ITALIAN PRIMARY IMMUNODEFICIENCIES STRATEGIC SCIENTIFIC COMMITTEE

WISKOTT-ALDRICH SYNDROME AND X-LINKED THROMBOCYTOPENIA

Recommendations for Diagnosis and Treatment

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AIM

The recommendations for the diagnosis and treatment of Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT) have been devised following the guidelines adopted by AIEOP in drafting protocols for the diagnosis and treatment of "orphan diseases" like primary immunodeficiencies.

Prompt registration of clinical and laboratory data is essential to check the validity of diagnostic criteria and establish the efficacy of available therapeutic strategies in patients with rare diseases for which controlled clinical trials have not yet been carried out and treatment recommendations are based on small patient cohorts. In particular, it is essential to keep updated registries for diseases like WAS and XLT which far from being distinct nosological entities increasingly appear to be the phenotypic end-points of a continuum of clinical disorders of varying severity linked to mutations of the same gene.
On the basis of these considerations, the key aims of these recommendations are to:

- Establish definite diagnostic criteria for the spectrum of diseases linked to mutations reducing or abolishing the function of the WASP protein and whose phenotypic spectrum extends from WAS to XLT
- Draw up and implement updated treatment recommendations applicable nationwide for patients with WAS
- Record the natural history of XLT, focusing on clinical evolution and immune status
- Devise possible therapeutic guidelines for less severe disease forms (XLT, possibly associated with minor immune changes), whose efficacy must be ascertained over time
- Adjust treatment protocols on the basis of prompt updates on clinical status and laboratory data for all patients enrolled in the Registry and the outcome of any controlled experimental trials and results obtained by other research groups.

The first part of these diagnostic and therapeutic recommendations outline the pathophysiological mechanisms and phenotypic spectrum underlying WAS and XLT. Available treatment options are also presented discussing any evidence of efficacy. The second part describes the diagnostic protocol common to WAS and XLT. *Inclusion criteria are listed in italics*. The third part summarises the treatment recommendations including the prevention of infection, surveillance and the therapeutic approach to the risk of haemorrhage, the treatment of autoimmune complications and indications for haematopoietic stem cell transplantation. *Key consensus recommendations are listed in italics*. The fourth part of the recommendations deals with the problems of genetic risk, including screening for carrier status in women at risk, and prenatal diagnosis. In this case, indications are merely informative since it is the individual patient's right to make an informed decision on whether to undergo genetic testing and embark on pregnancy. Lastly, the fifth part gives indications on the management of diseases commonly associated with WAS (eczema, tumours, rare infections).

1. INTRODUCTION

1.1 What are Wiskott-Aldrich syndrome and X-linked thrombocytopenia?

Wiskott-Aldrich syndrome (WAS) was described for the first time by Wiskott in 1937. In its clinically complete form, WAS presents with a classical triad of eczema, recurrent infections and bleeding (linked to thrombocytopenia). The X-linked nature of the syndrome was identified by Aldrich in 1954. Another less severe X-linked disorder mainly characterised by thrombocytopenia and a tendency to bleed (known as X-linked thrombocytopenia, XLT) was described for the first time by Wooley in 1956, but given its allelic nature with respect to WAS, XLT was only identified by Canales in 1967.

The pathophysiology of both WAS and XLT remained unsettled for a long time and was only identified in part when Derry et al. cloned the gene in 1994. The *WASP* gene (*standing for Wiskott-Aldrich Syndrome Protein*) codes for a protein selectively expressed in the haematopoietic system and involved in the cytoskeletal-organizing complex and the maturation, activation and transport of blood elements.

1.2 Genetics and pathophysiology of WAS and XLT

1.2.1 Structural and functional features of the WASP protein

WAS and XLT are allelic diseases caused by mutations of the *WASP* gene located on the short arm of chromosome X, in position Xp11.2-11.3. This gene codes for a protein (called WASP) of 502 aminoacids, organized in different functional modules (domains) and expressed exclusively in the cells of the haematopoietic system. The WASP protein is the founding member of a growing family of proteins involved in cytoskeletal rearrangement, namely actin nucleation and polymerization in response to cell activation stimuli.

From the extreme aminoterminal to the carboxyterminal domain the WASP protein includes the following functional domains: a) a pleckstrin-like domain (PH-WH1, Pleckstrin-Homology WASP-Homology 1); b) a basic domain (B); c) a domain binding GTPase (GBD, GTPase Binding Domain); d) a proline-rich domain (PPR, PolyProlin Rich); e) a verprolin and cofilin homology domain (VH-CH, Verprolin Homology Cofilin Homology); f) an acid domain (A). Under normal conditions, WASP has an auto-inhibitory conformation resulting from the link between the GBD and VC domains. Following activation stimuli, the complex formed by cdc42 (a small GTPase) and GTP bind to the WASP GBD domain, releasing the molecule from its inhibitory state and exposing the VC and A domains which interact with the proteins of the Arp2/3 complex involved in actin nucleation and polymerization. Through the proline-rich PPR domain WASP interacts with the SH3 domains of other proteins involved in the transport of activation signals (Nck, Grb2, PSTPIP, Btk) thereby mediating the coupling between cell activation and cytoskeletal rearrangement. WASP function is also modulated by the phosphorylation of the molecule at the level of thyrosine 291 (which stabilizes the active conformation of WASP) and the interaction between the PH-WH1 domain and the WIP molecule (which instead stabilizes the inactive conformation of WASP). Cell activation stimuli allow the bond between WASP and WIP to be released, thereby triggering the processes of cytoskeletal rearrangement.

1.2.2. Cell biology

At cellular level, WASP is known to play a role in the formation of immunological synapses (i.e. cell-cell contacts essential to the immune response) by redistributing the molecule that tends concentrate in the contact zones between cells. In addition, WASP

regulates the production of cell protrusions (podosomes, filopods, lamellipods) which play a key role in chemotaxis, chemokinesis and cell transport.

WASP mutations therefore have major effects on the function of cells in the haematopoietic system, as demonstrated both in WAS/XLT patients and in knock-out mice. In particular, T cells in WAS patients have stubby protrusions, an impaired proliferative response via CD3, calcium flow delayed and less sustained in time after cell activation and major defects in the formation of immunological synapses. NK cells in WAS patients also present impaired immune synapses and hence have a reduced cytotoxic activity. Major migration defects have been described for macrophages and dendritic cells as a result of the impaired formation of cell protrusions and a diminished response to chemotactic stimuli.

The migration defects of haematopoietic cells in WAS subjects are responsible for a biological event widely used in screening for carrier status in women at risk who are known to present unbalanced inactivation of the X chromosome in all blood cell populations. This unbalanced inactivation is also present in CD34+ spinal cord cells and reflects a homing and migration defect of stem cells during ontogenesis.

The role of WASP changes on the production and function of platelets is less clear. Platelet changes (thrombocytopenia with microthrombocytes) are the hallmark of the syndrome irrespective of its phenotypic severity (WAS or XLT). Apparently, the capacity of megakaryocytes to produce proplatelets remains intact in WAS/XLT subjects, but in response to adhesion stimuli they show major defects in filopod formation and F-actin polymerization and compartmentalization. In addition, it has been observed that in patients with severe disease (WAS) WASP protein expression varies in different lymphocyte subpopulations while it is more deficient in platelets. Thrombocytopenia has been accounted for by the fact that asymptomatic subjects present higher membrane levels of phosphatidylserine, a signal for phagocytosis and platelet destruction by macrophages. It is noteworthy that most mutations responsible for XLT are missense mutations involving the first three exons of the gene in the region corresponding to the PH-WH domain. This region is therefore likely to be critical for healthy platelet production and function.

1.2.3. Molecular genetics of WAS and XLT – Genotype-phenotype correlation

WASP gene mutations can impair all or part of the protein's expression and function. There is growing evidence that the severity of impaired protein expression is directly correlated to the severity of the clinical phenotype. In particular, missense mutations in exons 1 and 2 (which usually diminish but do not suppress protein expression) are associated with a mild phenotype, whose main feature is thrombocytopenia (XLT). Splicing mutations to donor and acceptor splice sites, especially outside the conserved positions (-2, -1, +1, +2), may also lead to low levels of protein expression with normal aminoacid sequence resulting in mild or moderate clinical phenotypes. Conversely, nonsense mutations or mutations impairing the reading frame of the RNA messenger, thereby disrupting the protein's aminoacid sequence, abolish or radically reduce protein expression and tend to be associated with a severe clinical phenotype (WAS).

The advent of cytofluorometric methods able to determine WASP protein expression have led to major advances in the study of the effect of different gene mutations. This assay is simpler than alternative methods (Western-blotting) and is widely used in the diagnostic work-up and in monitoring immunohaematological reconstitution after haemopoietic stem cell transplantation. In addition, there is much evidence that cytofluorometry is a more predictive index of genotype-phenotype correlation than simple mutation analysis.

Missense mutations within the GBD domain are a special case. These mutations can stop the protein assuming the functionally inactive conformation, thereby preventing the bond between the GBD domain and the VCA domains at the molecule's extreme C-terminal. Under these conditions the VCA domain is continually exposed and always ready to take part in actin enucleation and polymerization processes. These mutations have an activating role and determine a distinct phenotype characterized by neutropenia or X-linked myelodysplasia in the absence of thrombocytopenia, eczema or immunodeficiency. The treatment of these conditions is beyond the scope of these recommendations.

1.3 Epidemiology and phenotypic spectrum of Wiskott-Aldrich syndrome

1.3.1 Epidemiology - Mortality

On the basis of historical reports in various national registries for primary immunodeficiencies, the incidence of Wiskott-Aldrich syndrome has been gauged to be at around four cases per 1.000.000 live male births. Nowadays this figure appears largely underestimated following the many cases reported after gene cloning and the fact that the phenotypic spectrum of the syndrome is much wider than was previously acknowledged.

According to a large American multicentric study published in 1994, the average age at diagnosis was 21 months (range: birth-24.8 years); the average age at diagnosis was lower in patients with a positive family history than in sporadic cases (10 months vs. 24 months).

The mortality rate has progressively declined over the years thanks to improved diagnostic techniques and better treatment options. Retrospective figures published in 1980 reported an average survival of only eights months for male infants born before 1935, rising to 6.5 years for those born after 1964. One 1994 study reported an average survival of 11 years, whereas the latest studies published in 2003 reported an average age of survival of 14.5 years. These data, however, are not representative of the entire cohort of patients with WAS and XLT, but reflect higher inclusion rates for patients with the most severe clinical phenotype (WAS). The main causes of death in patients who do not undergo haematopoietic stem cell transplantation include: severe haemorrhage (23% of cases), infections (44%) and tumours (26%). No reliable data are available on mortality rates in patients with the mild phenotype (XLT).

1.3.2 Clinical and immunological phenotype

1.3.2.1 General remarks

WAS is characterised by the hallmark triad of eczema, thrombocytopenia with microthrombocytes and immune deficiency. However, even before gene cloning, a multicentric study disclosed that this classical phenotype is found in less than a third of all patients. Among the remainder, 5% have a clinical history dominated by infections, whereas the only clinical feature in 20% of cases is bleeding. The severity of immune deficiency varied from one family to another and within the same family. More light has been shed on the phenotypic heterogeneity of the disease since the *WASP* gene was cloned and XLT identified as an allelic variant of WAS. More recent cases have been described in which mutations of the *WASP* gene permissive for adequate protein expression with a single aminoacid substitution are associated with a phenotype of

intermittent thrombocytopenia representing the mildest extreme of the phenotypic spectrum. Broadly speaking, it is increasingly evident that WAS and XLT are not distinct nosological entities and that mutations in the *WASP* gene can give rise to a continuum of clinical disorders comprising oligosymptomatic forms (intermittent XLT, XLT) and clinically complete severe forms (WAS).

1.3.2.2 Bleeding

Bleeding is the most common symptom seen throughout the phenotypic spectrum of WAS. Varying degrees of bleeding manifestations occur in both WAS and XLT patients. Traditionally it was thought that the severity of bleeding did not differ in the two groups of patients, but recent reports suggest that severe bleeding is more common and occurs earlier in WAS patients who have a protein expression defect with respect to XLT patients whose protein expression is spared (Imai et al., 2003a). The average incidence of bleeding manifestations before diagnosis is more than 80% (**Table 1**), consisting of petechiae and ecchymoses in most cases (78%). Nonetheless a significant number of patients (30%) present severe bleeding episodes, the most common being haematemesis and melena. Bloody diarrhoea in newborns and infants should arouse suspicion. Intracranial bleeding is uncommon but constitutes a major finding for the purposes of prognosis.

The severity of bleeding is closely linked to the severity of thrombocytopenia. The number of significant bleeding manifestations is 3.85 per patient-year in patients with platelet values at onset below 10.000/μL, dropping to 1.08 episodes per patient-year in patients with platelet values at onset between 50.000 and 100.000/μL. This difference is associated with a different risk of intracranial bleeding and a different overall mortality rate. As a whole, bleeding is the cause of death in between 4 and 20% of patients in different series.

1.3.2.3 Infections

Patients with WAS are more susceptible to bacterial infections as well as viral and fungal infections. Polysaccharide-coated bacteria pose a special risk because of the impaired capacity to produce antibodies of the IgG2 subclass against polysaccharide antigens. The most common clinical manifestations of infection are listed in **Table 1**. Upper airway infections prevail, but invasive infections (meningitis, sepsis) are also common, mainly those caused by encapsulated bacteria.

Among viral infections, Herpes Simplex 1 and 2 may cause recurrent severe and/or disseminated infections. Contagious mollusc and verrucas are both common and difficult to treat. In addition, candida or fungal infections frequently arise. Opportunistic infections such as Pneumocystis carinii have also been reported and tend to arise during the disease following diagnosis and seldom in the early months of life.

1.3.2.4 Eczema

Eczema affects 80% of WAS patients during the disease. It is more common in the first two years of life and tends to subside over time. Reliable data on the incidence of eczema in XLT patients is lacking, but there are many reports of eczema also in these patients, but it is usually mild and lasts shorter periods of time. Eczema varies widely in WAS patients and from one family to another and within the same family, without obvious correlations to the genotype. Other genetic and environmental factors in addition to the mutation in the *WASP* gene presumably contribute to eczema pathogenesis. Eczema is

often, but not always, associated with raised total IgE serum levels and specific IgE positivity for different allergens.

1.3.2.5 Autoimmunity

Autoimmunity is one of the commonest and most significant complications of WAS (**Table 1**). Its incidence varies in different series, probably due to a patient selection bias (the varying number of XLT patients in different series obviously affects the incidence of autoimmune manifestations in a given population). In the multicentric series analysed by Sullivan et al. in 1994, 40% of patients had at least one autoimmune event and 25% had several manifestations. In a series of patients analysed in a single centre study (Hospital Necker, Paris) recently published (Dupuis-Girod et al., 2003), 72% of patients presented at least one autoimmune event and 36% had multiple manifestations. Autoimmune or inflammatory events were reported in 24% of patients in a recent study (Imai et al., 2003a). On the other hand, the Japanese Registry of mutations (also including patients of other ethnic origins) scored clinical severity in a total of 250 patients reporting the highest score (presence of autoimmunity and/or tumour) in only 23 cases (9% of the total).

Irrespective of the different incidence of autoimmunity, its clinical manifestations are similar in different series (**Table 2**): namely autoimmune haemolytic anaemia, cutaneous vasculitis (including the Schoenlein-Henoch syndrome), nephropathies and arthritis which together account for more than 80% of autoimmune manifestations. Recent emphasis was also placed on the role of chronic inflammatory bowel disease and post-splenectomy idiopathic thrombocytopenia purpura among the autoimmune events arising in WAS.

Historical reports and recent case series (Imai et al., 2003a) highlight IgA nephropathy as a common complication in the XLT subgroup of patients, usually presenting in the second decade of life.

Age at onset of autoimmune events varies in WAS patients (**Table 2**), but is always below five years in the case of autoimmune haemolytic anaemia.

Autoimmunity has a negative prognostic significance: 25% of WAS patients with autoimmunity develop tumours (against 5% of WAS subjects without autoimmune manifestations). Conversely, 75% of tumours in WAS patients arise in subjects who had developed autoimmunity. In addition, autoimmune disease carries an increased mortality risk especially in the case of autoimmune haemolytic anaemia and post-splenectomy recurrent thrombocytopenia.

1.3.2.6 Tumours

Together with severe bleeding manifestations and autoimmunity, tumours constitute the main complication of WAS (**Table 1**). The multicentric study published by Sullivan et al. in 1994 reported a 13% incidence of tumours in the WAS population (10% in the recent series reported by Imai et al.), with an average age at diagnosis of 9.5 years. Tumours comprise laeukemias, myelodysplasia and lymphomas in 90% of cases, highlighting the role of the WASP protein (expressed selectively in the haematopoietic system) in regulating the differentiation and function of blood elements.

It has already been emphasised that autoimmunity is a strong risk factor for the development of tumours. Recent evidence suggests that the genotype may represent a further element of risk. In fact, the incidence of lymphomas is 3.2% in WAS patients with missense mutations, 9.2% among those with nonsense mutations or reading frame shifts, and 12.1% in patients with splice mutations, namely those with splice mutations in exon 6 carry a high risk (44% of B-cell lymphomas) (Shcherbina et al., 2003).

By definition, XLT subjects should not present tumours (see below, genotype-phenotype correlation). However, prospective studies on XLT cohorts are lacking so that the risk of tumour degeneration cannot currently be defined with certainty in these subjects.

1.3.2.7 Changes in laboratory parameters

The only consistent change in laboratory tests encountered in the WAS/XLT population is thrombocytopenia with microthrombocytes. Thrombocytopenia tends to be chronic and the number of circulating platelets below 100.000/μL, but usually below 70.000/μL. Rare cases present periodically low platelet values interspersed with normal cell counts (X-linked intermittent thrombocytopenia). Platelet volume is typically reduced and is usually <5fL (n.v.: 7-10 fL).

In addition to these changes, other laboratory abnormalities are present in some patients, namely those with WAS rather than XLT. According to the multicentric study of Sullivan et al. (1994), significant lymphopenia (<1000/ μL) is found in 22% of WAS patients, whereas eosinophilia (>500 eosinophils/ μL) is seen in 31% of cases. Anaemia is common and is caused by microbleeds or autoimmune events. Average erythrocyte size is also reduced similar to what occurs in platelets.

Different immunological changes have been described in WAS patients. Classical features include reduced serum IgM levels, whereas IgA and IgE are elevated. However, these changes are not constant features and mainly affect older patients. A significant number of WAS subjects present normal or even raised IgM values, the latter being a risk factor for the development of autoimmunity. Immunoglobulins are subject to hypercatabolism in WAS patients who also commonly present an isohaemoagglutinin defect and an inability to produce antibodies against polysaccharide antigens which may account for the increased incidence of invasive infections by encapsulated bacteria. These changes are also inconstant: 13% of WAS patients (and most XLT subjects) have normal isohaemoagglutinin levels, whereas 31% of WAS patients produce anti-polysaccharide antibodies. Lymphopenia tends to worsen with age, especially in the WAS population and is more marked in the CD8+ T lymphocyte subpopulation which is diminished in 61% of patients. A defective proliferative response to mitogens is encountered in 46% of cases and is more common after CD3 stimulation. These laboratory changes correspond in part to morphohistological abnormalities: WAS patients often present hypoplastic lymph nodes with lymphoid depletion which becomes increasingly more evident with age. In addition the marginal zone of the spleen responsible for the formation of anti-polysaccharide antibodies is absent or severely hypoplastic.

1.3.2.8 Clinical classification of WAS-XLT

To overcome the difficulties of clinical classification and to draw up treatment guidelines targeted to the clinical phenotype, Zhu et al. proposed a classification system for WAS-XLT based exclusively on clinical features. Although this system (**Table 3**) is not perfect, it is the only tool used to date to attempt a genotype-phenotype correlation and is also the cornerstone of the various therapeutic strategies. Broadly speaking, the score devised by Zhu et al. addresses the following parameters:

- thrombocytopenia with microthrombocytes
- eczema
- immunodeficiency
- infections
- autoimmunity

- tumours.

Each patient is allotted a score between 1 and 5. Score 1 corresponds to the presence of only thrombocytopenia with microthrombocytes, possibly associated with minor clinically insignificant immunological changes. Score 2 corresponds to thrombocytopenia associated with mild readily treated eczema, possibly accompanied by mild infections (like those encountered in the general population). Score 3 corresponds to thrombocytopenia, eczema and infections. If the eczema is particularly difficult to treat or infections are severe and potentially life-threatening score 4 is allotted. Score 5 is given to patients developing autoimmunity and/or tumours, irrespective of the remaining clinical phenotype. XLT is classified as the phenotype corresponding to scores 1 and 2 in Zhu et al.'s classification, whereas WAS corresponds to scores 3 to 5 depending on clinical severity.

Although the classification devised by Zhu et al. has many merits, it also has major shortcomings some of which may be resolved by these recommendations. In particular, the scoring system has not yet been validated in large patient samples and there are no longitudinal studies establishing the consistency of the score over time. It is very likely that the patient's age has a major influence on the score attributed: autoimmunity and tumours arise mostly in the second decade of life, whereas infants assessed in the early months of life may still only have bleeding manifestations. Lastly, genetic factors other than mutations in the *WASP* gene may influence the development of eczema, autoimmunity and even the risk of tumours. Nonetheless, Zhu et al.'s proposed classification has been widely adopted as it helps to classify patients with different degrees of disease severity and hence serves to select candidates for more aggressive treatment protocols. As demonstrated for studies on genotype-phenotype correlation, newly available methods assessing protein expression and gene arrangement are proving useful adjuncts to the scoring system proposed by Zhu et al., especially in infants when the clinical phenotype may not be fully expressed.

A recent longitudinal study by Imai et al. (2003a) on 50 patients with a diagnosis established at molecular level and a well-characterised protein expression demonstrated that the absence of protein expression is associated with a significantly higher early mortality rate (0% survival at 30 years in WASP-negative patients with respect to 36.8% in WASP-positive patients). In addition, patients negative for protein expression are at greater risk of intracranial bleeding (% survival free from intracranial bleeding at 30 years: 36.8% in WASP-positive subjects vs. 0% in WASP-negative subjects). Likewise, bacterial infections are four times more common in patients negative for WASP protein expression, whereas severe or recurrent viral infections (namely HSV) are threefold more common in WASP-negative than WASP-positive subjects.

1.4 Treatment: available alternatives and evidence of efficacy

The rarity and heterogeneity of the WAS syndrome has hitherto precluded controlled clinical trials aimed at establishing the efficacy of different treatment strategies. Our experience is therefore based on small patient cohorts followed at single centres or retrospective multicentric studies assessing different treatments based on different inclusion criteria. With these limitations, literature reports on treatment of different aspects of the syndrome are discussed together with haematopoietic stem cell transplantation which currently remains the only possible cure.

1.4.1 Treatment of thrombocytopenia

In addition to haematopoietic stem cell transplantation (see below), different strategies have been devised to combat thrombocytopenia. According to the multicentric

study of Sullivan et al. (1994), cyclic administration of steroids proved effective in a third of patients raising the platelet count by at least 20.000/μL. However some patients had undergone splenectomy and subsequently developed autoimmune thrombocytopenia. In fact steroids appeared to be more effective in terms of percentage response and raised platelet count in splenectomized patients developing ITP than in non-splenectomized subjects. In addition, steroid administration is associated with fatal outcome (possibly linked to treatment) in 13% of patients.

According to the same study, administration of high doses of intravenous immunoglobulins without other treatments proved ineffective in all patients, leading to a raised platelet count only in splenectomized subjects who had subsequently developed ITP.

Splenectomy is a potentially effective treatment and will prolong survival (average survival in splenectomized patients: 25 years vs. 4 years in non splenectomized subjects; Mullen et al., 1993). In the series reported by Sullivan et al., 92% of splenectomized WAS patients had an increased platelet count >20.000/μL (in 68% the circulating platelet count reached values ≥100.000/ μL). Similar efficacy rates were reported in other American studies (Lum et al., 1980; Corash et al., 1986; Mullen et al., 1993) which probably shared some of the patients described. However the large number of patients with a satisfactory initial response after splenectomy is counterbalanced by the 13-22% who subsequently develop recurrent thrombocytopenia deemed similar to episodes of ITP. An even more important finding in these studies is that splenectomy was associated with a significant risk of sepsis (26% in the paper by Sullivan et al. and 30.7% according to Mullen et al.) and mortality linked to sepsis (13-15%) despite antibiotic prophylaxis administered in five out of the nine patients who died in Sullivan et al.'s report and two out of seven in the paper by Mullen et al. On the other hand, all splenectomized patients who did not receive antibiotic prophylaxis had at least one episode of sepsis.

There is an anecdotal report of significantly raised platelet count following administration of interleukin-11.

1.4.2 Preventing infection

Antimicrobial prophylaxis and intravenous immunoglobulin administration are the most common means of reducing the infection rate in WAS. Antimicrobial prophylaxis usually consists of co-trimoxazole. Although studies are lacking on the efficacy of this drug in reducing the number and severity of infectious episodes, the reduced risk of interstitial pneumonia caused by Pneumocystis carinii is well established. There is much discrepancy in reports from one centre to another, and within the same centre from patient to patient on the use of antifungal and antiviral prophylaxis. Antiviral drugs appear to be potentially important in view of the increased incidence of recurrent and severe infections caused by Herpes Simplex viruses 1 and 2.

Intravenous immunoglobulin (IVIG) administration is widely adopted to combat the risk of infection and justified by the defective antibody production, especially against polysaccharide antigens. Although solid data on efficacy are lacking, the retrospective study by Sullivan et al. reported that IVIG administration had no impact on the incidence of infections in 64% of patients treated, whereas the incidence was reduced in 28% and 8% of patients even had an increase in the rate of infections.

The report of effective administration of recombinant interleukin-2 in one WAS patient with severe Herpes Virus infection and chronic eczema is purely anecdotal.

1.4.3 Treatment of eczema

Eczema in WAS may vary in severity: treatment in most patients does not differ from that in the general population. Topical or systemic administration of corticosteroids is effective but the dose should be kept as low as possible because of the risk of infection. Dietary restrictions based on the frequent finding of specific IgE may have some benefit, but do not usually serve alone to prevent eczema or reduce its severity. On the contrary, antibiotic administration (both treatment and prophylaxis) has been demonstrated to reduce the extent and severity of eczema, suggesting that infections are involved in triggering and/or maintaining eczema.

1.4.4 Treatment of autoimmune reactions

The treatment of autoimmune manifestations in WAS was the topic of a recent review (Dupuis-Girod et al., 2003). Reference has already been made to the treatment of post-splenectomy ITP with steroids and high dose IVIG. Administration of steroids (2-5 mg/kg/die) is at least partially effective in 70% of WAS patients with haemolytic anaemia. Other treatments include cyclophosphamide (750 mg/m^2) and azathioprine (3 mg/kg/die per os) but evidence of efficacy is insufficient. Steroids, possibly associated with cyclosporine per os, also proved useful in 66% of patients with cutaneous vasculitis and 63% of patients with arthritis. There is insufficient evidence available on the administration of anti-CD20 monoclonal antibodies in severe forms of autoimmunity, also because this complication is usually an indication for stem cell transplantation.

1.4.5 Treatment of tumours

The treatment of tumours in WAS is the same as that in the general population but special care must be paid to both the prevention of infections and the risk of bleeding also outside the stages of chemo and radiotherapy.

1.4.6 Haematopoietic stem cell transplantation

Haematopoietic stem cell transplantation is the only possible cure for WAS. Although T cells are reduced, their function is largely spared in WAS patients so that cord blood stem cell transplantation requires vigorous myeloablative and immunosuppressive therapy. The best outcome has been obtained with transplantation from an HLA-matched family donor (**Table 4**). Long-term survival (3-5 years) after this type of transplant was 81% and 87% in European and American series respectively. Transplantation from a non HLA-matched family donor has not been as successful (45% and 52% survival rates in European and American Registries respectively). In addition, this type of transplant carried a high risk of EBV-related lymphoproliferative disorders in the WAS population. To remedy the not very satisfactory results of transplants from a non HLA-matched family donor, some groups have embarked on transplants from matched unrelated donors (MUD). Filipovich et al. reported a five-year survival of 71% after MUD transplant, with much better results in recipients <5 years (79% survival) than recipients >5 years of age (38% survival). Interesting findings have been reported more recently in a small cohort of patients transplanted with partially matched cord blood stem cells.

When successful, haematopoietic stem cell transplantation can normalize the platelet count and restore immune function (**Table 5**). When stable, even conditions of mixed chimerism can enhance patients' clinical conditions. The main causes of death in the subgroup receiving stem cell transplants from non-matched family donors included bleeding or infections due to graft failure, EBV-related lymphoproliferative disorder or graft

versus host disease, the latter being the main cause of death also in MUD transplant recipients.

1.4.7 Gene therapy

There is currently no evidence of gene therapy for WAS in man. Preliminary in vitro results show that transfer of the *wasp* gene in the haematopoietic stem cells of wasp$^{-/y}$ mice leads to full immunological recovery. In addition, human T lymphocytes from WAS patients translated with normal gene show normalization of the functional defect.

Table 1 – Clinical phenotype (%) in patients with Wiskott-Aldrich syndrome (from: Sullivan et al., 1994)

Manifestation	Before diagnosis	After diagnosis	During the disease course
Infection			
Otitis	64	78	
Pneumonia	25	45	
Infectious diarrhoea	10	13	
Sinusitis	8	24	
Sepsis	7	24	
Meningitis	4	7	
P. carinii pneumonia	0.6	9	
Contagious mollusc	0	9	
Wharts	1	7	
Yeast/fungal infections	10	12	
HSV-1,-2 infections	6	16	
Bleeding manifestations	84		
Eczema			81
Autoimmunity			40
Tumours			13

Table 2 – Percentage of patients with different forms of autoimmunity in Wiskott-Aldrich syndrome

Manifestation	*Sullivan (1994)*	*Dupuis-Girod (2003)*	*Imai (2003a)*
AHA	14	36	25
Cutaneous vasculitis	18	22	33
Nephropathy	12	3.5	41.7
Transient arthritis	11	29*	25
Chronic arthritis	10		
IBD	3	9	16.7
Dermatomyositis	0.6		
Cerebral vasculitis		7	

AHA: autoimmune haemolytic anaemia; IBD: chronic inflammatory bowel disease
*the paper by Dupuis-Girod et al. does not distinguish between transient and chronic arthritis

Table 3 – Classification of X-linked thrombocytopenia and Wiskott-Aldrich system by clinical score (from: Zhu et al., 1997)

Disease	XLT		WAS		
Score	1	2	3	4	5
Thrombocytopenia	+	+	+	+	+
Microthrombocytes	+	+	+	+	+
Eczema	-	(+)	+	++	+/++
Immunodeficiency	-/(+)	(+)	+	+	+
Infections*	-	(+)	+	+/++	+/++
Autoimmunity and/or tumours	-		-	-	- +

+:present; -: absent; (+): mild, easy to treat; ++: severe, potentially dangerous or difficult to treat

(*): severe potentially life-threatening infections

Table 4 – Survival rate (95% confidence interval) after haematopoietic stem cell transplantation in WAS

Registry	HLA-identical family donor	Non HLA-identical family donor	MUD
Europe*	81 (67-94)	45 (30-60)	Not known
USA°	87 (74-94)	52 (37-65)	71 (58-80)

*Antoine et al., 2003. Survival estimated 3 years after transplant
° Filipovich et al., 2001. Survival estimated 5 years after transplant.

Table 5 – Assessment of stem cell transplant efficacy in WAS patients (from: Filipovich et al., 2001)

Disease status	HLA-identical family donor (n=48)	Non HLA-identical family donor (n=25)	MUD (n=47)
Cured	30 (73%)	12 (57%)	22 (73%)
Improved	8 (20%)	5 (24%)	8 (27%)
Unchanged	3 (7%)	1 (5%)	0 (0%)
Worsened	0 (0%)	3 (14%)	0 (0%)
Not reported	7	4	17

2. DIAGNOSTIC PROTOCOL

The European Society for Immunodeficiencies (ESID) and the Pan-American Group for Immunodeficiency (PAGID) (Conley et al., 1999) have drawn up the following criteria for **definitive diagnosis** of WAS/XLT:

male infant with congenital thrombocytopenia (<70.000 platelets/μL), microthrombocytes and at least one of the following:

- mutation in the *WASP* gene
- absence of specific mRNA at Northern-blot analysis of lymphocytes
- absence of WASP protein in lymphocytes
- maternal male cousins, uncles or nephews with thrombocytopenia and microthrombocytes

The following criteria are indicative of **probable diagnosis** of WAS/XLT:

male infant with congenital thrombocytopenia (<70.000 platelets/μL), microthrombocytes and at least one of the following:

- eczema
- abnormal antibody response to polysaccharide antigens
- recurrent bacterial or viral infections
- autoimmune diseases
- lymphoma, leukaemia or brain tumour

In agreement with these definitions, the following inclusion criteria have been devised:

2.1. Inclusion criteria

<u>males</u> with the following laboratory findings:

– *thrombocytopenia* (*platelets* < *100.000/mm*^{3}), reconfirmed in two separate determinations, associated with

– *volume-reduced platelets* (< *6 fL*).

(N.B. Measurements must include platelet size distribution and not only the average platelet volume as platelets aggregates [disclosed by distribution analysis of platelet volume] can falsify the average platelet volume).

A registration form (**Form 1.01**) and a diagnosis form (**Form 25.01**) will be filled in for patients meeting these inclusion criteria (XLA phenotype). Annual follow-up forms (**Form 25.02**) will then be filled and sent to the AIEOP Operation Office in Bologna.
All subjects meeting the inclusion criteria will follow the set therapeutic recommendations.

2.2 Definitive diagnosis

Patients enrolled on the basis of the inclusion criteria belong to three categories:

2.2.1 Patients belonging to families in which the WAS phenotype is present in males belonging to different generations of the maternal line (positive family history). The inclusion criteria alone establish definitive diagnosis of WAS in these patients.

2.2.2 Patients in whom the WAS phenotype is present in several males in the same phratry and there are no males with the same phenotype in other generations. The inclusion criteria only establish a probable diagnosis of WAS in these patients.

2.2.3 Patients with sporadic presentation, i.e. patients with no other males with the same clinical-immunological phenotype in their pedigree (negative family history). The inclusion criteria only establish a probable diagnosis of WAS in these patients.

Definitive diagnosis of WAS can only be established for patients in categories 2.2.2 and 2.2.3 by:

- analysis of the mutation in the *WASP* gene (with demonstration of the mutation)

and

- analysis of WASP protein expression (with evidence of diminished or absent protein expression).

2.3 Sending samples

On request of a centre in the network, the Coordinating Centre or the Centres in Florence or Milan will undertake analysis of the mutation in the *WASP* gene and analysis of WASP protein expression for definitive diagnosis of WAS. Before samples are sent for analysis specific informed consent must be obtained from each family and filed by the centre in the network for each patient enrolled

The following must be sent for these tests:

- two test tubes each containing 3 ml blood in ACD.

The samples must be sent at room temperature to one of the following Centres:

BRESCIA

Prof. Luigi D. Notarangelo
Istituto di Medicina Molecolare “Angelo Nocivelli”
Clinica Pediatrica
Spedali Civili
P.le Spedali Civili 1
25123 Brescia

FLORENCE

Prof. Chiara Azzari
Laboratorio di Immunologia
Dipartimento di Pediatria
Ospedale A. Meyer
Via Luca Giordano 13
50132 Firenze

MILAN

Dr. Alessandro Aiuti
HSR TIGET
Via Olgettina 58
20132 Milano

- Samples must be accompanied by **n° 1 National Health Service request form** duly filled in (date of sampling, patient details with place and date of birth, place of residence, health card number, tax code number, reason: molecular analysis for a rare disease: congenital thrombocytopenia).

- Samples must also be accompanied by **Form WAS/A** duly compiled and sent to the charge of the Coordinating Centre via TRACO 10 service which guarantees delivery of samples by 10 a.m. on the following day.

- Samples must be sent from **Monday to Wednesday** each week.

- The outcome of mutation analysis will be notified within 2 months.
- The results of protein expression within 2 working days.

2.4 Tests to be done at onset and during follow-up:

At diagnosis:
Full haemochrome with platelet volume determination
Azotaemia, creatininaemia
Transaminase levels
IgG, IgA, IgM
Total IgE
Anti-tetanus antibodies (if not vaccinated: measure the antibody titre before and 3 weeks after vaccination)
Anti-pneumo antibodies (at the Central Laboratory: Dr. Quinti- Rome)
CD3, CD4, CD8, CD19, CD16
C3, C4, CH50
ANA, Coombs' test
HCV RNA, HIV RNA
Serum EBV

Proliferative response to mitogens (PHA, anti-CD3)*(not compulsory)*
EBV integration *(not compulsory)*

Every 6 months:
full haemochrome
pre-infusion IgG, IgA, IgM measurement
transaminase levels,
azotaemia, creatininaemia

Every 12 months:
full haemochrome
CD3, CD4, CD8, CD19, CD16
total IgE
C3, C4, (CH50)
ANA, Coombs' test
HCV RNA
proliferative response to mitogens (PHA, anti-CD3)*(not compulsory)*
EBV integration *(not compulsory)*
obviously in addition to the tests required every 6 months

- **Tests to be done when clinically indicated**
  specific IgE (in case of eczema)
  cultures (from biological fluids or swabs) when infections suspected
  autoantibodies (in relation to clinical symptoms suggestive of autoimmunity)
  OGDscopy and colonoscopy with bowel biopsy (if diarrhoea persists for more than 4 weeks)
  abdominal ultrasound (for suspected lymphoma)
  brain CT scan (for suspected lymphoma)

3. TREATMENT RECOMMENDATIONS

3.1 Defining the clinical phenotype

The WAS therapeutic protocol is based on the clinical phenotype, genetic defect and protein expression.

Patients with a definitive diagnosis of WAS are assessed at diagnosis and every six months using the scoring system proposed by Zhu et al. and summarised in **Table 3** and listed again below:

Table 3 – Classification of X-linked thrombocytopenia and Wiskott-Aldrich syndrome according to clinical score (from: Zhu et al., 1997)

Disease	XLT		WAS		
Score	1	2	3	4	5
Thrombocytopenia	+	+	+	+	+
Microthrombocytes	+	+	+	+	+
Eczema	-	(+)	+	++	+/++
Immunodeficiency	-/(+)	(+)	+	+	+
Infections*	-	(+)	+	+/++	+/++
Autoimmunity and/or tumours	-		-	-	- +

+:present; -: absent; (+): mild, easy to treat; ++: severe, potentially dangerous and difficult to treat

(*)severe potentially life-threatening infections

The type of mutation and protein expression are also considered for the purposes of phenotype classification.
On the basis of these elements, patients with certain diagnosis of WAS or XLT are divided into the following phenotypic categories:

A) Patients with definitive diagnosis of the WAS phenotype
This category comprises patients with definitive disease diagnosis and a clinical score (according to Zhu et al.) ≥3, irrespective of the type of mutation.

B) Patients with the XLT phenotype and severe molecular changes
This category comprises patients with a clinical score according to Zhu ≤2, but nonetheless have severe gene mutations (nonsense, frameshift mutation) and/or absent protein expression.

C) Patients with probable diagnosis of the XLT phenotype
This category comprises patients with a clinical score according to Zhu ≤2 and minor gene mutations (missense mutations) associated with residual protein expression.

The phenotype must be determined at diagnosis and every six months thereafter as the disease can undergo changes in phenotypic expression which require prompt and appropriate adjustments to the therapeutic protocol.

3.2 Treatment recommendations for patients with definitive diagnosis of the WAS phenotype and patients with the XLT phenotype associated with severe molecular changes

3.2.1 Controlling the risk of bleeding

The treatment of thrombocytopenia is closely linked to its severity as summarised in the box below.

Number of platelets (PTL/ mm^3)	Treatment
PTL < 50.000 / mm^3 but >20.000 / mm^3	*Protective measures* *No indication for splenectomy*
PTL < 20.000 / mm^3 (persisting for at least 6 months)	*Splenectomy**

*splenectomy can be avoided is haematopoietic stem cell transplantation is already planned

Protective measures include:
- Wearing a protective helmet to reduce the risk of intracranial bleeding caused by head injuries;
- Lining the baby's cot, bed and playpen;
- Avoiding contact sports or leisure activities potentially dangerous from the standpoint of injury (football, basketball, riding a bicycle or moped, skating, skiing)

Splenectomy is a potentially effective treatment and will prolong survival with respect to untreated patients (average survival in splenectomized subjects: 25 years vs. 4 years in non splenectomized subjects; Mullen et al., 1993). A large number of patients show a satisfactory initial response after splenectomy, but 13-22% subsequently develop recurrent thrombocytopenia, deemed similar to episodes of ITP. An even more important finding is that splenectomy carries a significant risk of sepsis (13-15% mortality linked to sepsis)

despite antibiotic prophylaxis administered in five out of the nine patients who died in Sullivan et al.'s report and two out of seven in the paper by Mullen et al. These findings were confirmed by Imai et al. (2003a) who reported that five out of ten splenectomized subjects (all given post-splenectomy prophylaxis) developed sepsis and/or meningitis. On the other hand, all splenectomized patients who did not receive antibiotic prophylaxis had at least one episode of sepsis.
Splenectomized subjects must always be given prophylaxis against infections, according to the protocols outlined below (see 3.2.1.5)

Platelet transfusions (collected by apheresis) should be avoided as they enhance the risk of alloimmunization and can trigger forms of ITP which are difficult to control. Platelet transfusions should be reserved for special cases, such as imminent surgery, etc.

3.2.2 Prophylaxis against infections

3.2.2.1 Antimicrobial prophylaxis with co-trimoxazole.
Although there is no evidence of the efficacy of this strategy in reducing the number and severity of infectious episodes, its capacity to reduced or eliminate the risk of interstitial pneumonia caused by Pneumocystis carinii is well established.

Co-trimoxazole: 6 mg/Kg/die trimethoprim per os once or twice daily.

3.2.2.2 Antiviral prophylaxis with acyclovir.
There is no consensus on antiviral prophylaxis, but it appears to be important given the increased incidence of recurrent and severe Herpes Simplex 1 and 2 infections. *Patients who have a history of at least one episode of severe HSV infection should be given antiviral prophylaxis with*

Acyclovir: 5 mg/Kg/dose every 8 hours per os.

3.2.2.4 Intravenous immunoglobulin replacement therapy (IVIG).
IVIG is widely used to combat the risk of infection and justified by defective antibody production, especially against polysaccharide antigens.
The therapeutic protocol prescribed is the same as that indicated in the recommendations for XLA and CVID.

Products: All products currently available in Italy can be deemed equally effective from the therapeutic standpoint. Therefore, if a product is well tolerated the patient should continue the treatment with the same product. Conversely, if a patient has severe adverse reactions or mild side-effects not controlled by the usual measures (reducing the speed of infusion, administration of antipyretics antihistamines or steroids) another IVIG product should be tried.

Dose: A dose of 400 mg/kg/month usually maintains serum IgG levels above 500 mg/dl, considered the protective limit for the main infections. If serum IgG levels are < 500 mg/dl after the first six months of infusions (the time usually required to reach a plateau), the interval between IVIG administrations should be shortened or the dose of IVIG increased maintaining the same treatment interval.

How to start treatment

Give a detailed explanation and ask for signed informed consent (for treatment with blood products). Take a blood sample when required and when clinically indicated

Record the type of product, batch number and expiry date in the patient's clinical records

If the patient weighs less than 20 Kg, infusion speed **must not exceed 60 ml/hour** as follows:

first hour: 10-15 ml
second hour: 20 ml
third hour: 30 ml
fourth hour: 45 ml
subsequent hours: 60 ml/h

Infusion speed should be gradually increased without hurrying but adapted to each individual patient. If the patient feels unwell during the infusion, especially during the first treatment sessions, the infusion should be immediately slowed down.

What to do at the first infusion:

-History-taking, physical examination, recording product, batch number and expiry date in the patient's records

Reactions to intravenous immunoglobulin administration

Intravenous immunoglobulin administration gives rise to two main side-effects:

1) Allergic and/or inflammatory reactions which may be vasoactive or anaphylactoid reactions or generalized anaphylaxis;
2) Intravenous transmission of infectious agents.

Vasoactive or anaphylactoid reactions usually appear within the first 30 minutes of infusion and are characterized by abdominal pain, low back pain, nausea and vomiting, fever, headache, muscle pain and weakness lasting up to several hours after the end of infusion. Dyspnoea and hypotension seldom occur.
Reactions usually arise during the first infusions and during multiple chronic episodes of infection since a Herxheimer reaction probably takes place with the massive release of endotoxins by the many bacteria destroyed by immunoglobulin infusion.

What to do

a) Suspend the infusion which can be resumed a few minutes later reducing the speed.
b) If fever and/or headache and/or muscle pain are present give salicylates (10-20 mg/Kg) or paracetamol (10 mg/Kg) before resuming infusion.

c) When a patient has presented systemic symptoms corticosteroids (hydrocortisone 10 mg/Kg) and antihistamines (clorphenamine 0.1 mg/Kg) should be administered intravenously about an hour before the start of subsequent infusions. If fever was the only symptom premedication with paracetamol is sufficient.
d) If the reaction was severe, a product prepared by a different method should be tried. The new product should be infused adopting the same criteria as for the first infusion.

Anaphylactic reactions presenting the classic symptoms of IgE-mediated anaphylaxis: dyspnea, rash, vomiting, cardiocirculatory collapse and loss of consciousness up to generalized shock are rare and usually arise during the first infusions at the start of infusion.

What to do
a) Suspend the infusion immediately and send for a resuscitation expert.
b) Administer adrenaline 1:1000 subcutaneously at a dose of 0.01 ml/Kg to be repeated 15 minutes later. If the patient's general and cardiocirculatory conditions fail to recover administer adrenaline 1:10.000 intravenously at a dose of 1 ml in bolus (irrespective of the patient's weight) followed by continuous intravenous infusion of 1-4 μg/Kg/minute of the same solution until arterial pressure is resumed.
c) It is essential to keep the venous access used for IgG infusion patent as it may be required in case of shock caused by administration of emergency fluids or drugs (other vasodilators and bronchodilators in addition to adrenalin).
d) IgG infusion must not be resumed on the same day even if the patient recovers promptly.
e) After an anaphylactic reaction subsequent intravenous immunoglobulin infusion should be undertaken in a facility with an intensive care physician present adopting the same criteria as for the first infusion and infusing a different product. If the reaction should recur, intravenous immunoglobulin treatment should be suspended and continuous antibiotic prophylaxis with a cephalosporin or co-trimoxazole instituted at half/third of the dosage taken in a single evening dose.
A specific form (**Form 25.03**) is available for patients presenting anaphylactic reactions and should be sent to the AIEOP Coordinating Centre: the data collected will constitute a database to devise specific laboratory tests, nationwide surveillance of adverse reactions to intravenous immunoglobulin administration and to plan safe and adequate intervention strategies.

3.2.2.5 Vaccinations
All recommended vaccinations should be given.
Systematic active immunoprophylaxis is particularly important to reduce the incidence of infections in WAS. Irrespective of the need to perform splenectomy, WAS subjects should be given the following vaccinations:

- *anti-pneumococcus vaccination*
- *anti-meningococcus vaccination*
- *anti-H. Influenzae vaccination*

In view of the diminished antibody response against polysaccharide antigens in WAS subjects, *conjugate vaccines should always be used.*

The following vaccination schedule is recommended according to the patient's age and vaccine characteristics.
- Conjugate anti-pneumoccocal vaccination is recommended for: infants under 12 months two administrations at one month intervals and a third dose in the second year of life; older babies and children two administrations with an interval of at least two months between doses.
- Conjugate anti-meningoccocal vaccination is recommended for: infants under 12 months three administrations at one month intervals; older babies and children a single administration.
- Conjugate anti- H. influenzae vaccination is recommended for: infants under 12 months three administrations (two does and one-two month intervals and the third dose at 15-18 months); older babies and children a single administration.

3.2.3 Treatment of autoimmune manifestations

The treatment of autoimmune reactions in WAS was the topic of a recent review (Dupuis-Girod et al., 2003). The following indications are not part of the protocol, but simply suggestions.

	Drug	Dose
1st choice	steroids	prednisone: 2 - 5mg/kg/die per os
2nd choice	steroids + cyclosporine	prednisone: 2 - 5mg/kg/die per os cyclosporine: 3-5 mg/kg/die per os
3rd choice	azathioprine	3 mg/kg/die per os
4th choice	cyclophosphamide	750 mg/ m^2 per os
5th choice	anti CD20 monoclonal antibodies	

High dose steroid boli can be given to treat forms particularly resilient to treatment (up to 10 mg/kg/die i.v.).

3.2.4 Haematopoietic stem cell transplantation

Haematopoietic stem cell transplantation is currently the only possible cure for WAS. When successful, haematopoietic stem cell transplantation can normalize the platelet count and restore immune function. The indication for transplant must be carefully assessed on the basis of clinical phenotype.

Patients with certain WAS clinical phenotype have an absolute indication for transplantation from an HLA-matched family donor.
In the absence of a matched family donor for a child younger than 5 years, a search should be made for a matched unrelated donor (MUD) or matched stem cells from cord blood. Cord blood should be cryopreserved and HLA matched whenever a new birth takes place in families with WAS or XLT children.
MUD transplantation should be entertained for children with a clinical score of 5 (autoimmunity or tumours) even when >5 years.

Lastly, transplantation from a non-matched family donor can be considered if an MUD donor is not available for a patient with severe autoimmune manifestations.

Patients with the XLT phenotype and severe molecular changes are indicated for transplantation from an HLA-identical family donor.
In the absence of a matched family donor transplant from an MUD or cord blood can be entertained as long as the patient is under 5 years of age.

3.3. Treatment recommendations for patients with probable XLT phenotype

The natural history of XLT is currently unsettled thereby precluding clear-cut treatment indications beyond controlling of the risk of bleeding.

3.3.1 Controlling the risk of bleeding

The treatment of thrombocytopenia is strictly related to its severity as summarised in the box below.

Number of platelets (PTL/ mm^3)	Intervention
PTL < 50.000 / mm^3 but >20.000 / mm^3	*Protective measures* *No indication for splenectomy*
PTL < 20.000 / mm^3 (persisting for more than 6 months or more)	*Splenectomy*

Protective measures include:
- Wearing a protective helmet to reduce the risk of intracranial bleeding caused by head injuries;
- Lining the baby's cot, bed and playpen;
- Avoiding contact sports or leisure activities potentially dangerous from the standpoint of injury (football, basketball, riding a bicycle or moped, skating, skiing)

Splenectomy is a potentially effective treatment and will prolong survival with respect to untreated patients (average survival in splenectomized subjects: 25 years vs. 4 years in non splenectomized subjects; Mullen et al., 1993). A large number of patients show a satisfactory initial response after splenectomy, but 13-22% subsequently develop recurrent thrombocytopenia, deemed similar to episodes of ITP. An even more important finding is that splenectomy carries a significant risk of sepsis (13-15% mortality linked to sepsis) despite antibiotic prophylaxis administered in five out of the nine patients who died in Sullivan et al.'s report and two out of seven in the paper by Mullen et al. These findings were confirmed by Imai et al. (2003a) who reported that five out of ten splenectomized subjects (all given post-splenectomy prophylaxis) developed sepsis and/or meningitis. On the other hand, all splenectomized patients who did not receive antibiotic prophylaxis had at least one episode of sepsis.
Splenectomized subjects must always be given prophylaxis against infections, according to the protocols outlined above (see 3.2.1.5)

Platelet transfusions (collected by apheresis) should be avoided as they enhance the risk of alloimmunization and can trigger forms of ITP which are difficult to control. Platelet transfusions should be reserved for special cases, such as imminent surgery, etc.

3.3.2 Prophylaxis against infections

There is no evidence that patients with the XLT phenotype benefit from antimicrobial prophylaxis or intravenous administration of immunoglobulins. Any prophylaxis must be based on the patient's clinical history (e.g. prescribing co-trimoxazole if the patient tends to have recurrent infections even when not severe).

3.3.3 Haemopoietic stem cell transplantation

Haematopoietic stem cell transplantation can be entertained when an HLA-matched family donor is available but only for subjects with persistent severe thrombocytopenia (<20.000 platelets/μL).

4. PREVENTION

4.1 Disease carrier status

Identification of disease carrier status is essential for genetic counselling and is indicated for the mothers of patients and collateral females of the maternal line of the patient's family.

4.1.1 When to identify disease carrier status

4.1.1.1 Identification of disease carrier status is indicated in both the mothers of patients and in collateral females of the maternal line of the family in patients with a negative family history (sporadic presentation) (section 2.2.2) or patients affected only in the same phratry and not in other generations (section 2.2.3).

4.1.1.2 Healthy carrier status is certain in the case of patients with a positive family history (affected males in different generations) (section 2.2.1) so that confirmation by molecular analysis is not needed. Identification of disease carrier status is nonetheless required in collateral females of the maternal line.

4.1.2 How to identify disease carrier status

4.1.2.1 Direct mutation analysis
This test is indicated whenever the patient's *WASP* mutation is known. If the same mutation is present in the heterozygous state in the mother, then she is a WAS/XLT carrier and there is a likelihood that other collateral females in the female branch of the family will also carry the mutation. Instead if the mother is homozygous for the normal sequence, she is not a WAS/XLT carrier and her son's disease is due to a de novo gene mutation.

4.1.2.2 Analysis of X chromosome inactivation
Analysis of X chromosome inactivation to identify carrier status is indicated for the mothers of males with sporadic disease presentation or with more than one son affected in the same phratry with no males affected in other generations if the patient's *WASP* mutation has not been determined. Mutation analysis need only be done later if necessary to study the whole gene.

4.2 Sending samples

At the request of centres in the network, the coordinating centre in Brescia and the Centres in Florence and Milan will undertake genetic testing to identify carrier status.

Testing requires a sample of:

- **5 ml of blood in EDTA** for identification of carrier status by mutation analysis of the *WASP* gene

- **14 ml blood in heparin** for identification of carrier status by studying the pattern of chromosome X inactivation.

Blood samples should be sent at room temperature to the following addresses:

BRESCIA

Prof. Luigi D. Notarangelo
Laboratorio di Biologia Molecolare
e Genetica Medica
Clinica Pediatrica
Spedali Civili
P.le Spedali Civili 1
25123 Brescia

FLORENCE

Prof. Chiara Azzari
Laboratorio di Immunologia
Dipartimento di Pediatria
Ospedale A. Meyer
Via Luca Giordano 13
50132 Firenze

MILAN

Dr. Alessandro Aiuti
HSR TIGET
Via Olgettina 58
20132 Milano

- Samples must be accompanied by **n° 1 National Health Service request form** duly filled in (date of sampling, patient details with place and date of birth, place of residence, health card number, tax code number, reason: investigation of WAS carrier status).

- Samples must also be accompanied by **Form A/WAS** duly compiled and sent to the charge of the Coordinating Centre via TRACO 10 service which guarantees delivery of samples by 10 a.m. on the following day.

- Samples must be sent from **Monday to Wednesday** each week.

- The outcome of mutation analysis will be notified within 2 months.

4.3 Prenatal diagnosis

Prenatal diagnosis of WAS/XLT requires certain diagnosis established in the family. All invasive prenatal diagnostic techniques (chorionic villi sampling, amniocentesis, umbilical cord blood sampling) carry a risk of pregnancy termination. Although this risk is low (from 0.5% for amniocentesis to 1.5% for umbilical cord blood sampling), it is only justified when there is clear evidence that the family is actually affected by WAS/XLT.

Before proceeding to prenatal diagnosis, the couple must be offered genetic counselling to give them a detailed picture of disease characteristics and currently available treatments.

Before prenatal diagnosis the Coordinating Centre should be contacted to establish the technical details of sampling and dispatch of samples. Some technical points are listed below.

For WAS/XLT families with a known mutation prenatal diagnosis is done by sampling the chorionic villus (from the 10^{th} week of pregnancy) or amniotic fluid (at the 16^{th}-18^{th} week).

This material is used for:

- DNA extraction from the specimen
- foetal karyotype analysis (on the same sample)
- if the foetus is male, a search for the mutation on the extracted DNA;
- it is important to rule out contamination by maternal tissues on the same DNA sample (by molecular analysis by highly polymorphic markers).

5. RECOMENDATIONS ON THE MANAGEMENT OF ASSOCIATED DISORDERS

5.1 Eczema

As outlined above, eczema is very common in WAS patients and in most cases treatment does not differ from that in the general population. Topical or systemic administration of corticosteroids is effective but the dose should be kept as low as possible because of the risk of infection. Dietary restrictions may have some benefit, but do not usually serve alone to prevent eczema or reduce its severity. On the contrary, antibiotic administration (both treatment and prophylaxis) has been demonstrated to reduce the extent and severity of eczema, suggesting that infections are involved in triggering and/or maintaining eczema.

5.2 Tumours

The treatment of tumours in WAS is the same as that in the general population so that the specific AIEOP protocol should be followed according to tumour type. Special care must be paid to both the prevention of infections and the risk of bleeding also outside the stages of chemo and radiotherapy.

5.3 Other infections

Bacterial infections in different organs and apparatuses should be treated according to the recommendations specified in the protocols for XLA and CVID.
Special care must be paid to persistent lymphadenomegaly: a search for EBV virus integration is recommended in these cases possibly with lymph node biopsy given the high risk of EBV-related lymphoproliferative disease.
Contagious mollusc and verrucas are also common and both are difficult to treat.
Fungal infections caused by candida and other fungi are also commonly reported, but there is currently no scientific evidence to support continuous systemic prophylaxis in patients with certain diagnosis of WAS.
For this reason it is reasonable to suggest that only WAS patients with established fungal infection received prophylaxis with Itraconazol at a dose of 5-10 mg/kg/die per os (up to a maximum of 200 mg/die).
Beyond the early months of life, opportunistic bacterial infections, including pneumonia caused by Pneumocystis carinii, may occur during follow-up and should therefore be strongly suspected.

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Form A/WAS

Patient's Surname_____ Name _____

Date of birth I_I_I_I_I_I_I
day month year

Referring physician : …….
Institution……...
Address ……
post code……. City……....
Tel…… Fax……..…
e-mail…….

Requests:

□ *WASP* gene mutation analysis

□ Analysis of WASP protein expression

Send to: _____
_____
_____
_____
_____
_____

Form B/WAS

Informed consent to intravenous immunoglobulin treatment of minors

I/We the undersigned……parent/s of……..
born in ……( ) on……. have been informed by Dr./Prof…… that the clinical conditions of my/our daughter/son require treatment with immunoglobulins and that such treatment is not completely free of risks (including the transmission of hepatitis, etc.) I/we have fully understood what has been explained to me/us by Dr./Prof……. both in terms of clinical conditions and the risks linked to the treatment and those which could arise by not receiving the treatment. I/we therefore **consent** / **do not consent** (*) to my/our daughter/son receiving at this institution the immunoglobulin treatment required throughout his/her disease course.

(*) delete as appropriate.

Withdrawal of informed consent

I/We the undersigned parent/s of ……. born in ……. ( ) on ……..**withdraw** my/our consent to immunoglobulin treatment signed by me/us on …….
date…….. Signature…….

Informed consent to intravenous immunoglobulin treatment of adults

I, the undersigned……..born in……( ) on……. Have been informed by Dr./Prof…… that my clinical condition requires treatment with immunoglobulins and that such treatment is not completely free of risks (including the transmission of hepatitis, etc.) I have fully understood what has been explained to me by Dr./Prof……. both in terms of clinical conditions and the risks linked to the treatment and those which could arise by not receiving the treatment. I therefore **consent/do not consent** to receive at this institution the immunoglobulin treatment required throughout my disease course.

(*)delete as appropriate.

Withdrawal of informed consent

I the undersigned ……. born in …… ( ) on ……..**withdraw** my consent to immunoglobulin treatment signed by me on …….
date…….. Signature…….

Form C/WAS
Patient's Surname: _____ Name _____
Date of birth I_I_I_I_I_I_I
day month year

Referring physician : …….
Institution……...
Address ……
post code……. City……....
Tel…… Fax……..…
e-mail…….

Surname_____ Name _____
Family relationship with the patient _____

requests:
□ Analysis of the Btk gene mutation
□ Inactivation of the X chromosome

Surname_____ Name _____
Family relationship with the patient _____

requests:
□ Analysis of the WASP gene mutation
□ Inactivation of the X chromosome

Surname_____ Name _____
Family relationship with the patient _____

requests:
□ Analysis of the WASP gene mutation
□ Inactivation of the X chromosome

Surname_____ Name _____
Family relationship with the patient _____

requests:
□ Analysis of the WASP gene mutation
□ Inactivation of the X chromosome

Surname_____ Name _____
Family relationship with the patient _____

requests:
□ Analysis of the Btk gene mutation
□ Inactivation of the X chromosome

Send to: _____
_____
_____
_____
_____
_____