

## CONCISE REPORT

# Fibrosis regression induced by intravenous gammaglobulin treatment

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**Objectives:** To review case histories of patients in whom fibrosis played a significant role in the pathogenesis of their disease, and to determine whether intravenous gammaglobulin (IVIg) contributed to the regression of their fibrotic condition.

**Methods:** Eight patients with excess fibrotic reaction in the course of diverse diseases were analysed; a tendency that reverted with different IVIg treatment options. Myelofibrosis was predominant in three patients (a patient with a myeloproliferative syndrome, one with systemic lupus erythematosus, and one with Sjögren's syndrome). Three patients had scleroderma as their main feature, one patient had hepatitis C cirrhosis, and one had idiopathic thrombocytopenic purpura.

**Results:** Fibrotic excess was reduced in all the patients by IVIg treatment. In five patients the disease as a whole benefited from the infusion of immunoglobulins.

**Conclusion:** IVIg may enhance resorption of fibrosis and promote healing in patients with fibrotic associated disorders.

Deposition of collagen, laminin, fibrinogen, and other molecules is fundamental to the processes of inflammation and healing. However, the accumulation of excessive amounts of these extracellular proteins may lead to malfunction of vital organs, resulting in myelofibrosis, cirrhosis, pulmonary fibrosis, retroperitoneal fibrosis, and other conditions. Recently, we reported the beneficial effect of intravenous gammaglobulin (IVIg) in the tight skin murine (Tsk) model. Tsk mice carry a dominant mutation in the fibrillin-1 gene on chromosome 2, which causes a systemic sclerosis-like disease, characterised by increased collagen deposition in the skin and visceral organs, starting two weeks after birth. By treating Tsk mice at the age of 4 weeks with IVIg for eight weeks we demonstrated decreased cutaneous collagen staining, abrogated collagen-I mRNA expression, and inhibition of transforming growth factor  $\beta$  and interleukin 4 (IL4) secretion by splenocytes.<sup>1</sup>

In this paper we present eight patients from different countries (some reported on elsewhere), in whom fibrosis regressed after treatment with IVIg. These observations may shed some light on the diverse mechanisms that are precursors to fibrosis, and may lead to an adjuvant treatment.

### PATIENTS

Table 1 summarises the clinical, aetiological, and therapeutic characteristics of the patients with different fibrotic conditions who were treated with IVIg.

#### Cases No 1–3: Resolution of systemic sclerosis<sup>2</sup>

Three patients with systemic sclerosis were admitted because of a progressive and rapidly deteriorating symptomatic skin

disease that did not respond to any conservative treatment. The duration of their illness ranged from less than one year to 20 years. All three patients had diffuse cutaneous sclerosis and reflux oesophagitis, of whom one also had a mild restrictive lung disease.

Six cycles of IVIg were given to two patients, resulting in an impressive decrease in their skin thickness score, from 26 before IVIg treatment to 16 after treatment in one patient and from 34 to 20 in the other, calculated by the modified Rodnan total skin thickness score. The third patient responded favourably to three cycles of IVIg treatment, with an increment of her pulmonary diffusion indices (carbon monoxide transfer factor) from 70% to 84% and with a concomitant decrease of skin thickness from 32 to 20.

#### Case No 4: Resolution of myelofibrosis

A 56 year old man presented with bilateral swelling of the parotid glands that caused some discomfort and gave a constant "mumps-like" look to his face. Shortly thereafter he developed progressive symptoms of dryness of the mouth and irritation of the eyes. A diagnosis of primary Sjögren's syndrome was made as at least two functional test results for the lachrymal and salivary glands were abnormal and in addition the focus score in the small salivary glands was >1 simultaneously with the presence of anti-SSA and -SSB autoantibodies.<sup>3</sup> The disease remained stable for 14 years, after which the patient developed generalised urticarial vasculitis with progressive anaemia (83 g/l) and thrombocytopenia ( $<60 \times 10^9$  cells/l). A bone marrow biopsy demonstrated myelofibrosis. The patient did not respond to subcutaneous injections of erythropoietin and glucocorticosteroids, and became dependent on blood transfusion. After a year, 2 mg/kg IVIg was given over two days, followed by three cycles at intervals of three weeks. Both haemoglobin and platelets levels rose to 108 g/l and  $175 \times 10^9$  cells/l, respectively. Three years later the patient still has normal peripheral blood cell values.

#### Case No 5: Resolution of cutaneous fibrosis<sup>4</sup>

A 67 year old woman with chronic idiopathic thrombocytopenic purpura, mild anaemia, leucopenia, and splenomegaly presented after three years of unsuccessful treatment with multiple conventional regimens. Intravenous infusions of the anti-D IgG preparation at 10 day intervals completely stopped the bleeding (the patient is Rh positive). Her slow platelet count rise was not affected by extending the infusion interval to 20 days ( $10 \times 10^9/l$  to  $70 \times 10^9/l$ ). She received treatment for eight years and, interestingly, her plantar calluses have completely disappeared.

**Abbreviations:** GM-CSF, granulocyte macrophage colony stimulating factor; IFN, interferon; IL, interleukin; IVIg, intravenous gammaglobulin; SLE, systemic lupus erythematosus; Tsk, tight skin

**Table 1** Clinical, aetiological, and therapeutic characteristics of patients with different fibrotic conditions who were treated with IVIg

Primary disease and No of case	Clinical and laboratory findings	Impact of IVIg treatment	Duration of IVIg treatment	Total amount of IVIg	Ref
Systemic sclerosis Case Nos 1–3	All patients had diffuse cutaneous sclerosis and reflux oesophagitis, one with a mild restrictive lung disease	Decrease of the skin thickness, improvement of pulmonary diffusion indices	Patients 1 and 2, 6 months Patient 3, 3 months	Patients 1 and 2, 720 g Patient 3, 56 g	2
Sjögren's syndrome Case No 4	Dryness of the mouth and eyes	Decrease in exocrine glandular symptoms and near normalisation of myelofibrosis—clinically and histologically. Normalisation of red blood cell and platelet counts 1.5 months	1.5 Months	420 g	
Idiopathic thrombocytopenic purpura Case No 5	Chronic thrombocytopenia After 10 years	Rise in platelet count, disappearance of plantar calluses, and rejuvenation of skin	Very low doses IV anti-D for almost 8 years	Anti-D 63 mg	4
SLE Case No 6	Weakness, anorexia, weight loss, arthralgia, marked hepatosplenomegaly, lymphadenopathy. Pancytopenia. Anti-dsDNA IgG, and IgM anticardiolipin, antihistone antibodies, anti-Ro and hypocomplementaemia and myelofibrosis	Resolution of marrow fibrosis	4 Months	720 g	5
Myelofibrosis Case No 7	Osteosclerosis, splenomegaly and pancytopenia. Trisomy 1 (karyotype: 47XY, +1) in haematopoietic cells	After resolution of fibrosis inside the bones, haematopoietic tissue fully repopulated its osseous habitat. Noteworthy reduction of palm callosities	6 Years high dose IgG, IgM, IgA	IgG 4.316 kg, IgM 647 mg, IgA 647 mg	6
Hepatitis C virus cirrhosis Case No 8	Splenomegaly pancytopenia, ascites and prominent collateral circulation, destabilising haemorrhages due to combined thrombocytopenia and K dependent coagulation factors' deficit	Bleeding stopped, recovery of liver function normalises K dependent coagulation factors. Complete ascites resorption—splenomegaly pancytopenia persisting	7 Years	Anti-D 6.6 mg, IVIg 4.5 kg	7

**Case No 6: Resolution of myelofibrosis<sup>5</sup>**

A 54 year old woman presented with exertional dizziness, general weakness, anorexia, weight loss, bilateral knee arthritis, and pleuritic chest pain with bilateral pleural effusion. Physical examination showed marked hepatosplenomegaly, axillary and groin lymphadenopathy. Along with pancytopenia, many burr cells, teardrop cells, and ovalocytes were seen on the peripheral blood smear. Serological tests were positive for antinuclear factor, anti-dsDNA (47%), anticardiolipin, antihistone antibodies, and anti-Ro, and negative for anti-La antibodies with concomitant hypocomplementaemia. In the bone marrow, increased deposition of reticulin and collagen with focal hypercellularity was noticed. Based on these findings the diagnosis of systemic lupus erythematosus (SLE) was established, and treatment with oral prednisone was started to no avail. Later, treatment with IVIg (400 mg/kg/day for five days) was begun. Within a week the haemoglobin concentration rose significantly and stabilised at near normal levels. Concurrently, the prednisone dose was gradually tapered. After the third course of IVIg treatment a second bone marrow biopsy showed marked regression of the fibrosis and regeneration of the normal marrow tissue. Five years after her fourth and last therapeutic course with IVIg the patient remains clinically, serologically, and haematologically stable.

**Case No 7: Resolution of myelofibrosis and cutaneous fibrosis<sup>6</sup>**

This 59 year old man developed painful osteosclerosis with splenomegaly and pancytopenia. After unsuccessful interferon (IFN)  $\alpha$ 2b monotherapy, a splenectomy was performed, which alleviated the mechanical burden. Improvement was noted after IVIg treatment was added. Within 30 days the thrombocytopenia and anaemia markedly improved, with a concomitant drop in the blast levels from 8% to normal values. After 14 months of combined treatment, marrow biopsy specimens from the same iliac spine that in the past yielded a

dry tap, demonstrated spongiosa-like tissue and harvested cell-rich haematopoietic material (karyotype: 47XY, +1).

After a follow up period of 71 months, the patient having received a total of 154 reinforcement doses of IVIg, normal haematopoietic tissue is maintained, which seems to be dependent on the continuation of this combination treatment. A six month period during which IFN was stopped provided evidence that the patient's wellbeing depended on the association of IFN with IVIg. After the drug was resumed the patient again started to do well. The size of the scar from splenectomy also decreased with time.

**Case No 8: Resolution of cirrhosis<sup>7</sup>**

This patient with chronic active hepatitis C virus infection has been described at length previously. On admission at the age of 70, the patient presented with prominent ascites with collateral circulation from breast to groin. Hypersplenism accounted for the pancytopenia. His profuse haemorrhagic syndrome was attributed to combined thrombocytopenia and vitamin K dependent coagulation factors' deficiency in the presence of portal hypertension.

Bleeding remitted with weekly intravenous infusions of small amounts of polyclonal anti-D IgG, 4.5–5.5  $\mu$ g/kg body weight (the patient was Rh negative). When after five months IVIg treatment became available, it replaced anti-D IgG. For seven years the patient enjoyed a full and active life. IFN  $\alpha$ 2b, three times a week, was intermittently added during the first years of treatment. His death was caused by hyperacute parkinsonism. The patient throughout the years refused a liver biopsy; however, imaging procedures detected findings compatible with cirrhosis. As a farmer he had worked in the open, but despite his extensive exposure to the sun his skin appeared to be less wrinkled during his last two years.

**DISCUSSION**

Several mechanisms of action that might be connected with inhibition or reversal of fibrosis have been attributed to IVIg.

IVIg treatment modulates the production, release, and function of proinflammatory cytokines. IVIg down regulates the production of IL2, IL3, IL4, IL5, IL10, tumour necrosis factor  $\alpha$  and granulocyte macrophage colony stimulating factor (GM-CSF) by peripheral blood mononuclear cells and attenuates the intensity of inflammation.<sup>8</sup> The administration of IVIg leads also to the production and secretion of natural cytokine antagonists such as the IL1 receptor antagonist which abrogates the activity of IL1 and IL8.<sup>9</sup> Anticytokine antibodies have been detected in the sera of healthy subjects, therefore it is not surprising that significant titres can be found in IVIg preparations. High levels of polyclonal anticytokine antibodies to IL1 $\alpha$ , IL6, IL10, IFN $\alpha$ , and GM-CSF have been reported.<sup>10</sup>

IVIg also impedes the rate of tumour necrosis factor  $\alpha$  or IL1 $\beta$  stimulated transcription of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 and of the chemokines monocyte chemoattractant peptide-1, M-CSF, and GM-CSF. These molecules have a significant role in the process of inflammation and fibrosis.<sup>11</sup> Recently, it has been shown that an anti-Fas (CD95/Apo-1) antibody can induce apoptosis in fibroblasts in a dose and time dependent manner. IVIg was found to induce apoptosis in some cell lines, involving, at least in part, the Fas (CD95/Apo-1) gene.<sup>12</sup>

Cases 5 and 8 deserve special attention: Intravenous polyclonal anti-D IgG preparations induced antifibrotic effects similar to those of IVIg. The efficacy of anti-D preparations in cases of idiopathic thrombocytopenic purpura stems from the FcR blockade they cause, sparing continuous destruction of platelets.<sup>13</sup> There is no controlled study comparing anti-D and IVIg treatment, but the former is less expensive. Patient No 8 had a favourable response to anti-D treatment even though he was RhD negative, implying that various mechanisms mediate its beneficial effect.<sup>14</sup>

In this communication we report on eight patients with phenomena related to fibrosis of diverse aetiologies. Regardless of the primary aetiology that stimulated fibroblast proliferation IVIg treatment reversed, to some extent, the fibrotic process in each patient. Owing to the relatively low incidence of side effects of IVIg treatment, these case histories underline the importance of evaluating the role of IVIg in these conditions which until now were regarded as incurable.<sup>15</sup>

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