X-linked thrombocytopenia (XLT) due to WAS mutations:
Clinical characteristics, long-term outcome and treatment options


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Abbreviations: X-linked thrombocytopenia (XLT), Wiskott-Aldrich syndrome (WAS), Wiskott-Aldrich syndrome protein (WASP), intravenous immunoglobulin (IVIG), antibiotics (AB), hematopoietic stem cell transplantation (HSCT), primary immunodeficiency disease (PID)
Abstract

A large proportion of patients with mutations in the Wiskott-Aldrich syndrome (WAS) protein gene exhibit the milder phenotype termed X-linked thrombocytopenia (XLT). Whereas stem cell transplantation at an early age is the treatment of choice for WAS patients, therapeutic options for patients with XLT are controversial.

In a retrospective multicenter study we defined the clinical phenotype of XLT and determined the probability of severe disease related complications in patients above the age of 2 years with documented WAS gene mutations and mild to moderate eczema or mild, infrequent infections.

Enrolled were 173 patients (median age 11.5 years) from 12 countries spanning 2830 patient years. Serious bleeding episodes occurred in 13.9%, life threatening infections in 6.9%, autoimmunity in 12.1%, and malignancy in 5.2% of patients. Overall and event free survival probabilities were not significantly influenced by the type of mutation or IVIG or antibiotic prophylaxis. Splenectomy resulted in increased risk of severe infections.

This analysis of the clinical outcome and molecular basis of XLT patients demonstrates excellent long term survival but also a high probability of severe disease related complications. These observations will allow better decision making when considering treatment options for individual XLT patients.
Introduction

In 1937 Wiskott described a clinical entity characterized by thrombocytopenia, eczema, bloody diarrhea, and recurrent otitis media in male infants. Following rediscovery in 1954 by Aldrich as an X-linked recessive disorder it was designated the Wiskott-Aldrich syndrome (WAS) \(^1-^3\). X-linked thrombocytopenia (XLT), sometimes associated with mild eczema and/or infections, was recognized in the 1960ies and suspected to be a variant of WAS \(^4-^6\). This was confirmed when patients with XLT were shown to have mutations in the Wiskott-Aldrich syndrome protein gene (\textit{WAS}) \(^7-^9\).

\textit{WAS} gene mutations result in three distinct clinical phenotypes: classic WAS, XLT and X-linked neutropenia \(^10,^11\) and a strong genotype phenotype correlation has been suggested \(^12-^15\). Mutations completely averting \textit{WAS} protein (WASP) expression typically lead to the classic phenotype. Missense mutations resulting in expression of defective WASP, often in reduced quantity, most often result in the XLT phenotype, sometimes with only intermittent thrombocytopenia \(^16\). X-linked neutropenia is caused by gain of function mutations resulting in constitutively activated WASP \(^17-^19\).

There are however exceptions to these rules, making it difficult to predict the clinical course of a male infant solely based on the type of \textit{WAS} gene mutation and its effect on WASP expression.

The classic WAS phenotype with microthrombocytopenia, severe eczema, increased susceptibility to pyogenic and opportunistic infections and increased risk of autoimmune disease and cancer usually leads to death in early childhood or adolescence if left untreated \(^20-^22\). Curative treatment by allogeneic hematopoietic stem cell transplantation (HSCT) should be offered to all such patients. The outcome is excellent if performed early in life from an HLA matched related or unrelated donor \(^20,^23-^25\).

Hematopoietic stem cell gene therapy might in the future offer an alternative approach in patients lacking a suitable donor \(^26-^28\).

Generally accepted treatment policies do not exist for patients exhibiting the XLT phenotype, in whom HSCT would seem like an excessively risky procedure if they suffer from thrombocytopenia and eczema only. While it has been assumed that patients with XLT have a lower risk for cancer or autoimmunity than those with WAS, this has never been formally examined. Therefore the risk-benefit ratio for HSCT is not known in XLT.
In this multicenter study we assessed retrospectively the spectrum of clinical phenotypes, the associated genotypes and the long term outcome of the largest cohort of XLT patients studied so far.
Patients and Methods

Data accrual

Questionnaires were sent out worldwide to major centers treating patients with primary immunodeficiency diseases (PID) asking to enroll their patients with the XLT phenotype and to provide data on the following disease parameters: infections, eczema, thrombocytopenia, bleeding, malignancy, autoimmunity, WAS gene mutation, WASP expression and type and extent of therapy. An alternative possibility was documentation online via the same questionnaire in the ESID registry (www.esid.org). Patients were anonymized by the submitting physician. The study was approved by the ethics committee of the University of Munich, Germany.

Patients

All submitted patient data were evaluated and patients were included as study patients by consensual decision of a central review board (M.H.A., T.C.B., B.H.B., H.D.O.). To be enrolled into the final study, patients had to fulfill all of the following criteria:

(I) confirmed mutation within the WAS gene,

(II) classified by their treating physician as XLT,

(III) with or without mild to moderate eczema or mild, infrequent infections not resulting in sequelae,

(IV) age above two years and

(V) no severe infection, autoimmunity or malignancy within the first two years of life.

Bleeding events before the age of two were no reason for exclusion from the study. Above the age of two, severe infections, the development of autoimmunity or malignancy was recorded and included in the analysis, but was no reason for exclusion from the study.

If patients underwent allogeneic HSCT, the transplantation was recorded as the last date of follow-up; the resulting events/outcome were not part of this analysis.
Definitions

Life threatening infections were defined as requiring hospitalization such as sepsis, meningitis, or pneumonia needing oxygen supply or mechanical ventilation. Serious bleeding was defined as a fatal or life-threatening bleeding episode resulting in hospitalization or red blood cell transfusion. Other serious complications were a diagnosis of autoimmunity, malignancy or death. If a patient experienced more than one serious event, only the first event was registered for the analysis of event free survival. Severity of thrombocytopenia was defined as follows: <20000/µl: severe; 20000 – 50000: moderate; >50000 or cyclic: mild. All patients with normal or reduced levels of WASP detectable by Western blot or FACS were designated as WASP positive; those with truncated (by Western blot) or undetectable protein were categorized as WASP negative. IVIG or antibiotic prophylaxes were defined as having had IVIG or prophylactic antibiotics more than once for any period of time. Mutations are reported according to the current nomenclature of the Human Genome Variation Society (www.hgvs.org) 29.

Statistical analysis

Kaplan Meier survival estimates and cumulative incidence rates were compared using the log rank test (Prism, GraphPad, La Jolla, CA, USA). Cumulative incidence for different events adjusting for competing risks was estimated using the statistics language R 30 with the cmprsk package employing the method by Gray et al 31. Other analyses utilized the chi square or Fisher exact test and were accepted as significantly different at a level of p<0.05.
Results

Study cohort

A total of 69 centers known to treat PID patients were contacted and 50 responded (72%). Of 213 completed forms representing 12 countries from 4 continents 173 (171 male, two female) patients from 128 families and 21 centers with a median age of 11.5 years (range 2.0 – 74.6) fulfilled the inclusion criteria, covering 2830 patient years. The two female patients of our XLT cohort had been reported previously, one with a homozygous missense mutation \(^{32}\) and one with a heterozygous missense mutation and skewed X-inactivation in favor of the mutated allele \(^{32, 33}\).

Mutations in XLT patients

We identified 62 unique mutations (Table 1) including three mutational hotspots, defined as affecting 10 or more non-related families with either the identical mutation or a missense mutation affecting the same amino acid. Two hotspots were located in exon 2 affecting either a valine at position 75 (p.Val75Met or p.Val75Leu; 23 patients) or an arginine at position 86 (p.Arg86Gly, p.Arg86Cys, p.Arg86His or p.Arg86Leu; 33 patients). The third hotspot mutation, located in intron 6 (c.559+5G>A) was found in 15 patients. Thus 41% of all patients had a hotspot mutation.

The majority of mutations was located in exon 1 (10% of all patients) and exon 2 (54%). Most mutations were missense (69% of all patients), followed by splice site mutations (19%), deletions (5%), insertions (3%), nonsense mutations (2%), and no-stop mutations (1%) (Supplemental figure 1). With few exceptions, patients with missense and splice site mutations expressed WASP in reduced quantity or in truncated form (Table1).
**Survival**

Without curative treatment classic WAS results in premature death, often during childhood\textsuperscript{22,34}. XLT patients are expected to have a better prognosis. To verify this perception we defined the probability of survival in our cohort of XLT patients.

Overall survival was excellent with 97% (95% confidence interval 95-100), 96% (91-100), 81% (66-97) and 81% (66-97) at 15, 30, 45, and 60 years, respectively and only slightly reduced when compared with the survival curve of the normal male German population\textsuperscript{35} (Figure 1A). However, survival probability without having experienced a severe disease related event was less favorable with 74% (65-82), 56% (43-70), 36% (20-53) and 27% (10-44) at 15, 30, 45, and 60 years, respectively (Figure 1B).

Thus the excellent survival in XLT patients is associated with a high rate of severe disease related events throughout life.

**Incidence of severe disease related events**

In order to better define the nature and occurrence of severe disease related events we analyzed the cumulative incidence rate of these events separately.

Median event free survival was 10.2 years (range 0.1 – 73.9 years). A total of 86 events in 47 patients were reported, some of them occurring in different event categories in the same patient (detailed in table 2). Cumulative incidences for each event category are detailed separately in figure 2. If events were analyzed honoring other events as competing, the cumulative incidences were slightly lower because later events in the same patient were ignored (data not shown).

Life threatening infections occurred at a median age of 24.8 years (2.0 - 73.9), three of which were fatal. There was no discernible effect of patient age on the incidence of infectious events (Figure 2A). In contrast, all but one serious hemorrhage occurred before the age of 30, at a median age of 5.7 years (0.1 - 74.6) (Figure 2B). Most serious bleeding events (18/33) were intracranial hemorrhages. Five bleeding episodes were fatal at a median age of 4.9 years (2.0 - 74.6). There was no correlation
between the recorded platelet counts and the incidence of severe or fatal bleeding which was 12.5% in mild, 9.7% in moderate, and 18.4% in severe thrombocytopenia (p=0.31). Autoimmune nephropathy and hemolytic anemia were the most frequent autoimmune manifestations, the former occurring more frequently in Japanese patients than in patients from other countries (5/28 versus 4/145; p=0.006). In general, autoimmune diseases were not significantly more frequent in Japanese patients (5/28 versus 16/145; p=0.34). Autoimmunity was not restricted to adult patients but occurred at all ages with a median of 12.2 years (4.9 – 56.0) (Figure 2C). Malignancies developed at a median age of 34.0 years (7.8 – 74.0) (Figure 2D), half of which were of lymphoid origin (5/10). Two patients died from their malignancies, two more went on to have HSCT and died from transplant related causes and two died from other complications.

In conclusion, with the exception of severe bleeding which seems to be limited to the first three decades of life, a relatively high rate of life threatening or fatal disease related events was observed in XLT at all ages.

**Influence of WAS gene mutation, protein expression, IVIG or antibiotic prophylaxis on overall and event free survival**

Because some XLT patients have a largely uneventful course of disease and a normal life expectancy while others suffer from severe or even fatal complications at any age, we asked whether individual WAS gene mutations, the presence or absence of WASP or the prophylaxis with antibiotics (AB) and intravenous immunoglobulin (IVIG) had any influence on outcome.

WASP expression, if assessed, was detectable in 98 patients and absent in 21. Presence or absence of WASP had no influence on overall and event free survival in patients with the XLT phenotype (Figure 3A). Similarly there was no significant impact on the incidence of disease related events (data not shown). The same was true when the influence of IVIG prophylaxis (n=39) was analyzed in comparison to patients having never received IVIG (n=134) (Figure 3B). AB prophylaxis had no positive influence on outcome (Figure 3C). Patients with hotspot mutations had no different overall and event free survival and event incidences when compared to others (data not shown).
In summary none of the tested outcome variables were of significance in this cohort of XLT patients selected based on their mild phenotype.

**Influence of splenectomy on infections and bleeding episodes**

Splenectomy in XLT/WAS patients usually leads to a sustained increase in platelet counts and is considered as an effective measure to control the bleeding predisposition. Therefore, splenectomy has been recommended by some investigators for WAS and XLT patients \(^{36,37}\).

A total of 41 patients (23.7\%) underwent splenectomy at a median age of 7.02 years (0.8 - 43.0). The indication for splenectomy was not reported but seven of these 41 patients had experienced a severe bleeding episode before splenectomy and 28/41 had had severe thrombocytopenia. All 13 patients in whom post splenectomy platelet counts were available had experienced an increase in platelet numbers, 7 having counts above 100000/µl. In the two patients who experienced a severe bleeding event after splenectomy, platelet counts were not reported. Therefore it cannot be excluded that these two may have had low counts despite splenectomy. The overall cumulative incidence rate of serious bleeding events in these patients after splenectomy compared to before splenectomy was reduced although not significantly (p=0.15). However, there was a significantly higher incidence of severe infectious events after splenectomy compared to before (p=0.005). This might possibly be due to negligent antibiotic prophylaxis in some patients. Of the 9 patients who did not receive antibiotic prophylaxis three had a severe (1 fatal) infection up to 53 years post splenectomy. This compared unfavorably, however not statistically significant, to splenectomized patients with antibiotic prophylaxis in whom only 5 of 32 (1 fatal) had such an event (p=0.34). Overall survival in splenectomized patients was not significantly different from non splenectomized patients (data not shown).

These data indicate that splenectomized XLT patients are at significant risk for severe infections and require life long antibiotic prophylaxis.
Discussion

WAS is a multifaceted disorder with a wide spectrum of disease severity. In contrast to classic WAS, patients with a mild clinical phenotype, termed XLT, require comprehensive assessment in deciding on the strategy to provide optimal treatment. This is true for children who often present with selective micro-thrombocytopenia and have an uncertain long term prognosis at a time when they are excellent candidates for allogeneic HSCT. Similarly, adult XLT patients who often are wrongly categorized as having chronic immune thrombocytopenic purpura and who may already have developed complications such as autoimmunity pose unique therapeutic challenges. This retrospective study was designed to better define the type of mutations and the clinical course of XLT patients, and to collect supportive evidence for optimal treatment choices.

The design of such a study requires a stringent definition of inclusion and exclusion criteria. The WAS scoring system has been used successfully in categorizing patients according to their disease severity. However, an individual patient is not expected to keep the same score throughout his life. Progression from a score of 1-4 to a score of 5 by developing cancer or autoimmunity can occur at any age and classic WAS patients often present with a relatively mild phenotype during infancy. We therefore chose inclusion criteria that best reflect the situation when XLT/WAS patients present in an immunodeficiency clinic. In addition to the classification as XLT by physicians experienced in treating patients with PID we deliberately chose stringent criteria to prevent the inclusion of classic WAS patients with few disease symptoms as maybe the case during the first two years of life. One possible drawback of this study could be its retrospective, cross-sectional design. It is likely that some events took place when medical care differed from that of today. Naturally the study design might encompass a bias by some confounding factors such as patient compliance, physician preference, choice of prophylactic measures and availability of HSCT. We can also not exclude some selection bias, missing very mild cases that are undiagnosed or misdiagnosed and not referred to an immunology center. But some older patients in this study had lived an uneventful life, before being diagnosed as XLT because their brothers, nephews or grandsons were discovered to have a WAS gene mutation. Of note, these older relatives’ outcome did not differ from that of the rest of the cohort (data...
not shown). At this time the retrospective study design seems to be the only possible means to assess the clinical characteristics of a large cohort of XLT patients. Having established this database of patients with XLT, we now have the opportunity to prospectively follow their course of disease.

Only 17.6% of evaluable XLT patients from this cohort lacked WASP expression. In contrast, the proportion of WASP negative patients from a multinational cohort of WAS/XLT patients with known WAS mutations was 57% (104/184) \(^{15}\). Some patients may in fact express WASP as the methods used to assess expression, e.g. Western Blot analysis, might not be sensitive enough to detect low protein levels. This possibility is supported by the fact that 10 patients who were WASP negative had mutations (missense and invariant splice site) expected to result in WASP expression. In this selected cohort of XLT patients, the clinical outcome of those who did not express WASP was not different from those who expressed WASP. Similarly we did not find any beneficial effect of IVIG or AB prophylaxis on overall and event free survival or on the incidence of life threatening infectious events. These results have to be interpreted with caution and a possible beneficial effect of these measures cannot be ruled out because data on AB and IVIG prophylaxis were very heterogeneous regarding dose and duration of treatment. They might solely reflect the fact that, by definition, most XLT patients can mount effective antibody responses and therefore don’t need IVIG or AB prophylaxis. It is possible that the initiation of these prophylactic measures might have been triggered by slightly more severe disease symptoms \(^{34}\).

In this cohort of 173 patients, 108 (62%) had missense mutations in the first four WAS exons; the remaining 38% (including 11 patients with missense mutations in exons 6-12) were spread over the entire gene, including 19% in non-coding regions. This is in line with previous reports of XLT \(^{13-15, 34}\). We could not detect any influence of the type of mutation on survival or on the incidence of specific disease related events. A mild phenotype despite a deleterious mutation might be due to other disease modifying genes, pathogen exposure or somatic mosaicism caused by in vivo reversion, leading to some WASP expression and thus a milder phenotype. Reversion is an event quite frequent in WAS \(^{38, 39}\), but it was not specifically analyzed in this cohort.

Forty-one patients (23.7%) had been splenectomized, reflecting the acceptance of splenectomy by some caretakers to reduce the risk of bleeding and thus improve quality of life in XLT patients \(^{37, 40}\).
Interestingly, there was only a non-significant reduction of severe bleeding episodes after splenectomy - possibly due to the low overall incidence which decreased with age. On the other hand the incidence of severe infections was significantly increased, especially in patients not receiving antibiotic prophylaxis. These data suggest that before splenectomy in XLT patients one needs to carefully weigh the pros and cons of this procedure. If performed i.e. in patients with recurrent episodes of serious bleeding, the family must understand the risk of infections and be willing to accept the need for antibiotic prophylaxis. In addition vaccination against pneumococci and meningococci has to be considered, given the fact that most XLT patients can be effectively immunized. The high incidence of severe infectious complications after splenectomy including adult patients highlights the importance of lifelong antibiotic prophylaxis in splenectomized XLT patients.

The excellent overall survival rate which is close to that of the normal male population supports the perception that XLT is a mild, chronic disease and that, as a rule, XLT patients do not require standard prophylactic interventions. Declining immune function has been observed in XLT and defective antibody responses may require prophylactic measures such as IVIG in some patients. However, the reduced event free survival demonstrates substantial risks for severe, life-threatening or potentially debilitating disease related complications. The cumulative incidence rate analysis of events revealed that serious bleeding episodes were generally restricted to the first 30 years of life. In contrast the risk of developing autoimmune disease, malignancy or having a life threatening infectious episode was rather constant throughout the patients’ lifetime. The prevalence of autoimmunity is 12% in our cohort, suggesting that this complication is less common than in classic WAS where it was reported to be as high as 40% to 72% 21, 41, 42. Interestingly, we found a significantly higher incidence of autoimmune nephropathy in Japanese patients. Similarly, the prevalence of malignancy was less in our XLT cohort (5%) than in classic WAS (13%) 21, 43. Considering the higher mean age of XLT patients as compared to untransplanted classic WAS patients these differences are even more significant.

The persistent morbidity associated with XLT might argue for HSCT as a treatment option for these patients. Given the excellent success in young children with classic WAS 24, 25, HSCT might be considered a viable option for XLT patients if an HLA-identical donor can be identified. However, when discussing HSCT, which requires full conditioning in both WAS and XLT patients, one needs to
carefully weigh the advantage of a possible cure against the acute risks and long term consequences of this procedure, such as risk for secondary malignancy and infertility. Thus HSCT in XLT has to be decided upon on an individual patient basis. In our cohort 25/173 patients underwent HSCT at a median age of 7.3 years (2.1-38.0) and 22 (88%) are alive after a median follow-up of 2.2 years (0.0 - 12.1). Of note, more than half of the patients received their transplant at an age greater than 5 years, when matched unrelated transplants in WAS may have a less favorable outcome.

Long term studies of HSCT in XLT patients, not available at the present time, are urgently needed. Since patients with XLT may present to different medical specialists it seems vital to raise awareness of this likely under- or misdiagnosed condition. While this study revealed a high overall survival rate of XLT patients, it also demonstrated that they are at risk for life threatening complications. By defining the natural course of XLT and recognizing the life long medical problems that affect the prognosis and quality of life of these patients, it has become possible to select safe and effective individualized therapies for this unique set of patients with mutations of the WAS gene that are generally expected to be less devastating.
Author contribution


Acknowledgments

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References


Table 1: WAS gene mutations in XLT patients:

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<tr>
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<th>coding DNA mutation</th>
<th>predicted protein change</th>
<th>mutation type</th>
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<th>WES score (nr. of pt.)</th>
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fam.: number of families with the respective mutation; Fr: France; Ger: Germany; Isr: Israel; JPN: Japan; ND: not done; NL: Netherlands; pt: number of patients with the respective mutation; Rus: Russia; Sp: Spain; Sw: Sweden; UK: United Kingdom; US: United States of America; 1→5: WAS score progressing from 1 to 5, due to either A: autoimmunity or M: malignancy.
Table 2: Disease related events:

a) | infection | total events | fatal |
--- | --- | --- |
| pneumonia | 6 | 0 |
| bacterial meningitis | 4 | 0 |
| sepsis | 4 | 2 |
| gastrointestinal (salmonellosis) | 1 | 1 |
| orchitis | 1 | 0 |
| tuberculosis | 1 | 0 |
| **nr. of events** | **17*** | **3*** |
| **nr. of patients** | **12** | **3** |

3 patients had more than one infectious event.
*: 8 events in previously splenectomized patients.
**: 2 events in previously splenectomized patients.

b) | bleeding | total events | fatal |
--- | --- | --- |
| ICH* | 18 | 3 |
| gastrointestinal | 6 | 1 |
| ear/nose/throat | 4 | 0 |
| pulmonary | 2 | 1 |
| traumatic, not ICH | 2 | 0 |
| retinal | 1 | 0 |
| **nr. of events** | **33** | **5** |
| **nr. of patients** | **24** | **5** |

4 patients had more than one bleeding episode.
ICH: intracranial hemorrhage.
* 15 spontaneous, 3 traumatic.

c) | autoimmunity | total events | fatal |
--- | --- | --- |
| nephropathy | 9 | 0 |
| AIHA | 6 | 0 |
| vasculitis | 3 | 0 |
| ITP | 4 | 0 |
| arthritis | 3 | 0 |
| colitis | 1 | 0 |
| **nr. of events** | **26** | **0** |
| **nr. of patients** | **21** | **0** |

3 patients had more than one autoimmune disease.
AIHA: autoimmune hemolytic anemia; ITP: immune thrombocytopenic purpura

d) | malignancy | total events | fatal |
--- | --- | --- |
| lymphoma/EBV-LPD | 4 | 1 |
| MDS | 1 | 0 |
| spinalioma | 2 | 0 |
| seminoma | 1 | 0 |
| ALL | 1 | 0 |
| pancreatic cancer | 1 | 1 |
| **nr. of events** | **10** | **2** |
| **nr. of patients** | **9** | **2** |

1 patient had two malignancies.
ALL: acute lymphoblastic leukemia; EBV-LPD: Epstein-Barr-Virus associated lymphoproliferative disease; MDS: myelodysplastic syndrome
Figures

**Figure 1: Overall and event free survival.**

- **A:** Kaplan Meier estimate of overall survival probability of all study patients compared to survival of the normal German male population 2006. B: Event free survival probability. Event defined as a severe or fatal infection, severe or fatal bleeding, autoimmunity, malignancy or death.

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  # at risk: 173
Figure 2: Cumulative incidence rate of severe events. Cumulative incidence of A: severe or fatal infectious episodes in the study cohort, B: severe or fatal bleeding episodes, C: autoimmune disease and D: malignancy, compared to cancer incidence in the US male population. 

| delineates a censored event. # at risk: number of patients at risk at indicated time point. 

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CI (percent)
Figure 3: Influence of WASP expression, IVIG or AB prophylaxis on overall and event free survival. Kaplan Meier estimate of overall survival and event free survival probability of A: WASP positive (n=98, dotted line) and WASP negative patients (n=21, solid line). B: patients receiving any IVIG prophylaxis (n=39, solid line) or no IVIG prophylaxis (n=134, dotted line) and C: patients receiving any antibiotic (AB) prophylaxis (n=16, solid line) or no AB prophylaxis (n=116, dotted line). Splenectomized patients were excluded from the analysis in C. vertical line delineates a censored event.
X-linked thrombocytopenia (XLT) due to WAS mutations: Clinical characteristics, long-term outcome, and treatment options