Title: How I manage patients with Wiskott Aldrich syndrome

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Key words

Wiskott Aldrich syndrome

X-linked thrombocytopenia

Immunodeficiency

Haematopoietic stem cell transplant

Acknowledgements:

This work was supported by funding from The Wellcome Trust (090233/Z/09/Z AJT and 201250/Z/16/Z ER), National Institute for Health Research UCLH Biomedical Research Centre (SB), and National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London.

We would like to thank our patients and families for providing consent for photographs to be used in this review and Dr Kimberly Gilmour, Great Ormond Street Hospital for critical review of the manuscript.

Summary

Wiskott Aldrich syndrome (WAS) is a primary immunodeficiency disease resulting in recurrent infections, eczema and microthrombocytopaenia. In its classical form, significant combined immune deficiency, autoimmune complications and risk of haematological malignancy necessitate early correction with stem cell transplantation or gene therapy. A milder form, X-linked thrombocytopaenia (XLT), shares similar bleeding risk from thrombocytopaenia but is not associated with other significant clinical features and is generally managed conservatively. Here, we detail our approach to the diagnosis and treatment of classical WAS and XLT.

Introduction

Wiskott Aldrich syndrome (WAS) is a rare X-linked primary immunodeficiency disorder, with an incidence between 1 in 50,000 and 1 in 250,000 live births (Perry*, et al* 1980, Puck and Candotti 2006). In its classical form, WAS presents early in life with a triad of recurrent infections, eczema and microthrombocytopenia caused by loss of function mutations in the *WAS* gene (Derry*, et al* 1994). Expressed only in haematopoietic cells, *WAS* encodes the Wiskott Aldrich syndrome protein (WASp), a key actin cytoskeletal regulator that coordinates assembly of actin filaments in response to cell signalling events (Machesky and Insall 1998). Defects in WASp function have been shown to impair cellular processes in myeloid and lymphoid lineage cells including cell adhesion and migration, phagocytosis, immune synapse assembly (reviewed in (Thrasher and Burns 2010)) and more recently autophagy and inflammasome regulation (Lee*, et al* 2017). The pathogenesis of the platelet defect remains only partially understood and is thought to result from a combination of megakaryocyte dysfunction leading to small/ abnormally formed platelets (Ingrungruanglert*, et al* 2015, Sabri*, et al* 2006) and increased platelet destruction in the spleen (Grottum*, et al* 1969). Megakaryocyte numbers in bone marrow are typically normal (Grottum*, et al* 1969, Ochs*, et al* 1980).

Recognition of WAS is important as curative stem cell and gene therapies are available, without which median survival is reduced to 10-15 years as a result of infections, severe bleeding, autoimmune complications and haematological malignancies (Sullivan*, et al* 1994). Milder forms, known as X-linked thrombocytopenia, present with a similar bleeding phenotype but without other significant clinical features (Villa*, et al* 1995) and can generally be managed conservatively.

When we suspect WAS/ XLT

Low number of platelets is a universal feature of WAS and XLT, usually presenting in the first year of life and typically causing petechiae, easy bruising, spontaneous or prolonged bleeding. Where no prior family history has impacted obstetric and neonatal care, cephalohematoma related to an instrumental delivery is not an uncommon first presentation, or prolonged bleeding if circumcision is undertaken (Ochs*, et al* 2009). In toddlers, bruising can be the presenting feature and may raise concerns about non-accidental injury. The degree of thrombocytopenia is variable and can be classified based on platelet count as severe (<20 x 10^9/L), moderate (20-50 x 10^9/L) or mild (>50 x 10^9/L). The finding of small platelets in the context of thrombocytopenia is pathognomonic for WAS/ XLT (Andres*, et al* 2018) and the newly described but functionally related ARPC1B deficiency (Kahr*, et al* 2017). Diagnosis can be achieved by an experienced haematologist on blood film, which we have found to be more reliable than routine full blood count parameters, where a normal haemocytometer MPV does not rule out the diagnosis. Occasionally, mild-to-moderate

thrombocytopenia can present later in childhood, mimicking idiopathic thrombocytopenia (ITP) but without response to oral steroids. There are also reports of intermittent thrombocytopenia in XLT but these represent a rare subgroup (Medina*, et al* 2017, Notarangelo*, et al* 2002).

In contrast with thrombocytopenia, eczema and/or recurrent infections are variable features (Sullivan*, et al* 1994), but their presence in association with low platelet counts should prompt consideration of WAS/ XLT (Case 1). Autoimmunity and haematological malignancy are rarely the presenting features of classical WAS but can complicate disease course (Case 2).

How we diagnose WAS/ XLT

While microthrombocytopenia is highly suggestive of WAS, genetic analysis is the gold standard for diagnostic confirmation and plays important roles in management decisions and family screening. Over 300 mutations are published (Burns*, et al* 2004) and this number is increasing with wider availability of genetic screening. Mutations of all types (nonsense, insertions, deletions, splice site and missense) occur throughout the whole gene, although clustering of missense mutations in the first four exons of the gene with a number of hot spots have been described (Jin*, et al* 2004, Schindelhauer*, et al* 1996).

Details of the genetic mutation alone are often insufficient to predict the severity of the clinical phenotype (although can be helpful if previously described), but a combination of information about the mutation plus its impact on WASp levels enable genotype phenotype correlation (Imai*, et al* 2004, Jin*, et al* 2004, Liu*, et al* 2015) (Table 1). Therefore, we perform analysis of WASp expression as part of our diagnostic work up. WASp quantitation by western blotting has been superseded in our laboratory by flow cytometry which has been shown to be a robust and rapid test (Chiang*, et al* 2018). It is important to note that protein expression alone cannot absolutely be relied on for diagnosis as missense mutations can sometimes preserve normal levels of functionally impaired WASp. Similarly, apparently absent WASp expression may arise from disturbance of the epitope recognised by the detecting antibody.

Female mutation carriers can also be detected using flow cytometry, confirmed by genetic sequencing. While WAS carriers are usually asymptomatic, clinical features have occasionally been reported in girls, where clinical manifestations occur as a result of non-random X-inactivation and extreme lyonisation, with preferential use of the mutated X chromosome (Andreu*, et al* 2003, Takimoto*, et al* 2015). A very rare phenocopy condition, caused by deficiency of the WASp regulating protein, WIP, is inherited in an autosomal recessive manner which can impact both boys and girls (Lanzi*, et al* 2012).

<u>Classical WAS or XLT?</u>

Assignment of classical WAS or XLT is ultimately a clinical classification. Scoring systems have been published (Ochs*, et al* 2009, Zhu*, et al* 1995) but in practice we consider the presence of severe infections, any autoimmunity or haematological malignancy to indicate classical WAS (Table 1). As clinical features evolve over time, a diagnosis of XLT can only definitively be made after the age of two years. However, gene mutation details and protein levels can help to predict disease course in a young child in whom full clinical phenotype has yet to evolve. Patients with mutations that result in absence of WASp expression are predicted to have a severe clinical course (Imai*, et al* 2004) and we assign a diagnosis of classical WAS to these patients at an early stage to direct management (see below). Preservation of partial WASp expression (usually with missense or splice site mutations) is

associated with a milder outcome in cohort analysis but not absolutely predictive for an individual (Imai*, et al* 2004). A number of common hotspot missense mutations are reasonably predictive of XLT but even within in this subset, some patients have been described to acquire additional complications such as autoimmunity at a later stage (Albert*, et al* 2010). Finally, even within one family, phenotype can be somewhat variable presumably due to the influence of other genes, infections or epigenetic factors, although overall severity is generally consistent. As an example, we have looked after brothers who both required transplantation for classical WAS but whose grandfather had a mild course, effectively restricted to features of thrombocytopenia.

Typically, patients with classical WAS are described as having low numbers of $CD8^+$ T cells and dysgammaglobulinaemia, where IgG, IgA and IgM levels can be low or high as a result of altered humoral function. IgE levels are also typically raised. T cell proliferative responses are usually normal to the mitogen PHA but absent/ reduced in response to anti-CD3 antibody stimulation, reflecting the fact that T-cell receptor signalling requires actin polymerisation. All of these parameters are less disturbed in patients with XLT, although anti-CD3 T-cell responses are also frequently impaired. Patients with classical WAS often have good initial vaccine responses to protein antigens but usually poor polysaccharide responses (for example to pneumococcal polysaccharides) and low levels of isohemagluttinins. Since polysaccharide responses are difficult to assess under the age of two, as a result of immunological immaturity, they are in practice rarely measured. In our experience, patients with XLT make normal responses to protein antigens found in regular childhood vaccines and do not show substantial increased risk of infection or susceptibility to opportunists, despite minor immunological abnormalities.

How we manage WAS

Definitive therapy

Arguably the most important management decision that needs to be made when a child is diagnosed with WAS, is whether definitive therapy is indicated; either haematopoietic stem cell transplantation (HSCT) or stem cell gene therapy (GT). Regardless of initial clinical presentation, we refer all children with absent WASp expression and a genetic mutation consistent with classical WAS for early consideration of definitive treatment and do not wait for emergence of a severe clinical phenotype. We aim for transplantation within the first two years of life with sub-myeloablative conditioning, with excellent outcomes (Elfeky*, et al* 2018). Outcomes for children with WAS undergoing HSCT are also excellent internationally with survival rates over 97% (European cohort 1979-2001 97% (Ozsahin*, et al* 2008), UK experience 100% (Elfeky*, et al* 2018, Slatter*, et al* 2018)). Whilst use of sub-myeloablative conditioning regimens have reduced long-term effects, a number of post-transplant complications appear to be higher in WAS, including graft-versus-host disease (GvHD), infection in the context of prior splenectomy and autoimmunity. Although possible to preserve fertility with sub-myeloablative conditioning, infertility remains a substantial and as yet unquantified risk.

Gene therapy trials are in progress for management of classical WAS, at present restricted to patients without a fully matched donor. Although early WAS studies were hampered by late onset of haematological malignancy related to insertional mutagenesis (Braun*, et al* 2014) associated with gammaretroviral vectors, vector design modifications have improved safety. Recently reported studies have demonstrated good outcome data with no reported vector-related toxicity with

resolution of eczema, infections and improved autoimmunity (Aiuti*, et al* 2013, Castiello*, et al* 2015, Hacein-Bey Abina*, et al* 2015).

Currently we do not recommend definitive treatment for XLT as medical management is available and definitive therapy can result in long term complications including GvHD, infertility, secondary malignancy and death. Instead, we advise a wait and watch approach, with a low threshold for referral for HSCT or GT if disease severity progresses (e.g. development of autoimmunity). If parents are keen to explore definitive therapy even in the context of mild disease, they are referred for a HSCT discussion so that they have full information. In general, we do not recommend gene therapy for XLT where bleeding is the main clinical phenotype as correction of platelet numbers has been variable in clinical trials (Hacein-Bey Abina*, et al* 2015).

Definitive therapy for adults with WAS/ XLT remains a management challenge. In practice, few patients with uncorrected classical WAS reach adulthood and HSCT is rarely offered in adult primary immunodeficiencies (PID) as outcomes were historically poor. However, we recently published excellent outcomes (85% long-term survival at 10 years post HSCT) for a cohort of adults with different types of PID, making this a viable treatment option for carefully selected patients in specialist centres (Davila Saldana 2018, Fox*, et al* 2018). We have also successfully treated one classical WAS adult with GT, which has achieved substantial clinical improvement and provided proof of principle that GT is a viable option even for adults (Kohn 2017, Morris*, et al* 2017). Given the advantage of using autologous stem cells, thus avoiding GvHD, we view GT as a good option for adults with classical WAS and accumulated comorbidities. We also consider definitive therapy, mainly HSCT, in adult patients with an otherwise XLT phenotype who develop later onset autoimmunity or haematological malignancy. To date, we have considered the risk-benefit ratio of HSCT unfavourable for uncomplicated XLT in adults.

<u>Supportive therapy</u>

Supportive therapy for patients with classical WAS consists of prevention of infection, management of thrombocytopenia, autoimmune and autoinflammatory symptoms prior to definitive treatment (Table 3). In our practice, patients with XLT require little supportive treatment, except for management of thrombocytopenia.

Non-autoimmune thrombocytopenia

Thrombocytopenia in WAS/ XLT is universal and bleeding risk is a major management challenge for both groups of patients. Although life-threatening bleeding episodes, in particular gastrointestinal or intracranial bleeding, have been reported in 10-30% of patients (Albert*, et al* 2010, Mahlaoui*, et al* 2013), in our own cohort severe bleeding episodes requiring medical intervention were substantially lower (6% for classical WAS and 3% for XLT) (Rivers*, et al* 2018), possibly due to earlier access to definitive treatment for classical WAS and specific criteria for assigning a diagnosis of XLT. In our experience, severe bleeding in classical WAS is almost universally associated with the onset of autoimmune platelet consumption in addition to the intrinsic defect (see below).

As a result, our mainstay of thrombocytopenia management in classical WAS is early definitive therapy, typically within the first 2 years of life, to correct the platelet count and avoid emergence of autoimmunity. In the absence of active bleeding or a significant increase in petechiae/ bruising, we do not actively support the platelet count with platelet transfusions (even when $< 10 \times 10^9$/L) and intentionally minimise platelet transfusions to limit development of anti-platelet and anti-HLA antibodies which can complicate HSCT.

Management of thrombocytopenia in XLT is a lifelong process in the absence of definitive therapy. Parents are given general advice about avoidance of high risk activities such as contact sports and to seek prompt medical assessment for any significant head injuries. While we do recommend appropriate use of helmets for activities such as scooting and cycling, we do not generally recommend protective headgear for day-to-day activities or for toddlers learning to walk, in part because of compliance and stigma and in part because we consider this risk to be low. We have not to date seen emergence of autoimmune thrombocytopenia (AIT) in our XLT cohort, although this can rarely occur even in adulthood (Albert*, et al* 2010). Anxiety associated with thrombocytopenia usually results in significant restriction of activities which can have a substantial impact on quality of life of the child. For this reason, in recent years, we have advocated splenectomy for patients with XLT who have no significant infectious history and are at an age where polysaccharide vaccinations can be given. All patients receive pre-splenectomy booster vaccinations against pneumococcus, meningococcus (ACWY and B) and haemophilus influenzae type B, with protective vaccine responses ensured before proceeding. Although an increased incidence of sepsis in splenectomised patients is described in WAS (Albert*, et al* 2010, Lum*, et al* 1980), this risk can be significantly reduced with strict adherence to prophylactic antibiotics. We do not see a significant risk of infection post splenectomy in our cohort (all receiving antibiotic prophylaxis) and all patients with splenectomy for thrombocytopenia in XLT have shown an immediate and sustained platelet response with no episodes of thrombocytopenia relapse (Rivers*, et al* 2018).

Severe bleeding in WAS/ XLT is a medical emergency and requires urgent assessment with fluid resuscitation and blood product support as needed. For minor prolonged nosebleeds, use of IV tranexamic acid topically may be of use in avoiding cautery or packing. Platelet agonists such as Eltrombopag have been reported to have some effect in elevating the platelet count in WAS/ XLT (Gerrits*, et al* 2015) but to date we have not found these to be successful in children and have not utilised these in patients planned for definitive therapy. We do consider platelet agonists in adults with XLT if thrombocytopenia impacts quality of life and there is reluctance about splenectomy, but to date there is not sufficient experience to recommend this as first line treatment.

Autoimmune thrombocytopenia

Recognising development of autoimmune platelet consumption (AIT) on top of baseline thrombocytopenia in WAS can be difficult, particularly due to the poor correlation with antiplatelet antibodies, but is of particular importance as onset of AIT is associated with highest risk of significant bleeding (Rivers*, et al* 2018). We suspect AIT with the onset of significant increase in bruising/ petechiae or spontaneous bleeding and acute drop of platelets (usually to $< 10 \times 10^9$/L) from baseline. To confirm AIT, we recommend platelet transfusion with one and 24 hour increment assessment. Where the platelet count has not incremented substantially at one hour, or fallen significantly again by 24 hours post transfusion, we consider this to represent the onset of AIT and recommend treatment with high dose immunoglobulin (IVIg) and prednisolone (Table 3). When assessing response to therapy, we consider the improvement in clinical symptoms separate to the rise in platelet count as a significant factor in guiding therapy, as medical management of AIT is unlikely to lead to sustained rise above the patient's pre-AIT baseline. We have a low threshold for second line treatment with Rituximab where there is failure of platelet control following IVIg and prednisolone, or for recurrence of thrombocytopenia. We consider splenectomy for AIT only in patients with severe thrombocytopenia refractory to first and second line treatments and where a delay in definitive treatment is likely.

Prevention of infection

Severe or recurrent infections are frequently seen in classical WAS, including bacterial, viral, fungal and opportunistic organisms reflecting the broad functional immune defect (Table 2 and Case 2).

All patients with classical WAS are commenced on immunoglobulin replacement treatment at diagnosis, even if total immunoglobulin levels and vaccine responses are in the normal range. Almost without exception, immunoglobulin is administered by the subcutaneous route to achieve a total monthly dose of approximately 0.4 g/kg (Table 3). Parents are trained to deliver this at home on a weekly basis and despite severe thrombocytopenia, we have not encountered significant difficulties with bruising or haematomas. No further vaccinations are given once immunoglobulin is started, with the exception of annual inactivated flu vaccine. All live vaccinations are contraindicated as there is a risk of vaccine strain infection. In addition, patients with classical WAS are commenced on prophylactic antibiotics, typically co-trimoxazole to provide broad-spectrum antibiotic cover and include protection against Pneumocystis jiroveci. Prophylactic antifungal or antiviral treatment is considered on a case-by-case basis, for recurrent candidiasis, prior CMV viraemia or recurrent HSV disease.

Patients classified as XLT at our centre do not have significant infections by definition and are therefore not commenced on immunoglobulin replacement, are rarely commenced on antibiotic prophylaxis and receive full routine vaccination including live vaccines and BCG where indicated. For adults with XLT who wish to travel overseas, we recommend standard travel vaccinations, with the exception of yellow fever for which there is no documented experience in mild forms of PID.

Eczema and atopy

Eczema is extremely common at presentation in classical WAS and is frequently extensive and difficult to manage. Atopy and food allergy are strongly associated, with cow's milk protein allergy found almost universally in infants presenting with eczema (Lexmond*, et al* 2016, Tuano*, et al* 2015). Mild to moderate eczema can also be seen in XLT. We recommend standard treatment with emollients and topical steroids (Table 3). Topical tacrolimus is an option as a steroid-sparing agent. In severe cases specialist input from dermatology should be sought and more intensive treatments such as wet wraps or even oral steroids considered. For significant or early onset eczema, we recommend early dietician input and trial of hydrolysed formula/ cow's milk exclusion diet.

Autoimmunity/ autoinflammatory features

Autoimmunity occurs frequently in classical WAS (over 40% in our cohort (Elfeky*, et al* 2018)). In addition to AIT, other cytopenias (haemolytic anaemia and neutropenia) are common and managed with supportive care and immunosuppression. Arthritis, vasculitis and inflammatory bowel disease are other recognised but less common autoimmune features found in WAS and are managed with input from other specialties. Large vessel vasculitis can occasionally lead to aortic aneurysms and these are typically detected incidentally on radiological imaging for another purpose (Pellier*, et al* 2011). In our own practice, we do not routinely screen for the presence of large vessel vasculitis, since early definitive treatment should significantly reduce susceptibility.

IgA nephropathy is of particular interest as it is one of the more common autoimmune features associated with WAS mutations, appears to have a higher prevalence in patients with residual WASp expression and may present later in patients with an otherwise XLT phenotype (Albert*, et al* 2010, Imai*, et al* 2004, Liu*, et al* 2013, Shimizu*, et al* 2012). Increasing serum creatinine and proteinuria or episodes of haematuria in acute flares are presenting features (Case 3). Diagnosis is confirmed on

renal biopsy and treatment may require use of immunosuppression in the first instance. Progression is variable and may occur over years, but where renal transplantation is needed, careful discussions around appropriateness and timing of HSCT are warranted to balance the risks of nephrotoxicity from conditioning agents. We recommend screening for serum creatinine, blood pressure and proteinuria at routine follow-up appointments for all WAS/ XLT patients and seeking specialist renal advice where appropriate.

In addition to classical autoimmunity, other inflammatory complications can be seen that resemble those seen in other autoinflammatory disorders, including intermittent rashes and arthralgia. We postulate these are related to inflammasome activation (Lee*, et al* 2017) and have used colchicine and the IL-1 receptor antagonist anakinra with marked benefit in some cases.

Malignancy

Haematological malignancy, specifically lymphomas and leukaemia, is estimated to occur in approximately 10-20% of patients with classical WAS over time in the absence of curative therapy (Imai*, et al* 2004, Sullivan*, et al* 1994). EBV infection is associated with development of lymphoproliferative disease and we have a low threshold for investigation of new lymphadenopathy, particularly where EBV viraemia has been documented. Ultrasound is useful as a first line investigation, with biopsy where abnormal architecture is found. In the context of normal lymph node architecture and absence of B symptoms (fever, nights sweats or weight loss), we recommend a two week trial of Co-Amoxiclav in case of occult bacterial infection and re-consider biopsy if there is no improvement. Unusual infections, including mycobacteria, are seen in WAS and therefore biopsy samples should be sent for full microbiological assessment as well as for histology. Usual oncology protocols are used for treatment of malignancy, with a plan to move to definitive therapy in early remission.

Conclusion

Both supportive care and definitive treatment for Wiskott-Aldrich syndrome have improved substantially over the last two decades. Outcomes for HSCT are excellent and gene therapy is emerging as an attractive alternative option. The key decision for patient management is whether the combined genetic, protein and clinical phenotype indicate classical WAS or XLT. While supportive care remains the mainstay for patients with XLT, all patients with classical WAS should be referred early for definitive therapy before establishment of significant co-morbidities. Greater awareness of this rare disorder is the main challenge for improving diagnosis, with an ongoing need for education of paediatricians and haematologists to whom patients are most likely to first present.

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