that in SM and myelofibrosis, microdissected mast cells harboured both the *KIT* and *JAK2* V617F mutations in four of five patients, and microdissected CD15+ myeloid cells had both the *KIT* and *JAK2* V617F mutations in one of five patients. This implies that both molecular defects can be expressed in both components of the disease.⁷ Recent data have also identified additional molecular abnormalities, including *SRSF2*, *ASXL1*, *RUNX1* and *TET2* mutations, which are often acquired by neoplastic stem cells prior to *KIT* mutation, suggesting a multi-mutated stem cell disease and step-wise process of oncogenesis.⁸ In 45% of core binding factor AML the *KIT* D816V mutation is also present⁸ and reports of the pathogenic leukaemic translocation t(8;21) being detected in the neoplastic mast cells also hint that the mast cells may have developed from the leukaemic clone.⁹

In contrast, SM with coexisting lymphoid or plasma cell malignancies are thought to be clonally separate as the *KIT* D816V mutation present in the neoplastic mast cells has not been detected in malignant lymphocytes.¹⁰ Neoplastic mast cells are known to be a source of multiple cytokine production and *in vitro* studies have demonstrated that the cells are able to support the growth of malignant lymphoid cells through CD154-CD40 signalling and plasma cells via the production of cytokines such as IL-1, IL3, IL-6 and SCF.¹¹ Identification of oligoclonal immunoglobulin bands in the sera of some patients with SM also indirectly supports the notion that there is increased proliferation of plasma cells through neoplastic mast cells.¹¹ It is also possible that there is no pathophysiological link between the SM and IgM MGUS. As the prevalence of MGUS increases with age, with up to 4.5% of patients aged 70–79 having a diagnosis of MGUS, the association with SM in this patient may be an incidental finding.¹² Further, the ET evolved over time as it was not evident at the time of the IgM MGUS or SM diagnosis given the initial normal platelet counts.

Lastly, this case also highlights the importance of careful inspection of other cell lineages not affected by SM and the need for ongoing monitoring for the development of other haematological malignancies.

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Thinking fast not slow: a fast-acting, high-titre acquired factor VIII inhibitor

Sir,

A 45-year-old male was brought to the emergency department in February 2020 following a long lie with a reduced level of consciousness. The patient had a past medical history of relapsing remitting multiple sclerosis (MS), diagnosed in August 2016 following a step-wise deterioration in gait and mobility over 16 months. His MS was initially managed with fingolimod. In August 2017, radiological disease progression on magnetic resonance imaging mandated escalation of disease modifying therapy and a first cycle of alemtuzumab was administered without complication. A second cycle of alemtuzumab followed in August 2018.

On examination, he had bruising and swelling of both the right knee and ankle. Ultrasound imaging was consistent with a right suprapatellar bursa haematoma and haemarthroses of the right knee and ankle joints. A computed tomography scan of the brain did not reveal evidence of an acute intracranial haemorrhage.

Investigations noted a normal full blood count (Table 1). His prothrombin time was 14.9 s, international normalised ratio 1.0 and activated partial thrombin time (APTT) prolonged at 98 s. The patient had no history of heparin or direct oral anticoagulant use. Immediate and extended mixing studies were performed. The immediate 1:1 APTT mix was 91 s with minimal change after extended incubation, indicating the presence of a fast-acting inhibitor. A lupus anticoagulant (LA) screen was negative by dilute Russell's viper venom time. The LA screen by high and low phospholipid APTT reagents showed a clear positive ratio. However, the incomplete correction of the clotting time to normal by the high phospholipid reagent was a clue that an inhibitor other than a LA may be present.

Table 1 Laboratory parameters on admission

Laboratory variable	Result	Normal range
White cell count	$7.5 \times 10^9/L$	$4-10 \times 10^9/L$
Haemoglobin	147 g/L	130–170 g/L
Platelet count	$175 \times 10^9/L$	$150-400 \times 10^9/L$
Thrombin time	15.4 s	14.0–20.0 s
Prothrombin time	14.9 s	12.0–15.0 s
Activated partial thrombin time	98 s	25–37 s
Historical activated partial thrombin time (August 2016)	30 s	25–37 s
Lupus APTT final ratio	1.63	<1.1
Dilute Russell viper venom screen time	39 s	<45 s
APTT mix: low phospholipid for LA test	95	
APTT mix: high phospholipid for LA test	52	
Historical lupus APTT final ratio (October 2019)	1.58 ratio	<1.1
PFA100 collagen/epinephrine CT	76 s	80–170 s
PFA100 collagen/ADP CT	100 s	60–120 s
vWF antigen	334 IU/dL	55–200 IU/dL
vWF activity	309 IU/dL	50–185 IU/dL
vWF activity: vWF antigen ratio	0.93	
Factor VIII: vWF antigen ratio	<0.001	0.75–1.70
Factor VIII level	<2.0 IU/dL	70–220 IU/dL
Factor IX level	111 IU/dL	70–150 IU/dL
Factor XI level	>27 IU/dL	55–130 IU/dL
Chromogenic Factor VIII level	<1.0 IU/dL	70–220 IU/dL
Bethesda assay	679 BU/mL	<0.5 BU/mL

The patient had (F)actor VIII, IX and XI levels performed, which showed FVIII <2, FIX of 111% and FXI of >29% (Table 1). A chromogenic FVIII was also performed and measured <1.0%. The FIX and FXI assays showed non-parallelism with increasing sample dilution, which when combined with the low FVIII level suggested presence of a FVIII inhibitor. Bethesda assay confirmed the presence a FVIII inhibitor at a titre of 679.0 Bethesda units/mL (BU/mL).

The patient's immediate bleeding complications were managed with recombinant FVIIa and tranexamic acid. He received three days of 1 g methylprednisone daily followed by a weaning course of prednisone over 8 weeks. He was commenced on rituximab ($365\text{mg}/\text{m}^2$) weekly. The patient received a total of four doses. His inhibitor titre response is shown in Fig. 1. He has had no further bleeding complications in the 12 months since presentation.

Fig. 1 Changes in FVIII inhibitor titre, FVIII levels and activated partial thromboplastin time (APTT) from presentation in February 2020.

A review of a previous presentation 3 months prior (October 2019) for diplopia and confusion, likely secondary to Wernicke encephalopathy, identified the presence of a prolonged APTT for which LA positivity by APTT tests was described (Table 1). However, additional haemostasis testing was not requested. The patient had no bleeding symptoms at that time.

Acquired haemophilia A (AHA) is a rare, acquired bleeding disorder characterised by the production of an autoantibody against FVIII.¹ AHA has been associated with pregnancy, drug exposures, autoimmune disease, and malignancy.² Alemtuzumab, a humanised anti-CD52 monoclonal antibody, is associated with a significant risk of secondary autoimmunity and has previously been linked to the development of AHA in a patient with MS.³ However, our case exhibits several key differences which are important considerations when suspecting any presentation of AHA.

Firstly, our patient had a fast-acting inhibitor. A mixing study is the recommended initial investigation for a prolonged APTT. Test plasma is combined with normal pooled plasma in a 1:1 ratio and an APTT measured. An extended mixing study involves incubating the test: normal pool plasma mix at 37°C for 1–2 hours prior to performing an APTT. A time and temperature independent inhibitor, such as a lupus anticoagulant or direct-acting oral anticoagulant, is present when the Rosner index is $\geq 12.5\%$ the local cut-off level, i.e., there is no correction of the abnormal APTT on immediate mixing. FVIII inhibitors are classically 'slow-acting' and suspected if the incubated mix APTT is ≥ 7 seconds longer than the immediate mix by local cut-off criteria. However, our patient's APTT 1:1 incubated mix sample did not show slow-acting characteristics, increasing by only 2 seconds above the immediate mix APTT. It is hypothesised that FVIII inhibitors are slow acting because they require dissociation of FVIII from von Willebrand factor before they can exert their inhibitory action. Previous research indicates that antibody specificity in AHA is primarily directed against the C2 and A2 domains of FVIII.⁴ We speculate that the fast-acting nature of our inhibitor may be due to its specificity being directed against an alternate 'exposed' factor VIII epitope.

Secondly, our patient's acquired FVIII inhibitor interfered significantly with FIX and FXI APTT-based one-stage clotting factor assays tested at the initial standard dilution. Assays for multiple factors each at multiple sample dilutions was the key to determining the FVIII specificity of the inhibitor. Inhibitors to single clotting factors usually result in a selective reduction of that factor assay relative to the other factors. However, an apparent reduction in other clotting factor one-stage assays may be seen if there is an additional inhibitory effect on the target factor contained in the deficient plasma used in those assays.⁵ The differential diagnosis for a non-selective reduction in all intrinsic clotting factor assays includes a strong lupus anticoagulant or an inhibitor to contact factors such as FXII or prekallikrein. To exclude these differentials, a chromogenic FVIII assay was performed resulting in a level of <1%. This is a two-stage assay that combines diluted test plasma with FIIa, FIXa, FX, phospholipid and calcium. The FXa generated and the rate of colour formation as it acts on a chromogenic substrate, is proportional to the amount of FVIII present in the sample plasma. This assay is independent of prekallikrein, FXII and FXI and confirmed the selectivity of our inhibitor for FVIII.

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