Spuriously low IgG levels in lupus-associated mixed cryoglobulinaemia type II complicated by iatrogenic immunoglobulin-induced immune complex vasculitis

Sirs,

A 45-year-old female patient with SLE, monoclonal gammopathy (MG), and cutaneous vasculitis was referred for further diagnostics and treatment. The diagnosis of SLE was made 3 years before and based on discoid skin lesions, butterfly-rash, arthritis, photosensitivity, positive ANA with Ro-specificity, and complement activation (C4 <0.06g/l [0.1-0.4g/l], C3d 11.1mg/l [<9mg/l]). At initial presentation there was no internal organ involvement detected. Laboratory examination revealed elevated concentrations of rheumatoid factor (RF) (7510IE/ml [<16 IE/ml]), IgM (3.49g/l [0.4-2.3g/l]), and C3d (12.5mg/l), whereas C4 (<0.06g/l) and IgG (1.9g/l [7-16g/l]) were markedly reduced. Serum albumin was normal, proteinuria absent. Mixed cryoglobulinaemia type II (MC) consisting of IgMκ and polyclonal IgG with an elevated cryocrit (6%) was detected. Hepatitis-B or -C virus (HCV) infections were excluded. Bone marrow biopsy was consistent with MG of undetermined significance. Due to low IgG concentration, possibly caused by previous immunosuppressive treatment, an intravenous Ig (IVIG) substitution had been performed one year ago without complications. Considering the recently even lower concentration of IgG, the normal serum viscosity, the requirement of aggressive immunosuppression for active disease and recurrent purulent infections of the paranasal sinuses, we decided to repeat IVIG substitution. One day after the application of 20g of IVIG the patient developed new cutaneous lesions at the left lower leg (Fig. 1A). Moreover, a worsening of the pre-existing lesions at the right toes (Fig. 1B), and an increase of creatinine (89.9µmol/l to 104.5µmol/l) and C3d (12.5 to 14.5 mg/l) occurred. Assuming immune complex (IC) vasculitis, immediate treatment with intravenous corticosteroids, cyclophosphamide, and iloprost was started and led to an improvement of local lesions and renal function (67.1µmol/l). There are some articles reporting IVIG-induced adverse reaction in patients with MC due to IC formation between the IgM paraprotein displaying RF-activity and the administered IgG and subsequent depositions in the skin and kidneys (1). In patients with HCV-associated MC the administration of rituximab, a chimeric IgG1κ antibody, may lead to a flare of vasculitis, also caused by IC-formation induced by IgMκ exhibiting RF-activity (2). Considering the relatively mild susceptibility to infections in our patient, despite his severe hypogammaglobulinaemia, we suspected a falsely low measurement of IgG due to IC-formation *in vitro*. Previously, it had been shown in a RF-positive patient with Sjögren's syndrome, that a monoclonal IgMκ could react with the Fc portion of IgG, leading to the formation of soluble complexes at 37°C and an insoluble state at temperatures lower than 33°C (3). Hence, we repeated measurements of Ig and viscosity in sera processed either at room temperature (RT) or 37°C, assuming a sedimentation of large Ig complexes in RT-processed samples into the pellet. Confirming our hypothesis, we measured markedly higher Ig levels when processing at 37°C in comparison to RT (IgG: 7.86 *vs*. 5.51; IgM: 3.83 *vs*. 1.87; IgA: 1.63 *vs*. 1.13 g/l). Incubation with anti-IgM-antibodies required for nephelometry, revealed a visible precipitation only in the sample handled at 37°C, indicating a higher Ig concentration (Fig. 1C). Moreover, there was a higher viscosity in the sample processed at 37°C as compared to the one processed at RT (1.33 *vs*. 1.17 mPas) (Fig. 1D). Repetition of Ig measurements 3 months after IVIG substitution confirmed our findings of false low Ig levels in RT-handled samples (IgG: 3.01 *vs*. 1.71; IgM: 5.58 *vs*. 2.93; IgA 1.59 *vs*. 1.24 g/l). Hypogammaglobulinaemia in MC and MG occurs most likely due to hypercatabolism secondary to *in vivo* IC-formation (3). In addition, during processing at ambient temperature cryoprecipitates may form and get lost by precipitation into the blood pellet. Ongoing further studies should reveal if false low Ig quantification due to *in vitro* cryoprecipitation is a general and relevant problem in patients with MC. The application of rituximab, which is an effective treatment in some patients with MC, is known to cause flares of vasculitis, particularly in patients with high cryocrit and low C4 (2). Our case is confirming these findings for the administration of Ig in lupus-associated MC and suggests that also low Ig levels predispose for this treatment complication. Further studies are needed to clearly define risk factors for *in vivo* IC-formation in these patients.

In conclusion, in a RF-positive SLE patient with MC hypogammaglobulinaemia likely due to *in vivo* consumption by IC formation may be further accentuated by temperature-dependent cryoprecipitation *in vitro*. Considering the possible complications and costs of IVIG substitution, we propose to regularly assess Ig levels and viscosity in warm samples, in order to avoid false indications for IgG replacement in these patients.

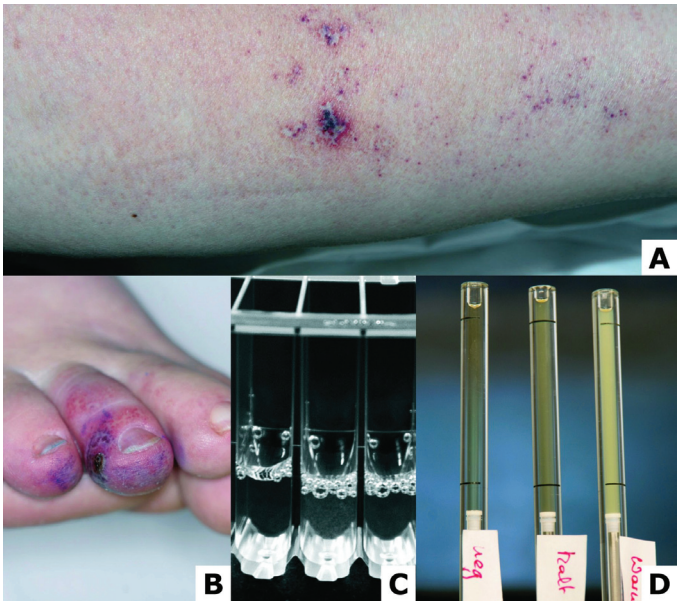

Fig. 1. Cutaneous vasculitis at the left lower leg (**A**) and right toes (**B**). A visible precipitation after incubation with anti-IgM-antibodies for nephelometric measurements occurred only in the serum sample which was processed at 37°C, which already indicated higher immunglobuline concentration (**C**, middle). There was a higher viscosity in nephelometric measurement of the sample handled at 37°C in comparison to the room tempered sample and the control (distilled water) (**D**, left: control, middle: sample processed at room temperature; right: sample processed at 37°C).

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