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## High intraplatelet cGMP levels in human sepsis

*Clin Microbiol Infect* 1998; 4: 163–165

Nitric oxide (NO) is synthesized from L-arginine by a group of enzymes, the nitric oxide synthases (NOS). Under normal circumstances the endothelial constitutive isoform releases small quantities of NO, which acts as an endogenous vasodilator and inhibits platelet adhesion and aggregation [1]. These actions are mediated by the activation of soluble guanylate cyclase which increases the concentration of cyclic guanosine monophosphate (cGMP) in target cells. Expression of the inducible NOS isoform is activated in various cells (endothelium, smooth muscle, macrophages, etc.) in response to inflammatory cytokines and bacterial lipopolysaccharide (LPS). This enzyme synthesizes large quantities of NO which play a significant role in the characteristic hemodynamic changes of septic shock [1]. There are indirect data supporting the idea of increased production of NO during sepsis. High plasma and urine levels of $NO_2/NO_3$ (inactive metabolic end products of NO) have been demonstrated in sepsis in both animals [2] and humans [3]. Both in endotoxin treated rats [4] and in human septic shock [5], an increase in plasma cGMP levels has been observed.

Keaney et al [6] have recently reported that LPS-induced shock in rabbits increases intraplatelet cGMP levels, reflecting the effect of high amounts of NO on these cells. Intraplatelet cGMP levels are considered to be a good index of the global NO pathway activity [1,7,8]. We hypothesized that the high production of NO in human sepsis should produce an increase in intraplatelet cGMP. We therefore measured intraplatelet cGMP levels in a group of septic patients and correlated these with other clinical and biochemical parameters.

Twelve patients with sepsis (five men and seven women), aged $61 \pm 20$ years (mean $\pm$ standard deviation), were studied. Patients were eligible for study if they fulfilled the sepsis criteria of the American College of Chest Physician Society and Critical Care Medicine Association [9]. Table 1 shows clinical and biochemical parameters. Patients with acquired immunodeficiency syndrome, active tuberculosis, neoplasia, autoimmune and hematologic diseases, and patients who had received antiplatelet drugs, glucocorticoids, immunosuppressants or antibiotics before the diagnosis, were excluded. The control group comprised 12 age- and sex-matched healthy volunteers (four men and eight women) aged $52 \pm 11$ years. Informed consent was obtained from all patients and volunteers and the research was approved by a local human investigations committee in accordance with the Helsinki Declaration.

Blood samples for cGMP determinations were collected into 0.105 M sodium citrate from the cubital vein, at diagnosis before starting antibiotic treatment (day 0), and 1 (day 1) and 7 days later (day 7). Plasma and intraplatelet cGMP was measured as described previously [7]. Serum creatinine levels were determined by the Jaffé kinetic method in Autoanalyzer Hitachi 737 (Boehringer Mannheim, Mannheim, Germany).

All data were analyzed using Statistical Package for Social Sciences software. Since the data distribution was non parametric, results are expressed as median and range. The Mann–Whitney rank-sum test was used for the comparisons between groups. Correlations were done with the Spearman test. Differences were considered significant if $p < 0.05$.

As shown in Figure 1, day 0 intraplatelet cGMP levels were higher in the septic patients (0.87 (0.35–5.06) pmol/$10^9$ platelets (median and range)), than in the control group, 0.23 (0.1–0.4) pmol/$10^9$ platelets; $p = 0.0001$). Plasma cGMP levels were also significantly increased in the septic patients (4.39 (1.59–13.78) nmol/L), when compared with the control group (1.87 (1.09–4.06) nmol/L, $p = 0.02$). We did not find significant correlations between plasma or intraplatelet cGMP levels and number of platelets, diastolic blood pressure (DBP), systolic blood pressure (SBP), APACHE II score or serum creatinine levels at day 0.

In septic patients intraplatelet cGMP levels were 1.06 (0.19–4.80) pmol/$10^9$ platelets at day 1, and 0.58 (0.32–2.60) pmol/$10^9$ platelets at day 7; plasma cGMP levels were 4.94 (2.21–11.96) nmol/L at day 1 and 2.82 (2.39–6.06) nmol/L at day 7. Intraplatelet cGMP levels did not change significantly after antibiotic treatment within the period of time studied. On the contrary, plasma cGMP levels of septic patients at day 7 had reached normal levels.

Our study demonstrates for the first time that intraplatelet cGMP levels are increased in septic

**Table 1** Physical characteristics and biochemical parameters of the patients

| Case | Age/Sex | Origin | Blood cultures | Pathogen | Apache II | Neutrophil count |
|---|---|---|---|---|---|---|
| 1 | 52/M | HP | – | ? | 3 | 13 604 |
| 2 | 67/F | HP | + | *Streptococcus agalactiae* | 15 | 13 776 |
| 3 | 84/F | Urine | + | *Escherichia coli* | 11 | 15 640 |
| 4 | 44/M | Urine | + | *E. coli* | 3 | 3 354 |
| 5 | 49/M | PA | + | Mixed | 4 | 4 872 |
| 6 | 47/F | Pneumonia | + | *Staphylococcus aureus* | 21 | 12 193 |
| 7 | 64/F | Urine | + | *E. coli* | 10 | 12 300 |
| 8 | 26/F | Meningitis | – | *Streptococcus pneumoniae* | 2 | 20 880 |
| 9 | 82/M | Urine | – | *E. coli* | 13 | 16 915 |
| 10 | 44/M | Pneumonia | – | ? | 0 | 8 160 |
| 11 | 91/F | Urine | + | *Staphylococcus epidermidis* | 9 | 3 321 |
| 12 | 83/F | Scar | + | Mixed | 6 | 24 640 |

HP, hip prosthesis; PA, perianal abscess.

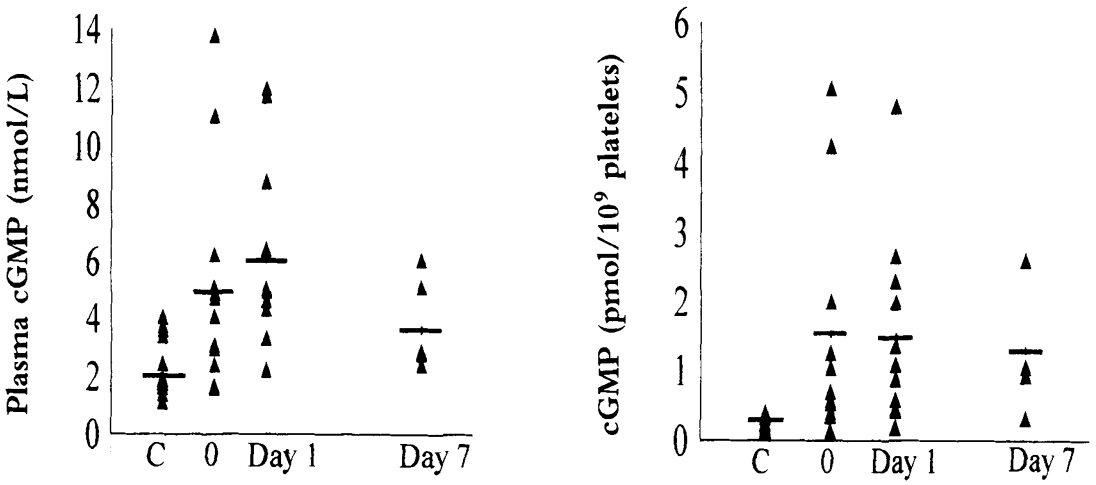


**Figure 1** Plasma and intraplatelet cGMP levels in control group (C) and in septic patients at 0, 1 and 7 days of evolution. (Horizontal line shows the mean of cGMP in each group.)

patients, as in LPS-induced shock in rabbits [6]. Thus, our data suggest that in human sepsis platelets experience high concentrations of NO. The origin of this NO may be the vessel wall, the circulating leukocytes, or the platelets themselves [1]. High levels of cGMP in the platelets probably play a protective role, inhibiting platelet adhesion and aggregation to the vessels, thus preventing intravascular coagulation [1,2].

At the end of the study, intraplatelet cGMP levels remained elevated, suggesting that the activation of NO synthesis was still operative. On the other hand, all but one of our patients did not have septic shock, so the activation of the NO synthesis is probably an early phenomenon in sepsis.

In agreement with other studies [4,5], we also found an increase in plasma cGMP levels in septic patients. The different time courses of plasma and intraplatelet cGMP levels suggest that different factors are responsible for these findings. In fact, it has been suggested that atrial natriuretic peptide (ANP) is responsible for the high plasma cGMP in sepsis [5]. Regrettably, we did not measure ANP levels in our patients.

We gratefully thank B. Paule, M. C. Rentería and J. Murat for their technical assistance. This study was supported by a Grant from Fondo de Investigaciones Sanitarias de la Seguridad Social, Ministry of Health, Spain (grant number 95/0928).

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Revised version accepted 10 October 1997

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## *Listeria monocytogenes* septic arthritis in a natural joint: report of a case and review

*Clin Microbiol Infect* 1998; 4: 165–168

In non-pregnant human adults, *Listeria monocytogenes* primarily causes meningitis, encephalitis, or septicemia. Focal infections caused by *L. monocytogenes* are rare, and usually result from a primary bacteremic phase.

We report the case of an 89-year-old male with diabetes mellitus and a long-standing history of bilateral knee arthrosis which had been periodically treated using non-steroidal anti-inflammatory agents. The patient was investigated following an increase in pain from the right knee plus epigastric pain. Gastroduodenoscopy showed hemorrhagic fundal gastritis and three duodenal ulcers. A joint aspirate from the right knee revealed a serosanguineous fluid, and intra-articular corticosteroid injection was performed. Over the following 3 days, the pain increased and radiated to the ankle.

The patient was then admitted to Trousseau Hospital, Tours, France, on 24 December 1996. On physical examination, the patient was alert and oriented. His temperature was 38.2 °C, his pulse was 88/min, and his blood pressure was 135/90 mmHg. The right knee was erythematous and warm, and had a marked effusion. Neurologic examination results were normal. Initial laboratory examination revealed hemoglobin 180 g/L, hematocrit 53, white blood cell count $10.7 \times 10^9$/L (86% polymorphonuclear leukocytes), glucose 8.50 mmol/L, and creatinine 75 μmol/L. A second joint aspirate was performed 3 days after the first, and revealed purulent fluid with a leukocyte count of $13.6 \times 10^9$/L (80% polymorphonuclear leukocytes). Gram-staining of the effusion showed Gram-positive rods. Culture of this fluid yielded small β-hemolytic colonies which were identified as *L. monocytogenes*, serotype 4b, lysovar 1317:340. Culture of the original aspirate also subsequently yielded Gram-positive rods. Manual susceptibility testing (Kirby–Bauer disk method) revealed that the bacterium was susceptible to ampicillin, gentamicin, erythromycin, chloramphenicol, tetracycline, trimethoprim–sulfamethoxazole and vancomycin. Blood cultures were performed but remained sterile. No lumbar puncture was performed, because neurologic examination was normal.

The patient was initially treated with intravenous amoxycillin and trimethoprim–sulfamethoxazole for 7 days; after this, the regimen was changed to intravenous amoxycillin alone. Two weeks after beginning antibiotic treatment, the patient had diarrhea, and *Clostridium difficile* with toxin A was found in his feces. Amoxycillin treatment was terminated and trimethoprim–sulfamethoxazole per os was administered (800 mg every 12 h). The patient was also treated with metronidazole for the following 14 days. After 6 weeks of antibiotic treatment, the septic arthritis had favorably resolved. The patient reported regularly consuming cheese prepared from unpasteurized goats' milk prior to onset of infection.

Septic arthritis is a relatively uncommon manifestation of listeriosis. A review of the English language