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MOLECULAR CHARACTERISATION OF MONOCYTE ESTERASE DEFICIENCY
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Monocyte specific esterase activity is a useful marker for identification of cells of the monocytemacrophage lineage. At present little is known about the in vivo role of MSE. Epidemiological evidence suggest a link between monocyte esterase deficiency (MED) and malignant neoplasia. Familial studies indicate that the trait may have an autosomal dominant pattern of inheritance, which is uncommon for an inherited enzyme deficiency. Monocyte esterase deficient cells have a reduced ability to lyse tumour cells in vitro. We are currently undertaking an investigation into the molecular defect responsible for MED. Sequence analysis of genomic DNA from affected individuals is being used to identify novel single nucleotide polymorphisms (SNPs) in the MSE gene, Ces 1, which may be linked to MED. We have identified two novel SNPs in intron nine of Ces 1 in two patients who originally presented with cancer of the gastrointestinal tract (GI) tract. Since growing evidence suggests that the MSE status of patients with GI cancer may be significant in determining an appropriate treatment strategy we aim to determine whether these SNPs will be clinically important.

PRIMARY CUTANEOUS LYMPHOMAS, THE UNFORGOTTEN LESSONS
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Skin is the second most frequent site for extranodal lymphomas following the gastrointestinal tract. Before immunohistochemistry was introduced in the late 1970’s, most cases were considered malignant proliferations of T-cells with mycosis fungoides/Sezary syndrome being the classical example. However, by using immunohistochemical stains, it has been found that at least 25% of all cases of primary cutaneous lymphomas are of B-cell origin. Even more recently, a small subgroup of primary cutaneous lymphomas was found to express the CD56 marker, and so was classified as primary cutaneous lymphoma of natural killer cells.

We are presenting three cases of primary cutaneous lymphomas, two of which are primary cutaneous B-cell lymphomas and a case of CD56 positive (natural killer) cutaneous lymphoma, with special emphasis on the clinical presentation and the histopathological and immunophenotypical patterns.

LYMPHOPLASMACYTOID LYMPHOMA AND HAEMOLYTIC ANAEMIA
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Lymphoplasmacytoid lymphoma is a low grade lymphoproliferative disorder that may be associated with cold haemagglutinin disease (CHAD). We described a case of CHAD in a patient with CD20 positive lymphoplasmacytoid lymphoma, resistant to standard therapies, treated successfully with Rituximab (anti-CD20 antibody).

A 75 year old man present to our Haematology outpatient department in March 1998 with weight loss, night sweats and increasing shortness of breath. Full blood count revealed anaemia (haemoglobin 6.2g/dL) with an elevated mean cell volume (100 fL). A cold agglutinin and serum IgMk paraprotein (13g/L) were present and the direct Coombs test was positive. Serum lactate dehydrogenase was markedly elevated. Bone marrow trephine biopsy showed an infiltrate of lymphoplasmacytoid cells which was paratrabeclar and at times diffuse. Immunohistochemical studies showed these cells
to be CD20 positive. Computerised tomography showed a few enlarged lymph nodes in the para-aortic and pelvic regions. Based on these results a diagnosis of lymphoplasmacytoid lymphoma with secondary cold haemagglutinin disease was made.

The patient was treated with several courses of chlorambucil and prednisolone. His lymphadenopathy resolved but he continued to haemolyze. Despite further courses of chlorambucil and prednisolone, maintenance prednisolone and warmed blood transfusions he remained anaemic.

Following several recent reports on the use of Rituximab in cold haemagglutinin disease we commenced this patient on Rituximab at a standard dose of 375mg/m² once weekly for four weeks (days 1, 8, 15 and 22). Rituximab therapy was well tolerated and has resulted in control of haemolysis with a normal haemoglobin, normal lactate dehydrogenase and decrease in paraprotein level (to 2 g/L) despite cessation of steroid therapy.

This case emphasises that cold haemagglutinin disease associated with an underlying lymphoproliferative disorder remains a difficult therapeutic problem and consideration should be given to therapy with Rituximab in patients whose disease is resistant to standard therapies.

FOLLICULAR DENDRITIC CELL SARCOMA OF THE MEDIASTINUM

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Follicular dendritic cell sarcomas are extremely rare, the majority occurring within lymph nodes. They have, however, been described at extra-nodal sites. We describe a recurrent follicular dendritic cell (FDC) sarcoma occurring within the soft tissues of the mediastinum in order to raise awareness amongst pathologists of this uncommon neoplasm.

An 84 year old lady presented with a posterior mediastinal mass. Nineteen years earlier a mass had been removed from the same location and had been diagnosed as a “neurogenic tumour”. The recurrent tumour was well circumscribed, and histologically was composed of bland spindle shaped cells with interspersed inflammatory cells, predominantly lymphocytes. Immunohistochemically the spindle cells were positive for S100 protein, LCA, CD68 and vimentin, but negative for CD21 and CD35 (markers of follicular dendritic cells). Ultrastructural examination demonstrated elongated cell processes joined by desmosome-like junctions. The ultrastructural features were diagnostic of follicular dendritic cells.

FDC sarcoma is a rare neoplasm and a high index of suspicion is required in order to reach a correct diagnosis. Immunohistochemistry and, or, electron microscopy is required for a definitive diagnosis. This case demonstrates that not all cases are immunoreactive with CD21 and CD35 and illustrates the role of electron microscopy in establishing a diagnosis.

AN UNUSUAL CASE OF ANAEMIA

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A fifty year old female presented with Coombs positive haemolytic anaemia. This was refractory to first line treatment. Search for a primary cause uncovered a solid area within the liver. Biopsy of this revealed a diffuse large cell B-cell Non Hodgkin’s Lymphoma. She received six courses of CHOP resulting in rapid remission of her haemolytic anaemia and lymphoma. Sixteen months later she developed a right-sided buccal swelling with simultaneous relapse of her haemolytic anaemia. Repeat biopsy proved this to be relapse of her large cell Non Hodgkin’s Lymphoma. Imaging showed no evidence of relapse elsewhere. She proceeded to salvage chemotherapy using the ESHAP regime and after one course her buccal swelling had reduced completely and her haemolytic anaemia entered a second remission. She proceeded to two further courses of ESHAP and an autologous peripheral blood stem cell transplantation.

There are less than 100 cases of primary extra nodal lymphoma arising in the liver reported and although the association between lymphoproliferative disorders and haemolytic anaemia is well recognised there are no recorded cases of primary B- cell Non Hodgkin’s Lymphoma of liver with associated haemolytic anaemia in the literature.

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AN UNUSUAL PAROTID SWELLING

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Unilateral parotid gland swelling when associated with facial nerve palsy is strongly suggestive of malignancy. We report a case in which the pathology indicated a very unusual presentation of Wegener’s granulomatosis in which the involvement was subsequently shown to be multisystem, in spite of the apparently localised clinical presentation.

The patient, a previously healthy 65 year old man, presented with a six week history of a clinically malignant swelling of his right parotid gland with right facial nerve palsy. The gland was surgically explored and histology revealed necrosis, active inflammation and granuloma formation, with a vasculitic process suggestive of Wegener’s granulomatosis. Subsequent serological investigation indicated a cANCA of 320 (normal 0-19) and PR3 ANCA 13.4U/ml (normal 0-2). The patient was diagnosed as Wegener’s granulomatosis with limited right parotid involvement. He was treated with corticosteroids, cyclophosphamide and voltarol. Two weeks later he collapsed and subsequently died following severe haematemesis.

Autopsy revealed massive gastrointestinal haemorrhage from a 5cm diameter, deep pyloroduodenal peptic ulcer with a bleeding artery at its base.

The artery did not show any histological evidence of vasculitis but there were lesions of Wegener’s granulomatosis in both lungs and in many other organs including the left parotid gland.

This case highlights the fact that parotid enlargement may be the presenting feature of Wegener’s granulomatosis and can mimic a malignant tumour. Pathologists should keep this in mind when faced with an unusual inflammatory process in the head and neck and recommend the appropriate serological investigations to help confirm the diagnosis.

SNOOKERED BY THE BLUES BROTHERS!

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Almost 60 years ago two brothers who were deeply cyanosed came to the attention of a local practitioner. He administered vitamin C to one brother and this alleviated the cyanosis. Initially this was thought to be due to congestive heart failure but cardiologists later ruled out that diagnosis. Analysis of the spectral properties of the brothers’ haemoglobin resulted in the detection of methaemoglobin. A local biochemist Dr Gibson, who was interested in the biochemical pathway controlling the reduction of methaemoglobin, intensively studied the erythrocytes from these patients. Gibson was able to elucidate the enzyme, now known as NADH-cytochrome b5 reductase, responsible for methaemoglobin reduction and was the first person to attribute a hereditary trait to a specific enzyme deficiency.

Since the characterisation of the NADH-cytochrome b5 reductase gene in the 1980s numerous mutations have been detected in with patients methaemoglobininaemia. To complete the study initiated by Gibson the genetic lesion was sought in the original blue brothers. Sequencing of genomic DNA detected a heterozygous mutation in exon 9 of the gene from one brother, which causes an amino acid substitution thereby making the enzyme less stable and hence non-functional. This mutation was also detected in an asymptomatic sibling. To complicate things the level of functional enzyme is 1% in the original brother therefore this mutation cannot account for complete loss of activity. Another mutation or loss of expression of the normal b5 reductase gene is being sought but remains elusive.

BASOPHIL-ACTIVATION MARKER ANALYSIS-A NEW APPROACH TO DIAGNOSE DRUG HYPERSENSITIVITY REACTIONS

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Type 1 hypersensitivity reactions to drugs are reported to occur in 0.1% and 0.01% cases in medical and surgical wards, respectively. Detection of specific IgE antibodies (RAST) and skin prick testing (SPT) is not helpful in all cases,
and diagnosis is usually made on clinical grounds. Flow cytometric assessment of drug induced basophil activation may offer an additional approach for diagnosis of these hypersensitivity reactions. We report the use of this methodology in the investigation of 2 cases of suspected drug-induced allergy.

**Case 1**: A 44-year old woman was referred to Immunology after she experienced a Grade IV reaction during exposure to general anaesthesia with Suxamethonium as a muscle relaxant. Elevated mast cell tryptase and urinary methylhistamine were demonstrated post-reaction. Suxamethonium-specific IgE antibodies were detected and SPT with Suxamethonium was positive. In vitro induction, by Suxamethonium, of CD63 expression on basophils was demonstrated by flow cytometry.

**Case 2**: A 30-year old woman with history of migraine was referred with recurrent episodes of urticarial rashes and swelling of eyelids after taking Paracetamol and Aspirin. RAST and SPT to Paracetamol and Acetyl Salicylic acid were negative. Drug challenge to both Paracetamol and Aspirin in controlled environment proved positive in two separate occasions. In vitro, induction by Paracetamol, of CD63 expression by basophils was demonstrated. Recently, drug challenge with Vioxx (COX-2 inhibitor NSAID) was negative.

Analysis of basophil activation may prove useful in the investigation of drug induced hypersensitivity reactions, particularly when SPT and RAST are negative or SPT cannot be performed or in cases where clinical and laboratory evidence is equivocal.

1. Vervloet D and Durham, S. Adverse Reactions to Drugs. BMJ 1998: 316 (7143) 1511.

**VASCULITIS PRESENTING AS PULMONARY THROMBOEMBOLIC DISEASE**

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Vasculitis and thrombosis are two conditions that can occur in the pulmonary circulation. However, an association of these two conditions is rare. Here we reported two cases of different types of vasculitis presenting as thromboembolic disease. The first case is a 34 year old female with a known history of Wegener’s granulomatosis who presented with shortness of breath. Investigations revealed thrombosis and stenosis in the main pulmonary arteries. Histological examination of these large elastic arteries showed features of Wegener’s granulomatosis. Presentation as thromboembolic disease and large elastic artery involvement by Wegener’s granulomatosis is unusual and has never been described before. The second case involves a 46 year old female who also presented with shortness of breath, and was clinically diagnosed and treated as chronic thromboembolic disease with a subsequent development of anti-coagulant and anti-cardiolipin antibodies. Her condition deteriorated and she died. Histological examination of autopsy specimens showed features of giant cell arteritis involving distal muscular pulmonary arteries. An association of giant cell arteritis with anti-phospholipid antibody syndrome has never been reported. These cases highlight the importance of consideration of vasculitis in pulmonary thromboembolic disease even when there is no prior suspicion of a vasculitic process.

**HER-2/NEU OVEREXPRESSION AND AMPLIFICATION IN BREAST CANCER: A DIRECT COMPARISON OF IMMUNOHISTOCHEMISTRY AND FLUORESCENCE IN-SITU HYBRIDISATION (FISH)**

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**Background**: her-2/neu status is an important prognostic and predictive factor in breast carcinoma, and is central to selection of patients for treatment with the humanised anti-her-2 monoclonal antibody (Herceptin). Accurate assessment of her-2 status is, therefore, important in the pathological evaluation of breast carcinoma. There is no consensus on the preferred methodology for assessment of her-2 status in...
Methods: her-2/neu status was evaluated in 83 consecutive unselected cases of invasive breast carcinoma. All biopsies were fixed in formalin, routinely processed and embedded in paraffin. her-2/neu status was assessed by immunohistochemistry (IHC) using the FDA approved Herceptest (DAKO) and by fluorescence in-situ hybridisation (FISH) using the PatVysion her-2 DNA kit (Vysis). IHC staining was evaluated by the manufacturer’s recommended scoring system (score 0 to 3+). Cases scoring 0 or 1+ were considered negative for her-2 overexpression, cases scoring 2+ or 3+ were considered positive. FISH was evaluated by the ratio of her-2 signals to chromosome 17 signals: a ratio of ≥2 was considered amplified.

Results: 31/83 (38%) cases were positive by IHC (11: 2+, 20: 3+), 52 were negative (32: 0+, 20: 1+). 17 (20%) cases exhibited her-2 amplification by FISH, 66 (80%) cases were not amplified. The results of IHC and FISH were discordant in 1/52 IHC negative cases (IHC score 0) and in 15/31 IHC positive cases. 10/11 (91%) of IHC score 2+ cases were negative for amplification, 5/20 (25%) of IHC 3+ cases were negative for amplification.

Conclusions: The results of IHC for her-2 using the DAKO Herceptest method correlate well with amplification status in negative (score 0/1+) cases. The majority of cases scored 2+ are not amplified and these are considered to represent IHC false positives. Cases scored 2+ on IHC should be further evaluated by FISH if therapeutic decisions are to be based on her-2 status.

HER-2 ANALYSIS IN TISSUE MICROARRAYS OF ARCHIVAL HUMAN BREAST CANCER: COMPARISON OF IMMUNOHISTOCHEMISTRY AND FLUORESCENCE IN SITU HYBRIDISATION

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HER-2 gene alterations have been shown to have prognostic and predictive value for the treatment of breast cancer patients with therapeutic agents, in particular, the monoclonal antibody, Herceptin. Because of this, the accurate and consistent evaluation of HER-2 status is crucial. HER-2 status is assessed at the protein level (overexpression) by immunohistochemistry (IHC) and at the DNA level (gene amplification) by fluorescence in situ hybridisation (FISH).

IHC is a reliable and economical test to assess HER-2 status while FISH is regarded as the gold standard method for detecting HER-2 amplification. Although the best approach is to combine both IHC and FISH assays this approach is not very practical or cost-effective for routine histopathological laboratories. The recent development of tissue microarray technology has allowed large-scale studies using formalin-fixed, paraffin-embedded material. We employed this technique to assess HER-2 status in a cohort of 54 invasive breast cancer cases by both IHC and FISH assays to determine if the results obtained were representative of the protein/gene expression patterns of the original whole tissue section.

Concordance for HER-2 IHC between the tissue microarray and full sections was 93%. Concordance between HER-2 FISH and HER-2 IHC on the tissue microarray was 98%. The use of three cores per tumour adequately represents the antigen expression on a whole tissue section.

To the best of our knowledge this is the first study to validate the use of tissue microarray for FISH assessment of HER-2. We conclude that tissue microarrays provide highly comparable results in the assessment of HER-2 protein levels and allow large-scale analysis of the HER-2 gene by FISH. The methodology described in this study for HER-2 analysis is both time saving and cost-effective and with minimal technical training is suitable for use in a diagnostic setting.

FIRST REPORT OF CANDIDA DUBLINIENSIS BLOODSTREAM INFECTION IN NORTHERN IRELAND

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A 35 year-old lady with metastatic carcinoma of the uterine cervix had an episode of *Candida dubliniensis* bloodstream infection (BSI) following a laparotomy performed to repair a broken down ileocolic anastomosis. She had received broad spectrum antibacterial therapy for two weeks prior to this for the treatment of a polymicrobial septicaemia. In addition, a central venous catheter had been placed and she had been receiving total parenteral nutrition. Unfortunately, the patient deteriorated and died three days postoperatively; on the same day blood cultures became positive with yeasts.

There were several interesting issues arising from this episode. Firstly, the described patient had several typical risk factors for common candidal BSI, but did not conform to any of the classical profiles (neutropenic, peri-transplant or HIV-seropositive) of patients with *C. dubliniensis* BSI described in the literature.

Secondly, the case outlined might serve as a reminder of the role for pre-emptive prescription of an antifungal agent in the regimen of any patient at risk of deep candidal infection failing to improve despite apparently appropriate antibacterial therapy.

Finally, the isolate was misidentified by routine phenotypic methods as *C. albicans*. The identification as *C. dubliniensis* was made, producing 100% homology, by molecular analysis. This raises the question whether more discriminating methods should be routinely used for the phenotypic speciation of germ tube positive yeasts. Alternatively one might suggest that the epidemiology of candidal BSI is important enough, and phenotypic identification difficult enough, to justify the routine use of molecular methods to identify such isolates from blood.

The aim of the study was to estimate the degree of error associated with phenotypic methods of identifying *Candida* spp.

Retrospective identification of 39 isolates from *Candida* stock was undertaken by sequencing of the large internal transcribed spacer (ITS) region (ie the ITS1-5.8SrRNA-ITS2 region) which had been initially recovered from blood between 1994-2000 and were initially identified by phenotypic methods. The molecular identity was taken to represent the true identity when >98% homology was achieved.

Of the 39 isolates tested, 5 were found to have been phenotypically misidentified. Critically, no recurring pattern of error, such as any particular species being repeatedly misidentified, was seen. An isolate which had been initially thought to be *C. krusei* was found to be *C. tropicalis*; an unspeciated isolate which was initially misidentified as *C. norvegensis* were found to be *C. krusei*. One isolate phenotypically identified as *C. glabrata* was found to be *C. parapsilosis*. Two isolates had been initially misidentified as *C. albicans*; one was genotypically identified as *C. glabrata* and the other, *C. dubliniensis*.

Our study estimated an error rate of one in eight phenotypic identifications; we consider this to be unexpectedly substantial. If the epidemiology of *Candida* bloodstream infections is of importance then we should further examine our phenotypic methods of identification and perhaps consider giving a role to molecular identification methods in routine practice.

**PHENOTYPIC MISIDENTIFICATION OF CANDIDA SPECIES**

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metastatic dissemination throughout the subarachnoid space in a 65 year old gentleman fifteen years after initial diagnosis. One of the metastatic foci caused a tumour mass completely filling the 4th ventricle and blocking the outflow foramina causing hydrocephalus.

ANGIOLYMPHOID HYPERPLASIA WITH EOSINOPHILIA, CASE REPORT AND REVIEW OF LITERATURE

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Angiolymphoid hyperplasia with eosinophilia is a rare disorder of uncertain histogenesis. It presents clinically as single or multiple nodules mostly around the head and neck region of adult patients. Because of the vascular component, it is usually misdiagnosed clinically as angiosarcoma. However, angiolymphoid hyperplasia with eosinophilia has characteristic microscopy with prominent nodular pattern. This is caused by florid proliferation of blood vessels lined by endothelial cells with a distinct epithelioid appearance. These blood vessels are surrounded by a mixture of inflammatory cells with prominence of eosinophils, thus the name.

We are reporting a case of an elderly lady who presented with several nodules on the back of several years duration. Histology showed the typical vascular pattern and the associated inflammatory component. Follow-up confirmed the benign nature of the lesions.