Autologous stem cell transplantation leads to a change in proinflammatory plasma cytokine profile of patients with juvenile dermatomyositis correlating with disease activity

Juvenile dermatomyositis (JDM) is a rare autoimmune disorder, affecting mainly muscles and skin. The mainstay of treatment is high dose corticosteroids, combined with other immunosuppressive drugs.[1] In about 30% of patients, the disease cannot be controlled despite multiple treatment interventions. Autologous stem cell transplantation (aSCT) has been reported as a last resort treatment in refractory patients with autoimmune

Table 1 Clinical characteristics of patients with JDM

	Patient 1	Patient 2	Patient 3
Age at diagnoses (years)	13.5	6.8	8.8
Age of aSCT (years)	16.3	7.8	16.6
Gender	F	F	F
Duration of disease to aSCT (years)	2.8	1	7.8
Follow-up after aSCT (years)	7	6	3
CMAS score (0–52), before aSCT (lowest measured)	13 (5)	11.5 (4)	38 (25)
MMT8 score (0–80), before aSCT or modified conditioning	36	19	52
PhyGloVAS score (0–100), before/after aSCT	40/10*	40/0	40/10
Major organ involvement	Absent	Absent	Absent
Autoantibodies before aSCT†			
ANA	+	+	+
anti-SSA-52, anti-SSA-60, anti-SS-B, anti-Sm, anti-nRNP/Sm, anti-AMA-M2, anti-Centromere-B, anti-dsDNA Lineblot, anti-Histones, anti-Jo-1, anti-Nucleosomes, anti-PM-Scl, anti-Ribosomal-P, anti-Scl-70	–	–	–
Autoantibodies after aSCT			
ANA	Weakly positive	Weakly positive	+
anti-SSA-52, anti-SSA-60, anti-SS-B, anti-Sm, anti-nRNP/Sm, anti-AMA-M2, anti-Centromere-B, anti-dsDNA Lineblot, anti-Histones, anti-Jo-1, anti-Nucleosomes, anti-PM-Scl, anti-Ribosomal-P, anti-Scl-70	–	–	–
Medication used before aSCT			
Prednisone (high dose)	Received	Received	Received
Methylprednisone (repeated pulses)	Not received	Received	Received
Methotrexate	Received	Received	Received
Ciclosporin A	Received	Not received	Received
Rituximab	Received	Received	Received
IVIG	Received	Received	Received
Tacrolimus	Not received	Received	Received
Mycofenolate mofetil	Not received	Not received	Received
Hydroxychloroquine	Not received	Not received	Received
Mesenchymal stem cell infusion	Received	Not received	Not received
Muscle enzymes: 2 weeks before aSCT			
CK (U/L)	54	73	100
AST (IU/L)	33	34	43
ALT (IU/L)	22	17	28
LDH (IU/L)	229	359	513
Inflammation markers: 2 weeks before aSCT			
CRP (mg/L)	0.06	16	<2
ESR (mm/h)	Not done	38	26
Stem cell mobilisation			
Cyclophosphamide	Received	Received	Received
G-CSF (Filgrastim) for 5 days			
Conditioning			
Cyclophosphamide (Endoxan)	Received	Received	Received
Fludarabin	Received	Received	Received
ATG	Received	Received	Received
Stem cell transplantation			
CD3/CD19 depletion	Done	Done	Not done
CD34+ selection	Not done	Not done	Done
Side effects of stem cell transplantation: short-term effects as severe infections and organ toxicity related to chemotherapy, long-term effects as malignancies or secondary autoimmunity	Autoimmune thyroiditis	None	None
Current immunosuppressive treatment	None	None	None

*PhyGloVAS increased in this patient due to fatigue related to an autoimmune thyroiditis, MRI did not show any sign for myositis.
†Weakly positive: 1/40–1/100, positive 1/100–1/1000 serum dilution.
ALT, alanine transaminase; ANA, anti-nuclear antibodies; aSCT, autologous stem cell transplantation; AST, aspartate transaminase; CK, creatine kinase; CMAS, Childhood Myositis Assessment Scale; CRP, C reactive protein; ESR, erythrocyte sedimentation rate; G-CSF granulocyte colony stimulating factor; IVIG, intravenous immunoglobulins; JDM, juvenile dermatomyositis; LDH, lactate dehydrogenase; MMT, manual muscle testing; PhyGloVas, physician's global assessment of the patient's overall disease activity on a 100 mm visual analogue scale (0–100).

Ann Rheum Dis: first published as 10.1136/annrheumdis-2014-206287 on 30 September 2014. Downloaded from http://ard.bmj.com/ on April 9, 2024 by guest. Protected by copyright.

diseases.[2] The main hypothesis for the underlying immunological mechanism is that aSCT resets the immune system and restores immune tolerance following profound lymphodepletion and immune suppression.[3]

Here, we report three patients with refractory JDM that received aSCT (Clinical information, table 1). A short-term follow-up with detailed clinical information (including imaging before and after aSCT, and immune reconstitution after aSCT) of two patients describing complete remission was reported previously.[4] The current follow-up of these patients is more than 5 years showing sustained remission. A third patient (#3) has a follow-up of nearly 3 years. Indication of aSCT for patient 3 was refractory muscle and skin inflammation comparable to the other two patients. Whole body MRI prior to aSCT confirmed active myositis. As muscle tests improved substantially after aSCT, MRI was not repeated post aSCT in this patient. Immune reconstitution was similar to those published.[4] In patient 3 myositis is controlled and the patient is without systemic treatment at the time of publication. However, skin disease persists including calcinosis and contractures. Interestingly, autoantibody levels decreased in those patients with excellent response to aSCT (patients 1 and 2), but remained stable for patient 3 (table 1).

We have recently reported a typical proinflammatory protein plasma profile in patients with active JDM with three markers

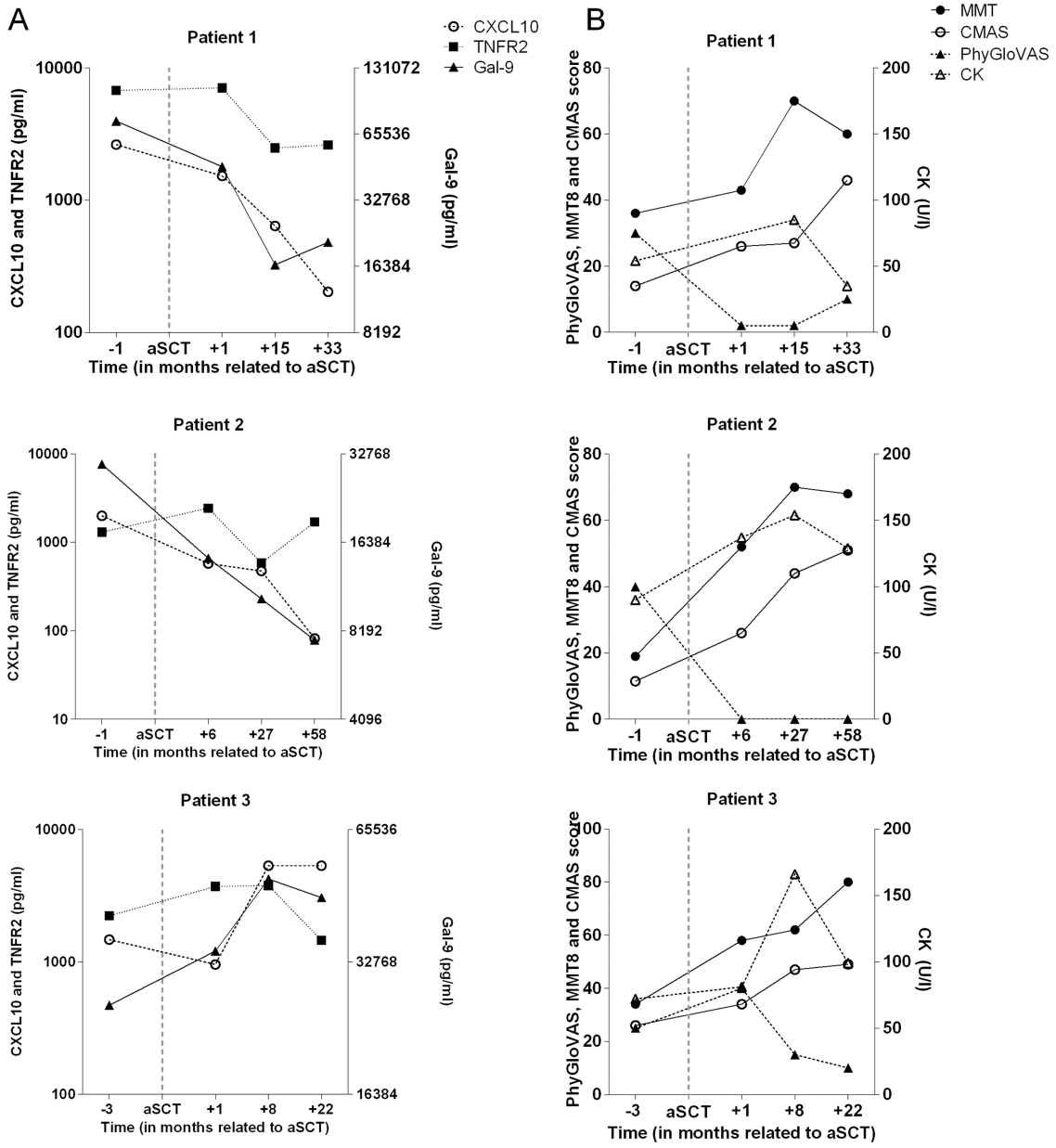

Figure 1 Protein levels and clinical scores over time in aSCT treated patients with juvenile dermatomyositis. (A) Levels of TNFR2, CXCL-10 and Gal-9 measured in serum (Patient 1) or plasma (Patients 2 and 3) by multiplex immunoassay as described previously.[5] In the same paper, cut-off levels for plasma values are described, cut-off values for serum are not yet described, but remission values were compared with those found in healthy adults (unpublished results). Evolution of protein profile over time shows decrease of all markers in patient 1, decrease of Gal-9 and CXCL10 in patient 2 and persisting high levels of Gal-9 and CXCL10 in patient 3. (B) Clinical scores and CK plasma levels. For patient 2, levels at 22 months after aSCT are comparable to those in patients in remission as described earlier.[5] CMAS, Childhood Myositis Assessment Scale; MMT, manual muscle testing; PhyGloVAS, physician's global assessment of the patient's overall disease activity on a 100 mm visual analogue scale; CK, creatine kinase; Gal-9, Galectin-9; aSCT, autologous stem cell transplantation.

Ann Rheum Dis: first published as 10.1136/annrheumdis-2014-206287 on 30 September 2014. Downloaded from http://ard.bmj.com/ on April 9, 2024 by guest. Protected by copyright.

being highly correlated to disease activity: CXCL10, TNFR2 and Galectin-9.[5] We measured these proteins before and at different time points after aSCT to determine kinetics and correlation to disease activity in the context of this intervention. Values of creatine kinase were not elevated in the weeks before aSCT and did not change after, indicating low correlation with actual disease activity. CXCL10, TNFR2 and Galectin-9 were elevated in the patients prior to the conditioning regimen, irrespective of maintenance treatment (figure 1A). After aSCT, these three markers decreased over time in patients 1 and 2 with the most pronounced reduction in levels of Galectin-9 and CXCL10. These levels remained low, even after full reconstitution of the immune system. In parallel, clinical scores improved over time as shown in figure 1B. Pre-existent severe calcinosis disappeared completely in patient 2 after aSCT[4] but is still persisting in patient 3. Importantly, only in this patient, CXCL10 and Galectin-9 levels remained elevated after aSCT.

In contrast to TNFR2, CXCL-10 and Galectin-9 are produced by immune and non-immune cells under inflammatory conditions.[6–9] As circulating immune cells are largely depleted during aSCT, our data suggest that CXCL10 and Galectin-9 are mainly produced by tissue cells or tissue infiltrating cells. Therefore, even during profound immunosuppression these markers may reveal ongoing disease activity in the tissues. This is also supported by the observation that CXCL10 and Galectin-9 levels dropped very gradually following aSCT in patients 1 and 2 and mirrored clinical disease improvement.

In conclusion these data demonstrate that aSCT can induce prolonged drug-free disease remission in refractory patients with JDM with regards to the myositis. Furthermore, we show that the proinflammatory signature as measured by TNFR2, CXCL-10 and Galectin-9 leads to a differentiated response after aSCT, with a marked decrease in the two patients with inactive disease but persistent elevation in a patient with skin involvement. Further studies are needed to determine the immunopathogenic role of these proteins in JDM.

F Bellutti Enders,[1,2] E M Delemarre,[1] J Kuemmerle-Deschner,[3] P van der Torre,[1] N M Wulffraat,[1] B P Prakken,[1] A van Royen-Kerkhof,[1,#] F van Wijk[1]

[1]Laboratory of Translational Immunology, Department of Pediatric Immunology, University Medical Centre Utrecht, Utrecht, The Netherlands
[2]Division of Allergology, Immunology and Rheumatology, Department of Pediatrics, University Hospital, Lausanne, Switzerland
[3]Division of Pediatric Rheumatology, Department of Pediatrics, University Hospital Tuebingen, Tuebingen, Germany

Correspondence to Dr A van Royen-Kerkhof, Laboratory of Translational Immunology, Department of Pediatric Immunology, Wilhelmina Children's Hospital, University Medical Centre Utrecht, Utrecht, Room KC 03.063.0, Lundlaan 6, Utrecht 3584 EA, The Netherlands; a.vanroyen@umcutrecht.nl

FBE, EMD, AvR-K and FvW contributed equally.

Acknowledgements The patients in this study gave informed consent to publication of the data. The authors thank Iris Haugg, Dr Julia Thomas and Dr Sandra Hansmann for providing clinical data and laboratory results.

Contributors Substantial contributions to the conception or design of the work, or the acquisition, analysis or interpretation of data; drafting the work or revising it critically for important intellectual content; final approval of the version published; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved: FBE, EMD, FvW, AvR-K, NW, BP, PvdT, JK-D.

Funding Ettore e Valeria Rossi Foundation; Bas Foundation, The Netherlands; ZonMw Veni grant from the Netherlands Organisation of Scientific Research (NWO); ZonMw AGIKO grant from the NWO; Reumafonds, the Netherlands.

Competing interests FBE is supported by Swiss Grants (The foundation of Ettore e Valeria Bossi). FBE is supported by the patient's society (Bas Stichting). FvW is supported by a ZonMw Veni grant from the Netherlands Organisation of Scientific Research (NWO). EMD is supported by a ZonMw AGIKO grant from the NWO. NW and BP are supported by the Reumafonds.

Ethics approval Netherlands, CCMO: NL34124.041.10.

Provenance and peer review Not commissioned; externally peer reviewed.

To cite Enders FB, Delemarre EM, Kuemmerle-Deschner J, *et al*. *Ann Rheum Dis* 2015;**74**:315–317.

Received 15 July 2014
Revised 12 September 2014
Accepted 13 September 2014
Published Online First 30 September 2014

Ann Rheum Dis 2015;**74**:315–317. doi:10.1136/annrheumdis-2014-206287

REFERENCES

1 Robinson AB, Reed AM. Clinical features, pathogenesis and treatment of juvenile and adult dermatomyositis. *Nat Rev Rheumatol* 2011;7:664–75.
2 Snowden JA, Saccardi R, Allez M, *et al*. Haematopoietic SCT in severe autoimmune diseases: updated guidelines of the European Group for Blood and Marrow Transplantation. *Bone Marrow Transplant* 2012;47:770–90.
3 van Wijk F, Roord ST, Vastert B, *et al*. Regulatory T cells in autologous stem cell transplantation for autoimmune disease. *Autoimmunity* 2008;41:585–91.
4 Holzer U, van Royen-Kerkhof A, van der Torre P, *et al*. Successful autologous stem cell transplantation in two patients with juvenile dermatomyositis. *Scand J Rheumatol* 2010;39:88–92.
5 Bellutti Enders F, van Wijk F, Scholman R, *et al*. CXCL10, TNFR2 and Galectin-9 correlate with disease activity in Juvenile Dermatomyositis. *Arthritis Rheumatol* 2014;66:2281–9.
6 Antonelli A, Ferrari SM, Giuggioli D, *et al*. Chemokine (C-X-C motif) ligand (CXCL)10 in autoimmune diseases. *Autoimmun Rev* 2014;13:272–80.
7 de Kivit S, Kraneveld AD, Knippels LM, *et al*. Intestinal epithelium-derived galectin-9 is involved in the immunomodulating effects of nondigestible oligosaccharides. *J Innate Immun* 2013;5:625–38.
8 Hirashima M, Kashio Y, Nishi N, *et al*. Galectin-9 in physiological and pathological conditions. *Glycoconj J* 2004;19:593–600.
9 Imaizumi T, Kumagai M, Sasaki N, *et al*. Interferon-gamma stimulates the expression of galectin-9 in cultured human endothelial cells. *J Leukoc Biol* 2002;72:486–91.

Ann Rheum Dis: first published as 10.1136/annrheumdis-2014-206287 on 30 September 2014. Downloaded from http://ard.bmj.com/ on April 9, 2024 by guest. Protected by copyright.