database against which the spectra is compared, as differing methods impact the identification power.

Other emerging technologies include the BioFire Diagnostics FilmArray Biothreat Panel (bioMérieux) which is a multiplexed, hemi-nested polymerase chain reaction (PCR) within a single pouch which includes two targets for *Y. pestis*. To date, there is only one published study evaluating the role of the FilmArray in the detection of *Y. pestis*.[12] Nevertheless, results are encouraging with sensitivity studies demonstrating detection down to 25 genome equivalents.[12] With minimal sample preparation and all reactions within the single pouch, the FilmArray may have utility as a supplementary test along with the MALDI-TOF MS in reference laboratories.

In conclusion, advances in organism databases and safe extraction techniques have allowed mass spectrometry to have a promising role in the identification of high-risk pathogens such as *Y. pestis*. However, the inability of mass spectrometry to reliably identify *Y. pestis* and other SSBA pathogens to a species level demands that further confirmatory testing remains necessary. It is likely that bacteriophage lysis testing, NAAT, and increasingly, WGS will continue to have a key role in definitively identifying *Y. pestis*, all of which can only be performed in Australia through the PHLN. Confirmatory testing for *Y. pseudotuberculosis* identified by MALDI-TOF MS should also be pursued, as even the enhanced 'Security Relevant' databases can miscall *Y. pestis*. Mass spectrometry will likely have an ongoing utility in ruling out rather than ruling in an SSBA pathogen.[7,9]

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

Susan A. J. Harch[1], Amy V. Jennison[2], Ivan Bastian[1]

[1]Department of Microbiology and Infectious Diseases, SA Pathology, Adelaide, SA, Australia; [2]Public Health Microbiology, Forensic and Scientific Services, Queensland Department of Health, Brisbane, Qld, Australia

Contact Dr Susan Harch.
E-mail: susan.harch@sa.gov.au

1. Public Health Laboratory Network. Australian department of health: plague laboratory case definition. Mar 2017; cited 18 Dec 2018. http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-phlncd-plague.htm
2. Hall M, Chattaway MA, Reuter S, *et al*. Use of whole-genus genome sequence data to develop a multilocus sequence typing tool that accurately identifies *Yersinia* isolates to the species and subspecies levels. *J Clin Microbiol* 2015; 53: 35–42.
3. Le Guern AS, Martin L, Savin C, *et al*. Yersiniosis in France: overview and potential sources of infection. *Int J Infect Dis* 2016; 46: 1–7.
4. Kaasch AJ, Dinter J, Goeser T, *et al*. *Yersinia pseudotuberculosis* bloodstream infection and septic arthritis: case report and review of the literature. *Infection* 2012; 40: 185–90.
5. World Health Organization. Plague fact sheet. Oct 2017; cited 4 Feb 2018. http://www.who.int/mediacentre/factsheets/fs267/en/
6. Achtman M, Zurth K, Morelli G, *et al*. *Yersinia pestis*, the cause of plague, is a recently emerged clone of *Yersinia pseudotuberculosis*. *Proc Natl Acad Sci USA* 1999; 96: 14043–8.
7. Rudrik JT, Soehnlen MK, Perry MJ, *et al*. Safety and accuracy of matrix-assisted laser desorption ionization-time of flight mass spectrometry for identification of highly pathogenic organisms. *J Clin Microbiol* 2017; 55: 3513–29.
8. Ayyadurai S, Flaudrops C, Raoult D, *et al*. Rapid identification and typing of *Yersinia pestis* and other *Yersinia* species by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. *BMC Microbiol* 2010; 10: 285.
9. Tracz DM, Antonation KS, Corbett CR. Verification of a matrix-assisted laser desorption ionization-time of flight mass spectrometry method for diagnostic identification of high-consequence bacterial pathogens. *J Clin Microbiol* 2016; 54: 764–7.
10. Drevinek M, Dresler J, Klimentova J, *et al*. Evaluation of sample preparation methods for MALDI-TOF MS identification of highly dangerous bacteria. *Lett Appl Microbiol* 2012; 55: 40–6.
11. Couderc C, Nappez C, Drancourt M. Comparing inactivation protocols of *Yersinia* organisms for identification with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Rapid Commun Mass Spectrom* 2012; 26: 710–4.
12. Seiner DR, Colburn HA, Baird C, *et al*. Evaluation of the FilmArray(R) system for detection of *Bacillus anthracis, Francisella tularensis* and *Yersinia pestis*. *J Appl Microbiol* 2013; 114: 992–1000.

DOI: https://doi.org/10.1016/j.pathol.2019.01.006

Transthyretin Val122Ile amyloidosis associated with isolated gastrointestinal disease and bowel rupture in a Caucasian woman

Sir,

An 84-year-old Caucasian woman was referred for gastroenterology review in December 2016 to investigate 6 months of diarrhoea. Her medical history included type 2 diabetes mellitus with nephropathy, chronic obstructive airways disease secondary to smoking, gout, depression, peptic ulcer disease, hysterectomy, right hip arthroplasty, spinal laminectomy and carpel tunnel release in August 2013. Her medications were glimpiride, allopurinol, ipratropium bromide, fluticasone, cholecalciferol, furosemide and duloxetine.

Shortly after the referral, the patient's diarrhoea and abdominal pain became worse. A computed tomography (CT) scan of the abdomen and pelvis was unrevealing. Bloods showed a raised C-reactive protein of 131.1 mg/L (normal <3.0 mg/L), normocytic anaemia (Hb 90 g/L, MCV 90 fL) with normal iron, B12 and folate levels and stable chronic renal impairment (creatinine 145 µmol/L, eGFR of 38 mL/min). Her albumin was 34 g/L and liver function tests were normal apart from a mildly raised ALP. Physical examination was unremarkable with the exception of nail dystrophy and carpal tunnel release scars.

A repeat colonoscopy was notable only for lax anal tone; colonic mucosa appeared normal which was confirmed on histology. Subsequently, the patient suffered a rectal prolapse that required surgical correction. Biopsies of the rectal mucosa demonstrated prominent eosinophilic deposits within muscularis blood vessel walls and nodular deposits surrounding perivascular tissues. These infiltrates were Congo red positive, consistent with amyloid. The patient was referred to the Victorian and Tasmanian Amyloidosis Service for evaluation.

At this review, the patient complained of inconsistent neuropathic pain in her hands and feet, and postural symptoms. On examination, however, there was no objective evidence of peripheral neuropathy or postural hypotension. Apart from nail dystrophy and carpal tunnel release scars, there were no other soft-tissue signs of amyloidosis, nor evidence of hepatomegaly or cardiac failure.

Investigations to determine the amyloid subtype and extent of systemic involvement were undertaken. There

was no detectable paraprotein. Both kappa and lambda serum free light chains were mildly elevated but with a normal light chain ratio, arguing against AL amyloidosis. An echocardiogram showed mildly impaired diastolic relaxation but normal left ventricular size, wall thickness and function. The interventricular septal thickness was 10 mm (normal <12 mm) and there was no impaired longitudinal strain. Cardiac biomarkers were mildly, but inconsistently, elevated over many months: NT ProBNP levels ranged from 34 pmol/L to 105 pmol/L (normal >75 years of age <52 pmol/L); similarly the troponin I was usually below the normal <0.04 μg/L, but once rose to 0.09 μg/L. The transient elevations in biomarkers were assessed as secondary to renal impairment, rather than amyloid cardiomyopathy. A technetium-99m pyrophosphate scan had no cardiac uptake. Immunohistochemistry on the rectal biopsy was positive for transthyretin (TTR), and negative for serum amyloid A protein, with an equal distribution of kappa and lambda light chains.

Further questioning revealed an intriguing, albeit incomplete family history: her ancestry was traced back to Ireland, with one of the patient's sisters also having suffered from protracted diarrhoea and abdominal cramping prior to her death. As a result, familial amyloid polyneuropathy associated with the mutant Thr60Ala TTR variant—which was originally described in Donegal County in North-West Ireland, but has since been reported in Caucasian populations around the world—was considered.[1] Genetic testing though, unexpectedly, revealed an isoleucine-122 mutation (Val122Ile) that was confirmed on repeat testing. The Val122Ile mutation is associated with West African heritage though the patient denied any African or Caribbean ancestry.

Treatment for TTR amyloidosis was initiated with doxycycline but was poorly tolerated due to photosensitivity and abdominal discomfort. Epigallocatechin-3-gallate (EGCG) was then commenced and well tolerated. Diflunisal was not offered initially due to renal impairment and history of peptic ulcer disease.

The patient remained well for 12 months, until she represented with severe abdominal pain, vomiting and a palpable mass. A CT scan confirmed small bowel obstruction with features of vascular compromise. At laparotomy, there was a 50–60 cm gangrenous mid-bowel segment secondary to right iliac fossa adhesions from previous surgeries. The segment was resected and bowel continuity re-established with an end-to-end anastomosis. The patient made a full recovery. Histology of the resection showed an abrupt transition point from normal small bowel mucosa to an area of transmural ischaemia (Fig. 1A) with extensive perivascular and lamina propria amyloid infiltration. Immunohistochemistry was again positive for TTR (Fig. 1B–F).

Following the bowel resection, the patient's diarrhoea settled and she was weaned off codeine. She remained on EGCG and well until she represented 6 months later with recurrence of diarrhoea and abdominal pain. At this time, based on risk/benefit discussions, she commenced diflunisal 250 mg twice daily. After 3 months, she is tolerating diflunisal and her symptoms have stabilised.

Amyloidosis refers to the pathological misfolding of proteins to form organised, fibrillar deposits within organs. The amyloidoses differ in the protein that is misfolded with resultant differences in the organs affected and clinical presentation of disease. To date, over 30 different proteins have been identified as causing amyloid. Although amyloid deposits have a characteristic appearance under electron microscopy, the most validated and common initial demonstration is via histological staining by Congo red dye, with 'apple-green' birefringence under polarised light.

Correct identification of the amyloid subtype is paramount to determine appropriate therapy. Immunohistochemical antibodies are the first step, but differ in sensitivity and specificity and are subject to interpretative variability. Laser microdissection and mass spectrometry based proteomic analysis provide greater accuracy but are limited by availability and cost. Genetic testing can be extremely helpful for the inherited amyloidoses, such as variant TTR, fibrinogen, apoliprotein A1 or lysozyme amyloidosis. In cases where doubt remains, referral to an amyloidosis specialist centre (http://amyloidosis.org/resources/#treatment-centers) is strongly recommended.

The organ distribution of amyloid can aid subtyping, albeit imperfectly since certain organs can be involved by numerous subtypes. Amyloid cardiomyopathy is most commonly seen with AL or TTR amyloidosis. Cardiac involvement can usually be demonstrated with echocardiography and/or cardiac magnetic resonance imaging (MRI), though neither technique is completely dependable with sensitivities of around 75% and 85%, respectively. For unknown reasons, bone seeking radiotracers accumulate in the myocardium of patients with TTR amyloidosis and cardiac involvement; however, they also accumulate in 10–20% of cardiac AL amyloidosis patients.[2] Nonetheless, the absence of a monoclonal protein or light chains in the serum or urine, and strong radiotracer uptake by bone scintigraphy (Perugini grade 2 or 3) can accurately diagnose cardiac TTR amyloidosis without the need for myocardial biopsy.[3]

In this case, the diagnosis of TTR amyloidosis was established from a rectal biopsy and bowel resection sample that stained strongly with TTR. The diagnosis was confirmed by the detection of the amyloidogenic isoleucine-122 mutation in the TTR gene. The detection of this mutation in a Caucasian woman with no myocardial uptake on bone scintigraphy is highly unexpected.

Transthyretin amyloidosis is divided into wild-type TTR amyloidosis (ATTRwt) and 'mutant' or hereditary TTR amyloidosis (hATTR). ATTRwt classically presents as late-onset cardiomyopathy—the majority of patients are over 70 years old—with a striking, unexplained male predominance. The reported male to female ratio varies between studies, but is at least 5:1.[4] Aggregate autopsy reports suggest that ATTRwt infiltration is present in the hearts of 14–25% of octogenerians, though symptomatic cardiomyopathy occurs in a smaller fraction.[4] Though ATTRwt is striking in its cardiac predilection, carpal tunnel syndrome precedes the diagnosis by 5–10 years in 50% of patients and, infrequently, infiltration of the ligamentum flavum can occur.[5,6] Renal impairment in ATTRwt has not been reported.

Hereditary TTR amyloidosis is caused by autosomal dominant mutations in the transthyretin gene, generating an unstable TTR protein that dissociates from its native tetrameric structure into amyloidogenic monomers that misfold and aggregate into amyloid fibrils. The fibrils deposit in various organs throughout the body, particularly the nerves and myocardium. TTR is generated mostly by the liver, hence the limited success of liver transplantation in treating hATTR, but local production also occurs in retinal pigment and choroid plexus epithelial cells. More than 100 inherited mutations in

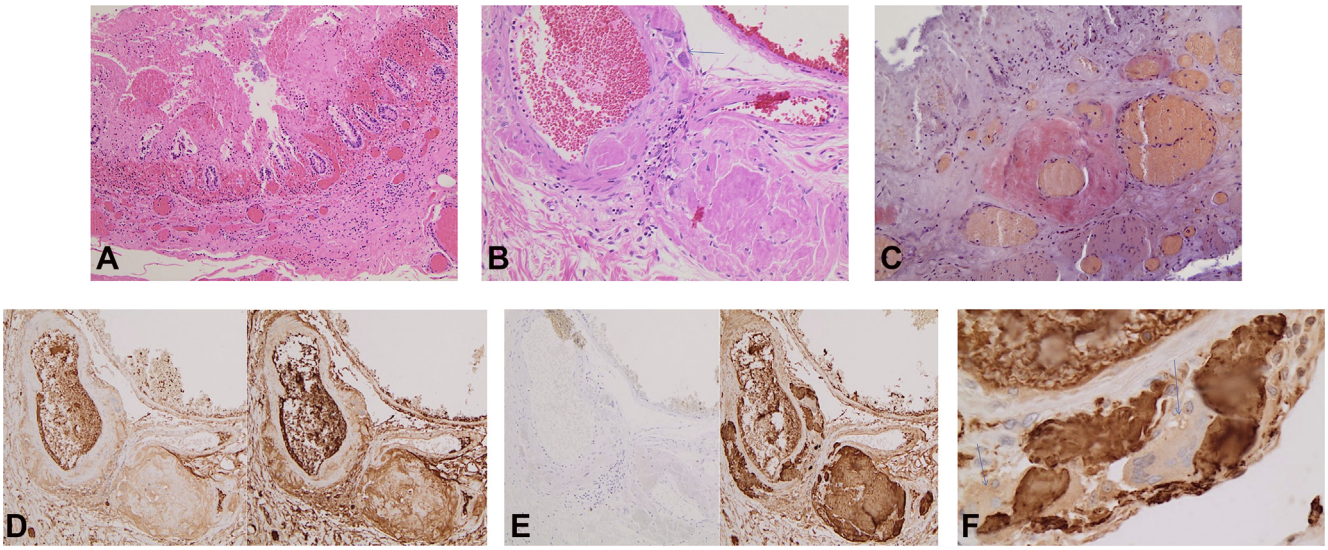

Fig. 1 (A–F) Small bowel resection. The specimen consisted of a segment of small bowel 42 cm in length, most of which was diffusely haemorrhagic and partly necrotic. There was 3 cm of normal small bowel at one margin and 5 cm at the opposite margin. A definite perforation was not identified. The mucosal surface was diffusely haemorrhagic with areas of necrosis. Microscopic sections showed predominantly mucosal but focally almost transmural necrosis within the haemorrhagic portion. Blood vessels within the bowel wall showed expansion of the wall by deposits of homogenous eosinophilic material, in some areas with an associated inflammatory cell infiltrate including histiocytic foreign body-type giant cells. Congo red stain was positive with green birefringence under polarised light consistent with amyloidosis. Immunohistochemical stains for amyloid classification showed the following results: kappa/lambda, background and non-specific staining without significant staining of amyloid deposits; amyloid A, negative; transthyretin, strong positive staining of the amyloid deposits. (A) Ischaemic bowel with surface ulceration and necrosis. (B) Blood vessels within the bowel wall showed homogenous eosinophilic material consistent with amyloid, in many areas associated with a histiocytic infiltrate including giant cells (arrow). (C) Congo red staining was strongly positive within the blood vessel walls and there was diagnostic green birefringence under polarised light. (D) Kappa (left) and lambda (right) show non-specific staining without significant staining in the amyloid deposits. (E) Amyloid A (left) was negative. Transthyretin (right) showed strong positive staining within amyloid deposits. (F) High power showing transthyretin stain with multinucleated giant cells (arrows) engulfing fragments of amyloid.

the TTR gene have been reported and there is considerable clinical heterogeneity depending on the mutation present.

The most common clinical phenotype of hATTR is a familial polyneuropathy characterised by progressive, axonal, sensorimotor and autonomic neuropathy beginning in the third to sixth decades.[7,8] Dysautonomic gastrointestinal symptoms are frequently reported. As with ATTRwt, carpal tunnel syndrome is common. A restrictive cardiomyopathy may develop later in the disease.[8] Worldwide, the Val30Met mutation is the most common inherited TTR mutation, though the geographic distribution of disease is uneven with endemic foci occurring in Portugal, Japan, Sweden and France. Each endemic cluster appears to have a unique age of onset.[7,8]

Other TTR mutations are associated with different clinical phenotypes. The alanine-60 TTR variant (Thr60Ala), the most common form of hereditary ATTR in the United Kingdom and Ireland, is predominantly a disease of the heart and autonomic nerves, with polyneuropathy present in only a quarter of patients.[9] For all the hereditary TTR amyloidoses, penetrance tends to be much higher in endemic foci. Even in these groups, however, it does not reach 100% and a family history is often absent.[7]

Less common inherited TTR variants are those that demonstrate a non-neuropathic phenotype, presenting with isolated cardiac amyloidosis (occasionally referred to as familial amyloid cardiomyopathy), or rarely, with leptomeningeal disease. The most common variant associated with dominant cardiac amyloidosis is the Val122Ile mutation which is carried by 5% of people of West African ancestry.[10] The carrier frequency in non-African American populations has never been described and the first reported case in a Caucasian man was in 1999, more than 11 years after the mutation had been described in African Americans.[11] In the United States, roughly 3.5% of African Americans are heterozygous for this amyloidogenic allele which may explain the nearly 4-fold increase in the prevalence of isolated cardiac amyloidosis in African Americans;[12] nearly 10% of African American patients with heart failure have the mutation.[12] However, overall penetrance is low; the recent Atherosclerosis Risk in Communities study which genotyped 3856 black participants found that amyloid cardiomyopathy affected only 7% of subjects with the Val122Ile TTR mutation.[12]

Our patient's case is unusual on multiple fronts. Firstly, the extent of amyloid infiltration seen within her bowel biopsies is rare. Despite many patients with systemic amyloidosis reporting autonomic dysmotility symptoms, biopsy proven gastrointestinal amyloidosis remains uncommon. Additionally, though hATTR can affect the gastrointestinal tract (seen in up to 40–50% of cases at post-mortem in a small series) exclusive gastrointestinal involvement is uncommon.[13] As far as we are aware, this is the first report of Val122Ile ATTR causing isolated symptomatic gastrointestinal amyloidosis, including bowel rupture. Secondly, the Val122Ile ATTR mutation in this woman, in the absence of cardiomyopathy and any known West African ancestry, is unprecedented. This case highlights our limited understanding of the epidemiology and extra-cardiac manifestations of Val122Ile ATTR, especially in non-African populations. Given that approximately 50% of patients with Val122Ile ATTR report gastrointestinal symptoms, it is probable that some degree of gastrointestinal amyloid is present in at least a proportion. For all the focus on cardiomyopathy in patients with Val122Ile ATTR, symptomatic cardiac failure only develops in a fraction of patients; the majority, like our patient, do not develop any cardiac manifestations.

Early diagnosis remains the greatest challenge in amyloid medicine. A low threshold to perform Congo red staining on any biopsy where the clinical presentation is not typical of a common disease is important. Immunohistochemistry, bone

scintigraphy and tests to exclude a plasma cell dyscrasia should be routine additional tests. In more difficult cases, laser-dissection with mass spectrometry and genetic testing provide further diagnostic accuracy. Most importantly, accurate diagnostic subtyping is the key goal as the treatment implications are highly significant, ranging from chemotherapy and stem cell transplantation for AL amyloidosis, to protein stabilisers, fibrillar disruptors and, most recently, novel antisense oligonucleotides for hereditary disease.[14–16]

Conflicts of interest and sources of funding: No pharmaceutical or industry sources of support were utilised for this manuscript. The authors state that there are no conflicts of interest to disclose.

Shreerang Sirdesai[1], Patrick Hosking[2], Graeme Stewart[3], Stephen Ting[1], David Booth[4], Eileen Fong[5], Simon Douglas John Gibbs[1]

[1]Haematology Department, Eastern Health, Victorian and Tasmanian Amyloidosis Service, Monash University Eastern Health Clinical School, Melbourne, Vic, Australia; [2]Pathology, Eastern Health, Melbourne, Vic, Australia; [3]Clinical Immunology, The Westmead Institute for Medical Research, The Westmead Amyloidosis Clinic, University of Sydney, Sydney, NSW, Australia; [4]Centre of Immunology, The Westmead Institute for Medical Research, Sydney, NSW, Australia; [5]Anatpath and Eastern Health, Melbourne, Vic, Australia

Contact Dr Shreerang Sirdesai.
E-mail: S.Sirdesai@alfred.org.au

1. Staunton H, Dervan P, Kale R, *et al*. Hereditary amyloid polyneuropathy. *Brain* 1987; 110: 1231–45.
2. Rapezzi C, Quarta CC, Guidalotti PL, *et al*. Role of 99mTc-DPD scintigraphy in diagnosis and prognosis of hereditary transthyretin-related cardiac amyloidosis. *JACC Cardiovasc Imag* 2011; 4: 659–70.
3. Gillmore JD, Maurer MS, Falk RH, *et al*. Heart failure nonbiopsy diagnosis of cardiac transthyretin amyloidosis. *Circulation* 2016; 133: 2404–12.
4. Gagliardi C, Dominguez F, Gonz E, *et al*. Clinical characteristics of wild-type transthyretin cardiac amyloidosis: disproving myths. *Eur Heart J* 2017; 38: 1895–904.
5. Nakagawa M, Sekijima Y, Yazaki M, *et al*. Carpal tunnel syndrome: a common initial symptom of systemic wild-type ATTR (ATTRwt) amyloidosis. *Amyloid* 2016; 23: 58–63.
6. Yanagisawa A, Ueda M, Sueyoshi T, *et al*. Amyloid deposits derived from transthyretin in the ligamentum flavum as related to lumbar spinal canal stenosis. *Mod Pathol* 2014; 28: 201–7.
7. Sekijima Y, Yoshida K, Tokuda T, *et al*. Familial transthyretin amyloidosis. In: Adam MP, Ardinger HH, Pagon RA, *et al*., editors. *GeneReviews*. Seattle, WA: University of Washington; 1993–2018. Cited May 2018. https://www.ncbi.nlm.nih.gov/books/NBK1194/
8. Plante-Bordeneuve V. Transthyretin familial amyloid polyneuropathy: an update. *J Neurol* 2018; 265: 976–83.
9. Sattianayagam PT, Hahn AF, Whelan CJ, *et al*. Cardiac phenotype and clinical outcome of familial amyloid polyneuropathy associated with transthyretin alanine 60 variant. *Eur Heart J* 2012; 33: 1120–7.
10. Jacobson DR, Alexander AA, Tagoe C, *et al*. The prevalence and distribution of the amyloidogenic transthyretin (TTR) V122I allele in Africa. *Mol Genet Genomic Med* 2016; 4: 548–56.
11. Gillmore JD, Booth DR, Pepys MB, *et al*. Hereditary cardiac amyloidosis associated with the transthyretin Ile122 mutation in a white man. *Heart* 1999; 82: e2.
12. Quarta CC, Buxbaum JN, Shah AM, *et al*. The amyloidogenic V122I transthyretin variant in elderly black Americans. *N Engl J Med* 2015; 372: 21–9.
13. Ikeda SI, Makishita H, Oguchi K, *et al*. Gastrointestinal amyloid deposition in familial amyloid polyneuropathy. *Neurology* 1982; 32: 1364–8.
14. Adams D, Gonzalez-Duarte A, O'Riordan WD, *et al*. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. *N Engl J Med* 2018; 379: 11–21.
15. Benson MD, Waddington-Cruz M, Berk JL, *et al*. Inotersen treatment for patients with hereditary transthyretin amyloidosis. *N Engl J Med* 2018; 379: 22–31.
16. Maurer MS, Schwartz JH, Gundapaneni B, *et al*. Tafamidis treatment for patients with transthyretin amyloid cardiomyopathy. *N Engl J Med* 2018; 379: 1007–16.

DOI: https://doi.org/10.1016/j.pathol.2018.12.423

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) in leukaemic phase without skin lesions: a diagnostic and management challenge

Sir,
We report a case of an 84-year-old female with blastic plasmacytoid dendritic cell neoplasm (BPDCN), having been diagnosed following an acute leukaemia-like presentation. There were no skin lesions. Full blood count was normal 7

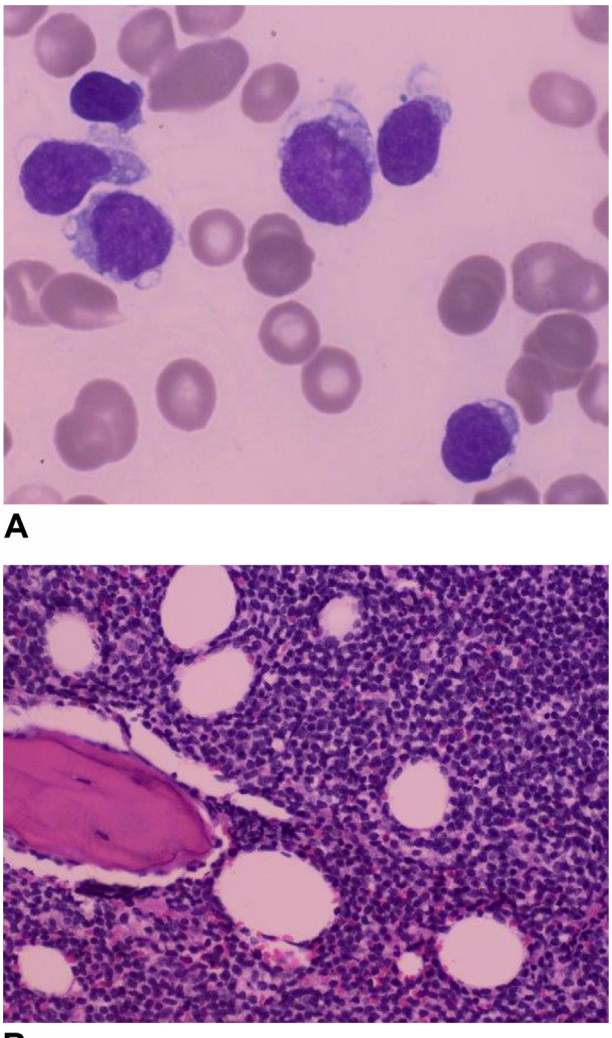

Fig. 1 (A) Bone marrow aspirate with peri-nuclear vacuolation – glycogen-containing microvacuoles in a pearl necklace pattern localised along the cell membrane. (B) Bone marrow trephine with heavy, diffuse infiltration of medium sized lymphoid cells.