Non–chemotherapy drug–induced neutropenia: key points to manage the challenges

Brian R. Curtis

Blood Research Institute, BloodCenter of Wisconsin, Milwaukee, WI

Non–chemotherapy idiosyncratic drug–induced neutropenia (IDIN) is a relatively rare but potentially fatal disorder that occurs in susceptible individuals, with an incidence of 2.4 to 15.4 cases per million population. Affected patients typically experience severe neutropenia within several weeks to several months after first exposure to a drug, and mortality is ~5%. The drugs most frequently associated with IDIN include metamizole, clozapine, sulfasalazine, thiamazole, carbimazole, amoxicillin, cotrimoxazole, ticlopidine, and valganciclovir. The idiosyncratic nature of IDIN, the lack of mouse models and diagnostic testing, and its low overall incidence make rigorous studies to elucidate possible mechanisms exceptionally difficult. An immune mechanism for IDIN involving neutrophil destruction by hapten (drug)-specific antibodies and drug-induced autoantibodies is frequently suggested, but strong supporting evidence is lacking. Although laboratory testing for neutrophil drug-dependent antibodies is rarely performed because of the complexity and low sensitivity of tests currently in use, these assays could possibly be enhanced by using reactive drug metabolites in place of the parent drug. Patients typically experience acute, severe neutropenia, or agranulocytosis ($<0.5 \times 10^9$ neutrophils/L) and symptoms of fever, chills, sore throat, and muscle and joint pain. Diagnosis can be difficult, but timely recognition is critical because if left untreated, there is an increase in mortality. Expanded studies of the production and mechanistic role of reactive drug metabolites, genetic associations, and improved animal models of IDIN are essential to further our understanding of this important disorder.

Learning Objectives
- Describe the clinical findings, incidence, and mortality associated with idiosyncratic drug-induced neutropenia (IDIN)
- List the drugs most frequently implicated in IDIN
- Discuss the current mechanisms proposed to explain IDIN and methods for drug-dependent neutrophil antibody testing

Granulocytes/polymorphonuclear leukocytes are a group of white blood cells (WBCs) that includes basophils, eosinophils, and neutrophils. Neutrophils are both the most abundant granulocyte and WBC in blood. A healthy human adult has 4.5 to 10 billion WBC/L, and ~60% are neutrophils. Neutrophils are critical to host defense against infectious agents such as bacteria and fungi. They phagocytize, kill, and digest these foreign invaders before they are allowed to multiply and cause disease. A reduction of neutrophils (segmented and band cells) in the blood to an absolute neutrophil count (ANC) of 1.5×10^9 cells/L is considered neutropenia. An ANC $<0.5 \times 10^9$ cells/L is considered severe neutropenia (often termed agranulocytosis), and individuals with an ANC $<0.1 \times 10^9$ cells/L are at severe risk of morbidity and mortality from infections.

Because there are numerous causes of neutropenia, making the determination of the possible cause in a patient is very difficult for clinicians. Some of these many possible causes are listed in Table 1.

Although it is not the most common cause of all neutropenias (ANC $<1.5 \times 10^9$ neutrophils/L), drug-induced neutropenia must be considered in patients with unexplained neutropenia. Drug-induced neutropenia is also a serious concern for the development of new drugs[1] because it can be missed in clinical trials if the incidence is low; in severe cases, it can even lead to withdrawal of a drug from the market.[2]

Drug-induced neutropenia

Drug-induced neutropenia is caused by decreased production or increased destruction of neutrophils. Decreased production is frequently a consequence of chemotherapeutic drugs that cause suppression of bone marrow myeloid progenitor cells. Chemotherapy drug–induced neutropenia has a very high incidence in oncology patients.[3] Idiosyncratic drug-induced neutropenia (IDIN) resulting from increased neutrophil destruction is commonly caused by adverse idiosyncratic reactions to nonchemotherapy drugs and is the focus of the present article. The topic is important because IDIN is a major source of morbidity and mortality, which adds significantly to the cost of health care.

IDIN incidence and mortality

Although IDIN is not the major cause of all neutropenias (ANC $<1.5 \times 10^9$ neutrophils/L), it is reportedly the cause in as many as two-thirds to three-quarters of severe cases of neutropenia/agranulocytosis (ANC $<0.5 \times 10^9$ neutrophils/L), and because most IDIN incidence data have been calculated for agranulocytosis

Table 1. Causes of neutropenia

Myeloid suppression: decreased production or direct cytotoxicity
1. 1. Inherited bone marrow failure syndromes such as severe congenital neutropenia, cyclic neutropenia, WHIM syndrome, Shwachman-Diamond syndrome, and GATA2 deficiency, and leukemia, acute leukemias, and large granular lymphocytic leukemia
2. Myelodysplastic syndromes
3. Other marrow infiltrative malignancies and disorders
4. Chemotherapy
5. Alcohol use disorder
6. Idiopathic neutropenia in adults
7. Vitamin B_{12} deficiency
8. Copper deficiency
9. Metabolic disorders (Pearson syndrome, Gaucher syndrome, acidemias)

Infection induced
1. Viral infections: hepatitis A, B, C; HIV/AIDS; EBV; CMV; HHV6
2. Lyme disease
3. Malaria
4. *Salmonella* infection
5. Mycobacterial infections
6. Fungal infections
7. Sepsis

Conditions that destroy neutrophils in the bloodstream and that can result in neutropenia include:

Immune mediated
1. Secondary to autoimmune disorders: rheumatoid arthritis, SLE, common variable hypogammaglobulinemia
2. Drug induced: antibiotics, antithyroid drugs, clozapine and others
3. Hypersplenism
4. Autoimmune neutropenia
5. Neonatal alloimmune neutropenia: maternal antibodies against human neutrophil alloantigens destroy fetal and neonatal neutrophils

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HHV6, human herpesvirus 6; SLE, systemic lupus erythematosus.

cases in adults, IDIN is the focus of the present article.[4-6] Recent data from the large, 10-year FAKOS (Berlin Case–Control Surveillance Study) of drug-induced agranulocytosis in adult patients (age range, 19-88 years) involving 51 hospitals in Berlin, Germany, showed that 72% of 88 cases of confirmed agranulocytosis were probably drug related.[7] The actual annual incidence of IDIN varies across different reports and ranges from 2.4 to 15.4 cases per year per million population.[4,8,9] The annual incidence of IDIN also increases with age and polypharmacy.[6,10,11] Reports of the incidence of IDIN in pediatric patients are scarce, but a recent report showed an annual incidence of 3.92 cases per 10 000 pediatric patients.[12]

Mortality associated with IDIN is currently estimated at 5%,[11] which is significantly lower than estimates 20 years ago, which were as high as 20%.[10] This finding is probably a result of improved education of both patients and physicians regarding the risk of IDIN, especially for commonly implicated drugs (eg, clozapine), which allows for early recognition and early initiation of optimum therapy.

Mortality rate also increases with age and with higher frequency in people aged >65 years; the incidence is also slightly higher in women up to age 65 years.[6,9] Other features associated with increased mortality in IDIN are neutrophil count $<0.1 \times 10^9$ cells/L, concomitant renal disease, septicemia, and shock.[4]

Most frequently implicated drugs

Numerous drugs have been implicated in IDIN across multiple drug classes. Of 63 cases of probable drug-induced agranulocytosis observed

in the FASKO study, the drugs most frequently implicated were metamizole (n = 10), clozapine (n = 6), sulfasalazine (n = 5), and thiamazole (n = 5).[7] The 5 drugs most frequently associated with IDIN in a recently reported study of 203 patients were carbimazole (n = 28), amoxicillin (n = 22), cotrimoxazole (n = 19), ticlopidine (n = 10), and valganciclovir (n = 9).[9] Table 2 lists the drugs frequently associated with IDIN from 4 different studies, including drugs most frequently suspected in patients tested for drug-dependent neutrophil antibodies at a large diagnostic neutrophil antibody reference laboratory.

Individual drugs such as poly propylthiouracil and other thioamides for the treatment of thyroid disorders and the atypical antipsychotic clozapine are reported to be more frequently associated with IDIN, with incidences of 0.2% to 0.5%[13] and 1%,[14] respectively. The anthelmintic agent levamisole and the antibiotic vancomycin are also often associated with neutropenia.

Propylthiouracil, carbimazole, and its active metabolite methimazole are used to treat hyperthyroidism (Graves' disease) by decreasing thyroid hormone levels. In a study of 754 Japanese patients who developed IDIN during treatment with primarily methimazole (96%), 24 patients (3.2%) died.[13] IDIN typically develops within 3 months of antithyroid treatment, but cases have been reported that developed within 5 days and after 10 years of treatment.[15] Mechanisms proposed for antithyroid IDIN are development of drug-dependent antibodies and antineutrophil cytoplasmic antibodies.

Clozapine, a dibenzodiazepine, is an atypical antipsychotic medication used in the treatment of schizophrenia resistant to conventional neuroleptic agents. Clozapine is perhaps the drug most often associated with IDIN. Clozapine-induced neutropenia occurs in ~1% of patients, usually during the first 3 months of treatment. The incidence of fatal neutropenia is reduced to 0.03% in patients whose WBC counts are monitored.[11] Clozapine carries 5 black box warnings alerting physicians to the risk of severe neutropenia and the requirement to monitor patients' WBC counts. The mechanisms responsible for severe neutropenia after exposure to clozapine include apoptotic destruction of neutrophils through adenosine triphosphate depletion by an active nitrenium metabolite[16] and inflammasome activation by reactive clozapine metabolites.[4]

IDIN caused by levamisole has been reported for >40 years, originally in patients treated for inflammatory conditions such as rheumatoid arthritis and nephrotic syndrome, and also when used as adjuvant chemotherapy in patients with colorectal cancer.[17] By 2003, cases of agranulocytosis were reported in individuals using cocaine adulterated with levamisole,[18,19] and the Drug Enforcement Administration reports in 2009 showed that 69% of seized cocaine was contaminated with as much as 10% levamisole.[20] Neutropenia reportedly occurs in as many as 61% of people exposed to levamisole-adulterated cocaine.[18] Neutrophil autoantibodies have been detected in sera of some patients with levamisole-related IDIN.[19]

Evaluation over a 7-year period of 114 patients receiving home intravenous vancomycin therapy found that 12% developed vancomycin-induced neutropenia and 3.5% had ANC $\leq 0.5 \times 109/L$.[21] A study of IDIN in cardiothoracic patients receiving vancomycin reported that 8% developed neutropenia.[22]

Proposed mechanisms for IDIN

The unpredictability of IDIN, lack of mouse models and diagnostic testing, and low frequency of occurrence makes systematic studies to

Table 2. Drugs most frequently reported to cause IDIN

Huber et al[7]	Medrano-Casique et al[5]	Andrés et al[9]	Curtis*
Carbamazepine†	Benzylpenicillin†	Amoxicillin†	Cefipime
Clozapine†	Cefipime†	Carbimazole†	Ceftriaxone‡
Metamizole (dipyrone)†	Linezolid	Clozapine†	Ciprofloxacin‡
Sulfasalazine†	Meropenem†	Cotrimoxazole†	Clindamycin
Thiamazole†	Metronidazole	Cefotaxime	Ibuprofen
	Piperacillin-tazobactam†	Noramidopyrine	Levetiracetam
	Teicoplanin	Piperacillin-tazobactam	Piperacillin-tazobactam‡
	Tobramycin	Salazopyrine	Quetiapine
	Torsemide	Ticlopidine†	Sulfamethoxazole/trimethoprim‡
	Vancomycin†	Valganciclovir	Tacrolimus
			Vancomycin‡
			Venlafaxine

*Drugs for which sera received during the period 2012 to 2016 from patients with suspicion of drug-induced immune neutropenia were tested for drug-dependent antibodies in a flow cytometry assay by Platelet & Neutrophil Immunology Lab, BloodCenter of Wisconsin, Milwaukee, WI. No drug-dependent antibodies involving these drugs were detected in any of the patients' sera.
†Five most frequently associated drugs.
‡Five most suspected drugs.

elucidate possible mechanisms extremely difficult. However, an immune-mediated mechanism is currently most often proposed to explain IDIN. Although an immune mechanism is frequently suggested, strong supporting evidence is lacking.[23] An immune mechanism for IDIN is consistent with the clinical presentation in which there is often a delay in neutropenia after exposure to drug in accordance with the time required to develop a primary immune response. The average time to develop IDIN after drug exposure is 1 to 6 months and varies with drug.[4] In addition, there is often a rapid onset of neutropenia on reexposure to the same drug in patients who previously developed IDIN, which is consistent with immunologic memory.[23] One of the most incredible in vivo demonstrations in humans that IDIN is immune in nature was performed in 1952. Investigators injected serum from a patient who developed agranulocytosis associated with aminopyrine exposure into a normal healthy subject together with aminopyrine; the healthy subject experienced acute, profound neutropenia (0.5×10^9 cells/L) after the serum injection.[24] The immune mechanisms for IDIN most frequently described are the hapten hypothesis and the danger signal hypothesis, both of which are discussed in detail in the following sections.

Hapten hypothesis

Haptens are low-molecular-weight (usually <5000 Da) molecules that are not capable of eliciting an immune response by themselves but can when coupled to a large carrier molecule, usually a protein. Antibodies formed against the hapten then bind the hapten–carrier complex and, in the case of IDIN, presumably neutrophil glycoprotein–drug complexes on the cell membrane surface leading to neutrophil clearance/destruction. Drugs such as penicillin and some cephalosporin drugs when covalently linked to cell surface proteins can elicit drug-specific/hapten antibodies. This mechanism is well described for drug-dependent antibodies (DDAbs) targeting red blood cells, which cause drug-induced immune hemolytic anemia.[25]

For the hapten mechanism to occur, the drug must bind tightly, usually covalently, to the protein carrier forming protein–drug adducts. Drug metabolism or biotransformation primarily occurs in the liver where the parent drug molecules are converted into water-soluble metabolites that can be eliminated in the urine to prevent cellular accumulation and toxicity. Biotransformation also produces both stable and reactive

intermediates and metabolites. Reactive metabolites can readily make covalent linkages with cellular proteins, including neutrophil membrane glycoproteins. Although the majority of drug biotransformation occurs in liver hepatocytes, there is increasing evidence that it can occur in other cells, including neutrophils.[4] Neutrophil enzymes such as myeloperoxidase are capable of producing reactive drug metabolites[20] that can form drug–protein adducts which attach to the neutrophil cell membrane with the potential to elicit drug-specific antibody production through a hapten mechanism (Figure 1). Antibody production would of course require presentation of small peptides from drug-modified carrier proteins by antigen-presenting cells (APCs) in the context of class II HLA to T helper (T_H/CD4+) lymphocytes. The majority of evidence in support of the hapten mechanism comes from studies of idiosyncratic drug reactions that cause liver injury and skin hypersensitivity reactions.[2,23,26] However, a large number of drugs associated with a significant incidence of agranulocytosis (including methimazole, propylthiouracil, captopril, levamisole, clozapine, procainamide, dapsone, sulfonamides, vesnarinone, amodiaquine, trimethoprim, diclofenac, carbamazepine, phenytoin, indomethacin, and ticlopidine) are all reported to be oxidized to reactive metabolites,[20,27] which can form drug–protein adducts that are believed to cause neutropenia by a hapten-type mechanism.[20,28] Hapten antibodies were reported to cause IDIN in patients exposed to flecanide.[29]

Danger hypothesis

Presentation of hapten-modified granulocyte proteins by APCs via major histocompatibility complex to T_H cells is referred to as "signal 1" (Figure 1).[4,26,30] For an immune response to proceed, APCs must be activated by signal 2; in the absence of signal 2, immune tolerance results. Signal 2 is the danger signal produced by injured cells that leads to co-stimulation of APC and T_H cells through interaction between B7 on APCs and CD28 on T_H cells, culminating in production of specific antibodies and cytotoxic T cells. There are numerous endogenous molecules (eg, heat shock proteins, hyaluronan fragments) reported to serve as ligands for pattern recognition receptors such as toll-like receptors on APCs that can serve as danger signals. It is not clear how a drug–protein adduct might serve as a danger signal. One possibility is that other danger signals work synergistically with drug danger signals because increased risk of IDIN has been linked to open-heart surgery and specific viral infections,[4] both of which create danger signals. Neutrophils "stressed" by exposure to reactive drug
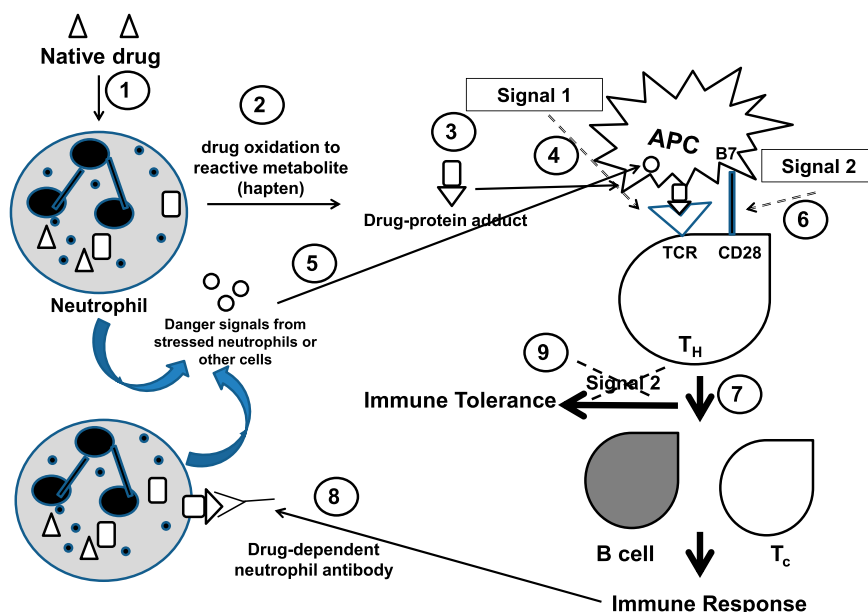
Figure 1. Hapten and danger hypotheses of IDIN immune mechanism. (1) Native drug in the bloodstream enters neutrophils, liver macrophages, and other cells. (2) Drug is oxidized to reactive metabolite(s). (3) Reactive drug (hapten) forms a covalent link with endogenous proteins, forming drug–protein adducts. (4) Drug–protein adducts are taken up by antigen- presenting cells (APCs) and peptide-drug presented in context of class II HLA to T helper (T_H) cells that engage through T-cell receptor (TCR): Signal 1. (5) Stressed neutrophils or other cells release danger signals that activate APCs. (6) B7 on activated APCs engages CD28 on T_H cell for co-stimulus that is Signal 2. (7) The activated T_H cell provides signals to B cells to produce drug-dependent neutrophil antibodies against drug/hapten and to stimulate hapten-specific cytotoxic T cells (T_c): Immune response. (8) Drug-dependent neutrophil antibodies bind drug–protein adducts on neutrophil membrane that cause neutrophil destruction/clearance. (9) In the absence of danger signals, there is no Signal 2, resulting in immune tolerance.

metabolites or reactions with drug-dependent neutrophil antibodies could theoretically release danger signal molecules as well. Another possibility is activation of inflammasomes by reactive drug–protein conjugates.[31] Inflammasome activation leads to production of inflammatory cytokines such as interleukin-1β and interleukin-18, resulting in cell death and release of danger signals. It is apparent that both the hapten and the danger hypotheses are required for an immune response to result in IDIN.

Other support for an immune mechanism of IDIN

Both class I and II HLA genes have been associated with several drug-induced adverse effects, which are also supportive of an immune mechanism.[23] Recently, 2 groups found a strong association between HLA genes and IDIN caused by thioamides. A Japanese study found an association between HLA class II DRB1*08032 and methimazole-induced agranulocytosis.[32] A separate study in Taiwan performed both direct HLA sequencing and a genome-wide association study of DNA from 42 thionamide-induced agranulocytosis patients and 1208 Graves' disease control subjects.[33] The study reported increased independent susceptibility for developing agranulocytosis and having HLA-B*38:02 and/or HLA-DRB1*08:03.

Testing for drug-dependent neutrophil antibodies

As discussed, the consensus is that the majority of IDIN is immune mediated. In this scenario, drug-dependent antibodies and/or cytotoxic T cells that target neutrophils or their precursors should be present in the patient's serum. One way to investigate an immune mechanism is to test the patient's serum for the presence of drug-dependent neutrophil antibodies. Laboratory tests for drug-dependent platelet antibodies[34] and red blood cell antibodies[35] show good sensitivity, but the same is not true when using similar assays for detection of drug-

dependent neutrophil antibodies. The reasons for this finding are unknown. Testing for drug-dependent neutrophil antibodies is rarely performed; the tests involving neutrophils are technically complex, primarily due to the daily requirement to use freshly isolated cells because neutrophils cannot be stored for long periods. This requirement demands the regular availability of large numbers of blood donors. When testing for neutrophil DDAbs is performed, the methods used are very similar to those used for detection of platelet DDAbs[34,36] with substitution of freshly isolated neutrophils for platelets. A typical assay consists of incubation of isolated neutrophils with the patient's serum in the presence of the implicated drug and also in the absence of drug; this is followed by washing of the cells incubated with drug with wash containing drug and those not incubated with drug just with buffer. Washed neutrophils are then incubated with fluorescent or enzyme-labeled anti-human immunoglobulin G and/or anti-human immunoglobulin M for detection of bound DDAbs. Increased neutrophil antibody reactivity in the presence of drug compared with reactivity without added drug is considered a positive result for drug-dependent neutrophil antibodies. Flow cytometry for immunofluorescent detection of DDAbs (Figure 2) and monoclonal antibody immobilization of granulocyte antigens assay for enzyme-linked immunosorbent assay detection and determination of the neutrophil glycoproteins targeted by DDAbs are methods that have been used.[19,37-40] Other methods include immunoblotting, the granulocyte agglutination test, and granulocytotoxicity.[41] Despite the availability of these various methods, neutrophil DDAbs, with the exception of those induced by quinine,[38,39,41-43] are rarely detected.[40,44-46] In the majority of cases, the antibody reactivity against neutrophils is not drug dependent, but the antibodies instead react against neutrophils isolated from all donors tested; this pattern of reactivity is suggestive of autoantibodies, perhaps induced by the drug. In a few cases when
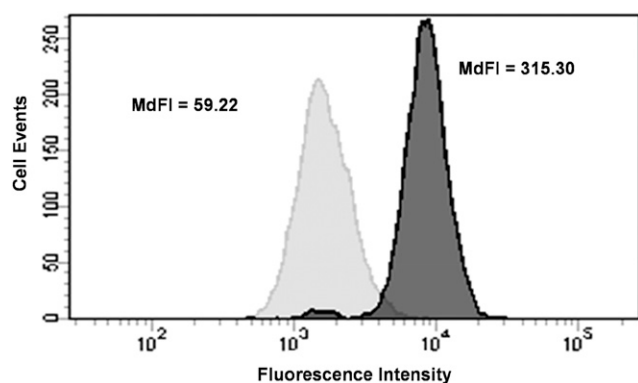
Figure 2. Fluorescence histograms generated from immunofluorescence detection of immunoglobulin G (IgG) drug-dependent neutrophil antibodies by flow cytometry. Isolated normal donor neutrophils were incubated with patient's serum and washed; neutrophil-bound antibodies were detected with fluorescent anti-human IgG. Results shown are for serum from a patient exposed to quinine with drug-induced neutropenia incubated with neutrophils showing higher IgG fluorescence (median fluorescence intensity [MdFI] = 315) with quinine (dark histogram) compared with patient's serum incubated with neutrophils and buffer/no drug (light histogram, MdFI = 59), indicating the presence of quinine drug-dependent neutrophil antibodies. MdFI values are shown for each histogram.

neutrophil DDAbs were detected, the glycoproteins targeted by DDAbs included CD177,[42] CD11b, CD35, and CD16.[40]

One possible reason for the low sensitivity observed for detection of drug-dependent neutrophil antibodies could be the need for reactive drug metabolites in the assay. Native drug is almost always used, and the assay conditions are not optimal to allow for the drug to be metabolized during incubation with neutrophils and patient's serum; it is therefore likely that if drug-dependent neutrophil antibodies are present, their specific antigen is not. This improvement alone to the test could be crucial to its accurate performance. Platelet and red blood cell drug-dependent antibodies that can only be detected in assays using drug metabolites have been described.[47-49] It could be difficult to obtain or produce reactive drug metabolites, but a good start might be to simply incubate the neutrophils to be used in the assay with native drug under conditions that promote oxidation by neutrophil myeloperoxidase; this method could include addition of supernatants from activated neutrophils as an additional source of enzyme.

Diagnosis and clinical management of patients

Because patients affected with IDIN are often asymptomatic, IDIN can be difficult to detect, and therefore, the early detection and timely correction of neutrophils to normal levels are critical to prevent severe infections. If infections are not treated aggressively, 60% of IDIN patients with severe neutropenia will develop septicemia.[11] IDIN frequently presents as an infection with symptoms of a sore throat and/or fever. In general, the diagnosis of IDIN should be made by following expert standards of care,[50,51] taking a complete medical history, performing a careful examination, and collecting laboratory results (including a complete blood count and review of a peripheral blood film). A lack of immature cells (eg, bands) often results in longer time to recovery of neutrophil levels.[11] A complete blood count for patients affected with IDIN will reveal a granulocyte

count $< 1.5 \times 10^9$/L (more often, 0.5×10^9/L), but other cell lines (platelets and red blood cells) are usually at normal levels.

The most important treatment of IDIN is discontinuation of the offending drug. Which drug can be difficult to determine if the patient is taking multiple agents, but initial consideration should be given to those medications frequently associated with IDIN (Table 2). After drug removal, most cases of neutropenia resolve over time, and only symptomatic therapy such as antibiotics for treatment and prophylaxis of infections and good hygiene practices are necessary. The average time for full recovery of the neutrophil count is 9 days (range, 9-24 days).[11] Patients experiencing prolonged neutropenia may require additional treatment with hematopoietic growth factors such as granulocyte-colony stimulating factor (G-CSF). Treatment of patients with severe neutropenia using G-CSF is controversial. Some reports show shortened duration of neutropenia,[9] antibiotic therapy, and hospital length of stay[11] with the use of G-CSF. Use of growth factors for treatment of IDIN was reported to reduce neutrophil recovery to normal levels in 8 days versus 9 days in control subjects.[52] A commonly reported dosage for treatment of adult patients is subcutaneous injection of 300 μg/d. A dose of 5 μg/kg per day until neutrophil counts are $>0.5 \times 10^9$ neutrophils/L has also been recommended.[53] The only prospective, randomized trial to date did not confirm the benefit of G-CSF therapy.[54] However, the patient numbers studied were small ($N = 24$), and the G-CSF dosage used was less (100–200 μg/d) than the 300 μg/d recommended by some experts. Patients with a neutrophil count $<0.1 \times 10^9$/L have been reported to have more infections and fatal complications than those with a higher nadir, and patients with a neutrophil nadir $\leq 0.1 \times 10^9$/L should therefore receive G-CSF 300 μg/d regardless of the presence of infection.[11]

Conclusions

Numerous reviews of IDIN have been published, several recently,[1,4,8,10,11,23,55] but reports of systematic studies of this relatively rare disorder are limited because of the very fact that such drug reactions are idiosyncratic; that is, they pertain to something peculiar to an individual. Therefore, it is difficult to fully understand the incidence, mortality, implicated drugs, and mechanisms responsible for IDIN. Antibiotics, antithyroid drugs, clozapine, and ticlopidine are frequently reported in IDIN cases. Accumulative evidence to date suggests that IDIN frequently occurs through an immune mechanism. Development of improved animal models and diagnostic testing for drug-dependent neutrophil antibodies, additional studies of drug metabolism, and further evaluation of genetic associations in IDIN are all important areas for the focus of future research efforts.

Correspondence

Brian R. Curtis, Diagnostic Labs and Blood Research Institute, BloodCenter of Wisconsin, PO Box 2178, Milwaukee, WI 53201-2178; e-mail: brian.curtis@bcw.edu.

References

1. Uetrecht J. Idiosyncratic drug reactions: current understanding. *Annu Rev Pharmacol Toxicol.* 2007;47:513-539.
2. Thompson RA, Isin EM, Ogese MO, Mettetal JT, Williams DP. Reactive metabolites: current and emerging risk and hazard assessments. *Chem Res Toxicol.* 2016;29(4):505-533.
3. Crawford J, Dale DC, Lyman GH. Chemotherapy-induced neutropenia: risks, consequences, and new directions for its management. *Cancer.* 2004;100(2):228-237.

4. Johnston A, Uetrecht J. Current understanding of the mechanisms of idiosyncratic drug-induced agranulocytosis. *Expert Opin Drug Metab Toxicol*. 2015;11(2):243-257.

5. Medrano-Casique N, Tong HY, Borobia AM, Carcas AJ, Frías J, Ramírez E. Non-chemotherapy-induced agranulocytosis detected by a prospective pharmacovigilance program in a tertiary hospital. *Basic Clin Pharmacol Toxicol*. 2015;117(6):399-408.

6. Ibáñez L, Vidal X, Ballarín E, Laporte JR. Population-based drug-induced agranulocytosis. *Arch Intern Med*. 2005;165(8):869-874.

7. Huber M, Andersohn F, Bronder E, et al. Drug-induced agranulocytosis in the Berlin case-control surveillance study. *Eur J Clin Pharmacol*. 2014;70(3):339-345.

8. Curtis BR. Drug-induced immune neutropenia/agranulocytosis. *Immunohematology*. 2014;30(2):95-101.

9. Andrès E, Mourot-Cottet R, Maloisel F, et al. Idiosyncratic drug-induced neutropenia & agranulocytosis. *QJM*. 2017;hcw220.

10. Rao KV. Drug-induced hematologic disorders, 24E. In: DiPiro JT, Talbert RL, Yee GC, et al, eds. *Pharmacotherapy: A Pathophysiologic Approach, 9/E*. Columbus, OH: McGraw-Hill Education; 2014.

11. Pick AM, Nystrom KK. Nonchemotherapy drug-induced neutropenia and agranulocytosis: could medications be the culprit? *J Pharm Pract*. 2014;27(5):447-452.

12. Medrano-Casique N, Tong HY, Borobia AM, Carcas AJ, Frías J, Ramírez E. Nonchemotherapy drug-induced agranulocytosis in children detected by a prospective pharmacovigilance program. *Pediatr Hematol Oncol*. 2016;33(7-8):441-456.

13. Nakamura H, Miyauchi A, Miyawaki N, Imagawa J. Analysis of 754 cases of antithyroid drug-induced agranulocytosis over 30 years in Japan. *J Clin Endocrinol Metab*. 2013;98(12):4776-4783.

14. Alvir JM, Lieberman JA, Safferman AZ, Schwimmer JL, Schaaf JA. Clozapine-induced agranulocytosis. Incidence and risk factors in the United States. *N Engl J Med*. 1993;329(3):162-167.

15. Vicente N, Cardoso L, Barros L, Carrilho F. Antithyroid drug-induced agranulocytosis: state of the art on diagnosis and management. *Drugs R D*. 2017;17(1):91-96.

16. Williams DP, Pirmohamed M, Naisbitt DJ, Uetrecht JP, Park BK. Induction of metabolism-dependent and -independent neutrophil apoptosis by clozapine. *Mol Pharmacol*. 2000;58(1):207-216.

17. Dahl O, Fluge Ø, Carlsen E, et al; Norwegian Gastrointestinal Cancer Group. Final results of a randomised phase III study on adjuvant chemotherapy with 5 FU and levamisol in colon and rectum cancer stage II and III by the Norwegian Gastrointestinal Cancer Group. *Acta Oncol*. 2009;48(3):368-376.

18. Larocque A, Hoffman RS. Levamisole in cocaine: unexpected news from an old acquaintance. *Clin Toxicol (Phila)*. 2012;50(4):231-241.

19. Czuchlewski DR, Brackney M, Ewers C, et al. Clinicopathologic features of agranulocytosis in the setting of levamisole-tainted cocaine. *Am J Clin Pathol*. 2010;133(3):466-472.

20. Wolford A, McDonald TS, Eng H, et al. Immune-mediated agranulocytosis caused by the cocaine adulterant levamisole: a case for reactive metabolite(s) involvement. *Drug Metab Dispos*. 2012;40(6):1067-1075.

21. Pai MP, Mercier RC, Koster SA. Epidemiology of vancomycin-induced neutropenia in patients receiving home intravenous infusion therapy. *Ann Pharmacother*. 2006;40(2):224-228.

22. Morris A, Ward C. High incidence of vancomycin-associated leucopenia and neutropenia in a cardiothoracic surgical unit. *J Infect*. 1991;22(3):217-223.

23. Uetrecht J, Naisbitt DJ. Idiosyncratic adverse drug reactions: current concepts. *Pharmacol Rev*. 2013;65(2):779-808.

24. Moeschlin S, Wagner K. Agranulocytosis due to the occurrence of leukocyte-agglutinins; pyramidon and cold agglutinins [article in undetermined language]. *Acta Haematol*. 1952;8(1-2):29-41.

25. Kerr RO, Cardamone J, Dalmasso AP, Kaplan ME. Two mechanisms of erythrocyte destruction in penicillin-induced hemolytic anemia. *N Engl J Med*. 1972;287(26):1322-1325.

26. Cho T, Uetrecht J. How reactive metabolites induce an immune response that sometimes leads to an idiosyncratic drug reaction. *Chem Res Toxicol*. 2017;30(1):295-314.

27. Tesfa D, Keisu M, Palmblad J. Idiosyncratic drug-induced agranulocytosis: possible mechanisms and management. *Am J Hematol*. 2009;84(7):428-434.

28. Lobach AR, Uetrecht J. Involvement of myeloperoxidase and NADPH oxidase in the covalent binding of amodiaquine and clozapine to neutrophils: implications for drug-induced agranulocytosis. *Chem Res Toxicol*. 2014;27(4):699-709.

29. Samlowski WE, Frame RN, Logue GL. Flecanide-induced immune neutropenia. Documentation of a hapten-mediated mechanism of cell destruction. *Arch Intern Med*. 1987;147(2):383-384.

30. Uetrecht JP. New concepts in immunology relevant to idiosyncratic drug reactions: the "danger hypothesis" and innate immune system. *Chem Res Toxicol*. 1999;12(5):387-395.

31. Weston JK, Uetrecht J. Activation of inflammasomes by agents causing idiosyncratic skin reactions: a possible biomarker. *Chem Res Toxicol*. 2014;27(6):949-951.

32. Tamai H, Sudo T, Kimura A, et al. Association between the DRB1*08032 histocompatibility antigen and methimazole-induced agranulocytosis in Japanese patients with Graves disease. *Ann Intern Med*. 1996;124(5):490-494.

33. Chen PL, Shih SR, Wang PW, et al. Genetic determinants of antithyroid drug-induced agranulocytosis by human leukocyte antigen genotyping and genome-wide association study. *Nat Commun*. 2015;6:7633.

34. Curtis BR. Drug-induced immune thrombocytopenia: incidence, clinical features, laboratory testing, and pathogenic mechanisms. *Immunohematology*. 2014;30(2):55-65.

35. Leger RM, Arndt PA, Garratty G. How we investigate drug-induced immune hemolytic anemia. *Immunohematology*. 2014;30(2):85-94.

36. Aster RH, Bougie DW. Drug-induced immune thrombocytopenia. *N Engl J Med*. 2007;357(6):580-587.

37. Bux J, Kober B, Kiefel V, Mueller-Eckhardt C. Analysis of granulocyte-reactive antibodies using an immunoassay based upon monoclonal-antibody-specific immobilization of granulocyte antigens. *Transfus Med*. 1993;3(2):157-162.

38. Stroncek DF, Herr GP. The chemical and immunoglobulin structural features necessary for reactions of quinine-dependent antibodies to neutrophils. *Transfusion*. 1995;35(3):247-253.

39. Gottschall JL, Neahring B, McFarland JG, Wu GG, Weitekamp LA, Aster RH. Quinine-induced immune thrombocytopenia with hemolytic uremic syndrome: clinical and serological findings in nine patients and review of literature. *Am J Hematol*. 1994;47(4):283-289.

40. Meyer O, Gaedicke G, Salama A. Demonstration of drug-dependent antibodies in two patients with neutrophenia and successful treatment with granulocyte-colony-stimulating factor. *Transfusion*. 1999;39(5):527-530.

41. Stroncek DF. Drug-induced immune neutropenia. *Transfus Med Rev*. 1993;7(4):268-274.

42. Stroncek DF, Shankar RA, Herr GP. Quinine-dependent antibodies to neutrophils react with a 60-Kd glycoprotein on which neutrophil-specific antigen NB1 is located and an 85-Kd glycosyl-phosphatidylinositol-linked N-glycosylated plasma membrane glycoprotein. *Blood*. 1993;81(10):2758-2766.

43. Hou M, Horney E, Stockelberg D, Jacobsson S, Kutti J, Wadenvik H. Multiple quinine-dependent antibodies in a patient with episodic thrombocytopenia, neutropenia, lymphocytopenia, and granulomatous hepatitis. *Blood*. 1997;90(12):4806-4811.

44. Salama A, Schütz B, Kiefel V, Breithaupt H, Mueller-Eckhardt C. Immune-mediated agranulocytosis related to drugs and their metabolites: mode of sensitization and heterogeneity of antibodies. *Br J Haematol*. 1989;72(2):127-132.

45. Fibbe WE, Claas FH, Van der Star-Dijkstra W, Schaafsma MR, Meyboom RH, Falkenburg JH. Agranulocytosis induced by propylthiouracil: evidence of a drug dependent antibody reacting with granulocytes, monocytes and haematopoietic progenitor cells. *Br J Haematol*. 1986;64(2):363-373.

American Society of Hematology

46. Wall JR, Fang SL, Kuroki T, Ingbar SH, Braverman LE. In vitro immunoreactivity to propylthiouracil, methimazole, and carbimazole in patients with Graves' disease: a possible cause of antithyroid drug-induced agranulocytosis. *J Clin Endocrinol Metab*. 1984;58(5):868-872.

47. Bougie DW, Benito AI, Sanchez-Abarca LI, Torres R, Birenbaum J, Aster RH. Acute thrombocytopenia caused by sensitivity to the glucuronide conjugate of acetaminophen. *Blood*. 2007;109(8):3608-3609.

48. Bougie D, Johnson ST, Weitekamp LA, Aster RH. Sensitivity to a metabolite of diclofenac as a cause of acute immune hemolytic anemia. *Blood*. 1997;90(1):407-413.

49. Bougie D, Aster R. Immune thrombocytopenia resulting from sensitivity to metabolites of naproxen and acetaminophen. *Blood*. 2001;97(12):3846-3850.

50. Dale DC. How I diagnose and treat neutropenia. *Curr Opin Hematol*. 2016;23(1):1-4.

51. Boxer LA. How to approach neutropenia. *Hematology Am Soc Hematol Educ Program*. 2012;2012:174-182.

52. Andersohn F, Konzen C, Garbe E. Systematic review: agranulocytosis induced by nonchemotherapy drugs. *Ann Intern Med*. 2007;146(9):657-665.

53. Andrès E, Maloisel F, Zimmer J. The role of haematopoietic growth factors granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor in the management of drug-induced agranulocytosis. *Br J Haematol*. 2010;150(1):3-8.

54. Fukata S, Kuma K, Sugawara M. Granulocyte colony-stimulating factor (G-CSF) does not improve recovery from antithyroid drug-induced agranulocytosis: a prospective study. *Thyroid*. 1999;9(1):29-31.

55. Uetrecht J. Idiosyncratic drug reactions: past, present, and future. *Chem Res Toxicol*. 2008;21(1):84-92.