Table 1 Characteristics of patients with faecal parasitic infections

Variable	Giardia lamblia (*n*=34)	Cryptosporidium spp (*n*=18)
Mean age, years ± SD	46.3 ± 26.5	24.2 ± 21
Length of stay, mean days	6.7	2.2
Overseas travel	2	4
Immunosuppression	2	2
Chronic/persistent diarrhoea[a]	11	0
Faeces microscopy (wet)	11	1
Another bacterial pathogen isolated on stool concurrently	2	1
Presentation with non-diarrhoeal symptoms[b]	11	2
Empirical therapy metronidazole	8	5
Definitive therapy metronidazole	10	0
Definitive therapy nitazoxanide	0	3
Symptomatic treatment only[c]	16	14
Patients who represented to ED following discharge due to ongoing symptoms	3	3
Resolution of symptoms[d]	21	11
Resolution attributed to antibiotics (impact)	9	3

[a] Chronic/persistent was mentioned on the patient history in eMR, pathology orderable or pathology request form.
[b] Fever, bloating and abdominal pain.
[c] Intravenous fluid, antispasmodic and antimotility drugs.
[d] Details from electronic record.

workflow of the microbiology laboratory would improve efficiency (high throughput and quicker turnaround time) and avoid the missed clinical cases seen with predefined criteria-based testing. The clinicians could then elect to treat those patients who do not resolve their symptoms quickly or those who are immunosuppressed. We acknowledge that in certain cases (returned travellers with diarrhoea, refugees/migrants), faecal multiplex PCR or EIA for *Giardia/Cryptosporidium* alone may not be adequate. These patients would benefit from extended faeces testing by microscopy with permanent stains and other tests for detection of other parasitic and helminth causes of chronic diarrhoea.

In summary, when using predefined clinical criteria to test for *Giardia* and *Cryptosporidium*, 70% of these cases would have been missed based on the laboratory request form. Even in cases where clinical information was actively sought from the eMR, about 52% of cases were missed. Not testing formed stool specimens would result in missed cases without too much reduction in the laboratory workload of faecal testing. We believe the only way of improving detection of *Giardia* and *Cryptosporidium* is to perform testing on all cases of community-onset diarrhoea. Although antigen detection/EIA may be useful in some small regional laboratories, broad panel multiplex PCR or faecal parasite PCR should be the preferred test in other laboratories.

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

R. Chavada[1], D. Maze[2], D. Dewit[1]

[1]Department of Microbiology and Infectious Diseases, NSW Health Pathology, Central Coast, NSW, Australia; [2]Department of Medicine, Gosford and Wyong Hospitals, Central Coast LHD, Gosford, NSW, Australia

Contact Dr Ruchir Chavada.
E-mail: ruchirchavda@gmail.com

1. Horton B, Bridle H, Alexander CL, Katzer F. Giardia duodenalis in the UK: current knowledge of risk factors and public health implications. *Parasitology* 2019; 146: 413–24.
2. Pönka A, Kotilainen H, Rimhanen-Finne R, *et al*. A foodborne outbreak due to Cryptosporidium parvum in Helsinki, November 2008. *Euro Surveill* 2009: 14.
3. Shane AL, Mody RK, Crump JA, *et al*. 2017 Infectious Diseases Society of America clinical practice guidelines for the diagnosis and management of infectious diarrhea. *Clin Infect Dis* 2017; 65: 1963–73.
4. Huang SH, Lin YF, Tsai MH, *et al*. Detection of common diarrhea-causing pathogens in Northern Taiwan by multiplex polymerase chain reaction. *Medicine (Baltimore)* 2018; 97: e11006.
5. Beatty N, Nix D, Matthias K, Al Mohajer M. Efficacy and cost comparison between a rapid multiplex polymerase chain reaction gastrointestinal pathogen panel versus conventional stool analysis techniques in suspected cases of infectious diarrheal disease at a tertiary medical center. *Open Forum Infect Dis* 2016; 3.
6. Currie S, Stephenson N, Palmer A, Jones B, Alexander C. Under-reporting giardiasis: time to consider the public health implications. *Epidemiol Infect* 2017; 145: 3007–11.
7. Lindo J, Levy V, Baum M, Palmer C. Epidemiology of giardiasis and cryptosporidiosis in Jamaica. *Am J Tropl Med Hyg* 1998; 59: 717–21.
8. Alexander CL, Currie C, Pollock K, Smith-Palmer A, Jones BL. An audit of Cryptosporidium and Giardia detection in Scottish national health service diagnostic microbiology laboratories. *Epidemiol Infect* 2017; 145: 1584–90.
9. Chalmers R, Atchison C, Barlow K, *et al*. An audit of the laboratory diagnosis of cryptosporidiosis in England and Wales. *J Med Microbiol* 2015; 64: 688–93.
10. Budu-Amoako E, Greenwood SJ, Dixon BR, Barkema HW, Mcclure JT. Foodborne illness associated with Cryptosporidium and Giardia from livestock. *J Food Protect* 2011; 74: 1944–55.
11. Amadi B, Mwiya M, Musuku J, *et al*. Effect of nitazoxanide on morbidity and mortality in Zambian children with cryptosporidiosis: a randomised controlled trial. *Lancet* 2002; 360: 1375–80.
12. Rossignol JF, Ayoub A, Ayers MS. Treatment of diarrhea caused by Cryptosporidium parvum: a prospective randomized, double-blind, placebo-controlled study of nitazoxanide. *J Infect Dis* 2001; 184: 103–6.
13. Granados CE, Reveiz L, Cuervo LG, Uribe LG, Criollo CP. Drugs for treating giardiasis. *Cochrane DB Syst Rev* 2009. https://doi.org/10.1002/14651858.CD007787
14. Wensaas K, Langeland N, Hanevik K, *et al*. Irritable bowel syndrome and chronic fatigue 3 years after acute giardiasis: historic cohort study. *Gut* 2012; 61: 214–9.
15. Escobedo A, Almirall P, Cimerman S, Rodríguez-Morales A. Sequelae of giardiasis: an emerging public health concern. *Int J Infect Dis* 2016; 49: 202–3.

DOI: https://doi.org/10.1016/j.pathol.2019.04.007

Lymphocyte involvement in nivolumab-induced autoimmune myositis

Sir,
Nivolumab is an IgG4 monoclonal antibody to PD-1 that is currently approved for treatment of patients with advanced non-small cell lung cancer (NSCLC) who experience progression of disease after platinum-based chemotherapy. Common immune mediated adverse events include rash, diarrhoea/colitis, hepatitis, pneumonitis and hypothyroidism. We report a case of biopsy proven immune-mediated myositis after a single dose of nivolumab.

A 61-year-old male with a background history of metastatic NSCLC (*KRAS*, *EGFR*, *ALK* and *ROS1* wild type) presented to a rural hospital with a 5 day history of upper and lower limb weakness, and difficulty swallowing after being

started on nivolumab 11 days prior. The patient had no significant past medical history. His only regular medication at the time of presentation was allopurinol for gout. He had no previous history of statin use.

His initial neurological examination demonstrated a pattern of weakness that was symmetrical, involving both upper and lower limb groups, with more pronounced weakness in the proximal muscle groups. He had normal power of his distal muscles. Throughout the next 5 days, he developed distal muscle weakness and swallowing difficulty. There was tenderness on palpation of the biceps, triceps and quadriceps muscles. However, he had a normal tone, non-exaggerated reflexes, sensation and co-ordination. He had no other neurological features of an upper motor neuron lesion. He also had no cutaneous features of dermatomyositis. Speech pathology assessment identified severe pharyngeal dysphagia. His ECG was sinus rhythm and did not show any previous myocardial infarction. A CT of the brain revealed no acute intracranial pathology. Initial blood tests were unremarkable other than a significantly raised creatinine kinase (CK) of 7540 U/L (normal 30−170 U/L).

A provisional diagnosis of autoimmune myositis was made. The patient was commenced on high dose corticosteroids and transferred to a tertiary centre for further management.

Video fluoroscopy confirmed severe pharyngeal dysphagia and supplementary nasogastric feeding was advised. A weaning dose of prednisolone was continued as the serum CK gradually fell, from a peak of 7540 to 153 one week later. The patient's muscle power and swallow both improved significantly and he was discharged home. Nivolumab was ceased indefinitely.

Further biochemical testing was arranged to determine the cause of myositis. ANA was mildly positive and ENA was negative. Antibodies to Ro52, EJ, OJ, PL-12, PL-7, SRP, Jo-1, PM-Scl 75, PM-SCL 100, Ku and Mi-2 were not detected, making a diagnosis of polymyositis, systemic sclerosis or anti-synthetase syndrome unlikely. Notably, anti-NT5C1A, anti-HMGCR and the anti-p155 antibodies were negative. These results did not support a diagnosis of inclusion body myositis (IBM), statin-induced myopathy or paraneoplastic myositis, respectively.

A muscle biopsy was performed during admission. Biopsies were obtained from the patient's left triceps and quadriceps. The light microscopy findings in both muscles were of multifocal myonecrosis, with numerous macrophages associated with the necrotic fibres, as well as lesser numbers of lymphocytes. There were numerous regenerating fibres. Focal lymphocyte invasion of non-necrotic fibres was seen in longitudinal muscle sections. There were no rimmed vacuoles or sarcoplasmic inclusions. The inflammation was confined to the skeletal muscle, without extension beyond the epimysium. On immunohistochemistry, there was sarcolemmal and sarcoplasmic MHC-I positivity within non-necrotic fibres as well as focal sarcolemmal MHC-II staining.

There was striking associated CD3+/CD5+ T-cell perivascular lymphocyte cuffing affecting small calibre endomysial and perimysial vessels (Fig. 1A,B), focally showing full-thickness extension through the vessel wall without associated fibrinoid necrosis. The T-lymphocyte population included small numbers of CD4+/FOXP3+ presumptive regulatory T-cells (Treg) and equal numbers of CD4 and CD8 positive cells. Cytotoxic CD8+ T-lymphocytes (CTLs) co-expressed granzyme B. PD-1 was co-expressed in 16% of T-lymphocytes. There was no difference in the immunophenotype of endomysial or perivascular lymphocytes.

MHC-I and MHC-II myopositivity were in keeping with an immune-mediated myositis. Despite a moderate increase in cytochrome c oxidase (COX) negative muscle fibres, there were no further features to support the diagnosis of IBM.

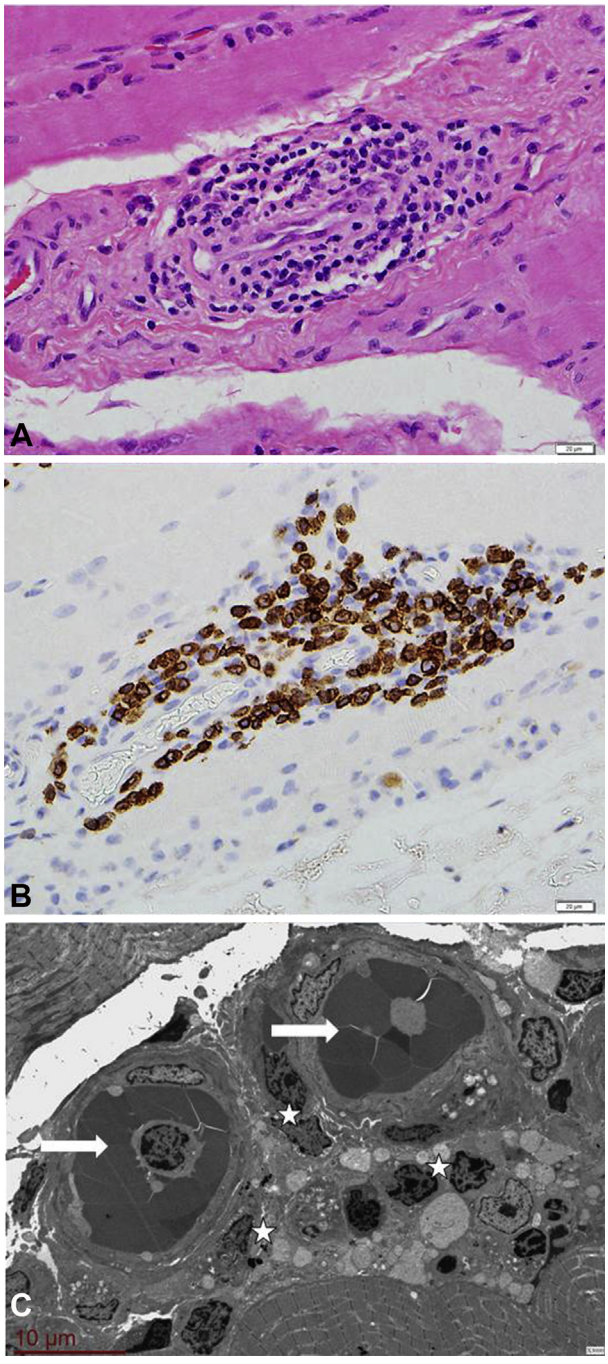

Fig. 1 (A) Small perimysial blood vessel showing concentric cuffing by small lymphocytes, with extension into the vessel wall (H&E). (B) Immunohistochemical staining for T-lymphocyte marker CD3 on a similar perimysial blood vessel to (A), highlighting the T-lymphocyte predominant vasculitis (CD3). (C) Electron micrograph on a similar perimysial blood vessel (lumina shown with white arrows) with T-lymphocytes present in a perivascular distribution and within the vessel wall (stars) (3000×).

C5b/9 (membrane attack complex) staining was negative in capillary endothelium and muscle fibres, excluding dermatomyositis. Further tests on serum for anti-U1RNP and anti-NXP2 antibodies were negative, and also did not support the diagnosis of mixed connective tissue disease or dermatomyositis.

Electron microscopy showed a monophasic process characterised by multifocal myonecrosis with early regenerating fibres, endomysial and perimysial lymphocytes and perivascular lymphocytes with invasion of the vessel walls and associated reactive endothelial changes (Fig. 1C). There were no tubulofilamentous inclusions or rimmed vacuoles to suggest a diagnosis of IBM.

Immune-related adverse events (irAEs) are being increasingly described for immune checkpoint inhibitors,[1] however myositis as a potentially life-threatening neurological irAE to nivolumab monotherapy is rare.[2] In the case described, we consider the findings of widespread perivascular T-lymphocytes and monophasic immune-mediated myositis to be part of the same process, presumptively caused by activated CTLs targeting muscle fibres and associated vasculature. The CD8+ effector lymphocytes were accompanied by a significant CD4+ T-cell population, including numerous presumptive Tregs. This has not previously been described in case reports of nivolumab-induced myositis,[3,4] despite the morphological inflammatory changes conforming to the polymyositis-like findings described in these case reports.[3] Alternative causes of immune-mediated myositis and connective tissue disorder overlap syndromes were considered as differential diagnoses. The light microscopy findings, immunophenotype of the inflammatory changes and additional investigation results, including negative myositis and other autoimmune auto-antibodies, excluded conventional immune-mediated myositides, such as dermatomyositis or IBM, and connective tissue disorders. The relatively monophasic nature of the histopathological changes also argued against malignancy-associated polymyositis. Due to the temporal relationship of onset of symptoms after nivolumab administration, lack of a known co-morbid myopathology and histological, biochemical and immunological features supporting T-lymphocyte mediated myositis, we believe that our findings are consistent with nivolumab-induced myositis.

With the increase in the number of approved indications for immune checkpoint inhibitors, clinicians need to be aware of rare and potentially life-threatening irAEs.

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

Benjamin Koh, Kilian Tuite, Adnan Khattak, Jason M. Dyke

Fiona Stanley Hospital, Perth, WA, Australia

Contact Dr Jason Dyke.
E-mail: jason.dyke@health.wa.gov.au

1. Tocut M, Brenner R, Zandman-Goddard G. Autoimmune phenomena and disease in cancer patients treated with immune checkpoint inhibitors. *Autoimmun Rev* 2018; 17: 610–6.
2. Astaras C, de Micheli R, Moura B, *et al*. Neurological adverse events associated with immune checkpoint inhibitors: diagnosis and management. *Curr Neurol Neurosci Rep* 2018; 18: 3.
3. Bourgeois-Vionnet J, Joubert B, Bernard E, *et al*. Nivolumab-induced myositis: a case report and a literature review. *J Neurol Sci* 2018; 387: 51–3.
4. Kimura T, Fukushima S, Miyashita A, *et al*. Myasthenic crisis and polymyositis induced by one dose of nivolumab. *Cancer Sci* 2016; 107: 1055–8.

DOI: https://doi.org/10.1016/j.pathol.2019.02.006

Urinary bladder stone due to adenine phosphoribosyltransferase deficiency: first genetically confirmed case in a Chinese patient

Sir,

Urinary stone disease is uncommon in prepubertal children. Its presence, if recurrent or with no apparent cause, is a strong indication for further investigations of a possible underlying inborn error of metabolism. Amongst these possible inborn errors, adenine phosphoribosyltransferase (APRT) deficiency (OMIM #614723) is an autosomal recessive inborn error of adenine metabolism, which has been reported globally in Japan, France and Iceland.[1] The deficiency of APRT activity is characterised by excessive production of 2,8-dihydroxyadenine (DHA) (Fig. 1). This water-insoluble compound is cleared in the kidneys, where it may further precipitate as crystals leading to urolithiasis or crystalline nephropathy, while some patients may be asymptomatic.[1]

It is important to diagnose APRT deficiency early because it is readily treatable with allopurinol, and if left undiagnosed patients may potentially develop devastating consequences such as recurrent urinary stone disease, end-stage renal failure requiring renal replacement therapy,[2] or even recurrent crystalline nephropathy in renal allograft.[3] Early diagnosis and treatment may improve renal function and achieve normal growth and development in children with APRT deficiency.[4]

Here we report the first Chinese family with genetically confirmed APRT deficiency. The proband was a 30-month-old Chinese boy born full term to non-consanguineous parents. His perinatal, developmental and family histories were unremarkable. He presented with recurrent urinary tract infection one week after initial presentation. There was no haematuria and no noted stone passage. Physical examination showed a round suprapubic mass which was also noted on plain radiograph (Fig. 2A), and confirmed by ultrasonography to be a bladder stone (Fig. 2B). The kidneys otherwise were normal in size and no renal mass lesion was detected. Initial blood tests showed elevated white blood cell count 18.8 $\times$ 10^9/L, predominantly neutrophils. Biochemical tests of the blood and urine, including blood gas, plasma calcium, phosphate, amino acids, and urine calcium, oxalate, cystine, organic acids and pH, were unremarkable (Supplementary Table 1, Appendix A). Urine microbiological studies showed white blood cell >100 per µL, and culture grew *Morganella morganii*. The patient was subsequently put on intravenous piperacillin and tazobactam, and arranged for surgical stone removal. Intraoperative cystoscopy showed a single