Alpha-methylacyl-CoA racemase is expressed in a majority of pancreatic neoplasms of neuroendocrine, acinar, and solid pseudopapillary differentiation

Sir,

Alpha-methylacyl-CoA racemase (AMACR) is an enzyme localised in peroxisomes and mitochondria, and involved in metabolism of branched chain fatty acids as well as some drugs. It is also a well recognised immunohistochemical (IHC) marker, particularly helpful in the diagnosis of limited prostatic adenocarcinoma. However, its usefulness in oncology and anatomical pathology practice may be much wider, as AMACR seems to be a good diagnostic and prognostic marker and, most importantly, a promising therapeutic target in many types of malignancies.

Several groups of investigators showed that AMACR is expressed in a minor portion of ductal adenocarcinomas of the pancreas (7.7–12.5%). In contrast, data on AMACR expression status in other types of pancreatic neoplasms are limited.

The aim of this study was to examine IHC expression of AMACR in non-ductal neoplasms of the pancreas: solid pseudopapillary neoplasms (SPN), neuroendocrine tumours (NET), neuroendocrine carcinomas (NEC), and acinar cell neoplasms (ACN, acinar cell carcinomas, mixed acinar-neuroendocrine carcinomas, and pancreaticoblastomas). Although all of these pancreatic tumours are considerably less frequent than conventional ductal cancers, they may still be difficult to differentiate from each other and, in a proportion of cases, to cure.

The tissue microarrays (TMAs) were prepared using formalin fixed, paraffin embedded tumour samples diagnosed using WHO reference book criteria. Fifty-seven tumour samples were included in the study: 12 cases of SPN, 29 cases of NET, seven cases of NEC, and nine cases of ACN (seven cases of acinar cell carcinoma, a single case of acinar-neuroendocrine carcinoma, and a single case of pancreaticoblastoma). Details on clinicopathological characteristics of the study samples are presented in Supplementary Table 1 (http://links.lww.com/PAT/A33). From each tumour sample 1–5 tissue cores (diameter 1.5 mm) were available for evaluation. Samples of normal pancreas were included in TMA blocks for control and orientation purposes.

For AMACR IHC assay, a previously validated rabbit monoclonal antibody (clone 13H4) from Dako (Denmark) was used. Three alternative detection systems (Envision FLEX+ (Dako), Envision (Dako), and Novolink Polymer Detection System (Leica, UK) were used. Details of IHC protocols are described in Supplementary Table 2 (http://links.lww.com/PAT/A33). Granular cytoplasmic reaction was considered positive. Stain intensity was interpreted as weak, moderate, or strong. Stain extent was recorded as a percentage of positive cells in 5% increments. Results of IHC assay were documented both qualitatively and quantitatively. Tissue sample was regarded as positive if at least 5% of cells showed expression (irrespective of stain intensity), in concordance with previous studies.

For statistical analyses, the highest histoscore value recorded among tissue cores from a particular case was taken as a representative for that case. Results of the study are summarised in Table 1 and Fig. 1 (results obtained using Envision FLEX+ detection system).

In samples of normal pancreas, diffuse and weak to moderate staining was seen in islets; some ductal cells were also weakly positive. This finding was observed irrespective of detection system used. Some tissue cores of normal pancreas stained using Novolink system showed weak background staining in acinar cells. Literature data on AMACR expression in normal pancreas are discrepant: some investigators did not observe any AMACR staining in normal pancreas, but others saw AMACR immunostain in pancreatic islets and ducts. In addition, low but still detectable levels of AMACR mRNA in normal pancreatic tissue were found.

All examined histotypes of neoplasms showed AMACR expression, albeit in slightly different extent and intensity.

Table 1 AMACR immunoreactivity across the study samples evaluated using Envision FLEX+ detection system (Dako)

<table>
<thead>
<tr>
<th>AMACR expression, irrespective of stain intensity (in at least 5% cells)</th>
<th>SPN (n = 12)</th>
<th>NET (n = 29)</th>
<th>NEC (n = 7)</th>
<th>ACN (n = 9)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMACR expression, moderate or strong intensity (in at least 5% cells)</td>
<td>12/12 (100%)</td>
<td>28/29 (96.6%)</td>
<td>77/77 (100%)</td>
<td>8/9 (88.9%)</td>
<td>p &lt; 0.032</td>
</tr>
<tr>
<td>AMACR stain heterogeneity</td>
<td>0/12 (0%)</td>
<td>2/26 (7.7%)</td>
<td>4/7 (57.1%)</td>
<td>4/9 (44.4%)</td>
<td>p &lt; 0.002</td>
</tr>
<tr>
<td>AMACR expression, median histoscore values</td>
<td>240.0</td>
<td>200.0</td>
<td>110.0</td>
<td>90.0</td>
<td>p &lt; 0.002</td>
</tr>
</tbody>
</table>

a Seven cases of acinar cell carcinoma, a single case of acinar-neuroendocrine carcinoma, and a single case of pancreaticoblastoma.

b Chi-square test.

c Stain heterogeneity was defined as a presence of at least single TMA core without AMACR expression among cores from a particular case.

d In two cases of NET only a single TMA core from a particular case was available for review; these cases were excluded from assessment of AMACR stain heterogeneity.

e A single case of NET and a single case of ACC without AMACR expression excluded from assessment of AMACR stain heterogeneity.

f Histoscore was calculated using a formula: H score = (a% * 1) + (b% * 2) + (c% * 3), where a, b, and c are percentages of cells which showed weak, moderate, and strong stain intensity, respectively.

g Kruskal–Wallis ANOVA test.

ACC, acinar cell carcinoma; ACN, acinar cell neoplasms; AMACR, alpha-methylacyl-CoA racemase; NEC, neuroendocrine carcinomas; NET, neuroendocrine tumours; SPN, solid pseudopapillary neoplasms; TMA, tissue microarray.

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(Table 1). Strong and diffuse AMACR expression was particu-
larly evident in SPN. Moderate or strong AMACR staining in
NET, NEC, and ACN was less frequent than in SPN, but still
not rare. Interestingly, stain heterogeneity was also not rare
among NET, NEC, and ACN cases.
In general, percentages of AMACR positive cases (irrespec-
tive of stain intensity) across SPN, NET, NEC, and ACN groups
were comparable. In contrast, percentage of cases with mod-
erate or strong staining as well as histoscores differed between
above diagnostic categories. Moreover, histoscores obtained
using Envision system in NET, NEC, and ACN (but not in SPN)
were slightly lower than those obtained used Envision FLEX+
and Novolink systems (Supplementary Fig. 4 and Supple-
mentary Table 4, http://links.lww.com/PAT/A33).
Irrespective of detection system used, IHC histoscores, in
particular tumour types, were not related to patient’s gender and
age, tumour localisation and diameter, presence of extrapan-
creatic extension/invasion, primary tumour stage according to
the European Neuroendocrine Tumor Society and American
Joint Committee on Cancer schemes, presence of lymph node
or distant metastasis, and World Health Organization (WHO)
grade (G1 versus G2, for NET only) (Mann–Whitney U tests,
Kruskal–Wallis ANOVA tests, Spearman’s rank correlation coefficients, as appropriate). Interestingly, two cases of ACN with nuclear β-catenin stain (acinar-neuroendocrine carcinoma and pancreatoblastoma) showed relatively high histoscore values (FLEX+ medians 200 and 300), but the number of cases did not allow statistical reasoning. Two cases of NEC with nuclear β-catenin stain showed lower histoscores (FLEX+ medians 0 and 130).

Extensive AMACR expression in SPN noted in the present study was in agreement with observation of Cavard et al.,11 who showed 6.8-fold increase of AMACR mRNA expression in SPN in comparison with normal pancreatic tissue in a microarray experiment (p = 2.89E-05). It was also concordant with recent results of Shen et al.,6 who examined AMACR expression in SPN using the same antibody, but different IHC protocol in comparison to the present report.

Data on expression levels of AMACR in NET, NEC, and ACN are contradictory. At least focal AMACR stain was seen in almost all NET, NEC, and ACN examined in the present series. Among three cases of ‘malignant carcinoid of pancreas’ stained using concentrated Dako antibody and accessible using Human Protein Atlas,3 one case showed weak, another weak to moderate, and the third strong staining (as scored by this author). AMACR expression was also observed in: (a) 37% (17/46) ‘carcinoid tumours’4; (b) 66.7% (6/9) gastric NET G2 and 90.2% (46/51) gastric NEC (but not in any of 22 gastric NET G1);5 (c) 11% (1/9) ‘lung, liver and gastrointestinal neuroendocrine carcinomas’4; and (d) 72% (31/43) of typical carcinoids, 52% (15/29) atypical carcinoids, 70% (16/23) large cell neuroendocrine carcinomas, and 51% (32/63) small cell carcinomas of the lungs.12 In contrary, Shen et al. did not see AMACR expression in 21 pancreatic neuroendocrine neoplasms (19 NET and 2 NEC) and seven ACC.6 It is difficult to explain differences between results obtained in this study and those reported by Shen et al.7 They may be related to the detection system used, other details of IHC protocol, pre-analytical factors, tissue representativeness, or study population.

In conclusion, AMACR is expressed in the majority of pancreatic neoplasms of neuroendocrine, acinar, and solid pseudopapillary differentiation. Therefore, AMACR IHC stain seems not to be useful in differential diagnosis of non-ductal neoplasms of the pancreas. However, AMACR expression in all examined histotypes of pancreatic non-ductal tumours suggests that AMACR may be an attractive target for anticancer therapy of these neoplasms. Further studies on the role of AMACR in pancreatic malignancies are needed.

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Solid-pseudopapillary neoplasm of the pancreas with extensive pleomorphic neuroendocrine differentiation

Sir,
Solid-pseudopapillary neoplasms (SPPNs) of the pancreas are rare low-grade malignant neoplasms with uncertain histogenesis. SPPNs occur predominantly in adolescent girls and young women and they are rare in men.1 The typical histopathological features are well described in the literature.1–2 Diagnosing conventional SPPNs is generally straightforward; however, SPPNs with unusual features often require an alternative diagnosis, which can be problematic to obtain. SPPNs are composed of primitive undifferentiated cells that express various elements of pancreatic differentiation. Neuroendocrine markers, including synaptophysin, neuron specific enolase (NSE), and CD56 are variably expressed in SPPNs, indicating possible neuroendocrine differentiation.1,3,4 Herein, we present an unusual case of SPPN which presented with massive pleomorphic pancreatic neuroendocrine tumour differentiation and we discuss a possible role for TP53 underlying this condition.

A 52-year-old man was admitted to the Department of Surgery at Chonbuk National University Hospital (Jeonju, Republic of Korea) to evaluate an asymptomatic pancreatic mass that was detected incidentally while he was receiving treatment for a fractured rib. The patient’s medical history was unremarkable and his serum tumour markers, including α-fetoprotein, carcinoembryonic antigen, and carbohydrate antigen 19-9 were within normal limits. Abdominal computed tomography (CT) revealed a 4.5 × 3.5 cm, out-pouching, lobulating mass with microcalcification located within the body of the pancreas (Fig. 1A). After performing a cytological analysis of the pancreatic mass using endoscopic ultrasound-guided fine
needle aspiration, we diagnosed the patient as having a pancreatic neuroendocrine neoplasm (PEN). Surgical exploration revealed a 4.3 × 3.5 × 2.5 cm, well-circumscribed, solid mass with firm consistency. Two-thirds of the total mass was located within the pancreatic parenchyma, while one-third of the mass protruded outside of the pancreas (Fig. 1B). Histologically, the portion of the tumour located within the pancreatic parenchyma had the appearance of typical SPPN. The growth pattern was heterogeneous, with a combination of solid and pseudopapillary structures. The solid and pseudopapillary areas were composed of poorly cohesive monomorphic cells that had eosinophilic or clear foamy cytoplasm and round to oval nuclei (Fig. 1C,D). The nuclei of the neoplastic cells had finely dispersed chromatin and they were often grooved or indented. The SPPN portion contained foci with giant-cell reaction to cholesterol crystals and scattered foamy histiocytes in the background. The neoplastic cells delicately infiltrated into the surrounding pancreatic tissue. The architectural features of the outside portion of the pancreatic mass resembled those of previously described pleomorphic PENs. The tumour cells revealed various growth patterns including solid, trabecular,
and haphazardly arranged infiltrating duct-like structures in a loose myxoid or fibrous stroma. The tumor cells had markedly enlarged, irregularly shaped, hyperchromatic nuclei with occasional bizarre forms. The pleomorphic cells had abundant amphophilic to eosinophilic cytoplasm and fine to coarse chromatin with centrally located, small nucleoli (Fig. 1E,F).

The tumor also contained gradual transition areas from the typical SPPNs toward morphologically different elements that had neuroendocrine features (Fig. 1B). To identify the nature of the tumour cells, we performed immunohistochemical staining; the results are summarised in Table 1. The tumour cells in both SPPN and pleomorphic PEN areas showed diffuse and strong immunoreactivity to vimentin, NSE, CD10, CD56, CD99, α-1-antitrypsin, and abnormal nuclear/cytoplasmic β-catenin expression (Fig. 2A). Characteristically, the tumour cells in the outside portion of the pancreas (pleomorphic PEN area) had a diffuse and strong positive reaction to synaptophysin and chromogranin A, but the tumour cells within the pancreas (SPPN area) were either not reactive to these molecules or had a focally weak reaction (Fig. 2B,C). Similarly, only the tumour cells in the pleomorphic PEN area showed a strong positive reaction to TP53 (Fig. 2D). The proliferation marker Ki-67 was positive in approximately 3% of tumour cells in both tumour areas. No obvious recurrence or metastasis of the tumour was detected post-operatively during the 3 years of follow-up.

SPPNs are uncommon pancreatic neoplasms of low-grade malignant potential, composed of poorly cohesive monomorphic epithelial cells that form solid and pseudopapillary structures. The characteristic pathological features and immunohistochemical findings of SPPNs are unique and well known.1–3 Almost all SPPNs harbour somatic mutations on exon 3 of CTNNB1, the gene encoding β-catenin. This mutation leads to nuclear translocation of the β-catenin-Tcf/LeF complex, as indicated by nuclear expression of β-catenin on immunohistochemical staining.1,5 Despite the well-described pathological and molecular features of this tumour, the cell of origin and true histogenesis are not well understood.

A particularly interesting finding in this SPPN was its extensive neuroendocrine differentiation, both histologically and immunohistochemically. Moreover, the cells with neuroendocrine differentiation formed a discrete out-pouching extra-pancreatic mass that could be seen macroscopically. To the best of our knowledge, such extensive neuroendocrine differentiation in a SPPN has not been previously reported. Similar to our case, focal nodules (2 mm and 5 mm at their widest diameters) that are within the SPPN and which cytologically stimulate PEN have been described.7 However, our case lesion is different from previously reported lesions because of its neuroendocrine marker expression and its size. Previously reported focal nodules had diffuse positive reactions to synaptophysin, diminished reactivity to CD56 and CD10, and a negative reaction to chromogranin A.7 Indeed, neuroendocrine differentiation in SPPN has been well described but it is generally thought to be a focal phenomenon in a limited number of cases. Neuroendocrine markers, including NSE, CD56, and synaptophysin, are variably expressed in SPPN, whereas chromogranin A is consistently negative or focally and weakly positive.6–8 Ultrastructurally, SPPNs have heterogeneous cell populations, including cells with endocrine features, but have never been shown to contain a neurosecretory product.9,10 These observations strongly suggest that neuroendocrine differentiation in SPPNs is one of the potential sources of tumour differentiation, rather than having a pure neuroendocrine cell origin. In this case, the tumour cells in both the SPPN and the pleomorphic PEN areas had overlapping immunohistochemical features between the two lesions, i.e., concomitant expression of vimentin, NSE, CD10, CD56, CD99, α-1-antitrypsin, and nuclear translocation of β-catenin in tumour cells, which is a characteristic immunohistochemical
The molecular mechanism(s) of transformation from SPPN. We also found that the tumour cells in the pleomorphic PEN area showed diffuse and intense expression of synaptophysin and chromogranin A, the most specific neuroendocrine markers, but the tumour cells within the SPPN area were negative or focally weakly positive for these markers. Additionally, we found gradual continuity in the elements between the two morphological areas of the tumour. These findings suggest a transformation of the tumour from a pre-existing SPPN to a pleomorphic PEN. A previous study reported the occurrence of melanocytic differentiation of a SPPN.11 The molecular mechanisms of transformation from SPPN to PEN are unclear due to an incomplete understanding of the histogenesis of SPPNs but ‘divergent transdifferentiation’ of primitive tumour cells could be a possible explanation for this intriguing phenomenon.11

Our case study has shown that the tumour cells in the pleomorphic PEN area have diffuse and strong immunoreactivity to TP53, while the tumour cells in the conventional SPPN area are focally and weakly reactive to TP53. The tumour suppressor TP53 plays an important role in tumour prevention and maintenance of genomic stability. Alteration of TP53 function commonly induces the initiation or progression of tumours.12 It is well known that a mutation in the TP53 gene often results in a prolonged half-life with accumulation of TP53 gene products in the nuclei, which yields positive nuclear staining on immunohistochemistry. Little is known about the role of particular genes and pathways in the development of neuroendocrine tumours from pre-existing tumours. Recent studies have demonstrated that alteration of the TP53 gene often involves or progression of neuroendocrine tumours of the stomach or pancreas.13–15 Gastric neuroendocrine carcinomas (NEC) are generated from precursor cell clones that arise from a preceding adenocarcinoma component. These precursors transform into NECs during rapid clonal expansion under the influence of an altered TP53 gene.14 The positive immunoreactivity rate of TP53 in gastric cancers with neuroendocrine differentiation was higher than that among gastric cancer cases without neuroendocrine differentiation.15 Based on similar microsatellite changes and TP53 mutations in both gastric neuroendocrine cells and adenocarcinoma cells, Wang et al. have proposed that neuroendocrine cells and adenocarcinoma cells are derived from the same stem cells.16 Hu et al. have suggested that the TP53 pathway is altered in PENs through aberrant activation of negative regulators, although they did not detect TP53 overexpression in PENs.17 Interestingly, Kim et al. have demonstrated that pleomorphic SPPNs have significantly higher TP53 expression than conventional SPPNs;17 however, they did not demonstrate neuroendocrine differentiation in their pleomorphic SPPNs. Because of several morphological features, including a low Ki-67 labeling index, lack of increased mitotic activity, and smudged chromatin patterns in pleomorphic cells, they suggest that nuclear pleomorphisms in SPPNs have a degenerative quality, rather than high-grade malignant potential.15 Although the functional role of TP53 overexpression in this pleomorphic PEN case is still unclear, these observations suggest a possible role for TP53 in the transformation of the tumour from a pre-existing SPPN to a pleomorphic PEN.

SPPNs show excellent prognosis after complete surgical resection. Approximately 5–15% of SPPNs may directly extend into adjacent organs or recur and metastasise.1–3,6–8 There are no empirically demonstrated biological or morphological predictors of outcome, although it has been suggested that muscular vessel invasion, tumour size, European Neuroendocrine Tumors Society stage grouping, as well as stage grouping by the American Joint Committee on Cancer, are the important predictors of disease-specific survival in patients with SPPN.2 Despite the finding of a pleomorphic PEN in our case, no obvious tumour recurrence or metastasis was detected during 3 years of follow-up, which is consistent with the results of previous studies and further demonstrates that nuclear pleomorphisms involving PENs or SPPNs do not influence prognostic significance.4–16 Although extensive neuroendocrine differentiation in SPPN is rare, identification of similar cases and further studies will enhance our understanding of the nature of this tumour.

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An unusual ampullary adenocarcinoma with Paneth cell and mucinous differentiation

Sir,
Carcinoma of ampulla of Vater is a relatively uncommon tumour, accounting for 6–20% of all periampullary tumours and represents 10–50% of cancers resected via pancreaticoduodenectomy.1 Ampullary carcinoma comprises of two main histological subtypes, the pancreatobiliary type and the intestinal type which have different pathogenetic and clinical characteristics.2 Determining the two subtypes is also important as prognosis and treatment protocols differ for these two subtypes. The intestinal subtype is correlated with a lower incidence of lymph nodal metastases, little or no invasion of the surrounding pancreatic parenchyma and a longer term survival after resection.3 Paneth cells are found in the mucosa of the entire small intestine, proximal large intestine and appendix. Their neoplastic transformation, described as single case reports at sites including small intestine,4 ampulla of Vater,5,6 large intestine,7 Meckel’s diverticulum8 and stomach,9 ranges from partial Paneth cell differentiation to Paneth cell-rich neoplasia. We describe a rare case of ampullary adenocarcinoma with dual differentiation comprising of Paneth cells and mucinous patterns in equal proportions. Paneth cells demonstrated features of malignancy and were clearly neoplastic. Additionally, IgG4 positive plasma cells characterised most of the tumour stroma. The current lack of data regarding this entity makes it difficult to draw prognostic conclusions and also the significance of IgG4 positive plasma cells within the tumour stroma is unclear. This dual differentiation pattern within ampullary carcinoma is exceedingly rare and is the first case of such a tumour in the literature, to the best of our knowledge.

A 58-year-old reformed alcoholic, a known diabetic and hypertensive on regular treatment for 5 years, presented with sudden onset epigastric pain radiating to the back of one day duration. He had a similar episode two and a half years previously which was diagnosed clinically as acute pancreatitis and treated conservatively. As his condition improved with empirical treatment, no radiological investigations or endoscopy were performed. He remained asymptomatic until the
present episode. The general and systemic examinations revealed pallor and palpable gall bladder, respectively. Investigations revealed anaemia (haemoglobin 9 g/dL) with a normal leucocyte count (7300/μL) and platelet count (249,000/μL). Serum amylase was elevated (422.5 U/L) as were the alkaline phosphatase (138 U/L) and lipase (76.8 U/L). Bilirubin and aminotransferase levels were normal. He underwent radiological investigations in which the contrast enhanced computed tomography (CECT) of the abdomen demonstrated dilated common bile duct and main pancreatic duct (Fig. 1A) due to a heterogeneously hypodense mass in the head and uncinate process of the pancreas with loss of fat planes in the second part of the duodenum (Fig. 1B). Magnetic resonance cholangiopancreatography (MRCP) demonstrated a mass in the periamputal region with abrupt cut-off of the main pancreatic duct and common bile duct and evidence of chronic pancreatitis (Fig. 1C). The carcinoembryonic antigen (CEA) and CA19.9 levels were within normal limits (2.13 ng/mL and 7.08 U/mL, respectively). Serum IgG4 level was elevated (96 mg/dL) when the patient was investigated for chronic pancreatitis. He underwent an endoscopic biopsy and a diagnosis of high grade adenoma was rendered as no definite invasion was identified. A partial Whipple’s procedure with end-side hepatico-jejunostomy was performed (Fig. 1D).

On gross examination, the periamputal region was remarkable with a 0.8 × 1.0 cm mass lesion and concomitant dilatation of the common bile duct. The pancreas, pancreatic duct, gall bladder and rest of the duodenum and jejunum were within normal limits. Microscopically, an invasive adenocarcinoma was identified occupying the duodenal mucosal segment of the ampulla filling up the ampullary lumen with early extension into the pancreas. The tumour cells demonstrated dual differentiation in equal proportions with the duodenal segment of the ampullary region exhibiting extensive Paneth cell differentiation characterised by bright eosinophilic granules filling up the cytoplasm, while the intra-ampullary portion had mucinous differentiation with both intra- and extra-cellular pools of mucin infiltrating into the pancreas (Fig. 2A–E). The transition between the two patterns was gradual with few glands featuring both Paneth cells and mucin secreting cells (Fig. 2F). Paneth cells arranged in glandular pattern exhibited features of malignancy with irregular glands, cellular pleomorphism, nuclear atypia and presence of atypical mitoses (Fig. 3A). Masson’s trichrome stain highlighted the dense granules within the Paneth cells (Fig. 3B). Immunopositivity with carcinoembryonic antigen (CEA) and IgG were detected within the cytoplasm of Paneth cells (Fig. 3C,D). Additionally, a dense plasma cell infiltrate was noted within the tumour stroma. IgG4 immunostain was performed and the ratio of IgG/IgG4 positive tumour infiltrating plasma cells was 0.66 (Fig. 3E). The adjacent pancreas revealed features of acute on chronic pancreatitis with squamous metaplasia identified within the lining of the common bile duct. The lymph nodes and resection margins examined were free of tumour. The patient was staged IB (T2N0M0). The post-operative period was uneventful and he is doing well on regular follow-up.

Paneth cells are the secretory epithelial cells forming a part of the normal lining epithelium in the fundus of the crypts of

**Fig. 2** (A) Low magnification demonstrating an invasive tumour centred at the ampulla of Vater containing abundant extracellular pools of mucin (H&E). (B) Glandular pattern of the ampullary tumour toward the duodenal segment (H&E). (C) Glandular pattern dominated by Paneth cells with bright eosinophilic granules filling up their cytoplasm (H&E). (D,E) Ampullary adenocarcinoma with extensive mucinous differentiation featuring both intra- and extra-cellular pools of mucin (D) H&E and (E) PAS-Alcian blue. (F) Neoplastic glands with both Paneth cells and mucin secreting cells (H&E).
Lieberkühn in the small intestine and proximal large intestine. These cells reside at the base of the crypts of Lieberkühn by escaping the upward migration which is destined for all other cells, thereby forming an integral part of the intestinal crypt stem cell niche providing essential niche signals to Lgr5⁺ crypt base columnar (CBC) cells. The Paneth cell granules stain brightly red with haematoxylin and eosin and Masson’s trichrome stains. They are immunoreactive with lysozyme antibody which is considered as the marker for normal and neoplastic Paneth cells. Primary Paneth cell tumours (both benign and malignant) or carcinomas of small and large intestine with Paneth cell differentiation and extra-intestinal tumours with Paneth cell differentiation are exceedingly rare and are seldom described in the literature. Most of these reports describe presence of Paneth cells within them as ‘dysplastic’ or ‘Paneth cell-rich’. Paneth cells as a part of the neoplastic process have been described in only two earlier reports in the duodenum and ampulla.

This case is a rare example of ampullary adenocarcinoma demonstrating two distinct patterns of differentiation—Paneth cell and mucinous cell types—present in equal admixture forming the tumour bulk. Paneth cells formed an integral part of the tumour with features of malignancy including disorderly arrangement, irregular glands, and presence of mitoses within the cells rich in eosinophilic granules. The other component of tumour, the mucinous pattern with both intra- and extra-cellular mucin, was identified within the intra-ampullary region. Other rare findings in the present case were a long history of symptoms in contrast to the early presentation in the intestinal type of ampullary carcinoma; obstructive jaundice was not a presenting symptom; laboratory finding of raised IgG4 levels (96 mg/dL) and absence of lymph nodal or distant metastases as opposed to the other two cases described in the literature. While Paneth cells constitute an integral part of the intestinal crypt stem cell niche, the two distinct patterns of differentiation within this tumour is strongly suggestive of its origin from the intestinal crypt stem cell or a totipotential cell. The significance of abundance of IgG4 immunopositive plasma cells within the tumour stroma is unclear; however, it is an important observational finding.

While the tumour is best classified as intestinal subtype of ampullary adenocarcinoma with Paneth cell and mucinous patterns, the biological behaviour of this tumour is uncertain due to the rarity of the lesion. With lymphatic vessel invasion reported in the earlier case of ampullary Paneth cell carcinoma suggesting its malignant potential, the absence of similar features in the present case makes it difficult to determine the potential outcome; however, the patient remains clinically well 3 months post-operatively.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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Fig. 3 (A) Neoplastic Paneth cells in a glandular arrangement with frequent mitotic figures (arrow). Note the tumour infiltrating plasma cells within the stroma (H&E). (B) Paneth cells granules highlighted as bright red with Masson’s trichrome stain. (C) Cytoplasmic immunoreactivity within the Paneth cells for CEA (immunoperoxidase). (D) IgG immunoreactivity within the cytoplasm of Paneth cells. The tumour infiltrating plasma cells act as internal control (immunoperoxidase). (E) IgG4 immunostain highlights some of the plasma cells within the stroma (immunoperoxidase). The ratio of IgG:IgG4 tumour infiltrating plasma cells was 0.66 (an average of 10 high power fields was calculated in a visual field area of 238 mm²).
Endometrial carcinosarcoma with prominent neuroectodermal component

Sir,

Primitive neuroectodermal tumours (PNET) are extremely uncommon in the female genital tract, with most reported cases occurring in the ovaries. The uterine corpus has given rise to fewer than 50 described examples, while the cervix and vulva are rarely the primary sites.

A 72-year-old woman presented with post-menopausal vaginal bleeding and clear-yellow discharge. Ultrasound examination documented three endometrial polyps, the presence of which was later confirmed on hysteroscopy (Fig. 1).

Fig. 1 Hysteroscopic image of multiple polypoid masses within the endometrial cavity exhibiting smooth contours and large surface vessels.

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Fig. 1 Hysteroscopic image of multiple polypoid masses within the endometrial cavity exhibiting smooth contours and large surface vessels.

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Endometrial curettage yielded multiple fragments of soft grey/brown tissue admixed with blood measuring $25 \times 20 \times 15$ mm in aggregate. Histological examination revealed a high grade carcinosarcoma (Fig. 2), with an intimate admixture of moderately to poorly differentiated endometrioid adenocarcinoma (Fig. 3) and a sarcomatous component which was dominated by heterologous neuroectodermal tissue. This included extensive neuroglia (Fig. 4), neuropil with neurones, some rosettes, small round blue cell areas consistent with PNET (Fig. 5), and possibly ependyma. No other epithelial or heterologous mesenchymal elements were identified. The patient subsequently underwent total hysterectomy with bilateral salpingo-oophorectomy and a staging procedure including pelvic and para-aortic lymph node dissection and omentectomy which exhibited no residual tumour in the endometrial cavity, despite extensive examination, nor in the adnexal structures, lymph nodes or omentum.

The immunoprofile of the tumour included focal GFAP positivity between the rosettes (Fig. 6A), NeuN reactivity in neuronal areas (Fig. 6B), neurofilament in neuropil, CD56 and FLI-1 in neuroectodermal stroma (Fig. 6C), and CK7, pan CK, EMA, and PAX-8 reactivity in the glandular areas, supporting their müllerian nature. Intriguingly, oestrogen receptor (ER) and progesterone receptor (PR) were expressed in the glands (as expected) but also in some of the neuroectodermal areas (Fig. 6D). The primitive neuroectodermal areas were non-reactive for CK7 (Fig. 7A) but quite strongly reactive for CD99 (Fig. 7B) and synaptophysin (Fig. 7C).

The term PNET was first coined in 1973 by Hart and Earle, to denote a group of tumours thought to be derived from fetal neuroectodermal cells and that had morphological features of small round cell tumours with variable degrees of neural, glial, and ependymal differentiation. There are two main categories of PNETs according to the cell of origin and location: central and peripheral. Central PNETs are derived from the neural tube and involve mainly the brain and the spinal cord. By contrast, peripheral PNETs are derived from the neural crest and occur outside the central nervous system, often involving the sympathetic nervous system or soft tissues and bones. Peripheral PNETs show typical EWSR1 gene rearrangement, while central PNETs lack the EWSR1 rearrangement. It has been suggested by Euscher et al. that tumours without rearrangement of the EWSR1 gene should be characterised as uterine tumours with neuroendocrine differentiation or alternatively central PNET, not otherwise specified, to avoid confusion with peripheral PNET as the latter group may become a candidate candidate.

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**Fig. 5** Small round blue cell area with attempted rosette formation, numerous mitoses and apoptotic bodies, and consistent with primitive neuroectodermal differentiation (H&E).

**Fig. 6** Selected immunoprofile of the neuroectodermal areas. (A) GFAP reactive fibrils; (B) NeuN nuclear reactivity in neuronal areas; (C) FLI-1 nuclear reactivity in neuroglial areas; (D) ER reactivity in both malignant glandular epithelium and adjacent neuroglial tissue.
for chemotherapeutic regimen used to treat pPNET outside of the female genital tract.3

Since the first reports of apparently primary neuroectodermal tumours in the uterus, various theories have been proposed as to the origin of the neuroectodermal tissues. Benign glial tissue is well described in the uterus, and it has been suggested that such tissue originated from fetal central nervous system tissue implanted at the time of miscarriage4 or secondary to ectopic migration of neural crest cells at the time of fetal development. Others suggest that the cells are of müllerian origin and have undergone ‘neometaplasia’ – the alteration of a neoplastic cell of one type to another type not normally found within the tissue of origin,5 and possibly supported in this instance by the neuroectodermal elements unexpectedly sharing the ER status of the malignant endometrial glands nearby (Fig. 6D). Uterine PNETs most commonly occur as pure PNET,6 in cases in which the neuroectodermal component is admixed with other epithelial components, it is possible that the neuroectodermal component represents a pattern of heterologous differentiation such as can be seen in malignant mixed müllerian tumours (MMMTs).3 Of the 44 cases of uterine PNETs reported in the literature,2,3,7 12 were associated with a müllerian neoplasm. Of these, the most common occurrence was endometrial carcinoma in six cases, followed by MMMT in three cases, and a case each of complex hyperplasia, endometrial stromal sarcoma and adenosarcoma.2,3,7

Uterine PNETs typically have areas displaying fibrillary background, ganglion cells, astrocyte-like cells, rosettes, ependymal and medulloepithelial differentiation. On immunohistochemistry, most of the uterine PNETs are variably positive for one or more neuroectodermal markers such as NSE, chromogranin, and synaptophysin, while cytokeratin ranges from negative to very focally positive.3 In this context, CD99 is a highly specific marker for peripheral PNET2 and was present in this example. Nuclear reactivity for FLI-1, by contrast, was present in neuroglial areas but not small round blue cell areas of the tumour. The significance of this is uncertain.9

The differential diagnoses of uterine PNET depend on the degree of differentiation. For less differentiated tumours showing small round blue cell morphology, poorly differentiated carcinoma, small cell neuroendocrine carcinoma and lymphoma need to be excluded. Tumours exhibiting well developed neuroectodermal elements need to be differentiated from mature glial tissue of ectocervix or endometrium, immature teratoma with glial tissue, pure uterine gliomas, carcinosarcoma with neuroectodermal differentiation and retinal anlage tumour.2

As neuroectodermal differentiation may be a marker for aggressive disease, its presence should be noted in the diagnosis.

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Fig. 7 Small round blue cell area consistent with PNET. (A) Non-reactive for CK7. Note strongly positive endometrial type gland in top left corner for comparison. (B) strong membranous staining for CD99; (C) synaptophysin reactivity.

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Endometrial polyp-like lesion arising in adenomyosis: a report of three cases

Sir,

Endometrial polyps (EPs) are amongst the most common benign pathological findings in biopsy and hysterectomy specimens, being identified in 13–17% of women.1 Histologically, EPs include glandular and stromal components, the latter typically comprising bland spindle shaped cells within a collagenous matrix. Cytogenetic investigations suggest that EPs represent a primary monoclonal proliferation of stromal cells whereas the epithelial component appears to be polyclonal and reactive in nature.1–5 Recent immunohistochemical studies have also shown that EPs are characterised by stromal p16 protein expression in contrast to normal endometrial stroma which is p16 negative, or shows staining in a minority of cells.6,7 This report presents three cases of an EP-like alteration that was confined to the myometrium and appeared to arise within foci of adenomyosis.

The patients were aged 60, 75 and 76 years, and they underwent hysterectomy as part of the surgical management of ovarian mature cystic teratoma, endometrial intraepithelial carcinoma (EIC, serous carcinoma in situ), and uterine prolapse, respectively. None of the patients was taking tamoxifen or other hormonal therapy. Macroscopic examination of all uterine specimens showed thickened and trabeculated appearances of the uterine wall, characteristic of adenomyosis with myometrial thickness ranging from 15 mm to 35 mm. All specimens also included endometrial polyps ranging from 7 mm to 20 mm extent as well as typical intramural leiomyomas. Histological examination confirmed adenomyosis which involved the inner half of the myometrium in two specimens and the outer half myometrium in one specimen. In each case there was an intra-myometrial alteration histologically reminiscent of an EP that appeared to have developed within foci of adenomyosis (Fig. 1 and 2). The EP-like lesions were approximately 2 mm, 2 mm and 4 mm in diameter and they were characterised by collagenous stromal appearances that contrasted with the adjacent ‘conventional’ adenomyosis, in which the stroma was more cellular, resembling that of normal basal endometrium. The EP-like foci were clearly separate from the endometrium (including the ‘eutopic’ EPs) and they were not related to the intra-myometrial portion of the fallopian tubes. On immunohistochemical staining, many of the stromal cells within the myometrial EP-like lesions were p16 positive (range 40–70%), whereas the stromal cells in the surrounding myometrium and adjacent areas of adenomyosis were unstained or showed minimal reactivity (<5% cells positive, Fig. 1 and 2). The adenomyotic glands which exhibited inactive or weakly proliferative endometrioid appearances also showed focal p16 expression similar to the findings in the corresponding basal endometrium.

Adenomyosis is a common uterine condition that rarely presents diagnostic concern. However, rarely neoplasms including adenocarcinoma and adenosarcoma appear to arise within foci of adenomyosis.8–12 In this report we describe an additional endometrial-type pathological process arising within adenomyosis, namely an EP-like alteration, a hitherto unreported finding to our knowledge. The stromal component of adenomyosis usually resembles that of basal endometrium and this was the appearance of the additional adenomyotic areas in our cases. Stromal fibrosis within adenomyosis has been reported in patients on tamoxifen therapy and this feature also characterises tamoxifen-related EPs.13 However, none of our

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Fig. 1 (A) Scanning magnification of the myometrium shows an expansile endometrial polyp-like lesion (right). There are adjacent cystically dilated adenomyotic glands with compressed stroma. The endometrium (left) shows attenuated atrophic appearances. (B) Higher magnification showing cellular and collagenous stroma with inactive endometrioid-type glands. (C) Many stromal cells in the polyp-like lesion (left) are p16 positive and there is also focal epithelial staining. Note that the stromal cells in adjacent ‘conventional’ adenomyosis (upper right) do not express p16.

Fig. 2 (A) Myometrium with two foci of adenomyosis. The lower focus shows an area of cellular stroma resembling that of an endometrial polyp in contrast to the upper focus which shows basal-type endometrial stroma. (B) Junction of myometrium (lower) and polyp-like lesion showing cellular and collagenous stromal appearances. (C) Many of the stromal cells express p16 in contrast to the unstained myometrium.
patients was on tamoxifen. Although cyogenetic studies were not performed in our cases the histological appearances in conjunction with immunohistochemical demonstration of stromal cell p16 expression would support a similar pathogenetic mechanism to that of conventional EPs.6,7 Interestingly, the uteri in all patients also showed ‘atopic’ EPs, one of which was complicated by EIC. Although EPs are usually solitary, multiple polyps are identified in up to 20% of patients.1

The current cases also present a particular semantic problem in that ‘polyps’ in general, including EPs, usually are defined as localised lesions which are elevated above or protrude from a normal mucosal or cutaneous surface. Such a definition is not readily applicable to the lesions described herein which were intra-myometrial in location and not in direct continuity with the endometrial cavity. However, since the histological and immunohistochemical findings were identical to those of EPs, we believe that ‘EP-like lesion’ is a reasonable descriptor of these cases.

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There was no evidence of local recurrence or distant metastases 3 months post-excision.

Case 2 was a 25-year-old male who presented with a slightly tender 26 mm subcutaneous mass overlying the trapezius muscle at the base of the neck. An incisional biopsy showed an unusual spindle cell tumour, difficult to characterise but raising the possibility of atypical fibrous histiocytoma. An excisional biopsy was subsequently performed revealing an infiltrative, cellular tumour centred in the subcutis, not extending beyond the deep fascia into the underlying skeletal muscle. The tumour was composed of spindled cells organised in storiform and fascicular arrangements, set in a mildly fibrous stroma. Occasional elongated and dilated thin-walled vessels were present within the tumour but thick-walled, hyalinised vessels did not feature. The tumour cells showed similar morphological features to the first case, including pleomorphic nuclei with intranuclear inclusions and abundant glassy eosinophilic cytoplasm, appearing glassy in areas and with occasional rhabdoid inclusions. Admixed with the tumour cells was a moderately dense inflammatory infiltrate of lymphocytes, mast cells and plasma cells. Mitotic figures were difficult to identify and numbered no more than one per 50 HPF (field area 0.23 mm²) and there was no necrosis. Lymphovascular invasion, however, was focally present.

Immunohistochemical stains again showed strong and diffuse positivity with CD34 and weak, focal staining with smooth muscle actin and calponin. A wide panel of cytokeratins and melanocytic markers were negative (Table 1). INI-1 expression was retained throughout.

There was no evidence of local recurrence or distant metastases 3 months post-excision.

Both cases were difficult to definitively classify on the initial small biopsy, but on the subsequent excision, were recognised to show features consistent with those of the recently described ‘superficial CD34-positive fibroblastic tumour’.1

In their series of 18 cases, Carter et al.1 report a mesenchymal neoplasm with distinctive morphological and immunohistochemical features, not shared by currently recognised diagnostic entities. The defining characteristics include striking cellular pleomorphism, an extremely low mitotic rate and strong diffuse CD34 positivity. These tumours affect adults with an age range of 20–76 years and typically occur in the lower limbs, particularly the thigh. By definition, the lesions are superficial to the subcutaneous fascia and do not involve the deep soft tissues or muscle. As seen in our cases, the tumour

Fig. 1 (A,B) Case 1 showing a circumscribed cellular tumour comprising pleomorphic spindle cells (A, H&E) with frequent intranuclear inclusions and abundant glassy eosinophilic cytoplasm, sometimes with rhabdoid inclusions (B, H&E). (C,D) Case 2 showing a cellular tumour infiltrating subcutaneous fat (C, H&E) comprising pleomorphic spindle cells with similar morphological features to Case 1 (D, H&E). Both tumours were characterised by strong and diffuse CD34 positivity (E), and an extremely low mitotic rate and Ki-67 proliferative index (F).
cells are typically spindled and arranged in sheets and fascicles, showing intranuclear pseudoinclusions with abundant fibrillary or glassy eosinophilic cytoplasm. In addition to CD34 positivity, limited expression of cytokeratin may be seen in up to 70% of cases and all consistently retain INI-1 reactivity. Although not included in the study by Carter et al., ultrastructural examination performed in one of our two cases confirms fibroblastic differentiation, thus supporting the histogenetic classification of this lesion as a fibroblastic neoplasm.

Of the 13 cases with available clinical follow-up in the original series, one patient developed regional lymph node metastases after incomplete excision of the primary tumour. There were no recurrences or metastasis in the remaining cases, with a median follow up of 24 months. Based on the limited available data, these tumours are presently regarded as of intermediate (borderline) malignancy with rare metastatic potential. Although there was no recurrence or metastasis in our two cases 3 months post-resection, we await long term follow-up with interest.

Whilst it could be argued that this entity is a heterogeneous collection of difficult to classify pleomorphic spindle cell tumours, we agree with the authors first describing this tumour that the morphological and immunohistochemical features are sufficiently unique to warrant classification as a new diagnostic entity. The importance of its recognition is to avoid misdiagnosis with other pleomorphic soft tissue tumours, especially high grade sarcomas with more aggressive biological behaviour.

Superficial CD34-positive fibroblastic tumour shares at least some histological features with undifferentiated pleomorphic sarcoma (formerly referred to as malignant fibrous histiocytoma or MFH), so much so that some cases in the series by Carter et al. had been previously oxymoronically diagnosed as ‘low grade MFH’. One of our cases reported herein was also informally regarded as ‘low grade MFH’ by one of the authors (BAW) prior to appreciation of this new entity. However, whilst undifferentiated pleomorphic sarcoma can show some CD34 positivity, strong and diffuse staining as seen in superficial CD34-positive fibroblastic tumour is distinctly unusual. A superficial location, extremely low mitotic rate and the absence of necrosis are also not typical features.

Table 1  Summary of immunohistochemical stains performed on both cases

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Clone</th>
<th>Dilution</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
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<tr>
<td>CD34</td>
<td>QB END-10</td>
<td>1:100</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD31</td>
<td>JC70A</td>
<td>1:150</td>
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<td>–</td>
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<td>ERG</td>
<td>EPR3664</td>
<td>Neat</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>CD68</td>
<td>PGM1</td>
<td>1:300</td>
<td>N/A</td>
<td>Background dendritic cells only</td>
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<td>F906.1</td>
<td>1:200</td>
<td>N/A</td>
<td>+</td>
</tr>
<tr>
<td>SM actin</td>
<td>IA4</td>
<td>1:1500</td>
<td>–</td>
<td>Weak, patchy</td>
</tr>
<tr>
<td>Desmin</td>
<td>D33</td>
<td>1:100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Calponin</td>
<td>CALP</td>
<td>1:800</td>
<td>–</td>
<td>Weak, patchy</td>
</tr>
<tr>
<td>Caldesmon</td>
<td>h-CD</td>
<td>1:50</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Myogenin</td>
<td>F5D</td>
<td>1:75</td>
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<td>–</td>
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<td>S100</td>
<td>Polyclonal</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>Melan A</td>
<td>MelA</td>
<td>1:50</td>
<td>–</td>
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<tr>
<td>HMB45</td>
<td>HMB45</td>
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<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SOX10</td>
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<tr>
<td>Ki67</td>
<td>MIB-1</td>
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<td>&lt;1%</td>
<td>&lt;1%</td>
</tr>
<tr>
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<td>MRQ-27</td>
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<td>+</td>
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<td>–</td>
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<td>CD21</td>
<td>2G9</td>
<td>1:50</td>
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<td>–</td>
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<td>CD35</td>
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<td>N/A</td>
<td>–</td>
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<tr>
<td>CD30</td>
<td>Ber-H2</td>
<td>1:100</td>
<td>N/A</td>
<td>–</td>
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<tr>
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<td>ALK-1</td>
<td>Neat</td>
<td>N/A</td>
<td>–</td>
</tr>
<tr>
<td>HHV-8</td>
<td>LNA</td>
<td>1:30</td>
<td>N/A</td>
<td>–</td>
</tr>
</tbody>
</table>

Of the 13 cases with available clinical follow-up in the original series, one patient developed regional lymph node metastases after incomplete excision of the primary tumour. There were no recurrences or metastasis in the remaining cases, with a median follow up of 24 months. Based on the limited available data, these tumours are presently regarded as of intermediate (borderline) malignancy with rare metastatic potential. Although there was no recurrence or metastasis in our two cases 3 months post-resection, we await long term follow-up with interest.

Whilst it could be argued that this entity is a heterogeneous collection of difficult to classify pleomorphic spindle cell tumours, we agree with the authors first describing this tumour that the morphological and immunohistochemical features are sufficiently unique to warrant classification as a new diagnostic entity. The importance of its recognition is to avoid misdiagnosis with other pleomorphic soft tissue tumours, especially high grade sarcomas with more aggressive biological behaviour.

Superficial CD34-positive fibroblastic tumour shares at least some histological features with undifferentiated pleomorphic sarcoma (formerly referred to as malignant fibrous histiocytoma or MFH), so much so that some cases in the series by Carter et al. had been previously oxymoronically diagnosed as ‘low grade MFH’. One of our cases reported herein was also informally regarded as ‘low grade MFH’ by one of the authors (BAW) prior to appreciation of this new entity. However, whilst undifferentiated pleomorphic sarcoma can show some CD34 positivity, strong and diffuse staining as seen in superficial CD34-positive fibroblastic tumour is distinctly unusual. A superficial location, extremely low mitotic rate and the absence of necrosis are also not typical features.

Atypical fibrous histiocytoma, also known as ‘dermatofibroma with monster cells’ and ‘pseudosarcomatous dermatofibroma’ also falls into the differential diagnosis but is classically based in the dermis. The tumour contains numerous large, bizarre cells and typically has a background of conventional benign fibrous histiocytoma, Circumscription, xanthomatous

![Fig. 2](image-url)
cells, peripheral dermal collagen trapping, epidermal alterations and the absence of diffuse CD34 positivity further distinguish this lesion from superficial CD34-positive fibroblastic tumour.\(^5\)

Our cases share a number of common features with pleomorphic hyalinating angiectatic tumour (PHAT) of soft parts including a superficial location, plump spindle cells with marked pleomorphism, intranuclear inclusions, noticeably low mitotic rate and CD34 positivity.\(^5,6\) However, the lack of thick-walled ecstatic vessels with prominent perivascular hyalination which is a defining feature of PHAT argues against this diagnosis.

Myxoinflammatory fibroblastic sarcoma is another tumour which occurs in the superficial soft tissues, contains pleomorphic spindle and epithelial cells and can show CD34 positivity.\(^3\) However, these tumours are most commonly seen in acral locations in association with tenosynovial structures, show a prominently lobulated architecture with paucicellular myxoid zones, characteristic large virocyte-like cells, ‘pseudolipoblasts’ and a more pronounced inflammatory infiltrate than that seen in our cases.

In summary, we report two new cases of the recently described entity, superficial CD34-positive fibroblastic tumour which is characterised by a superficial (suprafascial) location, marked cellular pleomorphism, extremely low mitotic rate and strong diffuse CD34 positivity. Based on limited available data, these tumours are presently best regarded as intermediate (borderline) malignancy with rare metastatic potential. We agree with Carter et al.\(^1\) that the histological and immunohistochemical features are sufficiently distinct to warrant separation from undifferentiated pleomorphic sarcoma and other currently recognised superficial pleomorphic soft tissue neoplasms.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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Follicular porokeratosis of the nose: two further cases of an emerging variant of porokeratosis

Sir,

Porokeratosis is a disorder of epidermal keratinisation with a low risk of malignant transformation. There are a number of clinical variants, including classic porokeratosis of Mibelli, disseminated superficial actinic porokeratosis, punctate porokeratosis, porokeratosis palmaris and plantaris disseminate and linear porokeratosis.\(^1\) All forms of porokeratosis share the common histological denominator of the cornoid lamella, which is characterised by a narrow column of parakeratosis which appears to be limited to the nose of young adults.\(^5,7\) We report two further cases of this entity that seems to be emerging as a rare but distinctive variant of porokeratosis.

Case 1 was a 26-year-old caucasian male who noted a solitary, asymptomatic and pigmented plaque on the nasal tip, present for 1 year. The lesion was static with no centrifugal growth, ulceration or bleeding. It did not change with sun exposure. His past medical history included a melanoma completely excised from the foot 1 year previously, which had shown no recurrence to date. He had also been diagnosed with cystic fibrosis. There was a family history of non-melanoma skin cancers. The patient worked as a mine-site rigger and was a non-smoker. Physical examination revealed a 3 mm, circumferential, pale-brown and smooth plaque on the nasal tip. There were no other skin lesions.

A 3 mm punch excision biopsy was sent for histopathological examination (Fig. 1A,B). This revealed severely sun-damaged skin with thick hyperkeratosis and parakeratosis. The epidermis showed a mild to moderate degree of acanthosis, with areas of dyskeratosis associated with loss of the granular layer and formation of a parakeratotic column involving the follicular infundibulum. The underlying dermal tissues showed severe elastosis associated with a scattered, mild lymphocytic infiltrate and focal telangiectasia. A diagnosis of follicular porokeratosis was made.

Case 2 was a 35-year-old caucasian female who noted a solitary, hyperkeratotic ‘scab’ on her nasal bridge, present for 8 weeks. The lesion first appeared on a sailing trip, starting as an erythematous nodule, thought to be a pimple. The lesion formed an overlying scab when it was traumatised and squeezed. After 4 weeks this scab was replaced by a skin-coloured, wart-like growth which eventually crusted to leave a
round, pale-yellow, scaly lesion approximately 3 mm in diameter. This was asymptomatic, static in size and did not bleed or ulcerate. There was no significant individual past medical history, but there was a family history of non-melanoma skin cancers. The patient regularly took the oral contraceptive pill, was a non-smoker and full-time office worker. Physical examination revealed a 3 mm circumferential, pale-yellow and scaly plaque on the superior nasal bridge. There were no other skin lesions.

A 3 mm punch excision biopsy was sent for histopathological examination (Fig. 1C,D). This revealed sun damaged skin with surface hyperkeratosis, as well as columns of parakeratosis. These appeared to overlie follicular structures, with loss of the underlying granular layer and dyskeratotic cells within the follicular epithelium. The features were consistent with cornoid lamellae formation restricted to the follicle, and a diagnosis of follicular porokeratosis was rendered. A mild dermal inflammatory infiltrate was also noted.

Porokeratosis is a disorder of epidermal keratinisation of unclear aetiology, first described in 1893 by Mibelli. Typical lesions are well-demarcated and annular with an atrophic or normal centre, surrounded by a distinctive hyperkeratotic ridge-like border. Several clinical variants of porokeratosis have been described based on age of onset, course, lesion size, number and distribution. Disseminated superficial actinic porokeratosis (DSAP) is the most common form and is characterised by multiple small plaques, less than 1 cm in diameter, which occur on sun-exposed sites, particularly the extensor surfaces of limbs, shoulders and back. The face is affected in only 15% and age of onset is usually the 3rd or 4th decade. Porokeratosis of Mibelli (PM) is the second most common form and is characterised by single or few lesions, greater than 1 cm in diameter, occurring on sun-protected areas with a tendency for centrifugal enlargement. Occurrence is frequently in childhood and greater in males.

The aetiology of porokeratosis remains unknown. Triggering factors include genetic factors, ultra-violet light, immunosuppression (particularly in the setting of organ transplantation), leukaemias/lymphomas, drugs, certain infections (including HIV) and trauma. The parakeratotic cells within the cornoid lamellae show abnormal epidermal DNA ploidy in association with increased DNA indices, and are said to be midway between normal cells and those seen in Bowen’s disease. The incidence of malignant change across all variants of porokeratosis is low (estimated as approximately 10% or less), and most commonly results in squamous cell carcinoma. Malignancies complicating porokeratosis are usually single, however multiple lesions are seen in one-third of cases, and lymph node metastases or death can occur. Other than the relatively low risk for malignant transformation, the prognosis for porokeratosis is generally favourable.

Across all the clinical variants of porokeratosis, the histological hallmark is cornoid lamellation, which correlates to the hyperkeratotic border of the lesions. Histologically, one observes tightly fitted and stacked parakeratotic cells, which are relatively well delineated from the rest of the corneocytes extending through the stratum corneum. Loss of the underlying granular layer is also seen, and dilated capillaries and lymphohistiocytic inflammation within the papillary dermis have also been described. Whilst cornoid lamellation is the characteristic feature of porokeratosis, its presence is not synonymous with this condition, having also been reported in association with other conditions including basal cell carcinoma, Bowen’s disease, solar keratosis and occasional inflammatory conditions. In many cases accurate diagnosis rests on careful clinicopathological correlation.

Follicular involvement, defined as cornoid lamellation arising from follicular infundibulae, has been documented in most variants of porokeratosis. In these cases the follicular cornoid lamellae are typically intermingled with more classical
cornoid lamellae in the interfollicular areas. However, cases in which the cornoid lamellae is only seen within follicular infundibulae are much rarer. In 2007, de Almeida et al. reported two cases of follicular porokeratosis, in the context of DSAP affecting the arms and legs and PM affecting the limbs. Since then, there has been one more case in the setting of PM and one case of follicular-centred papules on the trunk and extremities of a 40-year-old male which the authors proposed as a unique clinical and histological entity which they termed “follicular porokeratosis.”

In addition to the cases listed above, there have been recent reports of follicular porokeratosis occurring exclusively on the face of relatively young adults (aged 19–34 years). Of these, three report involvement limited to the nose, with clinical and histological characteristics almost identical to our cases. Facial involvement in porokeratosis is relatively uncommon, and exclusive involvement of the face is rare. In 2010, Wang et al. reported a 25-year-old Caucasian female with an asymptomatic, erythematous plaque on the nasal tip, which histologically showed cornoid lamellae centred exclusively within follicular infundibulae. Rocha-Sousa et al. described almost identical features in a 19-year-old Brazilian male with asymptomatic, keratotic papules 0.2–1.0 cm in size on the distal nose. Finally, Rifaoglu et al. reported a 34-year-old female with a 1 cm lesion on the distal nose, again demonstrating cornoid lamellae arising exclusively from follicular structures.

Our cases are remarkably similar to these in terms of the clinical features and histological findings, and thus may represent the fourth and fifth cases of a unique clinical and histological variant of follicular porokeratosis, which appears to be limited to the nose of young adults. These cases lack the typical clinical features associated with the currently recognised variants of porokeratosis, and they are unique in terms of their limited anatomical distribution as well as the restriction to follicular involvement. As with all forms of porokeratosis the aetiology remains obscure, but one might postulate that the condition results from a localised defect affecting keratinisation within the infundibular epithelium only. The documented cases of follicular keratosis are summarised in Table 1.

In conclusion, we have presented what we believe to be the fourth and fifth cases of a distinct form of porokeratosis, characterised clinically by lesions limited to the nose of young adults and histologically by cornoid lamellae formation restricted to follicular infundibulae. The diagnosis of this condition may be challenging histologically, particularly as cornoid lamellae arising within a follicle are often somewhat distorted and appear less striking than the archetypal epidermal example that is illustrated in textbooks. An increased awareness of the condition may in turn reveal that it may not be as uncommon as initially assumed.

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Table 1 Documented cases of follicular porokeratosis in the literature, describing exclusive localisation of cornoid lamellae to the hair follicles on histological examination, including the two cases from this report

<table>
<thead>
<tr>
<th>Age/Gender</th>
<th>Clinical variant/Diagnosis</th>
<th>Location</th>
<th>Clinical presentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>de Almeida et al.</td>
<td>DSAP</td>
<td>Arms and legs</td>
<td>Multiple asymptomatic lesions with keratotic ridges containing keratotic papules, in a sun-exposed distribution</td>
</tr>
<tr>
<td>Yong et al.</td>
<td>PM</td>
<td>Hand, ankle and arm</td>
<td>Three large (two lesions &gt;5cm), slowly enlarging and desquamative plaques with follicular accentuation inside a hyperkeratotic ridge Solitary large (3 x 3 cm), enlarging, pruritic, erythematous and scaly plaque</td>
</tr>
<tr>
<td>Pongpuapunth et al.</td>
<td>Follicular PM</td>
<td>Natal cleft</td>
<td>Multiple small (&lt;1 cm), static, follicular, pruritic, erythematous and scaly papules</td>
</tr>
<tr>
<td>Lee et al.</td>
<td>Not reported by authors; likely DSAP</td>
<td>Face: cheeks</td>
<td>Multiple, asymptomatic, brown, ring-like macules with raised hyperkeratotic borders on both cheeks; also a defined cluster on the right cheek</td>
</tr>
<tr>
<td>Rifaoglu et al.</td>
<td>Follicular porokeratosis of the face</td>
<td>Face: distal nose</td>
<td>Solitary (1 cm) asymptomatic, static plaque with hyperkeratotic ridge and depressed centre; not worse with sun exposure</td>
</tr>
<tr>
<td>Rocha-Sousa et al.</td>
<td>Follicular porokeratosis of the face</td>
<td>Face: distal nose</td>
<td>Multiple small (&lt;1 cm), asymptomatic, static, erythematous and keratotic papules with a raised double-edged border; not worse with sun exposure</td>
</tr>
<tr>
<td>Wang et al.</td>
<td>Follicular porokeratosis of the face</td>
<td>Face: nasal tip</td>
<td>Solitary asymptomatic, erythematous, thin plaque with an edge of scale; not worse with sun exposure</td>
</tr>
<tr>
<td>Current case 1</td>
<td>Follicular porokeratosis of the face</td>
<td>Face: nasal tip</td>
<td>Solitary small (&lt;1 cm), asymptomatic, static, pale-brown plaque; not worse with sun exposure</td>
</tr>
<tr>
<td>Current case 2</td>
<td>Follicular porokeratosis of the face</td>
<td>Face: nasal bridge</td>
<td>Solitary small (&lt;1 cm), asymptomatic, static, pale-yellow and scaly plaque</td>
</tr>
</tbody>
</table>

DSAP, disseminated superficial actinic porokeratosis; PM, porokeratosis of Mibelli.
Unusual presentations of lymphocytic phlebitis of the digestive tract

Sir,
Lymphocytic phlebitis is a rare vascular disorder of the digestive tract, mostly involving the intestines (hence the name enterocolic lymphocytic phlebitis, ELP). Reports of isolated extracolonic involvement are very rare.\(^1\)\(^-\)\(^3\) We report three cases of apparently isolated lymphocytic phlebitis, occurring in the duodenum and gallbladder respectively, and review the available literature.

Case 1 was an 83-year-old woman who underwent upper endoscopy for mild dyspepsia. She was otherwise well with no clinical features attributable to the lower gastrointestinal tract or autoimmune diseases such as connective tissue disorder, systemic vasculitis, inflammatory bowel disease (IBD) or sarcoidosis. Her white cell count was normal. The duodenal biopsy included mucosa and submucosa and showed phlebitis involving medium-sized submucosal veins (Fig. 1). The phlebitis was mostly lymphocytic with scattered small granuloma-like histiocytic aggregates and a few eosinophils. Plasma cells were not prominent. No fibrinoid necrosis, thrombi or endothelial changes were identified and no arteritis was seen. The lamina propria and submucosa showed mild prominence of eosinophils but were otherwise unremarkable. In particular, there was no acute inflammation, ulceration or fibrosis. The patient remained asymptomatic at follow-up 1 year later.

Case 2 was a 65-year-old woman who underwent cholecystectomy after presenting with typical symptoms of acute cholecystitis. She was otherwise well with no clinical features attributable to other gastrointestinal diseases or autoimmune diseases. The gallbladder was macroscopically unremarkable. Microscopy showed moderate chronic cholecystitis and florid subserosal phlebitis (Fig. 2 and 3). The phlebitis was predominantly lymphocytic with scattered histiocytes and numerous poorly-formed granulomas. Plasma cells were not prominent. The venous walls showed focal neutrophilic infiltration and fibrinoid necrosis. No arteritis was seen. The patient subsequently moved overseas and was lost to follow-up.

Case 3 was a 66-year-old diabetic woman who presented with septic shock and acute renal failure secondary to acute cholecystitis and urinary tract infection without localising...
In their review, the gender distribution was
reported under various names including ‘necrotising and
granulomatous phlebitis’, mesenteric inflammatory
veno-occlusive disease (MIVOD), granulomatous phlebitis and lymphocytic
venulitis. These cases (reported up to 2007) were reviewed by
Ngo and Chang. In their review, the gender distribution was
approximately equal. While most (80%) patients were older
than 50 years, ELP has been reported in patients as young as 25
years old.

Most patients present with acute or subacute abdominal
symptoms including pain, nausea and vomiting, diarrhoea and rectal bleed. The symptoms have been attributed to intestinal
ischaemia. Patients are often suspected to have inflammatory or
ischaemic bowel disease and are resistant to conventional
medical treatment. Uncommon presentations as a mass causing
stricturing stenosis or intussusceptions have been reported.1

Most cases of ELP are idiopathic and apparently isolated,
without a significant association with systemic vasculitides. Rare associations with hydroxethyl rutoside (used for varicose
veins), flutamide (an anti-androgen) and primary cytomegalovirus
viraemia have been reported but their significance is
unclear.6

ELP usually involves the large bowel (especially the right
colon), appendix and/or ileum in a segmental fashion. Rare cases
may result in pan-colonic necrosis.2 ELP of the upper gastroinestinal
tract or gallbladder appears to be much rarer. A case of
gastric and duodenal ELP has been reported.3 The patient pre-
sented with a Helicobacter-negative chronic gastric antral ulcer
and ELP was found in both the resected stomach and duodenum.
A case of ELP in the gallbladder (termed MVOD by the authors)
was reported in a patient with systemic lupus erythematosus
(SLE).6 An earlier case of ‘non-giant cell phlebitis’ of the
gallbladder in a patient without systemic vasculitis was reported
by Burke et al. in 1995 but scant details are available.2

Macroscopically, the bowel usually shows segmental haem-
orrhagic infarction, mural thickening, mass or mucosal
ulceration. The mass is due to bowel wall and serosal oedema
as well as mesenteric fibrosis secondary to pancricticulitis. Rare
cases appear macroscopically normal.5

The phlebitis of ELP involves veins of all sizes (from venules
to large veins) in the submucosa, muscularis propria, subserosa
or mesentery.5 Small submucosal veins and venules (<2 mm in
diameter) are most commonly involved2 whilst arteries are
invariably spared. The diagnosis is often made in resection
specimens as endoscopic biopsies are usually too superficial
and show non-specific acute or chronic ischaemic changes,
ulceration or mucosal necrosis only. The phlebitis is charac-
terised by a dense perivascular cuff of small lymphocytes which
also infiltrate the vessel wall. The lymphocytes are predomi-
nantly CD8-positive T cells and many have a cytotoxic
phenotype.7 There are infrequent admixed plasma cells and
eosinophils. The inflammation does not extend into the adja-
cent stroma or fat. The vessels may show endothelial swelling or
cyttoplasmic vacuolisation.8 In some cases there is granulo-
matous phlebitis with multinucleate giant cells. Other cases
show necrotising phlebitis characterised by neutrophil infiltr-
ation and fibrinoid necrosis of vessel walls.5 The vascular
lumina may be occluded by thrombi or, in chronic cases,
obliterative phlebitis secondary to endothelial and myointimal
hyperplasia. The venous occlusion initially causes mucosal
ulceration and necrosis. Later, bowel wall oedema, congestion
and ultimately haemorrhagic infarction may supervene.5 Phlebitis
may be seen in macroscopically normal bowel adjacent to the
ischaemic segments but these do not show luminal obliteration.4

ELP may coexist with other gastrointestinal pathology
including lymphocytic gastritis1 and enterocolitis.1 In the
former case there was no evidence of coeliac disease and the
lymphocytic gastritis resolved without dietary gluten restric-
tion. In a retrospective review of colectomy specimens by
Chetty et al.,5 ELP was seen in diversion colitis associated
with IBD (70–100%) or diverticular disease (17%). Lympho-
cytic phlebitis was noted in 10–20% of IBD without bowel
diversion, particularly in Crohn’s disease with transmural
inflammation. In such cases the phlebitis is part of the adjacent
inflammation and there is usually arteritis as well.6 Chetty et al.
also reported a case of ELP associated with lymphocytic
colitis.5 More recently, lymphocytic phlebitis with more pro-
minent plasma cells and meeting the criteria for IgG4-related
disease have been reported.8 Whether these conditions are
aetiologically related to ELP is uncertain.

ELP is a distinct pathological entity from gastrointestinal
vasculitis, which may be seen in systemic diseases such as
Henoch–Schönlein purpura, connective tissue diseases (includ-
ing SLE and rheumatoid arthritis), polyarteritis nodosa, ANCA
positive vasculitides and Behçet’s disease.2,9

The finding of phlebitis in these cases was either incidental
(Cases 1 and 2) or followed delayed surgery (Case 3). The last
case suggests that in some cases phlebitis may merely reflect
long-standing severe inflammation. These cases suggest that
ELP may be an organ-confined phenomenon of little clinical
significance, similar to some cases of arteritis limited to a single
organ. Isolated arteritis of the gastrointestinal tract and gall-
bladder has been reported. While most are usually self-limiting,
some are associated with systemic diseases.9 In all of these
cases the vasculitis mostly affects arteries with phlebitis being
at most a minor feature. Similar cases of incidental isolated
arteritis have also been reported in gynaecological organs.10
In all of these cases there were no histologically recognised
ischaemic changes in the organs.

Post-operative deaths due to severe intestinal ischaemia
have been reported in patients with ELP.11 Bowel resection is usually
considered curative although rare recurrences have been
reported. The recurrence may be related to phlebitis in the
remaining bowel segments which has occasionally been
reported.4 Follow-up for up to 15 years has not revealed
subsequent onset of systemic vasculitides.5

In contrast to the previously reported cases, our cases appear
not to be associated with systemic diseases, inflammatory
bowel disease or drugs. In addition, there was no evidence of
ischaemic complications.

In conclusion, we present three cases of extracolonic ‘enter-
ocolic’ lymphocytic phlebitis which has rarely been reported.
Lymphocytic phlebitis at these sites may have been under-
reported and their recognition may warrant a more inclusive
Granulosa cell tumour of the adrenal

Sir,

Treatment resistant hypertension in the context of an adrenal mass is often assumed to be adenocortical adenoma or phaeochromocytoma until proven otherwise. Histopathology may sometimes provide unexpected answers.

A 67-year-old female was referred by her general practitioner to a hypertension outpatient clinic for work-up of treatment resistant hypertension. Relevant background history included two cerebrovascular accidents (12 and 10 years ago), and a multinodular goitre (resected).

For the past 20 years she had refractory hypertension. On presentation, her blood pressure remained at 200/100 mmHg despite being on candesartan, perindopril, moxonidine and indapamide sustained release. The addition of spironolactone decreased her blood pressure to a systolic of 204 mmHg to 160 mmHg. She experienced headaches and visual symptoms whenever she did not take her antihypertensives. The patient was taking at least 3600 mg of potassium chloride tablets (Slow-K) a day in order to maintain her potassium within normal range. She had previously been intolerant to hydrochlorothiazide, verapamil, metoprolol, diltiazem and atenolol. She denied episodic sweating and palpitations, weight loss and polyuria. She did not complain of dysfunctional uterine bleeding, breast tenderness, vaginal secretions, hirsutism, acne and change in voice. There was no family history of breast or ovarian cancer. There was no smoking history. She was nulliparous and denied use of any oral contraceptives.

Her cardiovascular examination was significant for a hypodynamic apex beat. Abdominal examination also revealed bilateral bruits. She did not have signs or symptoms of hyperthyroidism. There was neither radio-femoral delay nor a differential blood pressure between upper limbs. She was euvolaemic and did not have signs of heart failure. There was no peripheral oedema.

On investigation, her urine protein to creatinine ratio was 17 mg/mmol. No casts were present. Her creatinine was 70 μmol/L (GFR 82 mL/min/1.73m²). Her sodium was 141 mmol/L, and bicarbonate 29 mmol/L. The patient’s morning cortisol, thyroid function and inflammatory markers (erythrocyte sedimentation rate, white cell count and C reactive protein) were normal. A CT renal angiogram revealed a well-defined solid mass from the left adrenal gland. It measured 4.3 cm in size with minimal calcification. There was no significant stenosis. The right adrenal was normal. Calcification was noted in the renal arteries without haemodynamically significant stenosis.

A 4.3 cm well-defined, minimally calcified 4.3 × 3.2 cm solid mass from left adrenal. Physiological tracer uptake on MIBG scan (Fig. 1). The right adrenal was normal. Calcification was noted in the renal arteries without haemodynamically significant stenosis.

Plasma metanephrine and normetanephrine levels, and urinary free noradrenaline, adrenaline, and dopamine were within normal range. She had previously been intolerant to hydrochlorothiazide, verapamil, metoprolol, diltiazem and atenolol. She denied episodic sweating and palpitations, weight loss and polyuria. She did not complain of dysfunctional uterine bleeding, breast tenderness, vaginal secretions, hirsutism, acne and change in voice. There was no family history of breast or ovarian cancer. There was no smoking history. She was nulliparous and denied use of any oral contraceptives.

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normal range. However antihypertensives may cause a false negative result. Episodic catecholamine release can also confound urine and plasma renin/aldosterone levels. Therefore the decision was made to continue treating as a presumed phaeochromocytoma. Spironolactone was changed to phenoxycbenzamine. This had a good effect, bringing the systolic blood pressure to 130 mmHg. After counselling of the advantages and disadvantages of further treatment, the patient elected for a left laparoscopic adrenalectomy. This proceeded without complications.

The resected adrenal tumour weighed 20 g and was 40 mm in maximum dimension. Histologically it demonstrated typical morphological features of granulosa cell tumour of adult type (Fig. 2A,B). Briefly, the tumour was composed of cords, trabeculae and nests of neoplastic cells (Fig. 2A). Many of the neoplastic cells demonstrated typical nuclear grooving and there were very occasional classical Call–Exner bodies (granulosa cells surrounding eosinophilic secretions, illustrated by arrow in Fig. 2B). By immunohistochemistry the neoplastic cells were negative for chromogranin (arguing very strongly against the possibility of phaeochromocytoma) but were positive for markers commonly seen in granulosa cell tumour including inhibin, calretinin and WT1. There was no vascular space invasion or perineural growth. Although the possibility of metastasis from an ovarian primary could not be excluded histologically, the favoured pathological diagnosis was a primary adrenal granulosa cell tumour of adult type. The patient subsequently underwent a dedicated pelvic ultrasound which failed to reveal any other ovarian or endometrial mass and further favoured the granulosa cell tumour as being of primary adrenal origin.

Interestingly, unilateral left adrenalectomy completely resolved the patient’s hypertension. All antihypertensives were ceased over the following 6 months post-operatively. Urine protein to creatinine ratio normalised and her hypokalaemia resolved. Her renal function remained stable at a creatinine of 120 μmol/L (GFR 41 mL/min/1.73 m²). She remained well on last review 28 months after initial presentation.

The ovaries and adrenal glands are embryologically related and rests of adrenal tissue are not uncommon incidental findings in normal ovaries and testis. Ovarian tissue or ovarian type neoplasms in the adrenal are less common, but are well recognised and in fact two previous cases of primary granulosa cell tumour in the adrenal gland have been reported. This case highlights the link between hypertension, renin and granulosa cell tumour. This has only been published in the literature once before. In that report electron microscopy was performed which suggested renin granules. Similar to our case, the patient had hypertension, high renin activity, hyperaldosteronism, hypokalaemia and a mass containing granulosa cells. Hypertension resolved after removal. Neither renin nor aldosterone levels were taken due to being on candesartan and perindopril. These antihypertensives confound the interpretation by blocking the renin-angiotensin axis. This increases the production of renin causing a falsely elevated result. It is recommended that antihypertensives be washed out for 2–3 weeks prior to testing. However, this was not feasible in our patient due to her refractory hypertension and risk of precipitating accelerated hypertension. Nonetheless, it is likely that our patient’s pathology was renin mediated. This is implied by her features of hypertension and hypokalaemia.

Granulosa cell tumours are usually associated with hyperoestrogenism. Oestradiol levels were taken due to being on candesartan and perindopril. These antihypertensives confound the interpretation by blocking the renin-angiotensin axis. This increases the production of renin causing a falsely elevated result. It is recommended that antihypertensives be washed out for 2–3 weeks prior to testing. However, this was not feasible in our patient due to her refractory hypertension and risk of precipitating accelerated hypertension. Nonetheless, it is likely that our patient’s pathology was renin mediated. This is implied by her features of hypertension and hypokalaemia.

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Colonisation with *Pneumocystis jirovecii* in Australian infants

Sir,

*Pneumocystis jirovecii*, a fungus with worldwide distribution, causes a severe inflammatory pneumonia in immunocompromised adults and children. The association or causal link between *P. jirovecii* and sudden unexpected infant death is controversial.1,2 Serological studies have indicated that exposure to *Pneumocystis* occurs at an early age.3,4 Such colonisation in healthy children may lead to later reactivation and overt disease, although *de novo* infection also occurs.5 The prevalence of colonisation in children, which appears to be higher than that in adults, is considered clinically relevant since colonised children are postulated to be a reservoir for *Pneumocystis*.6 Studies in Chile, the US, Zambia and Europe have examined for the presence of *Pneumocystis* using direct detection methods (immunohistochemistry or polymerase chain reaction, PCR) in children and reported prevalence rates ranging between 29% and 100%.1,3,6–9 However, there are no data on the prevalence of colonisation in Australian infants.

In this pilot study, we sought to determine if detection of *P. jirovecii* in young children without *Pneumocystis* pneumonia was feasible. We examined 50 non-duplicate nasopharyngeal aspirate (NPA) specimens that had been collected for routine virological testing, for the presence of *P. jirovecii* DNA. Nasopharyngeal aspirates from infants aged 2–8 months collected between December 2013 to March 2014 were retrieved from storage at −80°C. None of these patients had a clinical syndrome consistent with *P. jirovecii* pneumonia, nor were they HIV infected, but all had NPA specimens collected because they had respiratory symptoms. These specimens had been tested using the commercial Seeplex RV15 ACE Detection multiplex PCR assay according to manufacturer’s instructions (Seegene, Korea). This assay detects influenza A, influenza B, parainfluenza 1, 2, 3 and 4, respiratory syncytial virus A and B, rhinovirus, enterovirus, adenovirus, coronavirus, metapneumovirus, and bocavirus. As part of the study design, we randomly selected 25 specimens from patients in whom no respiratory viruses were identified and 25 specimens from patients who were infected with one or more respiratory viruses. Detection of *P. jirovecii* DNA by PCR was performed as previously described, with minor modifications.10,11 DNA was extracted from 500 μL of nasopharyngeal aspirate sample. Briefly, the assay is an ‘in-house’ real-time TaqMan PCR assay that targets the single copy β-tubulin gene, performed on the LightCycler platform, a closed amplification system (Roche Diagnostics, Germany). In anticipation of lower levels of *Pneumocystis* burden in comparison to patients with overt *P. jirovecii* pneumonia, an additional 10 cycles of amplification were performed in order to increase the assay sensitivity. Therefore, the cycling parameters were 95°C for 10 min, followed by 50 cycles of 95°C for 5 s, 58°C for 20 s and 72°C for 20 s. Analysis of DNA extracts by multi-locus sequence typing (MLST) of four genetic loci: (1) internal transcribed spacer 1 and 2 (ITS1/2) regions of the nuclear rDNA gene cluster; (2) the *P. jirovecii*-specific β-tubulin; (3) mitochondrial large subunit (mtLSU); and (4) dihydropterin reductase synthase genes, was performed as previously described.10

*Pneumocystis jirovecii* DNA was detected in seven NPA specimens (14%). As expected, the burden of *P. jirovecii* was low with PCR cycle threshold (Ct) values ranging between 38 and 50. In contrast, when using this assay to diagnose *Pneumocystis* pneumonia and other disease, samples with a Ct value <37.3 cycles are typically classified as highly suggestive for *P. jirovecii* infection.10

The mean age of colonised infants was 190.7 days (range 62–469 days). Four of these patients were co-infected with respiratory viruses including rhinovirus (*n* = 2), parainfluenza virus 3 (*n* = 2) and adenovirus (*n* = 1). In the other three patients, no respiratory viruses were detected. Attempts to perform MLST on the seven DNA extracts that yielded *P. jirovecii* DNA were unsuccessful for some of the loci despite multiple experiments. This was likely due to the low amounts of DNA template present in the extracts and was not further pursued. In contrast, the mtLSU gene target that is present in multiple copies in the *P. jirovecii* genome, was able to be amplified and sequenced in three of the DNA extracts.

In this study, we have demonstrated colonisation with *P. jirovecii* in Australian infants with a point prevalence of 14%, and as such, have opened up discussion on methodological issues that are relevant for future studies. Although reports from elsewhere have demonstrated a higher prevalence, there were a number of methodological differences in our study. Here, we used a single copy target for real-time PCR whereas other studies using PCR have employed multi-copy PCR amplification targets such as the mtLSU or the major surface glycoprotein (*MSG*) genes.3,6,9 In addition, previous studies reporting a significantly higher prevalence examined autopsy lung specimens.1,6 Because of the retrospective nature of the present pilot study, we were limited to using a small volume of specimen (500 μL) from the upper respiratory tract. Other studies using this specimen type have used between 200 and 3000 μL of sample for DNA extraction.1,12 The relative sensitivities of *P. jirovecii* PCR using different types of respiratory tract specimens is not well defined in children, but in adults lower respiratory tract specimens are preferred.13 However, in practice, when assessing for colonisation in infants, sampling the upper respiratory tract by the least invasive means is of high importance.

Previous studies have indicated that colonisation appears to be common in children during upper respiratory tract symptoms or infection.1 Our pilot study was not designed to determine any associations between *P. jirovecii* colonisation and co-infection with respiratory viruses, but this is an important consideration for future studies. *Pneumocystis jirovecii* may provide an alternative aetiological diagnosis for upper respiratory tract symptoms,3 and pathogens may co-exist and act synergistically in infection and disease. For instance, co-infection has been demonstrated to result in more severe disease caused by other
We have previously described an outbreak of *P. jiroveci* pneumonia in renal transplant recipients caused by two closely related genotypes of *P. jiroveci*, which subsequently spread to other institutions. Thus, we attempted MLST typing in this study to determine whether similar genotypes were present in infants. Unfortunately, we were unable to amplify all loci for all PCR positive specimens due to the assumed low fungal burden as also described by others. This indicates the need for larger volume sampling in future prospective studies examining for *P. jiroveci* prevalence in children.

In conclusion, although limited by a small sample size and the retrospective nature of the study, the results suggest that *Pneumocystis* colonisation does occur in infants, and provides a basis for further investigation into the prevalence and molecular epidemiology of *P. jiroveci* in the paediatric population, including longitudinal studies to determine the natural history and consequence, if any, of colonisation.

**Acknowledgements:** We thank Taryn Crighton from CIDMLS and Carolina Firacative from the Molecular Mycology Research Laboratory, CIDM, Sydney Medical School-Westmead Hospital, The University of Sydney, Westmead Millennium Institute, for technical advice.

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**Sternoclavicular septic arthritis caused by Neisseria elongata subspecies nitroreducens**

Sir,

*Neisseria elongata* subspecies *nitroreducens* is a rare pathogen, most commonly described as a cause of septicemia and endocarditis. It has also been isolated in the context of *P. jiroveci* pneumonia: lessons from a cluster in kidney transplant recipients. Transplantation 2011; 92: 1327–34.


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Gram negative cocccobacillus. The isolate was facultatively anaerobic, oxidase positive and catalase negative. Matrix-assisted laser desorption ionisation–time of flight mass spectrometry (Vitek MS: bioMérieux, France) identification was consistent with *Neisseria elongata* (confidence value 99.9%). Disc susceptibility testing was performed and demonstrated zone sizes of 38 mm and 36 mm for ceftriaxone and ciprofloxacin, respectively. Treatment was changed to ceftriaxone 2 g intravenously, daily.

Further testing was carried out at the reference laboratory. The isolate was urea, indole and aesculin negative. Carbohydrate utilisation tests were negative for glucose, lactose, maltose and sucrose; and nitrate and nitrite were both reduced. Negative results were also obtained for ornithine, lysine decarboxylase, hydrogen sulphide and ortho-nitrophenyl-β-galactoside. On this basis the isolate was identified as *N. elongata* subspecies *nitroreducens*. Unidirectional sequencing of the 16S rDNA region was carried out on the ABI 3130XL sequencer (Applied Biosystems, USA) and yielded an identification to the species level only: *N. elongata* (percentage identity ≥99.3%).

The patient received 2 weeks therapy with ceftriaxone followed by 2 weeks of oral ciprofloxacin, with complete resolution of the infection at 2-month follow up.

*Neisseria elongata* is a commensal of the oropharynx, classified in the family *Neisseriaceae* and is phylogenetically closely related to *Kingella* and *Moraxella* species. Of the three subspecies, it was originally believed that only *N. elongata* subspecies *nitroreducens* caused disease. Subsequently, both *N. elongata* subspecies *elongata* and *N. elongata* subspecies *glycolytica* have been isolated from clinical material in association with infection.

Among all three subspecies, which are asaccharolytic, oxidase positive Gram negative rods, *N. elongata* subspecies *glycolytica* is uniquely catalase positive. Only *N. elongata* subspecies *nitroreducens* reduces nitrate and nitrite, a key biochemical feature that was used for the subspecies identification in this case.

Partial sequencing of the bacterial genome, targeting the 16S rDNA and other regions has been highly successful for bacterial identification in difficult cases. However, there remain species and subspecies that even this level of resolution is unable to distinguish. As has been observed with previous infections due to *N. elongata*, traditional biochemical methods were required for subspecies identification in this case.

*Neisseria elongata* subspecies *nitroreducens* has been most commonly described as a cause of bacterial endocarditis. Interestingly, while the pathological process in many cases has been destructive, published cases reveal a low mortality rate. It is not clear whether this apparent phenomenon is due to publication bias or due to intrinsic characteristics of the organism. This subspecies has also been described less commonly in the context of osteomyelitis and spondyloarthropathies.

Only one previous case of septic arthritis at a non-vertebral site due to *N. elongata* appears in the literature; the agent was *N. elongata* subspecies *glycolytica*. Notably, this infection was also of the sternoclavicular joint, an uncommon site for septic arthritis.

Sternoclavicular joint septic arthritis comprises only 1% of septic arthritis overall. This figure reaches 17% in patients with a history of IVDU. Compared with septic arthritis of the large joints, it is more common in younger patients, with a mean age of 45 years. In addition to IVDU, risk factors include diabetes mellitus, central line infection, chronic renal failure, alcoholism and immunocompromise. Only 23% of patients have no apparent risk factors for infection. Around 50% of cases of sternoclavicular joint arthritides are caused by *Staphylococcus aureus*. *Pseudomonas* species, *Escherichia coli* and other Gram negatives comprise another 25%, with streptococci, *Mycobacterium tuberculosis* and polymicrobial infections accounting for 8%, 3% and 3%, respectively. Those patients with immunodeficiency or a history of IVDU are at particular risk from Gram negative organisms, although the incidence of sternoclavicular joint septic arthritis due to *Pseudomonas* species has declined since the end of an epidemic of pentazocine abuse in the 1980s.

Intra-arterial steroid injection is estimated to result in septic arthritis in 1 in 3000 to 1 in 100,000 cases. While rare, the potential for joint destruction makes this possible complication an important consideration prior to the procedure. Ensuring meticulous adherence to aseptic procedure is vital as is limiting the procedure to cases with well-established indications. It is commonly used for osteoarthritis and inflammatory arthritides such as rheumatoid arthritis, crystal arthropathies and spondyloarthropathies.

While the temporal relationship between the receipt of the intra-arterial injection and the diagnosis of infection in the present case makes this a possible route of infection, this cannot be definitively established. Additionally, infection due to haematogenous seeding is possible for a bacterial species most commonly found in the oral cavity, and this may have occurred as a result of his dental cleaning procedure. Joint damage, due to the initial injury, or the intra-arterial steroid injection, may have predisposed the patient to subsequent seeding of the joint during a period of bacteremia (focus minoris resistentiae).

In conclusion, we describe a case of sternoclavicular joint septic arthritis due to *Neisseria elongata* subspecies *nitroreducens*. To our knowledge this is the first reported case due to this agent.

**Acknowledgements:** The authors are grateful to the staff of the Microbiological Diagnostic Unit, Public Health Laboratory, University of Melbourne, for performing additional testing, including sequencing, in this case.

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Congenital analbuminaemia diagnosed in adulthood in an Australian family

Sir,

Congenital analbuminaemia (CAA) is a very rare autosomal recessive disorder arising due to a mutation in the human albumin gene, ALB.1–3 About 70 cases have been described world-wide, 40 of which were characterised at the molecular level, and until now there have been no reports of cases in Australia.4 Its prevalence is estimated at less than 1 in 1 million, apparently without gender or ethnic predilection.5 Homozygotes or, in a single case, compound heterozygotes demonstrate analbuminaemic trait, a mutation analysis of the albumin gene, with a nearly absent albumin band and raised alpha-2,45–6 Despite the fact that albumin is the most abundant serum protein in normal individuals, comprising approximately half the total serum protein concentration, the phenotype of CAA is apparently without gender or ethnic predilection.5

We report a case of analbuminaemia diagnosed in adulthood in an Australian family. A 23-year-old woman presented with a 5 year history of swelling in her legs, exacerbated by standing for long periods and hot weather. She was also concerned that she appeared to be gaining weight in an unusual distribution, primarily around her buttocks and lower limbs, with sparing of her upper body. She was born as a fraternal twin at very low birth weight, and developed ascites shortly after birth. Her parents were born in a small town in Lebanon, and were not known to be consanguineous. Due to language difficulties impairing communication with hospital staff, her mother discharged her early from hospital, and despite lack of medical follow-up, she thrived. She had been diagnosed with hypercholesterolaemia at the age of 16, however was on no regular medication. Her male twin, two older siblings and her parents were well. There was no family history of recurrent pregnancy loss or infant mortality.

On examination, she had marked deposition of fat in an unusual distribution over her buttocks, hips, thighs and calves, in the presence of a relatively slender upper body. Her body mass index (BMI) was 33 kg/m². Her blood pressure was 110/80 mmHg, and she had pitting peripheral oedema to mid-shin. There were no tendon xanthomata or xanthelasmata, and the rest of her examination was unremarkable.

Investigations (Table 1) were notable for a very low serum albumin, with a nearly absent albumin band and raised alpha-2, beta and gamma fractions on serum protein electrophoresis (Fig. 1). Total serum protein was only moderately reduced, while transferrin, fibrinogen, immunoglobulin M (IgM), cholesterol, low density lipoprotein-cholesterol (LDL-cholesterol), triglycerides and erythrocyte sedimentation rate (ESR) were elevated. A 24-hour urine examination excluded proteinuria, and faecal alpha-1 antitrypsin excluded protein loss from the gastrointestinal tract.

On the basis of the above reported clinical and biochemical findings, CAA was diagnosed in our patient. To confirm this diagnosis, in our continuing study of the molecular basis of the analbuminaemic trait, a mutation analysis of the ALB was carried out following the principles outlined in the Declaration of Helsinki. After we obtained informed consent, genomic DNA was extracted from whole blood of the proband, her mother, her brother, her sister, and two controls. Fourteen genomic fragments of the ALB, encompassing the coding exons and their intron-exon junctions, were polymerase chain reaction (PCR) amplified using specific primer pairs as described by Watkins et al. Genomic DNA from two unrelated healthy volunteers was available as a control. Heteroduplex analysis, performed as previously reported,3 clearly indicated that the only detectable change in all the members of the family occurred in the 356 bp long region amplified by using PCR primers A05A and A06A encompassing exon 3 and its splicing junctions (Fig. 2A). The proband (lane 1) is homozygous and all the other members of the family (lanes 2–4) are heterozygous for a mutation in this region of the molecule. The bands show an electrophoretic behaviour identical to that first reported for the Kayseri mutation.4 The 356 bp long fragment was then submitted to automated direct DNA sequencing. The results showed that the patient is homozygous for an AT deletion at nucleotide positions c. 228–229, which represent the 91st and 92nd bases of exon 3 (Fig. 2B). This mutation causes a frame

References

1. Kayseri mutation.
2. Watkins et al. Genomic DNA from two unrelated healthy volunteers was available as a control. Heteroduplex analysis, performed as previously reported,3 clearly indicated that the only detectable change in all the members of the family occurred in the 356 bp long region amplified by using PCR primers A05A and A06A encompassing exon 3 and its splicing junctions (Fig. 2A). The proband (lane 1) is homozygous and all the other members of the family (lanes 2–4) are heterozygous for a mutation in this region of the molecule. The bands show an electrophoretic behaviour identical to that first reported for the Kayseri mutation.4 The 356 bp long fragment was then submitted to automated direct DNA sequencing. The results showed that the patient is homozygous for an AT deletion at nucleotide positions c. 228–229, which represent the 91st and 92nd bases of exon 3 (Fig. 2B). This mutation causes a frame
shift leading to the presence of an anticipated stop codon, and the predicted translation product should consist of 54 amino acid residues. DNA sequencing of the other members of the family verifies heterozygosity for the same mutation (data not shown). Our results underline the inheritance of the trait and show that the mutation causing CAA in the Australian family is identical to the Kayseri defect (c.228_229delAT).² Six ty-two different mutations in the ALB gene have thus far been identified as causing CAA in humans.³ The Kayseri mutation found in this patient is the most common cause of this trait, accounting for about one-third of the cases characterised at the molecular level, and has occurred in seemingly unrelated individuals from several locations around the world.³-⁵

Interestingly, the serum albumin concentration in our patient was reported as low, but not absent, at 8 g/L. Measurement of serum albumin was made by a Dimension Vista multianalyser (Siemens Healthcare Diagnostics, USA), which uses a bromocresol purple dye-binding method. While the lower limit of detection for this assay is reported as 0 g/L, the lower limit of quantitation has been determined to be 6 g/L due to non-linearity below this concentration. Overestimation of serum albumin in analbuminaemic patients has previously been described, particularly when serum albumin is measured by dye-binding assays, due to non-linearity at low concentrations and weak reactions with serum albumin fragments produced by incomplete transcription and translation of the albumin gene.⁶

Despite a dramatic reduction in serum albumin, the total serum protein level in individuals with CAA is less markedly reduced, due to a compensatory increase in other serum proteins, including transferrin, fibrinogen and serum immunoglobulins, in order to maintain oncotic pressure.⁴ Serum elevations in IgM, which has the highest molecular weight of all immunoglobulin classes due to its pentameric structure, may contribute to oncotic pressure intravascularly. The erythrocyte sedimentation rate (ESR) is elevated in response to increased fibrinogen and immunoglobulins. There is a marked elevation of serum cholesterol, LDL-cholesterol, and apolipoprotein B.³,⁶ Lipoproteins, in particular the LDL and HDL₃ fractions, bind an increased amount of free fatty acids, demonstrating their carrying capacity in plasma in lieu of albumin in these individuals.⁴,⁵ Increased hepatic synthesis of serum proteins, including alpha-2 macroglobulin, transferrin and fibrinogen, and low density lipoproteins also occurs in hypoalbuminaemia due to nephrotic syndrome.⁹-¹¹ Clinical manifestations of CAA include mild peripheral oedema, fatigue and hypotension. Lipodystrophy affecting the lower limbs and hips has been reported, especially in women, and may respond to lipoplasty.¹² The reason for this lipodystrophy would seem to be the two-fold result of the lack of albumin. When the long chain fatty acids are cleaved from circulating lipoproteins by

![Table 1 Variations in serum protein profiles in the patient with CAA and her heterozygous brother, sister and mother](image)

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Patient</th>
<th>Brother</th>
<th>Sister</th>
<th>Mother</th>
<th>Units</th>
<th>Reference interval</th>
</tr>
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<tbody>
<tr>
<td>Serum albumin</td>
<td>8</td>
<td>33</td>
<td>34</td>
<td>33</td>
<td>g/L</td>
<td>38–50</td>
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<tr>
<td>Serum total protein</td>
<td>55</td>
<td>72</td>
<td>70</td>
<td>75</td>
<td>g/L</td>
<td>65–85</td>
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<tr>
<td>Alpha-1 globulins</td>
<td>4.4</td>
<td>1.9</td>
<td>2.2</td>
<td>2.3</td>
<td>g/L</td>
<td>1.9–4.6</td>
</tr>
<tr>
<td>Alpha-2 globulins</td>
<td>17.5</td>
<td>6.7</td>
<td>7.9</td>
<td>9.8</td>
<td>g/L</td>
<td>2.8–7.7</td>
</tr>
<tr>
<td>Beta globulins</td>
<td>17.8</td>
<td>8.4</td>
<td>7.8</td>
<td>10.8</td>
<td>g/L</td>
<td>5.1–14.0</td>
</tr>
<tr>
<td>Gamma globulins</td>
<td>15.4</td>
<td>10.4</td>
<td>11.3</td>
<td>7.5</td>
<td>g/L</td>
<td>5.1–14.0</td>
</tr>
<tr>
<td>Immunglobulin A</td>
<td>1.42</td>
<td>1.38</td>
<td>1.03</td>
<td>1.73</td>
<td>g/L</td>
<td>0.40–3.50</td>
</tr>
<tr>
<td>Immunglobulin G</td>
<td>10.9</td>
<td>12.0</td>
<td>12.3</td>
<td>8.5</td>
<td>g/L</td>
<td>6.6–14.9</td>
</tr>
<tr>
<td>Immunglobulin G1</td>
<td>6.7</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>g/L</td>
<td>3.5–11.0</td>
</tr>
<tr>
<td>Immunglobulin G2</td>
<td>3.9</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>g/L</td>
<td>1.2–7.6</td>
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<tr>
<td>Immunglobulin G3</td>
<td>1.21</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>g/L</td>
<td>0.15–2.50</td>
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<tr>
<td>Immunglobulin G4</td>
<td>0.15</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>g/L</td>
<td>0.06–1.50</td>
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<tr>
<td>Immunglobulin M</td>
<td>7.34</td>
<td>2.01</td>
<td>3.55</td>
<td>1.31</td>
<td>g/L</td>
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<tr>
<td>Transferrin</td>
<td>6.6</td>
<td>3.0</td>
<td>3.2</td>
<td>4.5</td>
<td>g/L</td>
<td>1.8–3.3</td>
</tr>
<tr>
<td>Fibrinogen</td>
<td>5.3</td>
<td>3.4</td>
<td>3.8</td>
<td>4.6</td>
<td>g/L</td>
<td>1.8–4.4</td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>8.2</td>
<td>7.5</td>
<td>4.3</td>
<td>5.5</td>
<td>mmol/L</td>
<td>3.0–5.5</td>
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<tr>
<td>LDL-cholesterol</td>
<td>6.1</td>
<td>4.9</td>
<td>2.4</td>
<td>3.6</td>
<td>mmol/L</td>
<td>&lt;3.5</td>
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<tr>
<td>HDL-cholesterol</td>
<td>1.5</td>
<td>1.0</td>
<td>1.6</td>
<td>1.3</td>
<td>mmol/L</td>
<td>&gt;1.0</td>
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<td>Triglycerides</td>
<td>2.4</td>
<td>3.48</td>
<td>0.69</td>
<td>1.38</td>
<td>mmol/L</td>
<td>&lt;2.0</td>
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<td>Serum calcium (uncorrected)</td>
<td>1.94</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>mmol/L</td>
<td>2.15–2.55</td>
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<tr>
<td>Erythrocyte sedimentation rate</td>
<td>97</td>
<td>5</td>
<td>18</td>
<td>45</td>
<td>mm/hr</td>
<td>3.0–19</td>
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<tr>
<td>C-reactive protein</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>&lt;3</td>
<td>g/mL</td>
<td>&lt;3</td>
</tr>
<tr>
<td>Urine albumin/creatinine</td>
<td>&lt;0.2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>mg/mmol</td>
<td>&lt;3.5</td>
</tr>
</tbody>
</table>

Results falling outside the reference interval are indicated in bold. NA, not available.

![Fig. 1 Serum protein electrophoresis of the patient with CAA (inset: lane 1, normal control; lane 2, proband) and its densitometric scanning (pale grey, normal control; dark grey, proband). The results show a marked reduction in serum albumin, and compensatory increases in alpha-2, beta and gamma fractions.](image)
It is interesting to note that this patient’s brother, sister and mother, all of whom are heterozygotes, each have unique increases in some, but not all, serum proteins, in particular IgM, fibrinogen, transferrin, and the alpha-2 proteins which typically include alpha-2 macroglobulin, caeruloplasmin and fibronectin. Small increments in these individual proteins may play a role in compensation for even a mild reduction in serum albumin concentration in individuals with heterozygous ALB mutations, as their total protein concentrations are normal and they are asymptomatic.

In summary, we present the first reported case of CAA in Australia, confirm that the common c.228,229delAT mutation is the cause of the condition in this individual, and demonstrate compensatory increases in other serum proteins in the patient and her heterozygous family members.

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Effect of HbE trait on measurement of HbA1c by three different methods

Sir,

HbA1c testing is mainly used for monitoring glycaemic control in patients with diabetes. However, the World Health Organization now recommends that HbA1c can be used as a diagnostic test for diabetes, provided that stringent quality assurance procedures are in place and assays are standardised to criteria aligned to the international reference values. Laboratories use many different methods for measuring A1C, but some of these methods can give inaccurate results when the patient has a variant haemoglobin. The effect of common haemoglobin variants (HbS, HbE, HbC and HbD) on HbA1c measurement has been previously studied. Descriptions of the method-specific analytical interferences of these Hb variants on A1C measurement are available on the National Glycohemoglobin Standardization Program (NGSP) website.

In an earlier study performed in our laboratory, a positive bias of ~10% was reported when glycated haemoglobin of patients with haemoglobin (Hb) E trait was measured using Bio-Rad Variant II Turbo 2.0 [cation-exchange high-performance liquid chromatography (HPLC) method; Bio-Rad, USA] compared to boronate affinity HPLC method. This was attributed to insufficient separation of A0 peak from HbE peak. Recently, Bio-Rad Variant II Turbo 2.0 introduced a new resin lot 11840 and dropline that separates HbA2 from HbA0 in the chromatogram, so it is expected that there should be no interference by the presence of common Hb variants like D and E which fall in the A2 region. Recently enzymatic HbA1c assay has been introduced in the Bio Majesty (BM) 6010/C automated chemistry analyser (JEOL, Japan). In this method, blood cells were first haemolysed, and haemoglobin was digested with protease to yield fructosyl amino acid. Fructosyl amino acid oxidase acts on the fructosyl amino acid and generates hydrogen peroxide, which reacts with chromogens in the presence of peroxidase. Capillary electrophoretic method uses the charge difference between HbA1c and other Hb fractions. Separation is achieved via a high-voltage electrical field and electro-osmotic flow. The Capillarys 2 Flex Piercing instrument (Sebia, France) uses capillary electrophoresis method and it separates normal and abnormal (or variants) levels of haemoglobin in the following order, from cathode to anode: A2/C, E, S, D, F, A0, other haemoglobins (including minor HbA1) and then A1C.

However, few reports are available about the effect of HbE on HbA1c measurement by these three methods. In our routine analysis of HbA1c by the ion exchange method, the most common variant observed is HbE. Hence, we evaluated the effect of HbE trait on the measurement of HbA1c by Bio-Rad Variant II Turbo 2.0 with the new resin lot 11840, Capillarys 2 Flex Piercing for HbA1c, and enzymatic method: BM Test HbA1c on JCA BM 6010/C. Boronate affinity method (Premier Hb9210; Trinity Biotech, USA) was used as the comparison method.

Samples that showed the presence of variant window when HbA1c was measured by Bio-Rad variant II turbo 2.0 were collected over a period of 3 months and were stored at −80°C until further analysis. These samples were screened for haemoglobin variant by the automated capillary electrophoresis, using the Capillarys 2 Flex Piercing instrument. A total of 233 samples that were identified as HbE trait by inspecting the chromatogram obtained by capillary electrophoresis and 153 samples of diabetic patients with homozygous A were included for the study.

For each method, results obtained for homozygous A and HbE trait were compared with boronate affinity method. An overall test of coincidence of 2 least-squares linear regression lines was performed using SPSS Software version 18 (SPSS, USA) to determine whether the presence of HbE caused a statistically significant difference (p < 0.05). Method bias was evaluated by Bland–Altman plot and Deming regression analysis using Analyse-it method evaluation software (Analyse-it Software, UK). Bias attributable to the presence of Hb variant was studied using ±5% [total allowable error by the Royal College of Pathologists of Australasia (RCPA)] at medical decision points of 6% and 9%.

![Fig. 1 Deming regression analysis between boronate affinity HPLC and (A) ion-exchange, (B) enzymatic and (C) capillary electrophoresis method.](image-url)
The linearity and assay imprecision for all the four methods were verified in our laboratory. The linearity ranged from 4% to 16% and the assay imprecision was <2%. Good agreement and correlation was observed for homozygous HbA samples between ion-exchange, capillary electrophoresis and enzymatic method with the comparative method, i.e., boronate affinity. Deming regression analysis for the HbE variant samples by the three methods showed a good correlation with the boronate affinity method (Fig. 1 and Table 1). Statistically significant difference (p < 0.05) was observed for ion-exchange and enzymatic method when compared to boronate affinity method. However, it is not clinically significant at the medical decision point of 6% and 9%. Table 1 lists the differences at 6% and 9% for the three methods. The bias for ion-exchange HPLC, enzymatic and capillary electrophoresis method for the variant samples when compared with the boronate affinity method were 0.2, 0.7 and 0.9%, respectively, which is less than the total allowable error by RCPA external quality assurance (Fig. 2).

Jeppsson et al. reported that different values for HbA1c can be obtained for the same sample by HPLC method depending on the kind of resin, resin lot, column size, buffer composition and elution times. Hence, we carried out this study to evaluate whether the change in the resin lot and peak integration in the chromatogram are effective in reducing interference by the presence of HbE trait. In the enzymatic method, glycated dipeptide (fructosyl-valine-histidine) is generated from the N-terminus of the beta-chain of haemoglobin by protease reaction. Since the substitution for glutamic acid by lysine is at position 26 of the $\beta$-globin chain in HbE, there should be no interference in the measurement of HbA1c by enzymatic method. Similar to the findings of Matsumoto et al., we also observed that BM Test HbA1c on JCA BM 6010/c is not affected by the presence of HbE trait. The non-glycated variant peak of HbE is well separated from A1C and A0 peak by capillary electrophoresis. Even though the glycated fraction of HbE is probably confounded with HbA0 peak, studies have shown that it does not interfere with HbA1c measurement. In the present study, none of the three methods evaluated showed clinically significant effects due to the presence of HbE trait. HbA1c measurement by Bio-Rad Variant II Turbo 2.0 did not show clinically significant interference due to the presence of HbE trait. This is probably due to the changes in the peak integration of A0 from A2 and also due to different resin lot.

In choosing the method, laboratory personnel must be aware of the effect of locally prevalent haemoglobin variant on the measurement of HbA1c; more so if HbA1c is used for the diagnosis of diabetes mellitus.

### Table 1

<table>
<thead>
<tr>
<th>Method</th>
<th>HbA1c MDP</th>
<th>Bias</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bio-Rad Variant II Turbo 2.0</td>
<td>6%</td>
<td>0.04</td>
<td>0.02 to 0.06</td>
</tr>
<tr>
<td>Capillary electrophoresis</td>
<td>9%</td>
<td>-0.01</td>
<td>-0.03 to 0.02</td>
</tr>
<tr>
<td>Enzymatic method</td>
<td>6%</td>
<td>0.08</td>
<td>0.06 to 0.1</td>
</tr>
<tr>
<td></td>
<td>9%</td>
<td>0.01</td>
<td>-0.03 to 0.05</td>
</tr>
</tbody>
</table>

CI, confidence interval; MDP, medical decision point calculated by Deming regression analysis.

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The authors state that there are no conflicts of interest to disclose.

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