Title: Presenting features and platelet anomalies in WAS: one centre's experience

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To the Editor,

In 2011, Moratto *et al*[1]. identified age at haematopoietic stem cell transplantation (HSCT) as a risk modifier for complications in Wiskott-Aldrich syndrome (WAS); of note, complications appear to be 1.5 times more likely in children transplanted at ages 2-5, compared to younger than 2 (RR = 1.53; CI, 0.90-2.58; P=0.130). This reinforces the importance of prompt recognition, diagnosis and assessment for HSCT in patients with suspected WAS.

We evaluated the presenting features in boys with *WAS* mutations transplanted at the Great North Childrens' Hospital, Newcastle-upon-Tyne between 1989 and 2015 in order to identify any delay in diagnosis and causative factors, including misdiagnosis, and clarify the role of mean platelet volume in making a diagnosis of WAS. We sought to identify key discriminating diagnostic features in order to aid timely diagnosis and transplantation of these patients.

Data was collected retrospectively from written and electronic medical records of 20 boys with *WAS* mutations, for which all families had given prior written consent. Information regarding date and nature of symptom onset, time elapsed until diagnosis, mean platelet volume at presentation, age at transplantation and outcome were recorded in an anonymised spreadsheet. Symptomatology was coded as one or more of the following: infectious and immunological, including autoimmunity; eczema; bruising and petechiae; and bleeding. No differentiation was made between WAS and XLT due to no significant difference in platelet counts, platelet size and bleeding episodes in these conditions.

Median age at symptom onset was 2 months (birth – 9 months) and at diagnosis was 1 year (2 months – 14.1 years). Median interval between symptom onset and diagnosis was 8 months (2 weeks – 13.8 years). The most common presenting symptom was bleeding (13 reports, 65%). The subsequent most common symptoms were bruising (11, 55%); eczema and skin disease (9, 45%) and immunological and infectious (7, 35%). Initial documented impressions included non-accidental injury (1), congenital cytomegalovirus infection (1) and suspected ITP (2).

The most common combination of symptoms at presentation was bruising with eczema, in 7 (35%), bleeding and bruising, 5 (25%); bleeding and eczema, 5 (25%); bleeding and infective/immunological, 4 (20%); bruising and infective or immunological, 4 (20%), infective or immunological and eczema, 1 (5%) patient. There was no documented family history of WAS or XLT.

Eight patients (40%) had a recorded mean platelet volume (MPV) following presentation to our unit. Median MPV was 4.85fl (range: 3.6 - 21); only 3 patients had a volume <5fl (Figure 1). One result was recorded as "low" and so was omitted from analysis.

Our results show a lag between onset of symptoms and confirmed diagnosis of WAS of 8 months. A diagnostic delay in WAS (and other primary immunodeficiency disorders) of >1 year is not uncommon; data gathered from the European Society for Immunodeficiencies online registry in 2004-6[2] suggests a median diagnostic delay of 1 year in WAS, based on a cohort of 51 patients. Other series report a median diagnostic delay of 1.5 years[3].

In order to expedite the diagnosis of patients with suspected WAS, factors that may aid recognition and diagnosis should be considered, as well as other causes of thrombocytopenia in childhood. Boys with WAS classically present with bleeding in infancy, followed by development of eczema, recurrent infection and eventual autoimmunity and malignancy. History of bleeding was reported in 13 (65%) of our patients, and was the sole symptom in 4

patients (20%). Bleeding diathesis may present in multiple ways: in our cohort, presentations included hematochezia, epistaxis, oral bleeding and one episode of bleeding from the umbilical stump. Although eczema was reported in 9 (45%) of patients, it did not occur in isolation and so may support, rather than raise, a diagnostic suspicion of WAS as in young children eczema is common.

The most common cause of isolated thrombocytopenia in children is immune-mediated thrombocytopenia, a diagnosis of exclusion characterised by a transient or persistent low platelet count. Boys with WAS are often diagnosed with ITP[4], and several patients in our cohort were initially given a diagnosis of ITP. However, ITP commonly presents later, has a shorter duration and is not associated with signs of eczema or infection. Blood film examination in ITP shows platelets that are larger than normal, while in WAS characteristic microthrombocytopenia is seen.

Classically, WAS may be differentiated from other platelet disorders through examination of MPV, which is commonly held to be <5fl[5], though without reference to data. As seen in Figure 1, only three of the eight patients who had MPV measured had platelets <5fl, and one had a MPV of 21 which is grossly outwith even the standard range of 7.2-11.7fl. This result may be explained by erroneous measurements by automated analysers, which measure platelets by the number of particles within a certain volume range. This poses an inherent problem in WAS, as platelets have abnormal morphology and volume leading to a falsely low platelet count and falsely elevated MPV. Therefore, mean platelet volume may not be useful in identifying WAS (although it may be helpful in distinguishing ITP from other, inherited causes of thrombocytopenia including WAS) and a blood film examination by a haematologist should always be considered.

This single-centre evaluation of the diagnostic journey of 20 boys transplanted for WAS supports existing data on diagnostic delay in primary immunodeficiency, especially in WAS and highlights some issues with using MPV to support a diagnosis of WAS. Our results were limited by the small number of patients studied, precluding the use of statistical tests; however, the centre is one of only two HSCT units in the United Kingdom that transplants such patients and in a condition as rare as WAS, cohort sizes are inevitably small.

Based on the common presenting features in this cohort and the symptomatology of WAS, the authors make the following suggestions to facilitate timely recognition of WAS and therefore improve potential outcomes from HSCT.

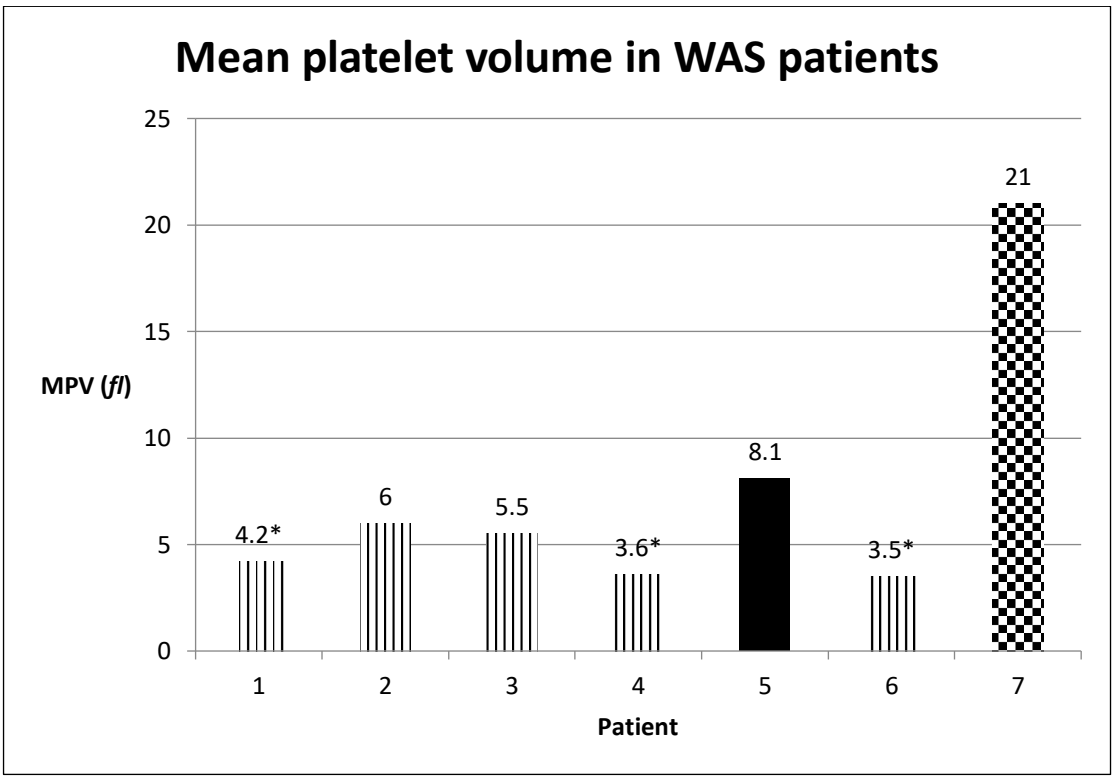
1. Consider WAS in boys with more than one of the following features:
    a. Family history of bleeding, recurrent infection or early death in childhood;
    b. Bleeding from more than one site, *or* significant haemorrhage (e.g. gastrointestinal or intracranial) from one site;
    c. Eczema or skin lesions in addition to history of bleeding or bruising;
    d. Symptoms of >6 months in duration, particularly if early in onset.
2. On suspicion of WAS, request examination of platelets on a blood film by an experienced haematologist and contact a regional centre with experience of paediatric immunology and/or HSCT for investigation of WASP expression by flow cytometry and/or *WAS* gene testing.
3. Low MPV may warrant further investigation for WAS, especially if platelet counts are low, but there are significant flaws in its calculation and so a normal or raised MPV should not exclude WAS.

References:

1. Moratto D, Giliani S, Bonfim C, et al. Long-term outcome and lineage-specific chimerism in 194 patients with Wiskott-Aldrich syndrome treated by hematopoietic cell transplantation in the period 1980-2009: an international collaborative study. *Blood*. 2011;118(6):1675-1684.
2. Eades-Perner A, Gathmann B, Knerr V, et al. The European internet-based patient and research database for primary immunodeficiencies: results 2004–06. *Clin Exp Immunol*. 2007;147(2):306-312.
3. Sokolic R, Oden N, Candotti F. Assessment of Immature Platelet Fraction in the Diagnosis of Wiskott–Aldrich Syndrome. *Front Pediatr*. 2015;3:49. doi:10.3389/fped.2015.00049.
4. Yazdani S, McGhee SA, Stiehm ER. *Chronic Complex Diseases of Childhood: A Practical Guide for Clinicians*. Universal-Publishers; 2011.
5. Thrasher AJ, Kinnon C. The Wiskott–Aldrich syndrome. *Clin Exp Immunol*. 2000;120(1):2-9. doi:10.1046/j.1365-2249.2000.01193.x

Figures:

Figure 1 - Mean platelet volume (in fl) at presentation as documented in the notes of 7 patients. One additional patient had MPV documented as "*low*" and so was excluded.



Black bars represent mean platelet volume within normal range (7.2-11.7fl). Striped bars represent mean platelet volume below the normal range. Checked bars represent mean platelet volume greater than the normal range. Asterisks (*) represent mean platelet volume <5fl.