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Rapid diagnosis of *Candida* mediastinitis by coagglutination

A rare case of mediastinitis and septicaemia caused by *Candida albicans* in a patient who had undergone cardiac surgery was diagnosed sooner by a *Candida* coagglutination test than by culture findings. The *Candida* agglutination test had been successfully used by us for the detection of antigenaemia in 10 patients with systemic candidiasis, where the coagglutination titres varied from 2 to 54.¹

In the case reported here, a 28 year old man who had had aortic and mitral valve replacement developed mediastinitis caused by *Candida albicans* infection. The *Candida* coagglutination test was extended for the detection of *Candida* antigen on the first sample of mediastinal fluid received on day 17 after surgery in the laboratory; simultaneously, culture was carried out. Both tests were carried out on the second sample of mediastinal aspirate after an interval of 48 hours.

Candida albicans antiserum used in the test had been raised in rabbits² against whole cell antigen of *C. albicans* serotype A (kindly provided by Dr AA Padhye, Centers for Disease Control, Atlanta) and this serum yielded indirect haemagglutination (IHA) titre of 1280.¹ Cowan I *Staphylococcus aureus* was grown in Todd hewitt broth at 37°C, formalin added, and washed as described previously by Christensen *et al.*³ and coloured as described by Koshi *et al.*⁴ The reagents were prepared by mixing 1.0 ml of 10% Cowan I staphylococcal cells and 0.1 ml of *C. albicans* antiserum and incubated at room temperature for 30 minutes. A 2% suspension was satisfactory for the test.²

For the *Candida* coagglutination test the mediastinal fluid was centrifuged at 2500 rpm for 15 minutes. Supernatant (400 µl) was mixed with 20 µl of 2 M sodium hydroxide and heated at 100°C for 30 minutes to remove non-specific reactions, centrifuged at 2500 rpm for 10 minutes, and subjected to the coagglutination test as described previously.¹ Fluid treated with heat and alkali (20 µl) was mixed with 40 µl of 2% *Candida* coagglutination reagent, as well as coagglutination reagent using Cowan I cells coated with normal rabbit serum (NRS) separately in a ceramic ring VDRL slide and rotated for three minutes.¹ The reactions were graded as 4+, 3+, 2+, 1+ and negative based on the formation and size of the clumps and clearing. The coagglutination reaction was considered to be satisfactory when the fluid did not cross react with staphylococcal cells coated with NRS.

To confirm the specificity of the coagglutination reaction a blocking test was carried out by mixing 50 µl of alkali heat treated mediastinal fluid with 50 µl of *C. albicans* antiserum, incubated at 56°C for 10 minutes and then again subjected for the coagglutination test. Patient serum was also treated and tested similarly for *Candida* antigen, prepared from *C. albicans* and 0.03 M phosphate buffered saline (pH 7.2), served as positive and negative controls, respectively.

The coagglutination detected mannan antigen and the test results were available one hour after receipt of the specimens. The titre was 64 in the first sample of mediastinal fluid, and a significant increase in titre to 256 was shown in the second sample. The serum also had a high coagglutination titre of 64, confirming a diagnosis of invasive candidiasis.

Budding yeast cells with pseudohyphae were detected in the Gram stained smear. Further confirmation of diagnosis was made by the isolation of *C. albicans* in scanty and heavy growth from the first and second samples of mediastinal fluid, respectively. Repeat blood cultures also yielded pure growth of *C. albicans*. No bacteria were isolated from the mediastinal fluid of blood.

The high antigen titre and increase in the *Candida* antigen titre in the mediastinal fluid and serum with severe candidiasis was probably indicative of a poor prognosis; the patient died four days after diagnosis. Prolonged treatment with broad spectrum antibiotics combined with belated aetiological diagnosis and consequent delay in starting antifungal treatment probably caused the fatality in this patient.

The *Candida* coagglutination test was a useful adjunct for the detection of *Candida* antigen in body fluids. The coagglutination test was as specific, evidenced by the absence of a cross reaction with sera obtained from a variety of patients with bacterial and fungal infection as well as rheumatoid factor positive sera.¹ The coagglutination test described here is recommended as a simple, cost effective, and specific test in the rapid detection of *Candida* antigen in serum or body fluids.

Since this letter was written the authors have been informed of the coagglutination test for the detection of *Candida* antigenaemia, first described by Anyiwo (1979).

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Intraepithelial malakoplakia

Most cases of malakoplakia, a chronic inflammatory condition first reported in 1902 by Michaelis and Gutmann, and in 1903 by von Hansemann, occur in the genitourinary tract, though gastrointestinal, respiratory, cutaneous, skeletal and even ocular disease have been recorded. Microscopically, the mucosal and cutaneous lesions are characteristically composed of large eosinophilic cells with abundant cytoplasm and small eccentric nuclei (von Hansemann cells) which are situated in the lamina propria or dermis and are either covered by intact epithelium or ulcerated. The presence of laminated, concentric, basophilic, intracytoplasmic and extracellular Michaelis Gutmann bodies is generally accepted as necessary for diagnosis. A comprehensive review in 1983 and the subsequent literature do not mention intraepithelial von Hansemann cells.¹

A 77 year old woman with a history of chronic urinary infections and a radiological appearance of xanthogranulomatous pyelonephritis, had, on cystoscopy, a mucosal appearance suggesting cystitis follicularis.

Histologically, the bladder mucosa showed diffuse infiltration of the stroma by sheets of von Hansemann-type macrophages, many containing characteristic basophilic cytoplasmic Michaelis Gutmann bodies. The overlying transitional cell epithelium, however, also contained von Hansemann macrophages, some containing Michaelis Gutmann bodies. Periodic acid Schiff and von Kossa stained these inclusions, with the periodic acid Schiff showing a targetoid appearance. Immunocytochemistry with MAC 387 clearly showed the intraepithelial macrophages, which gave a negative reaction with CAM 5.2.

That there is an intraepithelial component of malakoplakia has not previously been reported, it may well be that as the cells are macrophages they have a capacity for translocation, as do other inflammatory cells. Either transepithelial migration or ulceration may be responsible for the occasional reports of von Hansemann cells shed in urine.

The presence of large, pale, eosinophil intraepithelial cells associated with a history of chronic infection should raise the possibility of malakoplakia.

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Expression of epithelial membrane antigen by carcinoid tumours

Carcinoid tumours are normally identified by their distinct morphological appearance, affinity for silver stains, and, at an ultrastructural level, presence of dense core neurosecretory granules. In small, crushed biopsy specimens or fine needle aspirates recognition may not be so easy, and application of a panel of immunocytochemical reagents may assist diagnosis. Neuroendocrine tumours vary in reactivity with different markers of putative neuroendocrine derivation. Less well known is the occasional expression of epithelial membrane antigen.

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Positive reactions seen at each site (percentages in parentheses)

Tissue	Pancreatic polypeptide	EMA	PGP9-5	Serotonin	Grimelius
Small intestine	0 (0)	0 (0)	4 (75)	4 (75)	6 (100)
Pancreas	2 (29)	1 (14)	6 (86)	2 (29)	3 (43)
Lung	0 (0)	1 (50)	2 (100)	1 (50)	0 (0)
Stomach	1 (33)	2 (66)	1 (33)	0 (0)	2 (66)
Rectum	2 (40)	2 (40)	3 (60)	2 (40)	0 (0)
Appendix	0 (0)	1 (17)	4 (67)	6 (100)	6 (100)

Carcinoid tumours from various sites were withdrawn from the routine surgical pathology files of the General Infirmary at Leeds. These comprised six from the small intestine, two from the lung, three from the stomach, five from the rectum, six from the appendix, and seven islet cell tumours of the pancreas. Only those tumours were included in which the diagnosis was confirmed by either positive silver stains or demonstration of dense core neurosecretory granules by electron microscopical examination. All material was formalin fixed and paraffin wax embedded. One representative block from each tumour was selected.

Serial sections from each tumour were stained with haematoxylin and eosin and impregnated with silver by the Grimelius technique. Four immunohistochemical reagents were applied: PGP 9-5 (UltraClone Ltd, dilution 1 in 400), anti-human pancreatic polypeptide (Dakopatts, prediluted), serotonin (Seralab, dilution 1 in 100) and monoclonal anti-epithelial membrane antibody (Dako, dilution 1 in 10). Pretreatment with trypsin was used for all except PGP 9-5.¹ The predominant histological pattern of each tumour was determined according to Dawson's classification,² and positive or negative staining with each reagent noted. Focal staining was regarded as positive and clearly localised to tumour cells.

The results are shown in the table. A small proportion of carcinoid tumours give a clear positive reaction to epithelial membrane antigen (EMA). The most common pattern was a diffuse, granular cytoplasmic staining, but apical membrane staining was also seen around lumina. Positive staining showed no correlation with histological subtype, nor with site.

EMA is an antibody to a specific component of the human milk fat globule membrane.³ It has been shown to react with a wide variety of benign and neoplastic epithelia, and has proved a useful tool in the categorisation of undifferentiated tumours. Experience, however, has shown a more widespread distribution than was initially thought. Previous studies included very few carcinoid tumours. These were reported as unreactive^{4,5} or as showing isolated positive staining.^{6,7}

It is unwise to draw conclusions about cell of origin of a neoplasm from expression of antigen, but it is sometimes possible to predict antigen expression if the cell line is known. Carcinoid tumours are believed to originate from the mucosal endocrine cell. It is suggested that these in turn derive from a common endodermal stem cell. As most endoderm expresses EMA, occasional expression of EMA by carcinoid tumours comes as no surprise. It is important to be aware of this possibility, as otherwise the unexpected finding of a positive reaction to EMA with a dubious or negative silver stain may mislead the unwary.

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MATTERS ARISING

Nucleolar organiser regions in renal tumours

Bryan, Crocker, and Farr recently compared nucleolar organiser region (NOR) numbers in seven cases of renal "adenoma", nine cases of renal cell carcinoma, and five cases of xanthogranulomatous pyelonephritis.¹ Using the standard silver colloid staining technique they showed a significant difference between mean NOR values for renal cell carcinoma and xanthogranulomatous pyelonephritis but not between renal cell carcinoma and renal "adenomas". While not disputing the observations of these authors, we are of the opinion that their report does not provide an accurate indication as to the range of NORs seen in renal cell carcinoma.

Delahunt *et al* recently reported on NOR numbers in 75 cases of renal cell carcinoma, eight renal oncocytomas, and nine renal "adenomas".² They graded the renal cell carcinomas and found a significant difference in mean NOR numbers between each of the assigned grades of renal cell carcinoma and

between renal "adenomas" and oncocytomas when compared with grades II and III renal cell carcinomas. There was no difference in mean numbers of NORs of oncocytomas, "adenomas", and grade I renal cell carcinomas.

There is increasing evidence to suggest that the number of detectable NORs within nuclei is a reflection of the proliferative activity of the tissue examined.^{3,4} It has been further shown that higher grades of malignant tumours have greater detectable numbers of NORs, using the silver colloid stain, than similar tumours of lower grade.^{5,6}

Bryan *et al* did not grade the malignant tumours in their study, and the only indication they provide as to the grade of the renal cell carcinomas in their series is given in an illustration. This shows a low grade clear cell renal cell carcinoma which would be expected to have a mean NOR value within the range found in renal "adenomas".

The report of Bryan *et al* serves to emphasise that when NOR counts from different tumours are compared, the grade of the tumour should be taken into account and if this is not done then incorrect conclusions may be derived.

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Drs Bryan and Crocker comment:

We were interested to observe the comments of Delahunt *et al* from New Zealand regarding our study of AgNORs in renal neoplasms. We must make one or two points which will clarify the questions they have raised.

Firstly, we applied the AgNOR technique to renal cell carcinoma, adenoma, and xanthogranulomatous pyelonephritis in an attempt to facilitate differentiation. The continuous range in AgNOR counts shown by us in renal neoplasms suggested that this supported the notion of a continuous range of renal tumours. We therefore pointed out that the AgNOR technique is not able to distinguish tumours expected to behave in a benign manner from those expected to have a malignant natural history. The results of Delahunt *et al* are similar, with no significant difference in mean AgNOR counts for "adenoma" and grade I renal cell carcinoma. These observations would also support the hypothesis of a continuous spectrum of renal tumours. Naturally it is to be expected that high grade renal cell carcinoma would have a higher AgNOR score than low grade tumours, but conventional light microscopical examination