Table 1 Original bilirubin values, bilirubin values after addition of paraproteins (corrected) and percentage recovery

Original bilirubin, $\mu\text{mol/L}$	Bilirubin after IgA addition	Recovery %	Bilirubin after IgG addition	Recovery %	Bilirubin after IgM addition	Recovery %
43	48	111	54	126	4	10
39	44	113	48	123	24	69
52	56	108	44	85	4	8
39	36	92	36	92	12	31
68	46	68	48	71	16	24

The final IgA, IgG and IgM concentrations were 23 g/L, 13.6 g/L and 17.5 g/L, respectively.

The initial patient's results and the present study suggest that the Abbott Alinity c bilirubin assay is negatively affected at an IgM paraprotein concentration of 60 g/L (initial patient sample). The subsequent study showed that an IgM concentration of 17.5 g/L was capable of lowering the measured bilirubin values. A dilution series may be a useful method to evaluate an unexpectedly low bilirubin result in a sample with a paraprotein and to identify the dilution at which the paraprotein interference is eliminated. Additionally, when investigating a lower than expected bilirubin value measured using Abbott Alinity c assay, in a sample with a detected IgM concentration, it may be prudent to analyse bilirubin using another method.

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Restricted migration of polyclonal IgG on immunofixation gel electrophoresis in a case of IgG4-related disease

Sir,

A 71-year-old male was admitted to the hospital with cough, fatigue and left parotid and left submandibular gland swelling over the previous several months. He had a background

history of hypercholesterolaemia on atorvastatin, and hypothyroidism on thyroxine. His blood investigations were nondescript with a <4 mg/L C-reactive protein, and mildly raised erythrocyte sedimentation rate (44 mm/hr). His haematological and biochemical profiles were unremarkable. Fine needle aspirates of the salivary glands revealed reactive lymphoid hyperplasia with no evidence for malignancy.

He had a positron emission tomography (PET) scan which showed increased uptake in the parotid glands [standardised uptake values (SUV)max 7.1], the cervical lymph nodes (LN) (SUVmax 6.9), paratracheal LNs (SUVmax 4.1) and mediastinal lymph nodes (SUVmax 4.1). Peripheral blood flow cytometry did not reveal any evidence of B cell clonal disease. Due to concerns of an underlying lymphoproliferative disorder, he had exploratory surgery of his neck, and two enlarged lymph nodes were resected. The samples were sent for histological study and no features were seen to suggest lymphoma, granuloma or metastatic malignancy. However, sections showed reactive lymph nodes with marked capsular and mild parenchymal fibrosis with a background mixed chronic infiltrate including plasma cells, eosinophils and histiocytes. On IgG4/IgG staining, large numbers of plasma cells were present with an IgG4/IgG ratio of >70%. No storiform fibrosis was noted.

Serum samples were collected for protein electrophoresis (EPG) and immunofixation electrophoresis (IFE) analysis. This was initially diagnosed by an external laboratory as an IgG-kappa paraprotein. Repeat bloods were performed in our laboratory. The IFE showed a dense focal yet polyclonal migration of IgG in the fast gamma region (Fig. 1A). Subsequently, serum IgG subclasses were measured using SPAPlus turbidimetry which quantified a remarkable IgG4 level of 12.76 g/L (reference range 0.04–0.07). Serum free light chain studies demonstrated a normal kappa:lambda ratio of 1.57 (0.26–1.65), effectively ruling out a monoclonal IgG paraprotein.

Based on serological and histological findings, the patient was diagnosed with IgG4-related disease (IgG4-RD) and he was treated with prednisolone and methotrexate. Serial EPG/IFE and serum analysis revealed declining IgG4 levels and dissolution of the apparent restricted band (Fig. 1B,C). Six months following treatment, he reported resolution of his cough, fatigue and glandular swelling.

To further examine this laboratory phenomena, we examined a series of patients that had similar fast gamma region migrating IgG bands on IFE gel. Five patients were visually identified during a 3-month period in our laboratory. All of these patients were histologically and clinically diagnosed with IgG4-RD and had varying quantities of elevated serum IgG4 level ranging between 3 and 12 g/L. There were no light

Fig. 1 Serial immunofixation electrophoresis (IFE) gels. (A) Serum IFE gel showing a dense polyclonal IgG in fast gamma region at diagnosis (IgG4 level=12.76 g/L). (B) Serum IFE gel showing a reduction in dense polyclonal IgG, 2 months post-treatment (IgG4 level=5.75 g/L). (C) Serum IFE gel showing further reduction in dense polyclonal IgG, 4 months post-treatment (IgG4 level=3.50 g/L).

chain restrictions. We also examined two patients that had IgG4-RD (but IgG4 <2 g/L) that did not have an appreciable fast gamma region-migrating dense band, suggesting that the approximate threshold of visualisation on IFE is an IgG4 level around 2–3 g/L.

IgG4-RD is an immune-mediated chronic inflammatory condition of unknown aetiology presenting with mass-forming lesions. These can affect almost any organ and can mimic malignant, infectious and other inflammatory disorders.¹ IgG4-RD was first described in 2003 in unrelated conditions that shared elevated serum IgG4 levels and common histological findings which included lymphoplasmacytic infiltrate (>40% expressing IgG4 plasma cells), storiform fibrosis and obliterative phlebitis.² Histological examination of biopsy specimen is the gold standard in confirming IgG4-RD; clinical symptoms and serological findings are needed to confirm diagnosis. Serum IgG4 level is quantified in the laboratory using turbidimetry or nephelometry. About 60–70% of the patients suffering from IgG4-RD have an elevated polyclonal serum IgG4 level.³ Due to a variety of clinical manifestation of IgG4-RD and mimicking of other diseases, there is usually a delay in diagnosis of IgG4-RD.

During the workup of complex conditions, serum EPG/IFE may be requested. EPG/IFE is generally performed where symptoms suggest a monoclonal gammopathy. A monoclonal band, also known as an M protein, generally appears as homogenous, restricted, well-demarcated band in an EPG and can be further typed by IFE.⁴

IgG4 has a distinct pattern of migration in fast gamma region.⁵ Normally, IgG4 serum levels are the lowest in comparison to other IgG subclasses and therefore are invisible on EPG/IFE gels which have a lower limit of detection of about 1 g/L. In the above case, a dense polyclonal IgG is noted in fast gamma region on IFE which did not appear to be homogenous or monoclonal. A monoclonal IgG4 paraprotein, on the other hand, is homogenous in appearance and has light chain restriction (Fig. 2), therefore suggesting monoclonality.

Fig. 2 Clear distinct band of an IgG kappa paraprotein (IgG4=11.5 g/L). Note the kappa light chain restriction.

In conclusion, the appearance of dense focal polyclonal IgG in fast gamma region on IFE is suggestive of elevated IgG4. When this is noted, we would suggest reflex testing of serum IgG subclasses to assist clinicians in diagnosing the possibility of IgG4-RD, and consider adjunct testing (such as serum free light chains) to investigate for possible IgG4 paraprotein. However, raised serum IgG4 is not specific for IgG4-RD and may also be found in a variety of other disorders such as malignancies, infections, autoimmune diseases, primary immunodeficiencies, idiopathic interstitial pneumonitis and vasculitides.⁶ An IgG4 level of >1.35 g/L has a diagnostic sensitivity and specificity of 97% and 80%, respectively, for IgG4-RD.⁷ As a result of our observations, we now have a standardised comment that accompanies the IFE result, prompting clinicians to consider additional testing. This case report highlights the importance of the laboratory in identifying polyclonal IgG with restricted polyclonal fast gamma mobility to avoid a misdiagnosis of a monoclonal gammopathy.

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Cutaneous clear cell sarcoma with an epidermal component mimicking melanoma

Sir,

Malignant cutaneous tumours composed of spindled and epithelioid cells often present a diagnostic challenge, with a range of differential diagnoses including keratinocytic and adnexal carcinomas, melanoma and various sarcomas. Morphological clues should be sought to aid correct diagnosis, sometimes supplemented by the judicious use of immunohistochemistry or even molecular techniques. The presence of an epidermal component is usually interpreted as evidence of a precursor lesion in cases of melanoma or keratinocytic carcinoma and helps guide selection of further ancillary tests. Here, we present a case of primary cutaneous clear cell sarcoma with an intraepidermal component, initially considered to represent a nodular melanoma. To the best of our knowledge, this is the first reported case of cutaneous clear cell sarcoma exhibiting prominent Pagetoid spread, closely mimicking melanoma and highlighting the potential diagnostic pitfall of relying on intraepidermal growth patterns for the diagnosis of cutaneous tumours.

A 30-year-old man presented with an ulcerated nodular cutaneous lesion on the distal third toe, which had been present for several years. Excision revealed a 21 mm nodule eroding into bone (Fig. 1A) which histologically demonstrated a nested pleomorphic spindled and epithelioid cell tumour containing occasional multinucleated giant cells (Fig. 1B). Tumour cells had abundant amphophilic cytoplasm without discernible pigmentation (Fig. 1C). A nested junctional component was present, as well as single tumour cells showing Pagetoid spread (Fig. 1D) highlighted on immunohistochemistry for S100 (Fig. 1E). The tumour was also SOX10 positive with focal positivity for HMB45 at its deep and peripheral aspects (Fig. 1F,G). Immunostains for cytokeratins, Melan A, SMA and desmin were negative. The tumour was initially considered to represent a T4b nodular melanoma.

The tumour recurred and the patient developed regional nodal metastasis in six of 14 inguinal nodes. Mutation testing of *BRAF* and *NRAS* were negative, and he was commenced on adjuvant nivolumab. However, the patient recurred on treatment, developing pulmonary and bone metastases. An escalated regimen of combined ipilimumab/nivolumab was

poorly tolerated, with the patient developing severe, immune-related hepatitis. On further disease progression, he was treated with multiple lines of systemic therapy, including low dose ipilimumab, combination nivolumab and lenvatinib, and combination carboplatin and paclitaxel. Tumour tissue was sent for comprehensive targeted mutation testing through the Australian Genomic Cancer Medicine Centre's Molecular Screening and Therapeutics (MoST) Study which identified an *EWSR1-ATF1* gene fusion and a *TERT* promoter mutation.¹ In light of the new molecular findings, the original pathology was reviewed and considered to represent an *EWSR1-ATF1* fusion positive cutaneous clear cell sarcoma with an epidermal component. Crizotinib was initiated, but subsequent positron emission tomography/computed tomography scans showed disease progression.

Clear cell sarcoma was initially described as a deep-seated tumour arising from tendons and aponeuroses, with a predilection for the lower limbs of young patients.² Given the morphological similarities, stainable intracytoplasmic melanin pigment, diffuse S100 expression and the presence of ultrastructural melanosomes, clear cell sarcoma is sometimes referred to as 'melanoma of soft parts', and thought to be derived from the neural crest.³ Early case series found that the tumour rarely involved the subcutis or dermis, leading the authors to conclude that an absence of epidermal component or history of melanoma could strongly favour a diagnosis of clear cell sarcoma over melanoma.^{3–5}

First reported in 2009, there have now been 30 reported cases of primary cutaneous (dermal) clear cell sarcoma.^{6–8} The vast majority of cutaneous presentations showed typical morphological features of clear cell sarcoma; however, many of these features overlap with those of melanoma. Multinucleated floret-like giant cells are a characteristic feature of clear cell sarcoma and can be diagnostically helpful when present, but are not seen in all cases. Occasionally the tumour may expand the dermal papillae and encroach on the dermoepidermal junction. If this occurs, there is frequent discohesion and clefting artefact around individual nests, closely resembling an intraepidermal component.^{6,8} There have only been two reported cases of cutaneous clear cell sarcoma where single cell lentiginous growth along the junctional epidermal zone has been noted.^{6,9} To the best of our knowledge, our case here is the first to demonstrate prominent Pagetoid spread within the epidermis, closely mimicking an *in situ* component of primary melanoma. The extensive ulceration is also a unique feature of our case, suggesting a greater degree of epidermal destruction than previously described in clear cell sarcoma, and adding to the difficulty in distinguishing the tumour from an aggressive melanoma.

Morphological distinction of clear cell sarcoma from melanoma can be aided by appreciating the relative lack of intralesional heterogeneity and cytological atypia within the dermal component in clear cell sarcoma compared to the typical morphology of a thick melanoma. Multinucleated giant cells are more often seen in clear cell sarcoma and, when present in melanoma, usually display a more haphazard distribution of pleomorphic nuclei, rather than the floret-like arrangement of monomorphic nuclei seen in those of clear cell sarcoma.⁸