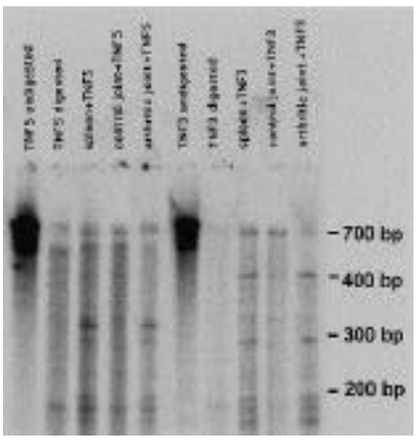


**Fig. 1.** RPA of TNF-α clones. *In vitro* transcription of TNF3 and TNF5 was performed with [$^{32}$P]CTP to obtain anti-sense probes. 20 µg RNA from spleen, healthy joints and arthritic joints was hybridised to the anti-sense probes from both TNF-α clones and digested with 40 pg/ml RNase A and 700 units/ml RNase T1. The purified RNA-RNA duplexes were analysed on an urea/acrylamide gel and subjected to autoradiography. Anti-sense probes hybridise to RNA from all tissues. Smaller bands indicate the presence of TNF-α variants with bp mismatches.

corresponded to previous results from rat TNF-α, excluding the stop codon that was not part of the PCR primer (7). The two clones differed at bp 365 (cytosine in TNF3 and thymidine in TNF5) and 568 (adenine in TNF3 and guanine in TNF5). Comparing TNF3 to the NIH database we found two cDNA and 3 DNA sequences with 12, in part repetitive, mismatches (NM_012675, AJ002278, D00475, L00981 and L19123). Eight nt changes (66.6%) were found at the third codon position without amino acid exchanges. Four nt replacements at the first and second codon positions resulted in amino acid exchanges such as proline versus leucine (bp 116 and 365), isoleucine versus threonine (bp 488), lysine versus glutamic acid (bp 566) and phenylalanine versus serine (bp 605). Both TNF-α transcripts were expressed in inflamed and control tissues as indicated by 705 bp RNA protected bands (Fig. 1) suggesting that the two clones are not selectively upregulated in arthritis. Furthermore, nucleotide variations present in arthritic joint-derived cDNA did not result from an error of the PCR-Taq polymerase. The RNase A and TI enzymes used to digest unhybridized RNA are capable of recognizing any single bp mismatches within the RNA-RNA hybrids. Smaller RPA bands indicate the presence of several minor variant sequences without a significant predominance for arthritic tissue.

Based on these results and the analysis from the NIH database, gene polymorphisms do not seem to be of biological relevance because most coding nt variations in the rat species occur without consecutive aa exchanges. Although some singular nt variations cause aa replacements, these changes are unlikely to result in relevant altered peptide confirmations. The described TNF-α polymorphisms thus appear to play a subordinate role in CIA.

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## Pancytopenia in ANCA associated vasculitis: Response to immunosuppressive therapy

Sirs,

We report two patients with antineutrophil cytoplasmic antibody (ANCA) associated disease, where severe pancytopenia was a disease manifestation that was improved by immunosuppressive therapy. Microscopic polyangiitis (MPA) is differentiated from polyarteritis nodosa (PAN) by small vessel involvement and the absence of microaneurysms (1). Rapidly progressive glomerulonephritis and pulmonary alveolar haemorrhage are often part of the disease and vasculitic lesions were found in a wide variety of organs (2). Wegener's granulomatosis is a small vessel vasculitis with upper and lower respiratory tract disease, and can have fatal progression in its generalised form (3).

A 37-year-old Caucasian male presented with anterior uveitis, ankle arthritis, recurrent mouth ulcers, nodular vasculitic cutaneous lesions, thrombophlebitis, peripheral sensory neuropathy and intermittent pulmonary shadowing with right pleural pain. Renal biopsy showed diffuse mesangial proliferative glomerulonephritis with no vasculitis or crescents and with no immune deposition. IgG pANCA were positive as were anti-myeloperoxidase antibodies. Antinuclear antibodies were negative. Coeliac axis and mesenteric angiography was normal. A variety of therapies were used starting with prednisolone, followed by intravenous and then oral cyclophosphamide, azathioprine, cyclosporin, thalidomide and methotrexate, over four years. He continued to have flares with cutaneous leucocytoclastic vasculitis lesions (immunofluorescence negative).

He then worsened clinically and presented with severe pancytopenia; haemoglobin (Hb) 6g/dl,platelet (Plt) 50,000/mm$^3$, white cell count (Wcc) less than 2,000/mm$^3$, neutrophils (Neu) 500/mm$^3$ and a MCV greater than 120 Femto/L. Bone marrow biopsy showed all cell lines present and maturing fully. Haematopoetic cells were numerous and adipocytes were sparse. Myelodysplastic changes, probably related to chemotherapy, were also present and included architectural derangement and an increase in immature precursors. On oral cyclophosphamide (100 mgs od) he gradually improved within four months with a Hb 7.3 g/dl,Wcc 4,500/mm$^3$ and Plt 153,000/mm$^3$. He is clinically stable on methotrexate (20 mg/week) but still has chronic anaemia, with normal reticulocyte numbers.

The second patient, a 60-year-old Caucasian male, presented with a flare of his inflammatory bowel disease, migratory superficial thrombophlebitis, recurrent non-

infective pneumonitis and pleurisy, arthralgia and sinusitis. He responded to methyl prednisolone pulses and prednisolone in increasing doses, and azathioprine.
During the following year he had recurrent red eyes, headaches, cough and breathlessness. An NW scan showed extensive sinus opacification. His status deteriorated and he failed to respond to prednisolone up to 25 mg/day and in turn oral cyclophosphamide, azathioprine and mycophenolate mofetil. C-ANCA was weakly positive and cryoglobulins were negative. His urine sediment showed red blood cells (RBC) and some RBC casts. Renal biopsy showed thin glomerular basement membrane disease with no other glomerular changes or vasculitis. He then worsened and developed pancytopenia: Hb 7.2 g/dl, Wcc 1,000/mm$^3$, Neu 900/mm$^3$ and Plt 48,000/mm$^3$. A nodule on the left forearm showed a granulomatous vasculitis characteristic of Wegener's granulomatosis (4). A bone marrow biopsy and trephine were hypercellular and all three cell lines were represented without granulomata or abnormal infiltrates.
He was started on etanercept (ENBREL®) alone, 25 mg twice a week, and dramatically improved in less than a week; FBC showed: Plt 79,000/mm$^3$, Wcc 1,900/mm$^3$, Hb 11.6 g/dl, Neu 1,200/mm$^3$. Methotrexate, 10mg/week, was then started (5-7). The patient continued on etanercept and methotrexate and is in clinical remission.
We have described here two patients with ANCA associated systemic vasculitis who developed pancytopenia that was apparently related to their disease activity. Neither patient had evidence of splenomegaly, viral infection, haemolysis, immune complex disease or drug toxicity and the bone marrow in each case was hypercellular. The rapid improvement in the peripheral blood count with oral cyclophosphamide or etanercept plus methotrexate strongly supports the idea that the pancytopenia was an autoimmune phenomenon and not related to the previous immunosuppressive therapy.
The second patient experienced a life-threatening relapse despite a variety of immunosuppressive agents, so etanercept was administered with excellent results. Whilst the haematological picture of pancytopenia with normal bone marrow is very well described in SLE, to our knowledge this is the first such report in two patients with ANCA associated vasculitis.

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## Successful treatment of chronic fatigue syndrome with midodrine: A pilot study

Sirs,
Systematic review of studies on treatment of chronic fatigue syndrome (CFS) revealed that only cognitive behavior therapy and graded exercise are consistently beneficial (1). Both of these modalities are palliative at best, however. Since dysautonomic cardiovascular reactivity is frequently present in CFS patients (2,3), we hypothesized that therapies directed to improve dysautonomia may also improve the symptom of fatigue. Midodrine HCl, a potent α-1-adrenergic agonist, is efficient in the treatment of dysautonomic syndromes such as orthostatic hypotension, vasovagal syncope and postural tachycardia syndrome (4, 5). We conducted an open study to test whether midodrine treatment could benefit patients with CFS. On review of the MEDLINE database we did not find a study describing midodrine treatment of CFS.
Ten patients with CFS meeting the Centers of Disease Control and Prevention criteria for CFS (6) were enrolled in this study, of whom 3 did not complete the treatment and were excluded from analysis. This group included 4 males and 3 females, their mean age was 27 years (range 16 to 29 years),and the mean duration of CFS was 11 months. The control group of 'non-CFS fatigue' patients had fatigue of similar severity to that of CFS patients, but did not otherwise meet the definition criteria of CFS. There were 2 males and 3 females, with a mean age of 42 years (range 26 to 72 years) and a median illness duration of 13 months. The presence of psychiatric disorders were exclusion criteria in both the patient and control groups (6).
The severity of the fatigue was estimated on a scale of 0 to 33 based on Chalder's fatigue severity questionnaire (7). The cardiovascular reactivity was evaluated according to the results of a 10-minute supine/30 minute head-up tilt test (HUTT) (8). Dysautonomic reactivity was recognized either when orthostatic hypotension, vasodepressor reaction,a cardioinhibitory reaction, or postural tachycardia syndrome occurred on tilt, or when a hemodynamic instability score (HIS) > -0.98 was found. The HIS was calculated according to a method recently described by us (8). HIS values >-0.98 strongly correlated with a distinctive cardiovascular reactivity in CFS, with 90.3% sensitivity and 84.5% specificity (8-10).
The patients were off medications for at least two weeks before entering the study. Midodrine treatment was started, p.o. 2.5 mg twice daily in patients and controls. Two weeks later, the HUTT was repeated. When

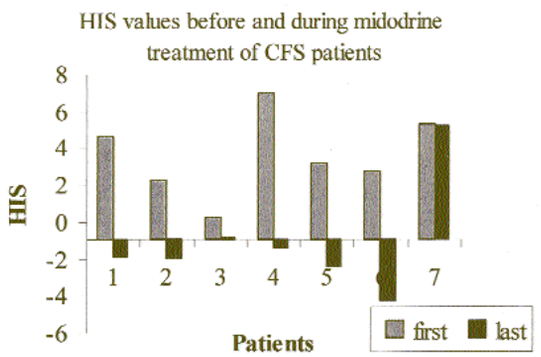


**Fig. 1.** HIS values before and during midodrine treatment of patients with CFS. Normalization of the HIS occurred in 6/7 patients.