Sirolimus for the treatment of multi-resistant Autoimmune Haemolytic Anaemia in children

Autoimmune Haemolytic Anaemia (AIHA) is a rare disease in children. Although steroid treatment, routinely used as a first line approach, is successful in about 80% of cases (Aladjidi *et al*, 2011), relapsed/resistant disease may become an important issue due to the absence of controlled studies on second and further line treatments. Moreover, children who experience a chronic clinical evolution of the disease often need long-term treatment with medium/low dosage of steroid. The possibility to use a mild and tolerable immunosuppressive therapy as maintenance in this setting could help to manage a steroid-dependent disease and reduce the associated side-effects. We describe four children with relapsed/resistant AIHA/Pure Red Cell Aplasia (PRCA) successfully controlled after sirolimus administration at the dose of 2 mg/m^2. Clinical data are shown in Table I and detailed below.

Case 1

The patient was diagnosed with primitive AIHA in another centre and unsuccessfully treated with prednisone. She then received immunoglobulin, rituximab (6 weekly infusions at the dosage of 375 mg/m^2) and ciclosporin (CSA) at 2 mg/kg/d, with temporary success. At 14 months from diagnosis and 1 month after stopping immunosuppressive treatment, the child was administered another course of rituximab and CSA for relapsed disease. Due to the persistent symptoms, prednisone was also added at 2 mg/kg and the patient reached another temporary response. In the meantime, a more detailed diagnostic work-up was performed and showed an underlying diagnosis of Autoimmune Lymphoproliferative Syndrome (ALPS). After 1 year, a second relapse occurred during CSA administration and was successfully

Table I. Clinical data and follow-up of patients.

Case number	1	2	3	4
Sex	Female	Male	Male	Female
Age at diagnosis (months)	5	11	25	11
Hb at diagnosis (g/l)	44	38	64	20
Reticulocytes ($\times 10^9$/l)	1050	664	3	1
DAT/IAT	+/+	+/+	+/+	+/+
Haemolysis indices (LDH, Haptoglobin, bilirubin)	Abnormal	Abnormal	Normal	Abnormal
Diagnosis	AIHA	AIHA	PRCA	AIHA/PRCA
Matching ALPS criteria	YES	NO	NO	NO
First-line therapy	PDN 4 mg/kg	PDN 2 mg/kg	PDN 30 mg/kg (3 d, then 2 mg/kg/d)	PDN 5 mg/kg
Prior further-line therapies	Rituximab, CSA, PDN	NO	MMF	Rituximab
Indication for sirolimus	Manteinance Steroid saver	Manteinance Steroid saver	Refractory	Manteinance Steroid saver
Time from diagnosis to sirolimus (months)	50	4	10	4
Response	CR	CR	CR	CR
Adverse events/side effects	No	No	No	No
Time to response (months)	1	8	3	1
Treatment duration (months)	42	30	30	9
Phase of treatment/ongoing therapy	Ongoing	Stop	Stop	Ongoing
Time to last follow-up from sirolimus (as of March 2014)	42	31	31	9
Status at last follow-up	CR	CR	CR	CR

Hb, haemoglobin; DAT, direct antiglobulin test; IAT, indirect antiglobulin test; LDH, lactate dehydrogenase; ALPS, autoimmune lymphoproliferative syndrome; AIHA, autoimmune haemolytic anaemia; PRCA, pure red cell aplasia; CSA, ciclosporin; MMF, mycophenolate mofetil; PDN, prednisone; CR, complete remission.

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treated with another course of rituximab. One year later, a further relapse was treated with high-dosage steroid. During steroid tapering, sirolimus was started and no other relapses have occurred.

Case 2

The child was diagnosed with severe AIHA and reticulocytopenia. First-line treatment was performed in another hospital and consisted of prednisone 2 mg/kg/d for 3 d and then tapered. However, transfusion-dependent anaemia persisted for weeks and the child was admitted to our centre. Diagnostic work-up excluded underlying infections/immunological disorders. Colony studies on bone marrow showed no inhibitory effect on growth of homologous/control erythroid progenitors. The patient quickly responded to a second course of high dose steroid treatment. Simultaneously, sirolimus administration was added in order to quickly taper the dosage. During the next 6 months, five steroid-treatment interruptions were followed by return of the disease, always related to an infective episode. In that period, sirolimus blood levels were never adequate and the dosage needed to be frequently modified. Finally, steroid therapy was maintained at low dose every 3 d for a further 6 months and sirolimus levels were maintained at adequate levels. No further relapses were documented.

Case 3

This patient was affected with severe hyporegenerative anaemia. Recent/ongoing infections and red cell membrane defects were excluded. Bone marrow evaluation showed normal cellularity and reduced maturation of erythroid cells. During the first weeks, anaemia was initially unsuccessfully treated with methylprednislolone bolus (3×30 mg/kg), i.v. immunoglobulin and transfusions. A bone marrow aspirate was then repeated and colony studies showed that patient's plasma had an inhibitory effect on growth of both homologous and control erythroid progenitors. Therapy with mycophenolate (750 mg/m^2 twice a day) was then started with a partial improvement of anaemia but persistent transfusion dependency, so it was substituted by sirolimus after 5 months. The anaemia promptly recovered and the bone marrow normalized.

Case 4

This patient was diagnosed with life-threatening acute anaemia, which appeared during a seizure episode. The diagnostic work-up clearly showed evidence of primitive AIHA with an intra-medullary involvement characterized by reticulocytopenia and severe red cell precursor hypoplasia in the bone marrow. Marrow progenitor colony studies showed that the patient's plasma had an inhibitory effect on the growth of both homologous and control erythroid progenitors. The child was successfully treated with prednisone 5 mg/kg, but this was changed to desametazone after 10 d due to a heavy neurological complication characterized by "status epilecticus" upon magnetic resonance imaging (MRI) that was initially compatible with acute disseminated encephalomyelitis (ADEM). Based on this additional diagnosis, the patient also received immunoglobulin. However, ADEM was not confirmed by further evaluations and MRI images became completely negative after some weeks. The complication was then interpreted as primary seizures. Due to the marked reticulocytopenia, the patient also received weekly doses of erythropoietin. Ten weeks after diagnosis, during steroid tapering, a relapse occurred and the child underwent 4 weekly administrations of rituximab at the dose of 375 mg/m$^{2.}$ In the meantime, sirolimus treatment was started with the aim of avoiding further steroid therapies. Clinical and haemato-biochemical response was prompt.

Sirolimus has been successfully used in children suffering from tacrolimus-associated post-transplant AIHA (Valentini *et al*, 2006; Acquazzino *et al*, 2013; Loar *et al*, 2013) and in 4 patients suffering from ALPS-related cytopenia (Teachey *et al*, 2009). Both abnormal lymphocyte apoptosis induction and increase of Tregs (Zhan *et al*, 2013), suggested that sirolimus might have also an activity on autoimmune cytopenias in non-ALPS patients. We experienced a successful outcome with sirolimus as maintenance treatment in a non-ALPS infant with multi-resistant AIHA/PRCA (Miano *et al*, 2014). To the best of our knowledge no other children with non-ALPS-related AIHA have been reported to receive sirolimus in the literature. Actually, one of our patients (Case 1) did not match the diagnostic criteria of ALPS at diagnosis, but was shown to be an ALPS-patient many years later, when the diagnostic criteria were reviewed by Oliveira *et al* (2010). She temporarily responded to rituximab, but remained CSA-dependent for many years before achieving a definitive remission with sirolimus. All of the patients in our series responded to sirolimus, including both children with intra-medullary involvement, underling the potential role of this drug in the autoimmune involvement of the bone marrow (Miano *et al*, 2014). In all cases, the drug was regularly monitored and maintained within normal ranges and was found to be well-tolerated without side effects.

Our experience may contribute to the knowledge regarding the administration of sirolimus to overcome steroid-dependency in children with AIHA and PRCA. These findings must be confirmed by further observations and trials to enable the use of sirolimus as a more up-front drug.

Author contributions

MM designed, performed the study and wrote the paper; MC, EP, FF, JS, MS, LB, GR, CM and CD provided essential clinical data and took care of patients; TL contributed essential laboratory tools.

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