Drug-induced agranulocytosis during treatment with infliximab in enteropathic spondyloarthropathy

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ABSTRACT
Agranulocytosis is a disorder characterized by a severe decrease in the number of granulocytes in blood, that frequently occurs as an adverse reaction to some drugs. By now, there are no reports in literature of agranulocytosis caused by tumor necrosis factor-α blockers.

We describe the case of a 20-year-old Caucasian male affected by enteropathic (Crohn’s disease) spondyloarthropathy HLA B27 negative, successfully treated with infliximab. After the second infliximab infusion, he was found to have a severe transient neutropenia (0.5 x 10^9/L). Routine serum chemistry and full blood cell count (apart from neutrophil count) were normal. Serology excluded an active infection. Bone marrow needle aspirate showed a normal trilineage differentiation. Autoantibody assessment showed negative ANA, anti-dsDNA, anti-ENA, and ANCA, but positive granulocyte-bound antibodies (GBA) and neutrophil-specific (CD 16+)-bound antibodies (anti-NA).

Ten weeks after infliximab infusion, neutrophil count and GBA and anti-NA assay returned spontaneously within normal range and we observed the same progress after every successive infliximab infusion we performed.

These data indicated that infliximab possibly triggered production of granulocyte and neutrophil autoantibodies with resultant autoimmune agranulocytosis.

Introduction
Agranulocytosis is defined as a decrease in the number of granulocytes in circulating blood, resulting in a neutrophil count of less or equal to 0.5 x 10^9/L. Most instances of neutropenia result from exposure to many drugs, and either the drug itself or a metabolite may be causative. Some of these drugs, such as chloralidone, clozapine, antithyroid drugs, sulfamethoxazole-trimethoprim, penicillins, benzodiazepines, antidepressants, and phenothiazines, are well-known causes of neutrophil count decrease (1); for others drugs, this is less certain. To date, in the medical literature, no cases of neutropenia related to treatment with tumor necrosis factor-α blockers have been reported.

We describe a case of a 20-year-old Caucasian male affected by enteropathic (Crohn’s disease) spondyloarthropathy, who developed a severe transient agranulocytosis, possibly triggered by i.v. infliximab treatment.

Case report
A 20-year-old Caucasian male was admitted with an 11-year history of Crohn’s disease (diagnosis performed by ileocolonscopic gut biopsy) and enteropathic spondyloarthropathy HLA B27 negative with significant axial involvement. He had been treated for 7 years with sulfasalazine (2000 mg daily) and nonsteroidal anti-inflammatory drugs (NSAIDs) and then with mesalazine (800 mg daily) and oral steroids for 4 years without significant improvement.

At the time of admission, the patient presented with active disease and a erythrocyte sedimentation rate (ESR) of 90 mm/hour, C-reactive protein (CRP) level of 9.3 mg/L (normal range < 1 mg/L), Bath Ankylosing Spondylitis Functional Index (BASFI) of 8, and Bath Ankylosing Spondylitis Disease Activity Index (BASDAI) of 8.2.

Because of lack of efficacy, mesalazine and oral steroids were discontinued and the patient was administered i.v. infliximab (5 mg/Kg). At the beginning of therapy, routine serum chemistry and full blood cell count were essentially normal (WBC: 6.00 x 10^9/L, with 61.8% neutrophils and 29.7% lymphocytes). Based on previous experience with onset of drug-induced lupus syndrome in a patient treated with infliximab (2), we decided to perform routine detection of antinuclear antibodies (ANA) and anti-double-stranded DNA (anti-dsDNA). Both tests, as well as rheumatoid factor, gave negative results.

Before performing the second infusion (2 weeks after the first one), we registered a significant improvement in the physical findings and axial involvement, with an ESR of 20 mm/hour, CRP of 0.9 mg/L, BASFI of 20, and BASDAI of 2.5. Full blood cell count was normal (WBC 5.59 x 10^9/L, with
51.8% neutrophils and 33.4% lymphocytes). Therefore, the second infliximab infusion was performed at the same dose. Two weeks later, clinical improvement was confirmed (ESR 12 mm/hour, CRP 0.8 mg/L, BASFI 22, and BASDAI 2.4). Complete blood cell count disclosed a WBC count of 5.82 x 10⁹/L (with 9% neutrophils [0.5 x 10⁹/L] and 82.5% lymphocytes), and a normal haemoglobin, red blood cell, and platelet count. Physical findings were grossly negative. There were no symptoms or signs that suggested any infective complication and serology excluded active infection with Epstein-Barr virus, Parvovirus B19, TORCH, Brucella, Salmonella, HIV, HBV and HCV; Mantoux test was negative. Chest X-ray, abdominal ultrasonic tomography, and routine serum chemistry were essentially normal, as well as serum complement, vitamin B12, folate and lymphocyte subpopulations. ANA, anti-ds-DNA, anti-extractable-nuclear antibodies (anti-ENA), rheumatoid factor, and ANCA were negative.

Bone marrow needle aspirate showed a myeloid/erythroid ratio of 1.4, and normal trilineage differentiation. No maturation arrest of granulocytes in bone marrow testing was observed. Colony-forming units of the bone marrow cells were within normal limits.

Detection by granulocyte immunofluorescence test (GIFT) of granulocyte-bound antibodies (GBA), serum granulocyte antibodies (SGA), neutrophil-specific (CD 16+) bound antibodies (anti-NA), and serum neutrophil-specific (CD 16+) antibodies was also performed on peripheral blood. As shown in Figure 1, both GBA and anti-NA IgG and IgM antibodies were strongly positive, whereas SGA and serum neutrophil-specific IgG and IgM antibodies were negative.

The WBC count was repeated and the general condition of the patient was monitored weekly for 2 months; the neutrophil count remained significantly low till the 8th week and then started to increase progressively, till normalization within the 12th week (neutrophils 2.8 x 10⁹/L). Autoantibodies detection, repeated at the 8th and 12th week, showed a significant progressive reduction of GBA and anti-NA levels, which were completely negative at the 12th week determination.

Based on the time-progress of neutrophil count and anti-granulocyte and anti-neutrophil antibodies demonstration, we conclude that neutropenia in this patient was probably autoimmune in nature and possibly triggered by infliximab.

Considering the significant improvement of the patient spondiloarthropathy and his good general conditions in spite of neutropenia, in this case we decided to continue with the infliximab therapy, periodically monitoring neutrophil count (Fig. 2).

After the 5th infusion, neutrophil count (0.4 x 10⁹/L) decreased so significantly that the patient was administered with human recombinant granulocyte-colony-stimulating factor (G-CSF, Filgrastim: 0.5 megaunits/Kg sc daily for 5 consecutive days), producing a shorter duration of neutropenia with faster haematological recovery of neutrophil count (Fig. 2).

### Discussion

Agranulocytosis is a life-threatening disorder characterized by a profound and transient decrease in the number of granulocytes in circulating blood, with a neutrophil count equal or less then 0.5 x 10⁹/L (3).

Most cases of agranulocytosis result from exposure to drugs, but other causes include infectious diseases (especially viral), immune disorders as autoimmune neutropenia, systemic lupus erythematosus, or Felty’s syndrome.
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Nutritional deficiencies (vitamin B12 and folate), haematological diseases (such as myelodysplastic syndrome, and congenital or chronic neutropenia (cyclic neutropenia, Kosinann's syndrome) (4).

Drug-induced agranulocytosis (DIA) is an uncommon event, the overall incidence ranges from 2.6 to 10 cases per million patients exposed to drugs per year (5). Diagnosis of DIA is based on both exclusion of an underlying disease and identification of temporal correspondence between onset and recovery of agranulocytosis and drug administration (6). Despite the existence of these consensus criteria of DIA, it is often difficult to establish a precise association and to evaluate the risk connected with a particular drug. Even if several drugs, such as chloralhydrate, clozapine, antithyroid drugs, sulfa-methoxazole-trimethoprim, penicillins, benzodiazepines, antidepressants, and phenothiazines, have been reported to be well-known causes of transient neutropenia (1), there have been to date no reports in the literature of infliximab-related DIA (7, 8).

In the case reported here, neutropenia was disclosed one week after the second infliximab infusion in a patient who did not present any other specific risk factor for agranulocytosis. He was and remained totally asymptomatic, as do a minority of DIA cases (9), despite the fact that most patients develop isolated fever, documented infections, septicaemia and septic shock or present general malaise, headache, chills, myalgia, and arthralgia (1).

Before making the diagnosis of DIA, we excluded every other cause of agranulocytosis by performing further tests. Bone marrow aspirate showed a normal total cellularity, as widely described in the literature (10); serology excluded active infection with any of the pathogen agents typically linked to DIA (11); no nutritional deficiency (vitamin B12 or folate) was found; autoantibody assays for ANA, anti-dsDNA, anti-ENA, rheumatoid factor, and ANCA excluded an underlying collagen autoimmune disease.

Direct toxicity and antineutrophil antibody mechanisms represent the two prevailing hypotheses to explain DIA and are supported by clinical and experimental data (12). In the first case, it is suggested that implicated drugs, or more likely their reactive metabolites, may interact with specific components of the bone marrow extracellular matrix (such as fibronectin, hemocitin, and other adhesion molecules) and may interfere with the normal regulation of granulopoiesis (13). In the second case, several immunological mechanisms, such as opsonizing neutrophils, neutrophil or early neutrophil precursors direct cytotoxicity, and neutrophil agglutination by antineutrophil antibodies, have been documented (14). Other mechanisms, involving cytotoxic T cells, haptens, autoreactivity and oxidative modification of drugs, have been evoked (15, 16).

Unfortunately, there is no way to determine for certain whether toxic or immune reactions (or both) cause DIA in a given individual.

In the case reported here, several factors may have led to an immunological reaction. First, the time-course of the appearance and re-occurrence of agranulocytosis upon re-exposure to infliximab. The neutrophil count decreased rapidly after every drug infusion, getting to the lower level within one week, excluding the possibility of bone marrow direct toxicity, which is instead typically characterized by a delay of 6-12 weeks between drug administration and occurrence of agranulocytosis (17).

Second, the detection of GBA and anti-ANA by GIFT, present in a high titre in the first 8 weeks after infliximab administration, progressively and spontaneously decreasing till normalization within 12th week, when clearance of infliximab was complete.

Finally, it has been demonstrated that infliximab can induce the production of a series of autoantibodies such as ANA, anti-dsDNA, by means of immunological mechanisms, which have not been yet completely clarified (2,18,19,20).

In the same way, it may be thought that the production of anti-granulocyte antibodies is triggered by infliximab and stopped exactly at the end of the period of infliximab clearance [8-10 weeks after the infusion (21)].

The management of DIA begins with the immediate withdrawal of any potentially causative drug. Measures to be undertaken concomitantly include the aggressive treatment of any diagnosed sepsis, as well as the prevention of secondary infections (5). In our case, the patient remained always asymptomatic and did not present any sign of any infective complication. Because of this fact and the significant improvement of the axial involvement, in this particular case we decided to go on with administration of infliximab, strictly monitoring neutrophil count and clinical findings.

The usefulness of human recombinant haematopoietic growth factors in agranulocytosis is described in drug-induced cases as well as in autoimmune neutropenia (22). As in our experience, Filgrastim is generally well tolerated, produces a shortening of the duration to blood count recovery and may prevent infective complications.

Therefore, the use of G-CSF can be considered a safe and effective procedure in all those infliximab-related DIA cases, in which treatment with infliximab is absolutely necessary.

References

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