Findings that shed new light on the possible pathogenesis of a disease or an adverse effect

Morning granulocytopenia in two patients with connective-tissue disease treated with oral corticosteroids

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Summary

The authors report two cases of morning granulocytopenia: a 73-year-old female with a long-standing, active rheumatoid arthritis (RA) and a non-tuberculous mycobacteria (*Mycobacterium avium*) pulmonary infection, and a 55-year-old female with longstanding systemic lupus erythematosus (SLE). After admission, the patients' peripheral neutrophil counts decreased with no associated symptoms. In both cases, The authors identified diurnal variation in peripheral blood neutrophil counts in blood samples collected at 3-h intervals. The white cell count (WCC) in the first case rose from 2500 cells/ μ l (neutrophils: 1512 cells/ μ l) at 08:00 to 6100 cells/ μ l (neutrophils: 5185 cells/ μ l) at 14:00, and from 2200 cells/ μ l (neutrophils: 319 cells/ μ l) at 06:00 to 2900 cells/ μ l (neutrophils: 2291 cells/ μ l) at 12:00 in the second case.

These cases are educational because necessary treatments would be stopped when morning granulocytopenia is not recognised. Morning granulocytopenia should be noted in the differential diagnosis in cases of incidentally found, asymptomatic granulocytopenia.

BACKGROUND

Agranulocytosis, which is a marked drop in the number of granulocytes, is a serious and often fatal condition. Therefore, it is important to identify any possible underlying conditions causing the granulocytopenia. Recently, several studies have reported that granulocytopenia develops in the morning due to the circadian rhythm.

However, there are few case reports of this condition and the mechanism by which granulocytopenia develops remains unclear. Here, we report two cases of morning granulocytopenia in which both patients had connective-tissue diseases and received oral corticosteroid therapy.

CASE PRESENTATION

Case 1

A 73-year-old female was admitted to the University of Tokyo Hospital for the treatment of refractory rheumatoid arthritis (RA) and non-tuberculous mycobacteria (NTM) chronic lung infection, which at age 69 was diagnosed as a *Mycobacterium avium* infection. She had been diagnosed with RA at age 59 and was treated with several antirheumatic drugs, including 7 mg/day of prednisolone (PSL). The disease activity of neither RA nor NTM had gotten neither better nor worse from the previous year.

white cell count (WCCs) were approximately 6000–7000 cells/ μ l (neutrophils: 5000–6000 cells/ μ l) from the previous year to the time of admission. Direct observation therapy was commenced upon admission, as we suspected poor medication adherence could be the cause of treatment-refractoriness with respect to both RA and the NTM infection. From the time of admission, the patient's WCC steadily decreased. Four days after admission, the WCC was 2900 cells/ μ l (neutrophils: 2610 cells/ μ l) and that at 7 days after admission was 2700 cells/ μ l

(neutrophils: 1890 cells/ μ l), although the patient experienced no symptoms. To exclude suspected drug-induced granulocytopenia, we stopped all drugs that could possibly cause granulocytopenia including sulfasalazine, ciprofloxacin, potassium l-aspartate, omeprazole, rebamipide, raloxifene and furosemide. However, no effect was seen. WCC decreased to 2400 cells/ μ l on admission day 11 and recovered to 7000 cells/ μ l at noon 14 days after admission. However, the WCC next morning was decreased to 2700 cells/ μ l. Because we usually perform inpatient blood collection before breakfast at 8:00, earlier than outpatient blood collection, we hypothesised that this change reflected blood collection time. We checked WCC repeatedly, and this morning-dependent granulocytopenic tendency was reproducible. We also compared the WCC and neutrophil counts from around 7:00 in the morning and around noon for the last 12 months. Mean WCC was 2833 cells/ μ l in the morning (n=9) and 6427 cells/ μ l around noon (n=11). Mean neutrophil count was 1992 cells/ μ l in the morning (n=7) and 5236 cells/ μ l around noon (n=11). The differences between morning and noon counts were statistically significant with t test in WCC (p=0.00000007) and neutrophil count (p=0.00000349, figure 1A). We hypothesised that the change in WCCs reflected the circadian rhythm, which is caused by intravascular granulocyte recruitment induced by PSL. Twenty-eight days after admission, we analysed the patient's WCC at 3-h intervals, which demonstrated a diurnal variation in the WCCs (figure 2A). We hypothesised that this variation in WCCs was caused by PSL administration. To prove this hypothesis, we analysed WCCs at noon in the absence of the morning PSL administration. Contrary to our expectations, the patient's WCC was 4700 cells/ μ l (neutrophil, 3384 cells/ μ l) in the absence of morning PSL treatment (figure 2A).

Figure 1 Graph of WCC data within 1 year stratified by blood sample collection time in case 1 (A) and case 2 (B). Error bars show 1 SD. Statistical analyses were done by t test and p value is shown.

Case 2

A 55-year-old female was admitted to the University of Tokyo Hospital for treatment of oesophageal candidiasis. She was diagnosed with systemic lupus erythematosus (SLE) with antinuclear antibody, malar rash, pancytopenia, arthralgias and low complement level at the age of 43 years and treated with methylprednisolone (mPSL) at 16 mg/day. After admission, itraconazole was administered to treat the candidiasis. The patient's WCC had been approximately 3000–4000 cells/ μl (neutrophils: 2500–3500 cells/ μl) during the previous year leading to the admission. Three days after admission, the patient's WCC began to decrease. The WCC 7 days after admission was 2200 cells/ μl (neutrophils: 432 cells/ μl) and 12 days after admission was 1800 cells/ μl (neutrophils: 360 cells/ μl). To exclude drug-induced granulocytopenia, we stopped all drugs that could possibly cause granulocytopenia including itraconazole, famotidine, potassium chloride, rabeprazole, sucralose, teprenone, mosapride and tocopherol nicotinate. Similar to case 1, this patient experienced no associated symptoms. To prevent any possible progression of SLE, we increased the dose of the mPSL administered. However,

increasing dose of mPSL had no effect. To treat the granulocytopenia, the patient was treated with granulocyte-colony stimulating factor (G-CSF) at a dose of 50 $\mu\text{g}/\text{day}$ for 2 days. WCCs recovered to 5200 cells/ μl (neutrophils: 4316 cells/ μl) when the patient was tested after discharge, although the effect of G-CSF was transient.

However, she was again admitted 1 month after discharge for the treatment of deep venous thrombosis. At this point, the patient's WCC was 2100 cells/ μl (neutrophils: 800 cells/ μl). We checked the WCC repeatedly, and the morning-dependent granulocytopenic tendency was reproducible. We compared peripheral blood WCC and neutrophil counts in blood samples from around 07:00 in the morning and around noon in the last 12 months, because we often perform inpatient blood collection before breakfast at 08:00, earlier than outpatient blood collection. Mean WCC was 2262 cells/ μl in the morning (n=8) and 3373 cells/ μl around noon (n=15). Mean neutrophil count was 646 cells/ μl in the morning (n=8) and 2869 cells/ μl around noon (n=14). The differences between morning and noon counts were statistically significant with t test in WCC (p=0.00000106) and neutrophil count (p=0.0000000163; figure 1B). We hypothesised that the cause of this asymptomatic granulocytopenia was morning granulocytopenia. Six days after admission, we analysed the patient's WCCs at 3-h intervals, which showed a diurnal variation (figure 2B).

DIFFERENTIAL DIAGNOSIS

Drug-induced, cyclic, congenital, nutritional deficiency, postinfectious, infectious, intrinsic bone marrow diseases and autoimmune diseases were considered in the differential diagnosis of granulocytopenia in both cases. The suspicion of drug-induced granulocytopenia was low because the granulocytopenia did not recover in spite of stopping all the drugs that potentially cause granulocytopenia in both cases. The patients had no history of transfusion or chemotherapy. Cyclic granulocytopenia is unlikely, because the cycle of their granulocytopenia was too short, being every 24 h. In both cases, the course of granulocytopenia was different from congenital granulocytopenia, and they had no familial history of granulocytopenia. Serum vitamin B-12, folate and copper levels were normal, and their liver function was normal. Postinfectious granulocytopenia was also different from the course of our cases because the granulocytopenia in the cases persisted for more than a year. They had no evidence of sepsis on vital signs, symptoms or laboratory data. hepatitis B virus, hepatitis C virus and HIV antibodies were negative, and antibodies to cytomegalovirus, herpes simplex virus and Epstein-Barr virus did not reveal an infection pattern in both cases. The patients had no skin rash at the time of admission. There were no characteristic physical signs of typhoid fever, Shigella enteritis, brucellosis, parasites, malaria or rickettsial infection, including sustained high fever or diarrhoea.

In case 1, we could not exclude intrinsic bone marrow disease because we did not perform a bone marrow aspiration analysis. However, the course and haematological data in this case were different from intrinsic bone marrow diseases. Although *M. avium* was positive in her sputum every time with repeated sputum Ziel-Nielsen stain (Gaffky 2–5), *Mycobacterium tuberculosis* was not detected with 8

Figure 2 Diurnal changes in WCCs (filled diamond) and neutrophils (filled squares) in case 1 (A) and Case 2 (B). (A) The WCC and neutrophil counts without morning prednisolone administration are represented by an open diamond and open square, respectively.

weeks of culture and repeated PCR analysis. RA may have played some role, but cannot explain such a phenomenon. Although we also considered Felty syndrome, hypersplenism was excluded by abdominal ultrasonography and CT scan. Moreover, since granulocytopenia for more than 3 months is in the diagnostic criteria of the Felty syndrome, we could not diagnose her as Felty syndrome because granulocytopenia recovered only after several hours. There were no characteristic physical signs and serum makers of other collagen disease such as SLE, including C3 72 mg/dl (normal range 65–140 mg/dl), C4 24 mg/dl (normal range 15–40 mg/dl) and double-stranded DNA (dsDNA) and antineutrophil antibodies were negative.

In case 2, there was no evidence of intrinsic bone marrow diseases because bone marrow aspiration analysis showed only slightly hypocellular bone marrow with no dysplasia. SLE may have played some role, but there was no evidence of SLE exacerbation other than granulocytopenia, because she had normal complement levels including C3 79 mg/dl, C4 22 mg/dl and CH50 40.7 U/ml (normal range 30–45 U/ml), and dsDNA antibody 5 IU/ml (normal range 0–10 IU/ml), C reactive protein 0.01 mg/dl (normal range 0–0.3 mg/dl) were within normal range. Only single-stranded DNA antibody 43 AU/ml (normal range 0–25 AU/ml) was slightly elevated. SLE was mostly controlled for several years in this case. Other blood cell counts, including haemoglobin 13.0 g/dl, RBC 3 970 000 cells/ μ l and platelet count 190 000 cells/ μ l were normal. Moreover, increasing the dose of mPSL had no effect. There were no characteristic physical signs or serum makers of other collagen diseases including rheumatoid factor or antineutrophil antibody.

OUTCOME AND FOLLOW-UP

Both patients remained asymptomatic and needed no treatment for granulocytopenia.

DISCUSSION

In healthy people, circulating blood cell counts change according to the circadian rhythm, although the range of variation is reported to be only approximately 10–30%,^{1–3} so it is generally acceptable to collect blood samples any time of day.

Morning granulocytopenia was first reported by Ahokas *et al* in 1999.⁴ Since that time, only seven cases have been reported. Four of these cases were associated with clozapine,^{4–7} one with risperidone,⁸ one with methimazole,⁹ and

Table 1 Reports of morning granulocytopenia

Author	Journal	Case	Disease	Possible cause
Ahokas and Elonen (1999) ⁴	<i>Psychopharmacology</i>	31-Year-old male	Schizophrenia	Clozapine
Nakamura <i>et al</i> (2001) ⁹	<i>Endocr J</i>	65-Year-old female	Graves' disease	Methimazole
Esposito <i>et al</i> (2003) ⁵	<i>World J Biol Psychiatry</i>	44-Year-old male	Schizophrenia	Clozapine
Esposito <i>et al</i> (2004) ¹¹	<i>J Clin Psychiatry</i>			
Esposito <i>et al</i> (2005) ⁸	<i>Am J Psychiatry</i>	33-Year-old female	Schizophrenia	Risperidone
Esposito <i>et al</i> (2006) ¹²	<i>Int J Neuropsychopharmacol</i>			
Sugimoto <i>et al</i> (2006) ¹⁰	<i>Mod Rheumatol</i>	35-Year-old female	SLE	PSL
Porter and Mohamed (2006) ⁶	<i>Int J Neuropsychopharmacol</i>	20-Year-old male	Schizophrenia	Clozapine
Ferreira <i>et al</i> (2010) ⁷	<i>World J Biol Psychiatry</i>	20-Year-old male	Schizophrenia	Clozapine
Case 1	This report	73-Year-old female	RA, NTM	PSL (?)
Case 2	This report	55-Year-old female	SLE	mPSL

mPSL, methylprednisolone; NTM, non-tuberculous mycobacteria; PSL, prednisolone; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

one with PSL treatment¹⁰ (table 1). Because morning granulocytopenia is asymptomatic and does not systematically predispose the person to an increased risk of serious infection,^{11 12} effective treatments for other conditions may not need to be stopped or changed.

Little is known about the mechanism by which morning granulocytopenia develops. The results of several studies indicate that the diurnal change in levels of serum G-CSF, granulocyte macrophage-colony stimulating factor (GM-CSF) or cortisol is related to morning pseudoneutropenia.^{8 9} In contrast, other reports suggest that morning pseudoneutropenia does not affect the normal circadian rhythm of G-CSF, GM-CSF and cortisol levels, and there is sufficient compensatory stimulation of granulopoiesis and preservation of the diurnal variation in circulating neutrophils.^{5 7} Although the relationship between morning granulocytopenia and cortisol, G-CSF and GM-CSF is still unclear, these reports indicate that the diurnal change in neutrophil levels in normal people versus those affected by morning granulocytopenia are qualitatively different.

The effects of corticosteroids on granulocyte kinetics are well known. Following corticosteroid administration, granulocyte counts in peripheral blood samples increase, and peak approximately 4–6 h after administration. This phenomenon is caused by both increased mobilisation of granulocytes from the bone marrow reserve pool into the peripheral blood and reduced egress from the circulation.¹³ These factors seem to explain a previous report of morning granulocytopenia in a patient with SLE who was being treated with PSL. These investigators hypothesised that morning granulocytopenia in that patient was caused by PSL insufficiency just before the administration of morning medication.¹⁰ However, the PSL insufficiency hypothesis cannot explain the phenomenon of case 1 (figure 1A). Increased PSL metabolism and endogenous cortisol induced by rifampicin may play some role in morning granulocytopenia in case 1.¹⁴ Additionally, chronic inflammation due to NTM infection and immunological dysregulation due to RA may contribute to the severe drop in neutrophils in this patient. However, further studies are needed to elucidate the exact mechanism of morning granulocytopenia.

Although we would like to investigate whether the two patients had normal adrenal function and circadian rhythm changes in plasma corticosteroid levels that fall within the normal range for Japanese women of that age, we could not because cortisol measurements can be affected by one or more of the synthetic or natural steroids. Thus, these measurements are unreliable in patients taking PSL (cross reactivity rate 77%) or mPSL (cross reactivity rate 43%). Moreover, we could not discontinue the oral corticosteroid treatment for several days to allow investigation of plasma cortisol levels, because we feared exacerbation of the connective tissue disease and occurrence of adrenal failure.¹⁵

To our knowledge, this is the first case report of morning granulocytopenia in a patient with RA (case 1), and the second case report of morning granulocytopenia in a patient with SLE (case 2).

Though circadian rhythms or just random undulation can be observed in most physiologic parameters, unnecessary tests such as bone marrow aspiration and unnecessary treatments, including G-CSF (case 2 and a previous report

of the case of Graves' disease)⁹ would be given when morning granulocytopenia is not recognised. Necessary drugs (sulfasalazine and ciprofloxacin in case 1, itraconazole in case 2, clozapine,^{4 5} risperidone⁸ for paranoid schizophrenia, methimazole for Graves' disease,⁹ zonisamide for the pain of Raynaud's phenomenon of SLE¹⁰) would also be stopped when morning granulocytopenia is not recognised.

While thought to be a rare entity, we speculate that there may be a substantial number of patients with morning granulocytopenia in cases of asymptomatic granulocytopenia, especially in cases refractory to G-CSF, as both our cases were diagnosed in a 25-bed unit of the Department of Allergy and Rheumatology, at the University of Tokyo Hospital within half a year. Although accumulation of more cases is necessary, we recommend considering morning granulocytopenia in the case of incidentally found, asymptomatic granulocytopenia and systematically repeating leucocyte counts in such cases.

Learning points

- ▶ Granulocytopenia can develop in the morning due to circadian rhythm
- ▶ Morning granulocytopenia should be considered in cases of incidentally found, asymptomatic granulocytopenia
- ▶ Leucocyte counts should be tested repeatedly and systematically in such cases.

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Competing interests None.

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