Thirdly, a LA had been identified on a sample taken 4 months prior in the setting of diplopia and confusion. Testing for a LA was specifically requested to complement a broader autoimmune screen. The LA positivity at that time was by APTT and based on non-correction in immediate mixing tests, with DRVVT screen being negative. Guidelines for LA testing state that positivity by only one test method is necessary for reporting LA presence.⁶ At that presentation, a routine APTT was not requested and the patient was not being investigated for bleeding symptoms. It is likely that the FVIII inhibitor was present at that time and misclassified as LA, in the absence of other haemostasis assay results.

Finally, the inhibitor had an exceedingly high titre at 679.0 BU/mL. The Bethesda assay is used to measure the strength of FVIII inhibitors. This functional assay involves mixing serial dilutions of patient plasma with normal pooled plasma, incubating at 37°C for 2 hours, and measuring FVIII activity by clotting time. One BU is defined as the amount of inhibitor that results in 50% residual factor VIII activity. The strength of our patient's inhibitor may explain the non-selective reduction in other intrinsic clotting factor assays. Interestingly, Bethesda inhibitor titre correlates poorly with treatment response and FVIII activity is a much better predictor of outcome.⁷ Our treatment approach of steroids and rituximab in patients with a FVIII <1 IU/dL or >20 BU/mL aligns with the recently published international guidelines by Tiede *et al.*⁸

The approved risk management plan for alemtuzumab mandates monthly blood tests for 48 months after the last infusion, to monitor for the development of more common autoimmune complications including thyroid autoimmunity and immune thrombocytopenic purpura. In Australia, these tests are facilitated by a nationwide automated monitoring and alerting system, the Bloodwatch Lemtrada Monitoring service. However, routine testing of coagulation factors is not mandated.

In summary, we present the case of a 45-year-old man who developed AHA 18 months after alemtuzumab therapy for MS. The inhibitor was fast-acting and a remarkably high titre, both atypical laboratory features. This case highlights the importance of vigilance for AHA in patients treated with alemtuzumab, and performing a complement of coagulation assays in the investigation of a bleeding phenotype and prolonged APTT.

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Paraprotein interference of automated total bilirubin measurement

Sir,

Paraproteins have been identified as a common cause of interferences in automated chemistry methods. Paraprotein interference in the total bilirubin assay on the Roche Modular¹ and Beckman Coulter AU5400 and AU2700² have been reported. Accordingly Roche³ and Beckman Coulter⁴ bilirubin assay reagent instructions for users include a statement that monoclonal gammopathy, especially IgM gammopathy may cause unreliable results. The Abbott Alinity c bilirubin assay has not been reported to be affected by paraprotein in the sample.

We wish to report monoclonal gammopathy as an interfering agent in the Abbott Alinity c total bilirubin assay (Diazo method)⁵ prompted by the following case history.

A 45-year-old male patient's serum specimen analysed on the Abbott Alinity c (Abbott, USA) automated instrument indicated a bilirubin result of <9 µmol/L [reference interval (RI) 4–20]. All other routine biochemistry results were normal except total protein 120 g/L (RI 60–85), albumin 38 g/L (RI 39–50) and globulin 82 g/L (RI 23–39). Previous results for bilirubin in this patient were <9 on several occasions but 12 months prior the bilirubin result was 54 µmol/L; with total protein, albumin and globulin levels of 116 g/L (RI 66–83), 37 g/L (RI 36–47) and 79 g/L (RI 26–41), respectively. At that time, the laboratory analytical system was Roche Cobas (Roche Diagnostics, USA). Therefore, an interferent in the Alinity c bilirubin assay was suspected, and the most likely candidate was thought to be paraproteins. Serum protein electrophoresis (capillary) and immunofixation (Sebia, France) indicated an IgG kappa band of 60 g/L (RI <0.1).

Five adult patient samples with bilirubin within the RI (4–20 µmol/L; NormBILI) and five samples with bilirubin levels above RI (HighBILI) were spiked with equal volumes of adult serum samples with known concentrations of paraproteins IgA, IgG and IgM (46 g/L, 27 g/L and 35 g/L, respectively). The bilirubin concentrations in the patient sera with high

paraproteins used to spike the samples were all within RI (4–20 $\mu\text{mol/L}$) as measured by the Abbott Alinity c assay. All spiked samples were analysed on the Abbott Alinity c modules for total bilirubin and the results were tabulated.

When sera with high paraprotein concentrations were added to samples, bilirubin values measured were lower than the expected values. In the NormBILI group, values measured after the addition of paraprotein rich samples resulted in little or no change for those with IgA and IgG additions, while the addition of IgM caused a marked drop in bilirubin. A correction for dilution was not used in these samples because the sera with paraproteins had bilirubin values also within the RI.

For the HighBILI group, a correction factor for dilution (1:2) was used when the paraprotein rich sera were added because the sera with paraprotein had bilirubin concentrations within the RI and much lower than those in the original samples.

Figures 1 and 2 depict the change in bilirubin when IgA, IgG and IgM rich sera were added to NormBILI samples and HighBILI samples, respectively. Table 1 indicates the original high bilirubin values in Fig. 2 (HighBILI), the measured values following correction for dilution and the percentage recovery after addition of each of the paraproteins.

The average recovery of bilirubin after the addition of IgA, IgG and IgM was 98%, 99% and 28%, respectively. IgM produced a more significant effect on recovery compared to that of IgA or IgG.

These findings suggest that the presence of paraprotein can affect the measured concentration of bilirubin using the Abbott Alinity assay. Furthermore IgM appears to exert a more significant effect on bilirubin measurement. It is likely that the IgM pentameric structure causes a greater disruption when measuring azobilirubin at the prescribed wavelength either by absorption or refraction.

Fig. 1 Bilirubin concentrations ($\mu\text{mol/L}$) after the addition of IgA, IgG and IgM rich sera to samples with bilirubin within the reference interval.

Fig. 2 Bilirubin concentrations ($\mu\text{mol/L}$) after the addition of IgA, IgG and IgM rich sera to samples with bilirubin higher than the reference interval (corrected for dilution).

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