Identification of the causative organism in invasive fungal infections is difficult. Accurate and reliable diagnostic methods are required in light of the increasing incidence of emerging fungal infections. We recently described a method for speciating fungi in formalin fixed, paraffin wax embedded tissue sections. DNA is extracted using a modification of the protocol for the TaKaRa DEXPAT Ribozyme kit (TaKaRa Biomedicals, Cambray Biosciences, Wokingham, UK), with additional steps using lyticase and ethanol precipitation. Amplification of the DNA is performed using panfungal primers and a method based on that described by Einsele et al. Identification to species level can be achieved by Southern hybridisation with a probe that binds Aspergillus fumigatus, A flavus, and A versicolor. Polymerase chain reaction products that do not hybridise with this probe are subsequently identified by sequencing analysis.

We have used this method to confirm the diagnosis in the case of a patient with acute myeloid leukaemia and pneumonia caused by Chaetomium globosum. We previously reported the details of this case and discussed the associated diagnostic difficulties. A computed tomography chest scan revealed cavitating lesions characteristic of an invasive fungal infection in the right upper lobe and a right lobectomy was performed. Histology demonstrated branching hyphae invading blood vessels that were “consistent with aspergillus”. However, many filamentous fungi resemble aspergillus species histologically, and identification relies on culturing the organism from the tissue. In this case, C globosum was cultured from the tissue sample, confirming the organism from the tissue. In this case, we were left with a presumptive diagnosis.

We have now achieved a definitive, molecular diagnosis of an invasive fungal infection caused by C globosum in this case using the method described above. Sections from the original paraffin wax embedded tissue were cut with a sterile microtome blade and two 10 μm thick sections were subjected to DNA extraction, after discarding the outer section. A positive result was obtained after polymerase chain reaction amplification with panfungal primers, but subsequent hybridisation with the aspergillus specific probe yielded a negative result. The DNA was purified using Wizard PCR Prep DNA purification system (Promega, Madison, Wisconsin, USA), according to the manufacturer’s instructions. The DNA was commercially sequenced (Cytomyx, Cambridge, UK), after which sequence analysis was performed with the EMBL software package (HGMP-RC: Medical Research Council, London, UK). The sequence was identified as C globosum using a BLAST search of Genbank and EMBL nucleotide sequence databases and subsequently submitted to the EMBL database (accession number AJ781794).

The C globosum isolate had been added to the UK National Collection of Pathogenic Fungi as NCPF 7115. We subcultured this isolate then extracted, purified, and sequenced the genomic DNA. The sequence was identical to that of DNA extract from the tissue sample, confirming the causative role of this organism in our case.

With the growing population of immunocompromised patients and the broadening spectrum of antifungal agents available it is imperative that we can accurately identify the organisms causing invasive fungal infections. This case illustrates the value of molecular tools to enhance the diagnostic process.

**References**


**Osseous metaplasia in a benign ovarian cyst in association with cloacal anomaly**

Osseous metaplasia has been described at many sites and in association with a large number of tumours. However, previous reports of osseous metaplasia in ovarian lesions are rare, there being only three published cases, which were associated with papillary serous carcinoma, thecoma, and endometrioma. We report a case of osseous metaplasia hitherto undescribed in a benign ovarian cyst in a girl with a complex urogenital malformation.

The patient was a 16 year old girl, who was diagnosed with a complex cloacal anomaly at birth, requiring posterior sagittal anorectovaginosurethreoplasty, followed by further lower urinary tract reconstructive procedures. At the time of surgery, normal fallopian tubes were noted. The right ovary was partially resected along with fallopian tubes were noted. She attained menarche at 14. Two years later she complained of lower abdominal pain and dysmenorrhoea. Ultrasound examination revealed a large cystic lesion in the right side of the pelvis. No calcification was seen on pelvic imaging and tumour markers were normal. Repeated aspiration did not result in long-term symptom relief and an open resection was performed. At surgery, a large multiseptated cyst was noted occupying most of the pelvis behind the augmented bladder on the right and crossing the midline, closely adherent to the right ovary and right fallopian tube. The right ovary was partially resected along with the cyst. Postoperative recovery was uneventful. Histological examination demonstrated a complex tubal cyst with chronic salpingitis, in addition to a simple follicular ovarian cyst, in the wall of which osseous metaplasia was noted (fig 1). The entire specimen was embedded and multiple sections examined, but there were no additional features present to suggest a teratoma.

The pathogenesis of osseous metaplasia is unclear, but in some circumstances appears to be an unusual reaction to tissue damage and repair. It is interesting that the previously reported case associated with an endometriotic ovarian cyst also occurred in association with locally abnormal pelvis, in a supernumerary ovary. Osseous metaplasia in a benign ovarian cyst appears to be an unusual incidental finding of unknown importance and aetiology, which is more common in
anatomically abnormal ovarian tissue in women of reproductive age.

P Godbole, A Outram, N Sebire
Departments of Paediatric Urology and Histopathology, Great Ormond Street Hospital for Children, London WC1N 3JH, UK, prasadgodbole@btinternet.com

References

Congenital bronchogenic cyst in the gastric mucosa

Bronchogenic cysts are congenital anomalies evolving from the ventral foregut between the 3rd and the 7th prenatal weeks. They are lined with cuboidal or pseudostratified ciliated epithelium and may or may not be surrounded by elastic fibres, smooth muscle, and cartilage. Bronchogenic cysts are divided into thoracic and abdominal.1 Abdominal bronchogenic cysts are rare, particularly those located exclusively within the confines of the gastric wall. Despite the fact that Ginsler and colleagues2 described the first case nearly 50 years ago, only two additional cases have been reported.3 Recently, we identified a new case of bronchogenic cyst in the gastric mucosa. The purpose of this letter is to draw attention to an important differential diagnosis between gastric congenital intramucosal cysts and acquired intramucosal cysts also lined with ciliated cells.4

A 26 year old Swedish man presented because of periodic epigastric pain. The pain began 18 months previously and was periodically treated with proton pump inhibitors. Palpation resulted in epigastric pain. Oesophageal manometry and pH were normal. Antral biopsy revealed grade 1 oesophagitis and normal intestinal metaplastic goblet cells that have irregular cilia. Cysts with ciliated metaplasia are usually located in the antrum and they are lined with a single row of gastric seromucinous cuboidal cells or with intestinal metaplastic goblet cells that have irregular cilia. Cysts with ciliated metaplasia evolve as a result of environmental factors, particularly in Asian patients harbouring a gastric carcinoma of the intestinal type.5

Expression of HIF-1α in human tumours

We have read with interest the recent paper by Jubb et al on the expression of hypoxia inducible factor 1α (HIF-1α) in human tumours.6 We note that they report only 5% of ductal adenocarcinomas of the breast to be HIF-1α positive. This proportion is unusually low compared with our own data and those of other workers. In our various studies, the proportion of HIF-1α positive breast cancers varied from 44% to 80%.6,7 From other groups, these percentages varied from 56% to 76%.8 We believe that this discrepancy may be caused by the use of tissue microarrays. In breast cancer, HIF-1α often shows pronounced intratumour heterogeneity because of focal perinecrotic staining, which is clinically highly relevant. Even patients with only 5% of cells overexpressing...
HIF-1α has a much worse prognosis. Thus, tissue arrays probably underestimate the true frequency of HIF-1α overexpression in breast cancer. Hence, data from studies on HIF-1α derived from tissue arrays are probably less reliable with regard to associations between HIF-1α and other biomarkers and prognosis for invasive breast cancer. Therefore, we believe that additional intentional tumour sections are superior for the assessment of HIF-1α overexpression in this type of cancer. For other cancers with a more diffuse type of staining this may be different.

P J van Diest
Department of Pathology, University Medical Centre Utrecht, PO Box 85500, Utrecht, The Netherlands
p.j.vandiest@kun.nl

M M Vleugel
Department of Pathology, VU University Medical Centre, 1007 MB Amsterdam, The Netherlands

E van der Wall
Department of Pathology, University Medical Centre Utrecht

References


text continues...
Osseous metaplasia in a benign ovarian cyst in association with cloacal anomaly
P Godbole, A Outram and N Sebire

J Clin Pathol 2005 58: 334-335

Updated information and services can be found at:
http://jcp.bmj.com/content/58/3/334.2

These include:

References
This article cites 3 articles, 0 of which you can access for free at:
http://jcp.bmj.com/content/58/3/334.2#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/