MAEDICA – a Journal of Clinical Medicine
2023; 18(2): 357-362
https://doi.org/10.26574/maedica.2023.18.2.357

MÆDICA - a Journal of Clinical Medicine

CASE REPORT

Unusual Extreme Leukocytosis in Metformin Overdose

Daniela RADULESCU[a, b], Elena CUIBAN[a, b], Ileana Adela VACAROIU[a, b]

[a]Clinical Department No. 3, "Carol Davila" University of Medicine and Pharmacy, 050474 Bucharest, Romania

[b]"Sf. Ioan" Department of Nephrology and Dialysis, Emergency Clinical Hospital, 421422 Bucharest, Romania

ABSTRACT

We present the challenging differential diagnosis of an extreme leukocytosis noted at hospital admission of a patient with severe lactic acidosis and acute kidney injury secondary to metformin overdose. Laboratory and imaging work-up ruled out an infectious or malignant cause for severely increased leukocytes count; thus, it was established that the process of leukocyte demargination was the main cause of increased count of leukocytes. Discussion of available literature data is also presented. Our case report highlights the importance for clinicians to be aware of this possible spurious increase of leukocytes in acute non-infectious stressful conditions in order to avoid unnecessary antibiotics.

Keywords: metformin overdose, lactic acidosis, acute kidney injury, leukocyte demargination.

INTRODUCTION

Metformin is the first line oral therapy for type 2 diabetes mellitus (1). It belongs to the class of biguanides and acts as an antihyperglicemic agent by decreasing glucose production in the liver and by enhancing insulin sensitivity in peripheral tissues (2). Metformin is excreted unchanged in urine, with its clearance being reduced in kidney dysfunction (3). It has a small molecular weight and a reduced binding with serum proteins; therefore, in intoxication cases, it can be removed from the blood with various types of extracorporeal therapies (4).

Metformin overdose has been increasingly reported in the last decades. According to a recent meta-analysis (5), in the majority of cases, toxicity is the result of gradual accumulation of metformin due to previous chronic kidney disease (CKD) and/or in the case of superimposed acute kidney injury (AKI); almost one half of cases are reported to be secondary to acute ingestion of high doses, mostly in suicidal attempts. Usually, clinical presentation consists of digestive symptoms like abdominal pain, nausea, vomiting and

Address for correspondence:
Daniela Radulescu, MD, PhD
Tel: 0040 751 109 295; email: daniela.radulescu@umfcd.ro

Article received on the 6th of April 2023 and accepted for publication on the 23rd of June 2023

diarrhea, and clinical exam may reveal hypothermia, tachycardia and hypotension; in severe cases, alteration of mental status is noted (5, 6). Lactic acidosis is the hallmark of metformin intoxication in laboratory work-up, but AKI is fairly often associated secondary to dehydration and hypotension (6). Hypoglycemia is usually noted in patients receiving metformin in combination with other hypoglycemic drugs, but in monotherapy it may be absent even in acute severe intoxications (7). In cases with previous normal kidney function there are several predisposing factors, including severe liver or heart failure, treatment with angiotensin-converting enzyme inhibitors (ACEIs), non-steroidal anti-inflammatory drugs or concurrent alcohol consumption (5, 6). As there is not antidote for metformin, the treatment consists in oral activated charcoal to decrease metformin absorption in the gut, parenteral hydration and sodium bicarbonate infusion. In severe cases, conventional or continuous extracorporeal therapies with bicarbonate buffer are recommended (4).

The effects of therapeutic doses of metformin on leukocytes function have been extensively studied in both diabetes patients and other diseases associated with chronic inflammation and increased peripheral resistance to insulin such as polycystic ovary syndrome, hyperinsulinaemic hyperandrogenism, metabolic syndrome or obesity (8-11). In these diseases, metformin corrects the previously increased number of leukocytes, especially in the group of neutrophils, and the effect is considered secondary to its ability to reduce abnormal activation of neutrophils and their adherence to the endothelium (8). Nevertheless, in chronic long-term use for diabetes, treatment with metformin was not associated with significant blood cell count abnormalities (12, 13).

Abnormalities in leukocytes count in metformin overdose have not been studied, although some case reports of metformin intoxication noted increased leukocyte and neutrophil count (14-16) and some authors reported that antimicrobial empiric treatment was given upon admission but it was stopped later, after sepsis was ruled-out (14).

Here we present the case of a type 2 diabetes patient hospitalized for severe lactic acidosis and acute kidney injury in the settings of metformin overdose, who presented with extreme leukocytosis at admission. We discuss the challenging differential diagnosis of leukocytosis in the case of multicomorbidity association. ❑

CASE PRESENTATION

Reasons for presentation. A 53-year-old male was admitted to the emergency room (ER) for shortness of breath, vomiting, diarheea and confusional state.

History of past illness. The patient had a three-year history of diabetes mellitus treated with metformin 2 g/d and systemic hypertension treated with enalapril 10 mg/d. He was also known with paranoid schizophrenia, for which he received treatment with risperidone, duloxetine and gabapentine, but, according to his family, he discontinued medication several months ago. He was a non-smoker and did not abuse alcoholic beverages.

History of actual sufferance. The patient's niece stated that two hours before hospital arrival she found him fainted on the floor with vomit around, confused, with accelerated breathing and an empty bottle of metformin nearby; she appreciated that about 20-25 tablets of metformin 1 g each were missing. Partial digested capsules were present in the evacuated vomit. She could not offer details regarding the duration of the sufferance since she had left home about eight hours ago.

Clinical exam and work-up. At the time of presentation, the patient was confused and lethargic (GCS13). He was an obese person, severely dehydrated, with decreased skin turgor, dry axillae and oral mucosa, with blood pressure (BP) 98/56 mm Hg, regular tachycardia 108 beats/min, tachypnea 28 breaths/min; no edema, rales or other abnormal clinical signs were found. The oxygen saturation of 82% in the atmospheric air at the time he was taken over by the ambulance was corrected to 98-100% with two liters/hour of oxygen per mask. Abnormal findings in laboratory work-up upon admission are summarised in Table 1. The patient was hospitalized in the nephrology department.

A peripheral blood smear and serial hemograms were repeated; the results were similar and no immature white blood cells were found (*i.e.*, myelocytes, metamyelocytes or band neutrophils).

TABLE 1. Abnormal blood tests at presentation and discharge

Blood tests	Admission	Discharge
Hemogram		
Hb (g/dL)	16.1	12.1
Ht (%)	51.4	35.7
MCV	103.2	92.90
MCH	32.8	31.3
MCHC	31.3	33.9
WBC (No*10^3/μL)	60.7	7.74
Neutrophils	42.2	5.91
Lymphocytes	9.9	1.13
Monocytes	7.4	0.64
Eosinophils	0.3	0.05
Basophils	0.9	0.01
Platelets (No*10^3/μL)	417	164
Glucose (mg/dL)	199	108
HbA_{1c} (%)	9.4	
Urea (mg/dL)	120	50.4
Creatinine (mg/dL)	2.6	1.21
Uric acid (mg/dL)	15	11
GOT (IU/L)	71	37
GPT (IU/L)	74	27.3

Blood tests	Admission	Discharge
Venous blood gas		
pH	6.876	7.414
pCO_2	15.6	36.60
pO_2	111.4	78.50
HCO_3	2.8	23.20
BE	- 30.5	- 0.90
Na (mmol/L)	137.6	135.8
K (mmol/L)	6.44	3.83
AnGap (mmol/L)	38	17.8
Lactate (mmol/L)	19.36	1.2
Presepsin (pg/mL)	2729	165
D-dimers (μg/mL FEU)	0.706	0.47
Procalcitonin (ng/mL)	5.58	0.034
Phosphate (mg/dL)	14.57	4.4
Calcium (mg/dL)	7.65	9.23
CK (IU/L)	710	69
CK-MB (IU/L)	23.60	11.80
Tryglicerides	466.7	389.7

Extensive investigations were performed for the presence of infection, but all results were negative. Native thorax, abdomen and pelvic computer tomography (CT), abdominal ultrasound, pulmonary radiograph and cardiac ultrasound were in normal limits, except a slightly increased fatty liver and moderate left ventricular hypertrophy. Blood culture, urine culture, feces culture, *clostridium difficile* were all negative. Markers of viral hepatitis were absent.

Other performed blood analysis, including erythrocyte sedimentation rate, fibrinogen, amylase, lactate dehydrogenase, albumin, alkaline phosphatase, cholesterol (total, fractions), bilirubin, thyroid-stimulating hormone and cancer markers were within the normal range. Serum osmolality upon admission was 289 mmol/L. Urine toxicology screen was negative. Urine exam showed pH 5, specific gravity 1025, rare isomorphic erythrocytes in the sediment. No abnormal proteinuria was present and the result for ketone bodies in urine was negative.

Treatment and evolution during hospitalization. Oral activated charcoal was administered in the Emergency room (ER). Intensive parenteral hydration with normal saline 3500 mL and molar sodium bicarbonate solution 300 mL were administered in the next six hours, with no improvement. We noted symptom aggravation in parallel with lack of correction of acidosis, an increased level of creatinine to 4.52 mg/dL and oligoanuria. Urgent hemodialysis with bicarbonate was initiated along with continuing parenteral hydration. No antimicrobial therapy was administered. Evolution was favorable with increasing of urine output, decreasing nitrogen waste products and full correction of blood gas and electrolytes. Leukocyte count decreased rapidly, with full normalization at day 3 after admission. The patient needed a total of six hemodialysis sessions, two hours each. For diabetes, insulin therapy was initiated according to the specialist recommendations. He was discharged from hospital after nine days. ❑

DISCUSSIONS

We described the case of a patient with metformin overdose secondary to suicidal attempt. Although in our hospital measuring metformin blood levels was not available, the diagnostic of metformin overdose was clearly sustained by anamnesis, clinical exam and severe lactic acidosis. Development of acute kidney injury in the settings of dehydration and previous treatment with an ACEI led to further serum accumulation of metformin with not only aggravation of lactic acidosis but also superimposed renal-induced metabolic acidosis. Urgent hemodialysis along with parenteral hydration were performed, with full recovery of kidney function and normalization of blood gas and electrolyte panel. Moderately increased transaminase levels were considered rather secondary to severe dehydration and transient liver ischemia, less likely secondary to an idiosyncratic reaction to metformin, which is a rare compli-

cation (17); at day 3, the levels of transaminases were normalized. Modest increase in CK was also considered to be secondary to severe dehydration.

The most challenging differential diagnostic upon admission regarded the severe increase of leukocyte count with important neutrophilia. Neutrophilia can be classified into three categories: factitious neutrophilia, primary neutrophilia and secondary neutrophilia (18).

Factitious neutrophilia, although not so severe as in our patient, may be secondary to inadequate anticoagulation of blood sample or to presence of cryoglobulins in blood (18). We excluded the first possibility by repeating analysis several times with adequate collection of blood sample. The titers of cryoglobulins have not been searched as neutrophil count rapidly returned to normal values.

Primary neutrophilia is seen in hematologic diseases with increased marrow production (*i.e.*, acute or chronic leukemia) and it is associated with the presence of immature white blood cells in the peripheral smear (19), which were not present in our patient. Nevertheless, in chronic stages of chronic myeloid leukemia, the examination of peripheral blood smear may not reveal immature neutrophils (20). Moreover, in chronic myeloid leukemia, increase counts of basophils, eosinophils and monocytes are usually present, similarly to our patient. As such, upon admission we could not exclude a chronic myeloid leukemia complicated with lactic acidosis (*i.e.*, Warburg effect) (21), but after three days of hospitalization the neutrophil count was fully corrected, as were the counts of basophils, eosinophils and monocytes. As a consequence, leukemia was ruled out definitively.

Hemoconcentration secondary to severe volume depletion could be one explanation for the presence of leukocytosis, because there was an increase not only in all types of WBC, but also in hematocrit and hemoglobin. Yet, we considered this to be only a minor contributor to the severe increase of leukocytes.

Secondary neutrophilia is usually noted in sepsis, inflammatory systemic diseases, neoplasis, after some drugs (anticonvulsivants, lithium, corticosteroids, cathecolamines etc), after emotional or physical stress (*i.e.*, surgery, intense exercise, etc) (18).

No inflammatory chronic conditions were present in our patient and no medication listed above was used prior to hospital admission.

We took into consideration sepsis as the main cause for leukocytosis with neutrophilia in the case described here. In clinical practice, the presence of neutrophilia is very often considered a sepsis-related finding, and empiric prescription of antibiotics is especially noted in critically ill patients (22, 23). Risk factors for sepsis were present in our patient, including diabetes, obesity and acute kidney injury (24). Some features revealed by the clinical presentation could have been considered part of sepsis clinical picture – tahycardia, hypotension – but no abnormal body temperature was noted. Also, markers of sepsis such as procalcitonin, presepsin and C-reactive protein were increased upon admission. Yet, laboratory and imaging work-up excluded sepsis. Increased serum levels of sepsis markers such as presepsin and procalcitonin in either AKI (25, 26) or CKD (27, 28) in the absence of sepsis have been reported to occur as a result of diminished renal clearance, but the cut-off levels for various degree of kidney dysfunction are yet to be defined. With regard to C-reactive protein, it is already known that its levels are increased in AKI irrespective of the presence or absence of sepsis (29). Therefore, in the absence of proven infection we did not administer antimicrobial therapy for our patient.

It is tempting to speculate that severe acidosis is the main cause of leukocytosis. As stated above, most authors of published metformin intoxication cases reported increased leukocyte count without an explanation for this finding. Our patient associated type B lactic acidosis secondary to metformin overdose, type A lactic acidosis secondary to dehydration and systemic hypoperfusion and also anionic gap metabolic acidosis secondary to acute renal failure (30, 31). Nevertheless, there is evidence that, along with multiple deleterious consequences on immune system, acidosis per se has an inhibitory effect on leukocyte motility and it is associated with reduced rather than increased leukocyte count (32). Leukocytosis may be a feature of diabetic ketoacidosis in the absence of infection (33-35) and it is considered secondary to leukocyte demargination (see below) or to increased production of neutrophils in the bone marrow; we per-

formed urine nitroprusside test in our patient and the result was negative.

The entire presentation and clinical picture of our patient can be classified as an acute stress by the association of intense dehydration, hypotension, acute kidney injury, severe acidosis and suicidal attempt. Acute stressful conditions may be associated with abnormal increase of leukocyte count in the blood in the absence of sepsis, chronic inflammatory conditions, neoplasia or hematologic diseases (36). The cause is mainly represented by leukocytes demargination from the vessel wall, but a stress-induced mobilization of leukocytes from hematopoietic tissues into the blood may also be a contributor (37). Leukocytes demargination is very often seen in clinical practice after initiation of treatment with glucocorticoids (38), but it was proven to arise also after infusion of noradrenaline or adrenaline (39). Dehydration, hypotension, trauma, surgery, intense exercise, convulsions are just few examples of acute stress conditions which are accompanied by increased endogenous secretion of both cortisol and cathecolamines (40, 41). Demargination of leukocytes in acute stress is characterized by an increase of the granulocyte subset of white blood cells (WBC), especially of neutrophils (36, 37), as it happened in our patient. In experimental studies, neutrophil demargination secondary to cathecolamines or glucocorticoids has been proven to be the combined result of decreased neutrophil stiffness (42) and downmodulation in expression of adhesion molecules (43). Taking into account that, in the case reported by us, the neutrophil count was corrected in parallel with treatment of all stressful conditions listed above, we believe that this was the major mechanism of neutrophilia in our patient. ❑

CONCLUSIONS

We reported the case of extreme leukocytosis occurred as a reaction to a cumulus of multiple and severe acute conditions, including metabolic acidosis, acute kidney injury, severe volume depletion, hypotension and suicidal attempt. This finding was considered secondary to massive demargination of neutrophils into the blood stream. The leukocyte count has rapidly normalized after correcting the above-mentioned conditions and in the absence of antimicrobial therapy. Our case report highlights the importance for clinicians to be aware of this possible spurious increase of leukocytes in acute non-infectious stressful conditions in order to avoid unnecessary use of antibiotics. ❑

Conflicts of interest: none declared.

Financial support: none declared.

Institutional review board statement: Ethical review and approval were waived as the present work was a case report.

Informed consent statement: The patient read and signed an informed consent at hospital admission. Written informed consent has been obtained from the patient also for publishing this report.

References

1. **Buse JB, Wexler DJ, Tsapas A, et al.** 2019 Update to: Management of Hyperglycemia in Type 2 Diabetes, 2018. A Consensus Report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetes Care* 2020;43:487-493.
2. **Rena G, Hardie DG, Pearson ER.** The mechanisms of action of metformin. *Diabetologia* 2017;60:1577-1585.
3. **Cheng AY, Fantus IG.** Oral antihyperglycemic therapy for type 2 diabetes mellitus. *CMAJ* 2005;172:213-226.
4. **Calello DP, Liu KD, Wiegand TJ, et al.** Extracorporeal Treatments in Poisoning Workgroup. Extracorporeal Treatment for Metformin Poisoning: Systematic Review and Recommendations From the Extracorporeal Treatments in Poisoning Workgroup. *Crit Care Med* 2015;43:1716-1730.
5. **Juneja D, Nasa P, Jain R.** Metformin toxicity: A meta-summary of case reports. *World J Diabetes* 2022;13:654-664.
6. **Chu J, Stolbach A.** Metformin poisoning. 2022. Available at: https://www.uptodate.com, accessed February 2023.
7. **Dunn CJ, Peters DH. Metformin.** A review of its pharmacological properties and therapeutic use in non-insulin-dependent diabetes mellitus. *Drugs* 1995;49:721-749.
8. **Chow E, Yang A, Chung CHL, et al.** A Clinical Perspective of the Multifaceted Mechanism of Metformin in Diabetes, Infections, Cognitive Dysfunction, and Cancer. *Pharmaceuticals* (Basel) 2022;15:442.
9. **Zak K, Orlenko V, Popova V, et al.** The role of the immune system in mechanism

of metformin therapeutic effect in patients with type 2 diabetes. *International Journal Of Endocrinology* (Ukraine) 2021;13:340-346.
10. **Orio F Jr, Palomba S, Cascella T, et al.** The increase of leukocytes as a new putative marker of low-grade chronic inflammation and early cardiovascular risk in polycystic ovary syndrome. *J Clin Endocrinol Metab* 2005;90:2-5.
11. **Ibáñez L, Jaramillo AM, Ferrer A, et al.** High neutrophil count in girls and women with hyperinsulinaemic hyperandrogenism: normalization with metformin and flutamide overcomes the aggravation by oral contraception. *Human Reproduction* (Oxford, England) 2005;20:2457-2462.
12. **Smaoui O, Hadj Ali Z, Samti G, et al.** Metformin and blood cell count (BCC) abnormalities. Abstr. IDF21-0368 in: *Diabetes Research and Clinical Practice* 2022;186:109735.
13. **Manssor ARJ, Rajab HK.** The effects of anti-diabetic agents on white blood cells. *Tikrit Journal of Pure Science* 2011;16:1813-1662.
14. **Iftikhar H, Saleem M, Kaji A.** Metformin-associated Severe Lactic Acidosis in the Setting of Acute Kidney Injury. *Cureus* 2019;11:e3897.
15. **Nisse P, Mathieu-Nolf M, Deveaux M, et al.** A fatal case of metformin poisoning. *J Toxicol Clin Toxicol* 2003;41:1035-1036.
16. **Timbrell S, Wilbourn G, Harper J, et al.** Lactic acidosis secondary to metformin overdose: a case report. *J Med Case Rep* 2012;6:230.
17. **Miralles-Linares F, Puerta-Fernandez S, Bernal-Lopez MR, et al.** Metformin-induced hepatotoxicity. *Diabetes Care* 2012;35:e21.
18. **Tahir N, Zahra F.** Neutrophilia. (Updated 2022 Nov 26). In: *StatPearls* (Internet). Treasure Island (FL): StatPearls Publishing; 2022 Jan-. Accessed February 2023.
19. **Sawyers CL.** Chronic myeloid leukemia. *NEJM* 1999; 340(17): 1330–1340.
20. **Van Etten RA.** Clinical manifestations and diagnosis of chronic myeloid leukemia. 2023 Available at: https://www.uptodate.com, accessed February 6, 2023.
21. **Padda J, Khalid K, Kakani V, et al.** Metabolic Acidosis in Leukemia. *Cureus* 2021;13:e17732.
22. **Haburchak DR, Alchreiki M.** Inpatients With 'Unexplained' Leukocytosis. *Am J Med* 2020;133:508-514.
23. **Kollef MH, Sherman G, Ward S, et al.** Inadequate antimicrobial treatment of infections: a risk factor for hospital mortality among critically ill patients. *Chest* 1999;115:462-474.
24. **NICE guideline (NG51).** Sepsis: recognition, diagnosis and early management. Available at: https://www.nice.org.uk/guidance/ng51/chapter/recommendations#risk-factors-for-sepsis, accessed February 2023.
25. **Nakamura Y, Ishikura H, Nishida T, et al.** Usefulness of presepsin in the diagnosis of sepsis in patients with or without acute kidney injury. *BMC Anesthesiol* 2014;14:88.
26. **Chun K, Chung W, Kim AJ, et al.** Association between acute kidney injury and serum procalcitonin levels and their diagnostic usefulness in critically ill patients. *Sci Rep* 2019;9:4777.
27. **Nagata T, Yasuda Y, Ando M, et al.** Clinical impact of kidney function on presepsin levels. *PLoS One* 2015;10:e0129159.
28. **Wu SC, Liang CX, Zhang YL, et al.** Elevated serum procalcitonin level in patients with chronic kidney disease without infection: A case-control study. *J Clin Lab Anal* 2020;34:e23065.
29. **Tang Y, Mak SK, Xu AP, et al.** Role of C-reactive protein in the pathogenesis of acute kidney injury. *Nephrology* (Carlton) 2018;23(Suppl 4):50-52.
30. **Seheult J, Fitzpatrick G, Boran G.** Lactic acidosis: an update. *CCLM* 2017;55:322-333.
31. **Burger M, Schaller DJ.** Metabolic Acidosis. 2022. In: *StatPearls* [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan–. PMID: 29489167. Bookshelf ID: NBK482146. Available at: https://www.ncbi.nlm.nih.gov/books/NBK482146/, accessed June 2023.
32. **Lardner A.** The effects of extracellular pH on immune function. *J Leukoc Biol* 200;69:522-530.
33. **Karavanaki K, Kakleas K, Georga S, et al.** Plasma high sensitivity C-reactive protein and its relationship with cytokine levels in children with newly diagnosed type 1 diabetes and ketoacidosis. *Clin Biochem* 2012;45:1383-1388.
34. **Kayashima T, Yamaguchi K, Akiyoshi T, et al.** Leukemoid reaction associated with diabetic ketoacidosis--with measurement of plasma levels of granulocyte colony-stimulating factor. *Intern Med* 1993;32:869-871.
35. **Xu W, Wu HF, Ma SG, et al.** Correlation between peripheral white blood cell counts and hyperglycemic emergencies. *Int J Med Sci* 2013;10:758-765.
36. **Ince LM, Weber J, Scheiermann C.** Control of Leukocyte Trafficking by Stress-Associated Hormones. *Front Immunol* 2019;9:3143.
37. **Dhabhar FS, Malarkey WB, Neri E, et al.** Stress-induced redistribution of immune cells--from barracks to boulevards to battlefields: a tale of three hormones–Curt Richter Award winner. *Psychoneuroendocrinology* 2012;37:1345-1368.
38. **Nakagawa M, Terashima T, D'yachkova Y, et al.** Glucocorticoid-induced granulocytosis: contribution of marrow release and demargination of intravascular granulocytes. *Circulation* 1998;98:2307-2313.
39. **Benschop RJ, Rodriguez-Feuerhahn M, Schedlowski M.** Catecholamine-induced leukocytosis: early observations, current research, and future directions. *Brain Behav Immun* 1996;10:77-91.
40. **Katsu Y, Baker ME.** Subchapter 123D – Cortisol. In: Ando H, Ukena K, Nagata S. *Handbook of Hormones* (Second Edition), Academic Press. 2021, pp 947-949.
41. **Paravati S, Rosani A, Warrington SJ.** Physiology, Catecholamines. (Updated 2022 Oct 24). In: StatPearls (Internet). Treasure Island (FL): *StatPearls* Publishing; 2022 Jan-. Accessed February 2023.
42. **Fay ME, Myers DR, Kumar A, et al.** Cellular softening mediates leukocyte demargination and trafficking, thereby increasing clinical blood counts. *Proc Natl Acad Sci USA* 2016;113:1987-1992.
43. **Trabold B, Lunz D, Gruber M, et al.** Immunomodulation of neutrophil–endothelial interaction by inotropes. *Injury* 2010;41:1079-1083.