Acquired Glanzmann's thrombasthenia with IgG and IgA against activated αIIbβ3

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Introduction

Glanzmann's thrombasthenia (GT) is a rare haemostatic disorder characterised by an impaired function of integrin αIIbβ3. We present a case study of an acquired GT and the modification of two assays; flow cytometry and ELISA, to confirm the presence of autoantibodies against **αIIbβ3.**

Background

84-year-old male with known chronic lymphocytic leukaemia (CLL) presented with 6 week history of extensive bruising. No previous history of bleeding tendencies and no family history. Six years prior to presentation the patient was diagnosed with extranodal marginal zone lymphoma and received six cycles of rituximab-bendamustine to complete remission. Biopsy two years ago confirmed CLL diagnosis and assigned watch-and-wait approach. CLL is known to be associated with autoimmune complications but it is believed that this is the first case documented of acquired GT associated with CLL. [1]

The patient's platelet count of 167 x10^9/L was within normal range (150 - 400 x10^9/L), however the white blood cell (WBC) count was abnormally high at 111.9 x10^9/L (normal range: 3.0 – 10.0 x10^9/L). Extrinsic and intrinsic factors were normal, apart from Factor VIII which was mildly raised. Von Willebrand factor (VWF) screen was also raised.

Materials & Methods

Platelet Aggregation

Platelet aggregometry analysed using a PAP8E profiler with platelet rich plasma obtained from sodium citrate samples. The following platelet agonists were employed at specific concentrations: arachidonic acid (1mM), adenosine diphosphate (1, 2.5 & 5μM) collagen (1 &4μg/ml) and ristocetin (0.5 & 1.5mg/ml). The maximum aggregation (MA) is recorded in percentage after 6 minutes of aggregation at 37°C.

Flow Cytometry

Citrate whole blood collected from patient and healthy controls. Platelets isolated from healthy controls were incubated with control and patient plasma in the presence/absence of platelet inhibitors (apyrase and prostaglandin E1) or collagen. Gated through platelet markers: CD42b, CD41 and CD61.

ELISA

Plate coated with purified human αIIbβ3 activated by LIBS2. Control and patient plasma incubated on the plate and analysed following ELISA steps, absorbance at 490nm.

Results

- Severe abnormality detected via platelet aggregation (figure 1). MA to all agonists were reduced (<50%). Healthy normal control run alongside patient and produced normal platelet aggregation results; thus excluding laboratory error.
- IgA and IgG only detected on the surface of the activated platelets (without inhibitors ± collagen). Suggesting that the antibodies target the active conformation of αIIbβ3. (Figure 2A&B)
- IgA and IgG present in patient plasma against platelets (confirmed by ELISA). Levels of IgA and IgG decreased over time from presentation (Figure 3A&B).

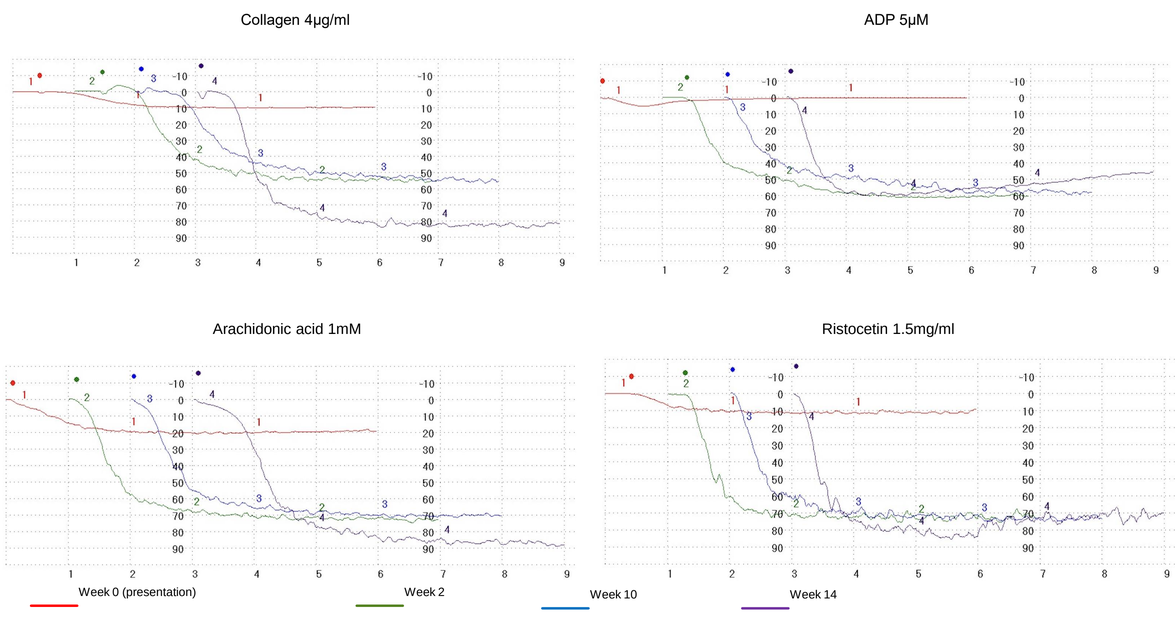

Figure 1. Platelet aggregation over time.

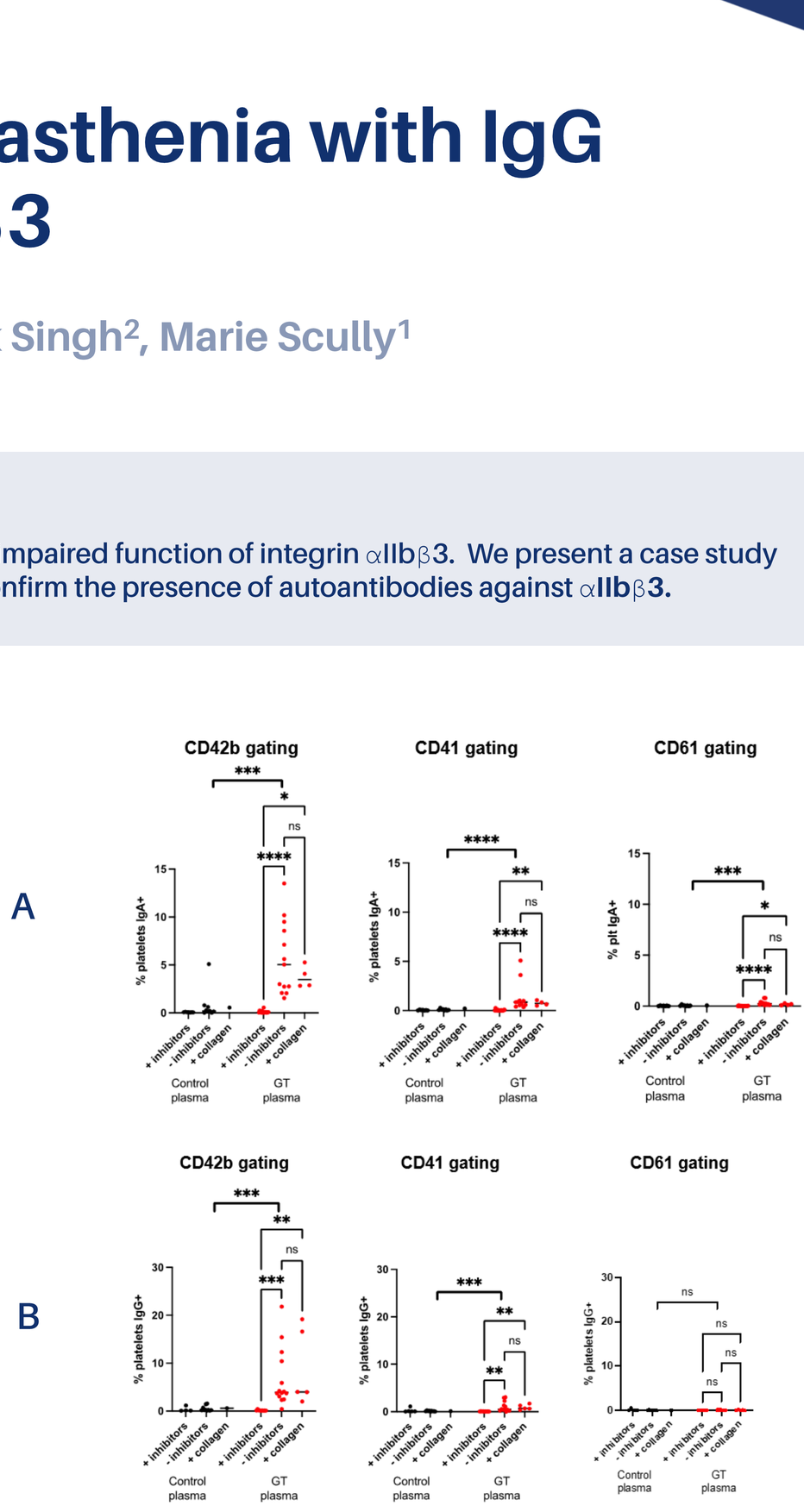

Figure 2. Presence of IgA and IgG against activated αIIbβ3 demonstrated through flow cytometry and ELISA. (A, B) Presence of IgA (A) or IgG (B) on control platelets after incubation with control/GT plasma, in the presence or absence of inhibitors (apyrase and prostaglandin E1) or collagen, and when gated through three different platelet markers—CD42b, CD41 or CD61. Data analysed using Kruskal–Wallis and unpaired student's t-test. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$

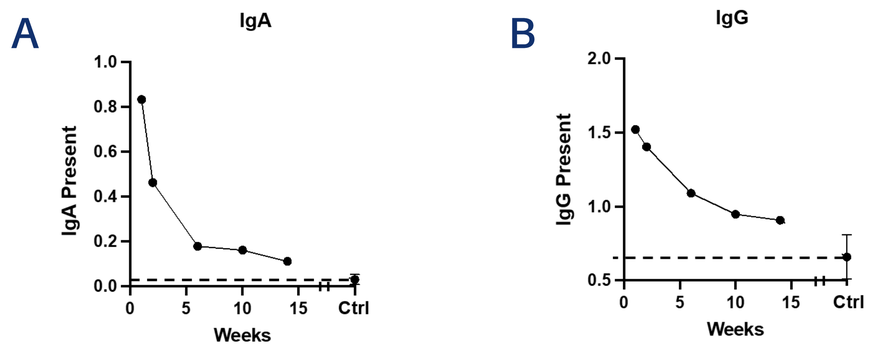

Figure 3. The levels of IgA (A) and IgG (B) in the patient's plasma over time. ELISA method against control plasma.

Conclusion

- The IgA and IgG autoantibodies target the activated platelets and not in their resting state. Evidence supported through the platelet count of the patient – was not thrombocytopenic.
- New case of acquired Glanzmann's thrombasthenia.
- To our knowledge, this is the first case to report the presence of IgA in acquired Glanzmann's thrombasthenia.
- A new insight into the pathogenesis of Glanzmann's thrombasthenia and how it can be approached for effective treatment.

References

1. Tsang, M. and Parikh, S.A., (2017) A concise review of autoimmune cytopenias in chronic lymphocytic leukemia. *Current hematologic malignancy reports*, *12*, pp.29-38.
2. Constantinescu-Bercu, A., McCann, S., Hmaid, A., de Groot, R., Singh, D., Gohil, S.H., Thomas, M., Scully, M. and Westwood, J.P., (2023) Acquired Glanzmann's thrombasthenia with IgG and IgA against activated αIIbβ3. *British Journal of Haematology*.