However, focal areas with typical histological features of leiomyosarcoma—namely, fascicles of spindle cells with cigar-shaped nuclei and brightly eosinophilic cytoplasm—can usually be identified.

Fibromatosis-like or hypocellular fibrous IMTs may show histological overlap with desmoid fibromatosis or calcifying fibrous tumour, depending on the degree of stromal hyalinisation. Desmoid fibromatosis, particularly if located in the mesentery, not infrequently shows focally fasciitis-like features, enhancing the histological resemblance to IMT. In classic areas, however, the spindle cells of fibromatosis are arranged in characteristic long fascicles, in contrast to the short fascicular or storiform patterns of IMT, and although a lymphocytic infiltrate may be present in desmoid tumours, plasma cells are infrequent. Furthermore, these lesions generally show aberrant nuclear positivity for  $\beta$ -catenin. Calcifying fibrous tumour is a rare benign neoplasm with a predilection for young patients and a wide anatomical distribution. Some authors have proposed that this lesion represents a late stage of IMT, while others have found no convincing link between the two entities. Histologically, calcifying fibrous tumours are uniformly hypocellular, in contrast to the variable cellularity of IMTs, and they usually contain scattered psammomatous or dystrophic calcifications.

The principal entity in the differential diagnosis of IMTs dominated by the myxoid/vascular pattern is nodular fasciitis. Clinical information is often helpful in this

differential, as nodular fasciitis usually appears rapidly over a few weeks to months, is rarely larger than a few centimetres in size, and typically arises in subcutaneous tissue or skeletal muscle, where IMT is very uncommon. Histologically, IMTs contain a much more prominent inflammatory infiltrate than nodular fasciitis and usually show collagenous storiform or fascicular areas, which are not seen in the latter. Occasionally, reactive processes dominated by granulation tissue may mimic IMTs with the myxoid/vascular pattern. In the absence of a supportive clinical history, histological clues to a reactive/post-traumatic process include an organised vascular pattern and fat necrosis in the adjacent soft tissue. Fasciitis-like IMTs arising in the GU tract should be distinguished from pseudosarcomatous myofibroblastic proliferations (see below).

### **Inflammatory pseudotumour versus IMT**

As discussed above, IMT was formerly buried within the broad category of non-neoplastic fibroinflammatory and neoplastic lesions referred to as inflammatory pseudotumour. The use of these terms synonymously in the literature has led to confusion regarding the true incidence and behaviour of, and the diagnostic criteria for, IMT at a variety of sites, particularly the lung, liver, spleen, lymph node, and bladder.

Pulmonary inflammatory pseudotumour (also often referred to as plasma cell granuloma) encompasses a variety of entities, including IMT and post-infectious/reparative processes, of which the latter are likely more common. Twenty to 30% of patients with pulmonary inflammatory pseudotumour report a history of lower respiratory tract infection, while others have a history of pulmonary infarcts or prior radiation therapy. Organising pneumonia is frequently identified within or at the periphery of these lesions, and many cases show features not seen in IMT, such as granulomatous inflammation, abscess formation, and numerous lymphoid follicles with germinal centres. A recent study reported increased IgG4-positive plasma cells and obliterative phlebitis in a series of plasma cell-rich pulmonary inflammatory pseudotumours, suggesting that some cases may have an autoimmune aetiology and form part of the spectrum of the recently recognised "systemic IgG4-related sclerosing disease". The broader age distribution and lower recurrence rate for pulmonary IMT as compared to extrapulmonary tumours may be due in part to the inclusion of non-neoplastic processes in prior studies. That said, the lung remains a common site for true IMT, and, conversely, IMT is one of the most common primary pulmonary neoplasms in children.

Similar to those in the lung, hepatic "inflammatory pseudotumours" represent a diverse group of lesions with infectious, autoimmune and neoplastic aetiologies, the latter including inflammatory pseudotumour-like FDC sarcoma and IMT. A bacterial or fungal cause has been identified in several cases of hepatic inflammatory pseudotumour, and in some reports, the masses resolved following antibiotic therapy. Prior reports of EBV in hepatic inflammatory pseudotumours are now believed primarily to represent inflammatory pseudotumour-like FDC sarcomas. This distinctive variant of FDC sarcoma characteristically arises in the liver and spleen of female patients, frequently presents with systemic symptoms, and shows a consistent association with EBV. Histologically, inflammatory pseudotumour-like FDC sarcomas are composed of spindle cells with

vesicular nuclei and palely eosinophilic cytoplasm and show a marked lymphoplasmacytic infiltrate, thus closely mimicking IMT. Immunohistochemistry is often required to distinguish between these two neoplasms: the spindle cells of inflammatory pseudotumour-like FDC sarcoma express at least one of the FDC markers (CD21, CD23, and CD35), are negative for ALK, and are consistently positive for EBER (EBV-encoded mRNA). Finally, recent studies suggest that hepatic inflammatory pseudotumours dominated by lymphoplasmacytic inflammation with a minimal myofibroblastic component may be a manifestation of the systemic IgG4-related sclerosing disease (see above). Similar to the pancreatic lesions associated with this disorder ("autoimmune" or lymphoplasmacytic sclerosing pancreatitis), the "lymphoplasmacytic type" of hepatic inflammatory pseudotumour consistently shows increased IgG4-positive plasma cells, obliterative phlebitis, and periductal inflammation with concentric fibrosis, whereas these findings are less common or absent in the "fibrohistiocytic type" of hepatic inflammatory pseudotumour.

Inflammatory pseudotumour of lymph node and spleen is a non-neoplastic entity distinct from IMT. Most affected patients are adults, and those with lymph node lesions frequently present with systemic symptoms and/or laboratory abnormalities similar to those seen in a minority of patients with IMT. Inflammatory pseudotumour of lymph node has a characteristic distribution, preferentially involving the connective tissue framework of the node (the capsule, trabeculae, and hilum) without forming a discrete mass, although in later stages, the lesion may efface the nodal architecture. Vascular changes, including perivascular fibrosis and vasculitis, are often seen within medium-sized vessels of the hilum or capsule, and extension into perinodal soft tissue is common. EBV has been detected in small lymphocytes in several cases of nodal inflammatory pseudotumour, but the spindle cells are EBV negative. In contrast to nodal lesions, splenic inflammatory pseudotumours are often asymptomatic, form a discrete mass, and usually lack associated vascular changes. Furthermore, splenic lesions are rarely associated with nodal lesions and vice versa, suggesting that the two entities may be biologically unrelated, despite their histological similarities. Some investigators have detected EBV in the spindle cells of a subset of splenic inflammatory pseudotumours, whereas others reported no evidence of EBV infection. The spindle cells in some of the EBV-positive cases also expressed FDC markers, and thus represent EBV-associated inflammatory pseudotumour-like FDC tumours, as discussed above. Inflammatory pseudotumours of lymph node and spleen are consistently negative for ALK

The family of idiopathic fibrosclerosing lesions, including sclerosing mesenteritis, idiopathic retroperitoneal fibrosis, sclerosing mediastinitis, and orbital inflammatory pseudotumour, may also be considered within the umbrella of inflammatory pseudotumour. These lesions may occur synchronously or metachronously in the same patient. Recently, an association with elevated serum IgG4 levels and other autoimmune disorders, particularly autoimmune pancreatitis, has been described in some patients with fibrosclerosing diseases, suggesting that a subset of these cases may be manifestations of the systemic IgG4-related sclerosing disease mentioned above in the discussion of pulmonary and hepatic inflammatory pseudotumours. Grossly, the mass lesions formed by these processes are ill-defined and often encase adjacent structures, in contrast to the

relatively circumscribed margins of IMT. Microscopically, the spindle cell component of fibrosclerosing lesions is usually significantly less cellular than that seen in even the most hypocellular IMTs, and the storiform, fascicular and myxoid/vascular patterns of IMT are absent. In addition, the inflammatory infiltrate is often patchy and perivascular, in contrast to the diffuse infiltrate seen in IMTs, and lymphocytic infiltration of blood vessel walls may be present in up to 50% of cases.

Finally, perhaps the most confusing topic in the recent literature on IMT is the distinction between pseudosarcomatous myofibroblastic proliferations (PMPs) and IMTs in the GU tract. Pseudosarcomatous spindle cell lesions in the GU tract have been described under a variety of names, including pseudosarcomatous fibromyxoid tumour, inflammatory pseudotumour, pseudosarcomatous myofibroblastic tumour/proliferation, pseudomalignant spindle cell proliferation, and postoperative spindle cell nodule for those arising following instrumentation. There are no histological differences between those that occur following trauma and those that arise spontaneously, the latter being more common. PMP usually occurs in adults, is not associated with systemic symptoms, and may recur locally in 10–20% of cases but does not metastasise. Although some authors do not distinguish between IMT and PMP in the GU tract, we and others believe that they are distinct entities that are separable on morphological grounds, and, in our experience, most fasciitis-like myofibroblastic lesions in the GU tract are PMPs. Histologically, PMPs are composed of a haphazard to loose fascicular arrangement of spindle cells, many of which have elongated bipolar cytoplasmic processes that are often brightly eosinophilic, mimicking rhabdomyoblasts. The stroma is typically oedematous to myxoid with prominent vascularity and a variably dense acute and/or chronic inflammatory infiltrate. PMP may show focally more cellular fascicular areas, particularly in the deeper portion of the lesion away from the mucosal surface, but the storiform and hypocellular fibrous patterns of IMT are rarely seen. In addition, the inflammatory infiltrate of PMP is generally less dense than that seen in IMT and lacks a prominent plasma cell component. As both IMT and PMP are myofibroblastic in nature, there is considerable immunohistochemical overlap between the two lesions. PMP expresses SMA in approximately 70% of cases, desmin in 35–60%, keratin in 42–94%, and may also be positive for ALK, although the molecular basis for this is unclear. One study documented ALK positivity in 10 of 21 PMPs, but found no evidence of *ALK* gene rearrangement in the 6 ALK-positive cases examined by FISH. Another group similarly found ALK expression in nearly 50% of PMPs (12 of 26) but also identified *ALK* gene rearrangements in 4 of the 6 ALK-positive cases examined, raising the possibility that these lesions may be neoplastic. Alternatively, this study (and others) may have included a heterogeneous group of lesions, both PMPs and "true" IMTs. However, there are certainly rare examples of bona fide IMTs that arise in the region of the bladder. Although the aetiology of PMP (reactive or neoplastic) and its possible relationship to IMT remain controversial, we believe there is sufficient data with respect to differences in clinical behaviour to warrant their separation.

In summary, IMT is a distinctive myofibroblastic neoplasm that has a predilection for the lung, abdomen, and pelvis of children and young adults, shows a tendency for local recurrence, and shows a characteristic cytogenetic abnormality in approximately half of

the cases. IMTs should be distinguished from the variety of neoplastic and reactive lesions included under the umbrella term "inflammatory pseudotumour". To avoid ambiguity, the latter designation is best avoided, with the exception of inflammatory pseudotumour of lymph node and spleen and orbital inflammatory pseudotumour, which are distinct clinicopathological entities. Finally, one should keep in mind that IMT is a diagnosis of exclusion in middle-aged or older adults, and in skin and somatic soft tissue, and the presence of nuclear hyperchromasia, atypical mitoses, or more than mild nuclear atypia argues strongly against the diagnosis.

### Take-home messages

- The unqualified designation "inflammatory pseudotumour" is best avoided.
- Inflammatory myofibroblastic tumour is a distinctive neoplasm of intermediate biological potential with a predilection for the abdominopelvic region and lung of children and young adults.
- Histological features in inflammatory myofibroblastic tumours do not correlate well with clinical behaviour.
- Chromosomal translocations leading to activation of the ALK tyrosine kinase (and overexpression of the ALK protein) can be detected in approximately 50% of IMTs, but are uncommon in older patients.
- Inflammatory myofibroblastic tumour is a diagnosis of exclusion in middle-aged or older adults, and in somatic soft tissue, and the presence of more than mild nuclear atypia argues against the diagnosis.

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## **A Survey of the Myriad Forces Changing Anatomic Pathology and Their Consequences**

**Bruce A. Friedman, MD**

My assigned role at this conference was to identify what I considered to be the 10 major forces that are impacting anatomic pathology, and I discovered that one of my major challenges was to limit this list to 10. There is no magic associated with the number 10, and others may not agree with my selection of these forces. My major goal is to stimulate thought among pathologists about some of these forces and how they might affect pathology..

I would like to briefly discuss this method of communication, using my own blog, "Lab Soft News," as a means to illustrate the possible effects of blogging on the future of pathology.

I started blogging about 17 months ago almost on a kind of whim, but it has become an important part of my life. My blog, Lab Soft News, is located at [www.labsoftnews.com](http://www.labsoftnews.com) (accessed September 23, 2007). It is what I call a professional blog, as opposed to a “Hi, Mom blog.” Monday through Friday, I post a blog entry containing about 300 or 400 words. So, basically this amounts to about 90 000 words a year.

I believe that professional blogging will emerge as an important intermediate step between hallway conversations and formal publications. In the same way as this conference has the ability to present a new agenda for anatomic pathology, professional bloggers in our field also have an opportunity to be change agents and help to transform pathology by promoting new ideas.

Before I sketch out the forces that are changing anatomic pathology, I begin by defining anatomic pathology as consisting of surgical pathology, cytopathology, and autopsy pathology.

Surgical pathology is a subjective discipline supported by what I would describe as aging technologies. Histopathology remains largely a subjective skill. As a recent National Cancer Institute grant proposal addressing precancer also refers to surgical pathology as being “highly subjective,” I am not alone in this opinion.

Many of the underlying technologies for surgical pathology such as tissue stains and paraffin embedding have been in use for many decades. The notion of histopathologic diagnosis as a qualitative and subjective discipline is reinforced by our training methods. There may be wide variation among the experts about which diagnosis to assign to a particular lesion, reinforcing this notion of subjectivity.

However, histopathology remains the gold standard for tissue diagnoses. It is irrefutable that histopathology and cytopathology now reign supreme as the best means for diagnosing tissue lesions and will continue to reign supreme for some time in the future, despite the fact that they are subjective disciplines. In addition to being highly accurate, histopathologic diagnosis is also relatively inexpensive and very rapid, which reinforces the fact that it is indispensable.

We now have emerging an imaging technique, molecular imaging, which is in its early phase but which potentially could be a competitor to surgical pathology and histopathology in terms of tissue diagnosis. Because histopathology is the gold standard, however, the radiologists and the companies that are pursuing research in molecular imaging on the radiology side will need surgical pathologists to validate the diagnoses arrived at using molecular imaging.

Put very succinctly, the early health model refers to presymptomatic, preclinical diagnosis, and I would now add to that list the diagnosis of precancer. I briefly referred before to a new grant opportunity with National Cancer Institute, dealing with the diagnosis of precancer.

The concept of the early health model is going to shake up a lot of important constituencies in health care, such as health insurance companies, clinicians, and pharmaceutical companies. We are going to begin to diagnose presymptomatic, preclinical disease. Clinicians are not trained to diagnose preclinical and presymptomatic disease. The pathologists and laboratory scientists can and should be at the epicenter of this revolution because the diagnosis of preclinical and presymptomatic disease is commonly arrived at using biomarkers. My own belief is that it will be much more cost-effective to diagnose preclinical and presymptomatic disease using serum rather than sending the patients for complex, expensive imaging studies. I believe that the early health model will involve periodic surveillance with large panels of biomarkers. Positive results from such serum screening will then prompt the clinician, who is directing the workup of the patient, to order focused imaging studies to confirm a suspected diagnosis.

No. 2: Molecular medicine becomes a major driver in health care. In the same way that this early health model is largely being driven and popularized by General Electric, molecular medicine and so-called full-service diagnostics is being popularized by Siemens. This molecular medicine involves the analysis of the molecular basis of disease and manipulation of those molecules to improve the diagnosis, prevention, and treatment of disease.

This notion of molecular medicine and full-service diagnostics is quite synergistic with the early health model because essentially molecular medicine is the tool by which we will execute the early detection and treatment of disease.

Molecular medicine also enables the monitoring of treatment efficacy, using biomarkers and medical/molecular imaging, and this segues into a discussion of so-called personalized medicine. This discussion about molecular medicine and the early health model expands the potential for screening programs and assessments of genetic predisposition. A key question at this point is how anatomic pathology and surgical pathology can be converted from a morphology-driven to a molecular-driven medical specialty.

No. 3: Clinicians will seek key indicators of prognostic and therapeutic efficacy. There is a major shift underway on the part of clinicians from the emphasis on the diagnosis to the prognosis assessment and monitoring the effectiveness of therapy. The diagnosis shortly will become something that can be arrived at, particularly with large panels of biomarkers, even in an early stage, very quickly.

The shift is being spawned by sophisticated medical imaging and molecular diagnosis. In other words, to put it very bluntly, diagnosis is becoming fast and accurate and that would be the very early beginning of the process. What is going to be most significant is prognosis and therapeutic recommendations, and I see the pathologist and the laboratory scientists as working at the center of this process.

With personalized medicine and targeted chemotherapy, we will be able to change drugs midstream if no observed, beneficial effects occur. With sophisticated imaging, we will

be able to observe minute shrinkage of tumors very quickly to indicate that we are on the correct therapeutic path. For pathologists, the opportunity exists to change the emphasis of reports, make them more consultative than a terse description of the diagnosis, and better respond to the clinician's needs. Here is what they are going to be interested in: "What is the prognosis of this patient, and am I pursuing the correct therapeutic path?"

No. 4: Constant pressure for more cost-effective health delivery. This is one of those huge macroforces that will be difficult to grapple with. The key question for pathology and laboratory medicine is how to lower the cost of health care delivery as diagnoses and therapeutic interventions become more sophisticated. These are issues that are driving a lot of the insurance payors to distraction with the increased emphasis on large panels of biomarkers and sophisticated algorithms, which are associated with expensive molecular testing. How do we transition to this early health model when the payors are already spending a lot of money on treating, sometimes ineffectively, an existing disease? My belief is that much of the cost of this health surveillance testing with large panels of biomarkers will be out-of-pocket, but I am not sure how this is going to play out.

For neoplasms, the earlier diagnosis and targeted therapy may avoid expensive surgery and prolonged hospital stays, but this advantage may be offset by the increased cost of complex testing and the cost of some of the novel new drugs that are now coming to market.

Wellness monitoring and healthy lifestyles may avoid the complications, so in the long run this early health model may cost less, but it may cost more in the short run. How are we going to deal with this? One of the solutions is to anticipate greater out-of-pocket costs for consumers.

On my blog, I have discussed some new models for health care delivery such as the walk-in clinics that are being opened in retail drugstores and big-box discount stores such as Wal-Mart. These walk-in clinics, which I believe offer a new paradigm for routine health care delivery, will begin to offer Clinical Laboratory Improvement Amendments-waived laboratory testing for routine problems. You are going to hear much more about these walk-in clinics in the future.

No. 5: There is early interest in the merger/convergence of pathology and laboratory medicine with radiology. The rationale for such a change is buttressed by economic, political, strategic quality, and organizational considerations.

Medical imaging is, I believe, on a collision course with surgical pathology in the sense that medical imaging will be increasingly able in the future to arrive at a concrete diagnosis. This is where molecular imaging is headed. What I also realized, after going into this topic in some depth, is that radiology is losing control over some of the imaging procedures, and therefore revenue, to clinical specialists such as those in cardiology and emergency medicine. One medical school, with a grant from General Electric, is equipping all of their medical students with portable ultrasound devices. These devices will substitute for the stethoscope and allow very rapid diagnoses at the bedside for many

conditions. Thus, you have technology driving and causing “porosity” of the boundaries between specialties, with radiology losing some of its imaging procedures to other specialties.

For me, the most important rationale for this idea of conversion/merger between pathology and laboratory medicine and radiology is the quality advantages for the 2 groups and for the patients that they serve. I have this image of an office radiologist sitting next to a pathologist and collaborating and looking at the whole historical record for patients while correlating imaging results with surgical pathology and cytopathology results.

No. 6: Multiplex biomarker panels will deliver early diagnosis and wellness monitoring. I believe such panels are much more comprehensive and sensitive than our current methods, such as the yearly, cursory physical examination accompanied by a small set of laboratory tests. What I hear over and over again is that health care consumers today want to know what is going to happen to them in the future on a 3- or 5-year horizon. I know there are a lot of practical and ethical considerations to be considered here and we will need to deal with false-positives. However, I believe that this approach to health care correlates well with early intervention, and I believe that ultimately, early intervention will be more cost-effective.

This approach to wellness monitoring is predicated on the knowledge that diseased cells and most notably neoplastic cells communicate with each other by the elaboration of proteins. This is a similar story to that of the Rosetta stone: All we have to do is better understand the meaning of these subtle changes in the level of proteins circulating in the body. This will allow us to arrive at earlier diagnoses assisted by the use of algorithms. That latter step is critical because these data are going to be so complex that we cannot just scan it like in the old days to arrive at the correct diagnosis.

No. 7, 8, and 9: Digital pathology begins to emerge as a fully matured discipline; direct searching of image databases becomes practical and commonplace; and hyperspectral imaging supplements brightfield microscopy. I am not going to dwell on these topics because there are too many experts in the room who can discuss them better than I.

If and when there is a merger between radiology, pathology, and laboratory medicine, digital pathology will emerge as one of the critical factors in such a partnership. If this conversion were to take place, laboratory data and medical imaging will form the basis for some 80% of all diagnoses rendered and medical consultation will be available on a global basis.

This conference features a speaker who discusses NightHawk Radiology (Coeur D'Alene, Idaho). This company is a striking example of the changes that can be effected when a specialty such as radiology goes completely digital. This company has evolved purely on the basis of digital radiology and is responsible for moving images around the world. What is interesting about NightHawk is that the company initially developed software to support and complement its global model of moving radiology images around the world.

However, it has now evolved in part into a software company, and it is licensing the software that it created for its own use internally, as part of its business model, to radiology groups that are colocated, allowing these other groups to manage images, reports, and personnel in multiple locations.

I believe that direct searching of image databases will become a reality in the very near future. In other words, as pathology becomes fully digital, we will have these valuable databases containing multiple images, and we will be able to search these image databases on a routine basis. We will have real-time access to differential diagnoses of prior cases based on regions of interest in a slide that we are currently studying. There will also be an opportunity for direct searching of image databases to assign a diagnosis to lesions of low incidence. For rare diseases, this will allow faster recognition and understanding that a cohort of distinct cases exist. This will result in simplified consensus generation for rare lesions.

We have this new phenomenon emerging of hyperspectral imaging that will be used to enhance and supplement brightfield microscopy in surgical pathology. This will allow us to leverage our existing low-cost, conventional histochemical stains to add significant diagnostic power. This hyperspectral imaging will allow us to apply multiplexed staining to brightfield microscopy using 5 to 8 immunostains in a section.

No. 10: We have need for a strategy to counteract the current commoditization of laboratory medicine.

In my view, the antidote to this challenge will be genomic and proteomic testing, particularly coupled with sophisticated algorithms that allow the interpretation of these test results. We also have an opportunity to provide very sophisticated laboratory consultations that are correlated with medical imaging to offset this commoditization. The secret will be staying ahead of the competition.

Another very important phenomenon that is now occurring is so-called medical tourism. Medical tourism involves patients both in the United States and other Western countries traveling to India, Singapore, and Thailand, particularly Bumrungrad Hospital in Bangkok, for surgical procedures that are very expensive in their home countries. For example, the cost of a total hip replacement in India or in Thailand is about one fifth of the cost in the United States. A whole range of services are being outsourced today and health care is not immune to this phenomenon.

In conclusion, there are major forces now bearing down on pathology that will greatly disrupt our historical franchise and the professional lives of our practitioners. We need to adapt to many of these changes or become irrelevant. The first reform that I believe is necessary will be closer integration of anatomic pathology with clinical pathology. This is critical because genomics and proteomics will be the basis for new biology. The second reform will be closer integration with radiology, and I have personally been an advocate of this conversion of merger, or at least some form of closer interoperation between radiology and pathology.

## **Sentinel Lymph Nodes: An Introduction**

**Geisinger, Kim R.; Levine, Edward A**

The word sentinel is derived from the Latin to feel and converted in both Italian and French to watch. In oncology, sentinel lymph nodes (SLNs) refer to the first lymph node or initial small group of nodes that receive lymphatic drainage from tumors. Hence, they are watching or guarding the regional lymphatic system. During the last decade, SLNs have evolved from a curious clinical investigative tool to the standard of care in the management of patients with breast cancer and cutaneous melanoma. It has also been evaluated as a tool to guide resections for most solid tumors.

Basically, the evaluation of SLNs is used to predict the metastatic status of other (nonsentinel) lymph nodes in the nodal basin at risk. This is predicated on the premise that if the SLN is positive for metastasis, then there is a significant risk that adjoining lymph nodes may also be positive; this prompts the need for a lymph node dissection of the nodal basin. When nodal metastasis is found, the completion lymphadenectomy serves both as therapy for nodal disease and supports more accurate staging. On the other hand, if the SLNs are free of metastasis, then it is highly unlikely that other nodes are involved, permitting the patient to avoid the additional dissection and its attendant morbidity. This issue of Pathology Case Reviews is dedicated to describing a number of aspects of the clinical usage and morphologic interpretation of these nodes and potential advantages and disadvantages associated with these harbingers of neoplastic spread. The lead-off contribution, authored by 2 active surgical oncologists, details the successful evolution of the clinical techniques to detect SLNs, concentrating their efforts on breast carcinoma and melanoma. In addition, they describe the different efforts to examine pathologically these nodes, including intraoperative assessment and molecular analysis. Howard-McNatt and Levine also touch upon SLNs in other malignancies, for example, adenocarcinomas of the gastrointestinal tract. All of this is wrapped in terms of the clinical results from a number of published studies worldwide.

This is followed by an article by McCarty and Silver discussing the intraoperative use of the reverse-transcription polymerase chain reaction analysis of SLNs to increase significantly the diagnostic sensitivity for metastasis in the setting of carcinoma of the breast.

Many laboratories use cytologic touch or scrape preparations for the intraoperative assessment of SLNs. Although these techniques possess several important advantages over frozen sections, cytology also suffers from several problems. The latter are both technical and interpretive, including the lack of architectural arrangements and cellular locations of the nonlymphoid elements. Parsons and colleagues emphasized this difficulty via the evaluation of SLNs in a patient with established malignant melanoma. Two SLNs were identified surgically and examined intraoperatively by touch preparations using both

Diff-Quik and hematoxylin and eosin stains. One node was obviously benign cytologically, but the other yielded clusters of cells suspicious but not diagnostic of metastatic melanoma; as is customary in their laboratory, a negative diagnosis was rendered intraoperatively (as a malignancy was not clear cut), and the complete dissection was not performed. The final diagnosis of both of the nodes was benign, but the one that was worrisome intraoperatively contained a capsular nevus. This benign proliferation of nevus cells was the source of the diagnostic dilemma during surgery. As capsular nevi are relatively common, especially in the SLNs of patients with melanoma, this could arise as a potential problem for others. Thus, this case report provides awareness of the source of an interpretive challenge.

Using evidence based medicine as his foundation, Wick deftly and deeply dissects into the clinical value of SLNs in patients with cancer, and in particular, melanoma and carcinoma of the breast. Clearly, this article should be carefully perused at least twice by thinking, practicing pathologists. The first time through provides a terse but excellent overview of the concept of evidence based medicine itself. This discussion is highly worthwhile for those readers for whom this construct is not well known. The second reading should be aimed at acquiring the evidence based medicine data on SLNs and recognizing that clear-cut information exists that does not support the use, at least routinely, of such procedures in the majority of all individuals with melanoma and a proportion of women with breast cancer. Interestingly, one of the patient cases in this article by Wick is an individual with melanoma in whom diagnostic challenges arose in the setting of a capsular nevus, highlighting again the difficulties that may occur in this specific setting.

Another aggressive cutaneous neoplasm is Merkel cell carcinoma. This cancer is also experiencing a progressively increasing utilization of SLNs in its clinical management. Young and colleagues describe a young woman with a Merkel cell carcinoma of the back who underwent SLN evaluation with intraoperative touch preparations. This, of course, raised the differential diagnosis of a small blue cell neoplasm versus benign lymphoid elements. In our experience, however, this cytologic distinction is not too difficult if one adheres to classic cytomorphologic criteria. Young and colleagues discuss in detail the advantages and disadvantages of the intraoperative examination of SLNs by both cytology (touch preparations) and frozen sections in their contribution.

Using 2 cases of early carcinoma of the uterine cervix as their spring board, Popa and colleagues launch into a detailed discussion of many facets of SLNs in women with early cervical disease. In contrast to many other tumor types, SLNs must be detected and examined bilaterally in these women. In many series dealing with the cervix, SLNs are sampled laproscopically. Both of these patients had false negative interpretations of intraoperative frozen sections of their SLNs, but small metastases were detected by an exhaustive histologic protocol of permanent sections and cytokeratin immunohistochemistry. Their literature review demonstrated an overall very high level of diagnostic sensitivity for frozen section detection of cervical metastasis. However, in their experience, false negative frozen section diagnosis occurs almost exclusively with very small metastasis, and thus the authors propose the possibility of a 2 step procedure:

if a macro-metastasis is suspected in a SLN, it is submitted for frozen sections; if metastases are not clinically apparent, then the SLN should be submitted solely for permanent histologic examination. If positive, the patient will return to the operating room for an additional procedure, namely, complete dissections.

Swing completes this issue with a case report involving a 17-year-old patient with an anal alveolar rhabdomyosarcoma who underwent bilateral inguinal SLN examination before receiving chemotherapy prior to definitive surgery. This included intraoperative cytologic evaluation of the SLNs by touch preparations. Only a small proportion of sarcomas metastasize to lymph nodes with any regularity; this includes rhabdomyosarcoma. Consequently, although uncommon, pathologists will, on occasion, encounter SLNs in pediatric patients and in individuals with a diagnosis of certain soft tissue malignancies.

Overall, we believe that this microtome will support the understanding of the principals and practice of modern SLN mapping for the pathologist. This technique appears to represent a great step forward in nodal staging with significant benefits accruing to patients. It is best practiced with continuing dialogue between surgeon and pathologist.

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## **Fine Needle Aspiration Cytology of Papillary Lesions of the Breast – V. 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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## **CLINICAL PATHOLOGY**

### **Automated Blood Cell Counts: State of the Art**

**Buttarelo M, Plebani M.**

The CBC count and leukocyte differential count (LDC) are among the most frequently requested clinical laboratory tests. These analyses are highly automated, and the correct interpretation of results requires extensive knowledge of the analytic performance of the instruments and the clinical significance of the results they provide. In this review, we analyze the state of the art regarding traditional and new parameters with emphasis on clinical applications and analytic quality. The problems of some traditional parameters of the CBC count, such as platelet counts, some components of the LDC such as monocyte and basophil counts, and other commonly used indices such as red cell volume distribution width and platelet indices such as mean platelet volume and platelet distribution width are considered. The new parameters, evaluated from analytic and clinical viewpoints, are the available components of the extended differential count (hematopoietic progenitor cells, immature granulocytes, and erythroblasts), the immature reticulocyte fraction, the reticulocyte indices, the fragmented RBCs, and the immature platelet fraction.

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### **Laboratory Assessment Of Nutritional Status**

**Prof. A. Shenkin**

It is widely accepted that patients in hospital who are undernourished are more likely to develop clinical complications and have a relatively poor outcome, with increased length of stay and higher mortality, than well nourished patients. Provision of adequate nutritional support reduces the complication rate and improves outcome. Considerable efforts have therefore been made in recent years to identify patients at risk of malnutrition, with a view to provision of nutritional support at an early stage. This review will consider some of the approaches to this identification currently in use, especially in relation to the role of the laboratory. Although aspects of protein-energy malnutrition (PEM) are of particular interest, it must not be forgotten that vitamin and trace element malnutrition are also likely in many patients and the laboratory can play a key role in identifying and managing such problems.

## **Plasma proteins and other laboratory tests of protein energy status**

Nitrogen balance has been the cornerstone of most nutritional research and recommendations of dietary protein requirements, and is still regarded as the reference method against which other tests of adequacy of recent protein–energy are compared. However, the limitations in obtaining complete collections of urine, and knowledge of all intakes, as well as the time-consuming technology, have meant that this remains a research rather than patient care technique. A number of plasma proteins have been proposed both for screening for malnutrition and monitoring of progress of nutritional support.

**i) Albumin** Serum albumin is of virtually no value in assessment and monitoring of nutritional status, but is included here for completeness, and to emphasise the limitations in interpreting serum albumin concentrations. The major factor affecting plasma albumin concentration in sick patients is the rate of transcapillary escape into the interstitial fluid - this is markedly increased in disease (as part of the acute phase response, or systemic inflammatory response syndrome SIRS) leading to a fall in concentration. It is therefore inevitable that sick patients with severe infection or who are post-operation will have a low concentration, and that the lower the albumin, the worse the prognosis. Albumin concentration is also affected by hydration status and liver function. There is also the possibility of some reduction of albumin as a result of chronic malnutrition, since the rate of albumin synthesis is about 5% of the total body pool, but this will be very slow, and a small component of the total change. Indeed, even in patients with anorexia nervosa serum albumin is likely to be only minimally reduced.

**ii) Transthyretin and retinol binding protein (RBP)** Transthyretin (prealbumin) binds to retinol binding protein to form a complex, and under normal conditions about 50-70% is in this complexed form. It is synthesised in the liver and catabolised in the kidney, its half-life in plasma being about two days. As a result of this short half-life, it is sensitive to changes in protein- energy status, and it closely reflects recent dietary intake rather than overall nutritional status. RBP also has a short half life, but since it is present in lower concentration and is more affected by renal failure, most studies have focused on transthyretin. Transthyretin concentration is also affected by changes in transcapillary escape, hence interpretation is difficult in patients with infections or following trauma.

A number of recent studies have evaluated the potential role of transthyretin in screening for malnutrition and monitoring progress. The results suggest a place for transthyretin measurement early after admission, but much more work is needed to clarify the precise place of this measurement in assessment, and how it fits into a nutritional care pathway. In particular, the interpretation of transthyretin concentration in relation to C-reactive protein (CRP) remains a fundamental issue, since transthyretin is as much affected by disease and inflammation as albumin.

**Transthyretin in monitoring** Very low transthyretin concentrations are typical in seriously ill patients, and are inversely related to CRP. However, an increase in transthyretin in response to feeding might be interpreted as a sign of improved metabolic

and nutritional status. An important observation was that patients in ITU receiving an approximately adequate intake showed a rise in transthyretin level of about 40 mg/L over one week, whereas a control group receiving an inadequate intake still showed a rise, but rather smaller at 20 mg/L, whilst CRP level was falling substantially. Similarly, in one ICU study, a loss of total body protein was observed, whilst an increase in transthyretin and a fall in CRP were observed. As has been noted recently, it is quite possible that a daily deficit of 200-400 Kcals may not prevent the improvement in visceral protein levels associated with the reduction in the acute phase response, but this would not permit muscle protein anabolism. Interpretation of transthyretin with respect to nutritional status in patients with an ongoing SIRS must therefore be cautious.

**Laboratory assessment of minerals, trace elements and vitamins** The role of the laboratory in assessing sodium, potassium, calcium, phosphate and magnesium is outside the scope of this review, but the status of these major minerals is frequently abnormal as a result of poor intake and increased losses, and the risk of redistribution as part of the refeeding syndrome. Careful and regular monitoring with adequate replacement is essential. The main trace elements of concern in patients who are sufficiently malnourished to require nutrition support, are zinc, iron, selenium and copper. For all of these, plasma measurements are substantially affected by redistribution resulting from SIRS, so any measurement must be accompanied by a measurement of CRP. Trends in results are easier to interpret than one-off analyses. Similarly for many of the vitamins, a SIRS markedly reduces the plasma concentration. The main vitamin measurements of use in nutrition support are folate and vitamin B12 status, and vitamin D [5]. Laboratory testing is especially useful in stable patients without SIRS, but where a single micronutrient deficit is considered likely. In such patients, plasma measurement of e.g. zinc/copper/folate/ascorbic acid may confirm the diagnosis and the likely pathophysiology, and provide an important baseline for monitoring. NICE has made recommendations of the type of trace element and vitamin measurements which ought to be performed in patients receiving nutrition support at home or in hospital. These recommendations are shown in Table 1, together with some notes on possible frequency of measurement and guides to interpretation.

## Conclusions

Transthyretin is currently the serum protein of most interest with regard to screening for malnutrition and monitoring progress. However, its concentration is affected, as is that of other proteins, by the severity of disease and the presence of a systemic inflammatory response (acute phase response). A rising serum concentration of transthyretin can be regarded as a favourable sign, as would a fall in CRP concentration, although this does not necessarily reflect an improvement in overall nutritional status or in muscle function. Similarly, laboratory tests of trace element or vitamin status are frequently affected by a SIRS. Such tests will be of greatest value in patients on long term nutritional support in whom there is no SIRS, or where the trend in results can be interpreted in the light of the trend in CRP concentrations. It is clear that the sensitivity and specificity of the various laboratory analytes varies both with the analyte and the clinical condition of the patient.

However, provided there is a good understanding of the limitations of these tests, they can play an important role, both in diagnosis and monitoring of nutritional problems and therapy.

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## MICROBIOLOGY

### Urine Culture Contamination: A College of American Pathologists Q-Probes Study of 127 Laboratories

Leonas G. Bekeris,; Bruce Allen Jones; Molly K. Walsh et al

**Context.**—While urine culture contamination may not be completely avoidable, some laboratories have lower contamination rates than others. A College of American Pathologists (CAP) 1998 Q-Probes study showed that many interventions commonly assumed to reduce contamination were not demonstrably effective. This article revisits the issue.

**Objective.**—To examine the frequency of urine culture contamination, review current laboratory practices in the collection of urine culture specimens, and determine practice characteristics that may be associated with the contamination rate.

**Design.**—Laboratories participating in a CAP Q-Probes study were required to prospectively collect data on 120 consecutive urine culture specimens and provide information on the patient's demographics (age and sex), the location where the specimen was collected, how the specimen was handled, the number of isolates in quantities greater than or equal to 10 000 colony-forming units (CFU)/mL, and whether the laboratory considered the specimen to be contaminated. Specific inclusion and exclusion criteria were provided to the participants. Each laboratory completed a supplemental questionnaire that probed for specific laboratory urine culture collection practices.

**Results.**—One hundred twenty-seven laboratories participated in the study. Results from a total of 14 739 urine specimens were received. For the purpose of this study, a urine specimen was determined to be contaminated if the culture yielded more than 2 isolates in quantities greater than or equal to 10 000 CFU/mL. Using these criteria the median institution had a contamination rate of 15.0%. Laboratories in the 10th percentile (low performance) had an average contamination rate of 41.7%, while laboratories in the 90th percentile had an average rate of 0.8%. The collection site had no influence on the contamination rate, but postcollection processing, especially refrigeration of the specimen, had a substantial effect. Providing instruction to patients produced a statistically significant lowering of contamination rates for specimens from male patients ( $P = .006$ ) but not for female patients, except when written instructions were provided in the emergency room, in which case specimen contamination rates for both male and female patients dropped ( $P = .01$ ).

**Conclusions.**—The median contamination rates remain at a level comparable to the results seen in a previous Q-Probes study, and some laboratories have very high contamination rates. Specimen refrigeration is associated with lower overall urine culture specimen contamination rate. Providing patient instruction is also associated with lower contamination rates under specific circumstances.

Some contamination of urine specimens may be unavoidable. A College of American Pathologists (CAP) Q-Probes study published in 1998 found that the contamination rate was as high as 36.8% for some institutions. The same study found that the use of central processing areas, refrigeration, urine screening systems, specimen preservatives, provision of written collection instructions or special collection kits, and thermally insulated specimen transport containers were not found to be associated with low specimen contamination rates in a multivariate analysis.

It is not cost-effective for a laboratory to provide definitive workup of contaminated cultures because repeat cultures yield better results. Inappropriate reporting of contaminated urine culture results may lead to inadequate therapy, increased costs, and poor patient outcomes. Repeat visits to re-collect a urine specimen will lead to patient dissatisfaction and delay of treatment.

We report the results of the CAP QP-052 Q-Probes study designed to investigate anew the frequency of contamination in outpatient urine culture specimens collected or processed at the participating laboratory's facility. The intent of the study was to assess whether progress has been made since the last study and reexamine which practices led to improved performance as measured by lower contamination rates. This study provides insight into how the participating laboratories handle their urine culture specimens.

## **COMMENT**

The first objective of specimen collection is to assure that the utmost quality of specimen is preserved during collection and handling. Urine culture is probably the most common microbiology procedure where the specimen is self-collected by the patient. Contaminated urine specimens have an impact on patient care by introducing delays and increasing costs.

Comparison of the overall contamination rates of the present study with those of the 1998 Q-Probes study suggests that no progress has been made; the median contamination rate of both studies is similar, and in the present study the high end of the spectrum is even higher than in the previous study. Yet, in both studies there are a significant number of participating laboratories with consistently low contamination rates. The site of collection did not have an impact on overall contamination rates, but post collection handling of the specimen did. The most prominent effect occurred with refrigeration of the specimen, which reduced the overall contamination rates by about 50%. The use of centralized specimen processing areas in the laboratory decreased the contamination rates for specimens from female patients. We do not understand why the same effect was not noticed with specimens from female patients.

Unless someone introduces microorganisms during processing, specimen contamination occurs at the time of collection. A priori wisdom dictates that providing proper instructions for midstream collection and proper cleansing should decrease contamination rates. Some articles support this argument. It has been reported that best results are obtained when patients receive instructions; patients who did not receive any instructions had the highest contamination rates. Many other articles have been published indicating that this may not be the case, but if specimen contamination was a completely random event, we would expect that contamination rates would be uniform across all laboratories, which is not the case.

In this study, when male patients were verbally instructed in the proper collection of the specimen, the contamination rate decreased by about half. Written instructions provided in the emergency department lowered the rate for both male and female patients. The issue may not be whether or not instructions are given, but the quality of the instruction process, the abilities of the instructor, and the setting in which the instructions are given. Personnel may be reticent to give a detailed description to a female patient on how to collect a urine culture specimen; this could be especially true if patients are receiving instructions across a counter in an open public area. Laboratories may consider including personnel retraining as part of the annual competency assessment. Also, posted instructions may provoke negative reactions from patients and visitors and may have been placed in a location where they are not easily readable.

Laboratory Practices	No. of Institutions	Percentage of Institutions
Refrigeration of urine specimens		
Yes, most sites (>75%)	62	50.4
Yes, some sites (25%–75%)	11	8.9
Yes, few sites (<25%)	3	2.4
No	9	7.3
Not applicable	7	5.7
Unknown	31	25.2
Preservatives, stabilizers, or preservation devices used >25% of the time*		
Boric acid solution	52	42.6
Media paddle dipped and removed from urine specimen	4	3.3
Other, not including refrigeration	4	3.3
None of the above used >25% of the time	65	53.3
Laboratory provides instructions on how to collect uncontaminated urine culture specimens		
Yes	101	84.9
No	18	15.1
Urine culture specimens transported by courier to laboratory		
Yes	111	93.3
No	8	6.7
Thermally insulated containers used if urine culture specimens are transported by courier		
Yes	100	90.9
No	10	9.1

\* Multiple responses allowed.

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