

CLINICAL STUDY

Nitric oxide as an indicator for severity of injury in polytrauma

Beitl E¹, Banasova A^{2,3}, Vlcek M¹, Mikova D⁴, Hampl V⁴Motol University Hospital, 1st Orthopaedic Clinic, Prague, Czech Republic. evzen.beitl@fnmotol.cz**ABSTRACT**

BACKGROUND: Patients with injuries to multiple organs or organ systems are in a serious risk of shock, multiorgan failure and death. Although there are scoring systems available to assess the extent of polytrauma and guide the prognosis, their usefulness is limited by their considerably subjective nature. As the production of nitric oxide (NO) by many cell types is elevated in tissue injury, we hypothesized that serum concentration of NO (and its oxidation products, NOx) represents a suitable marker of polytrauma correlating with prognosis. We wanted to prove that nitric oxide could serve as an indicator for severity of injury in polytrauma.

METHODS: We measured serum NOx and standard biochemical parameters in 93 patients with various degrees of polytrauma, 15 patients with minor injuries and 20 healthy volunteers.

RESULTS: On admission, serum NOx was higher in patients with moderate polytrauma than both in controls and patients with minor injury, and it was even higher in patients with severe polytrauma. Surprisingly, NOx on admission was normal in the group of patients that required cardiopulmonary resuscitation or died within 48 hours after admission. In the groups, where it was elevated on admission, serum NOx dropped to normal values within 12 hours. Blood lactate levels on admission were elevated in proportion to the severity of subsequent clinical course.

CONCLUSION: Elevated serum NOx and blood lactate in patients with polytrauma are markers of serious clinical course, while normal NOx combined with a very high lactate may signal a fatal prognosis (*Fig. 4, Ref. 8*).

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KEY WORDS: marker, systemic inflammatory response syndrome, shock, multiorgan failure.

Abbreviations: CVP – Central Venous Pressure, Hb – Haemoglobin, iNOS – Inducible Isoform of the NO Synthase Enzyme, ISS – Injury Severity Score, INR – International Normalized Ratio, MAP – Mean Arterial Pressure, NO – Nitric Oxide, NO₃⁻ – Nitrates, NO₂⁻ – Nitrites, NOx – oxidation products of Nitric Oxide, ppb – parts per billion, SIRS – Systemic Inflammatory Response Syndrome

Introduction

Polytrauma is defined as the combination of injuries to two or more organs or organ systems, at least one of which is fatal without a prompt treatment. A severely injured patient is always at risk of developing shock, which can rapidly lead to death. Even if shock is adequately managed, polytraumatized patients remain at the risk for the development of a systemic inflammatory response syndrome (SIRS) at the early stages, with the possibility of sub-

sequent multiorgan failure. A perilous manifestation of SIRS is coagulopathy, which consequently leads to diffuse hemorrhage. This hemorrhage is surgically untreatable and may hasten the patient back to the state of shock.

A number of classifications have been used to assess the severity of polytrauma, of which perhaps the most useful and established is the Injury Severity Score (ISS) (Baker et al, 1974, Copes et al, 1988). All the scoring methods, however, share the disadvantage of a significant subjective element of the judgment of the evaluating physician. As the result, a discrepancy between the apparently satisfactory initial clinical state of the patient and the subsequent severe course is a frequent experience. A great contribution for the clinician therefore would be a discovery of objective indicators that would correlate with the severity of injury, and would, to a certain extent, serve as a warning signal for a potentially complicated course.

Tissue injury is associated with an increased production of numerous mediators. Notable among them is the overproduction of nitric oxide (NO), which is the key signaling and effector molecule in a number of biological processes (Moncada et al, 1993, Hampl et al, 2006, Gross et al, 1995). An increased expression of the inducible isoform of the NO synthase enzyme (iNOS) in mast cells, hepatocytes, and vascular smooth muscle is responsible for the overproduction of nitric oxide in trauma. For these reasons we hypothesized that changes in serum concentration of NO and its oxidation products (NOx; because of the very fast oxidation

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of NO in oxygenated blood) shortly after suffering a polytrauma may represent a useful marker for the severity of patient injury after polytrauma, with a prognostic value. Therefore, we investigated the relationship between NOx just after polytrauma and the patient outcome.

Materials and methods

Totally, 108 patients were examined over the period of 30 months. Of this cohort, 93 patients fulfilled the criteria for polytrauma. They presented with major hemorrhage resulting from intrathoracic or intraabdominal organ injuries and from fractures of the pelvis and/or long bones.

The severity of patient injury was assessed according to the ISS scoring system. Based on their ISS score and the subsequent progression of clinical state, patients were divided into the four groups: 1. Patients with a mild course (ISS score between 16 – 25, i.e. minor polytrauma, n = 30), 2. Patients with a severe course (ISS score > 25, i.e. those with severe polytrauma, n = 40), 3. Patients with critical injuries necessitating cardiopulmonary resuscitation and/or high volume blood substitutions (n = 12), 4. Non-surviving patients – those who died within 48 hours after admission to the critical care unit (n = 11).

The remaining 15 patients not fulfilling the criteria of polytrauma were assigned to a separate group (“minimal injury”). Twenty healthy volunteers formed a control group.

Basic characteristic of the patients groups is in the Table 1.

All patients received the standard therapy as needed. Central venous pressure (CVP) and the mean arterial pressure (MAP) were followed in all subjects. Initial blood tests were taken to assess the serum concentration of NOx, coagulation parameters, hemoglobin, lactate levels, and glycaemia. Blood tests were performed during patient admission and at intervals of 12, 24, and 48 hours after admission.

As in an aqueous solutions (such as blood) NO quickly oxidizes to form nitrites (NO_2^-) and nitrates (NO_3^-), the NO levels in serum were assessed by measuring the sum of NO, NO_2^- and NO_3^- (NOx). This was done by exposing the samples to highly reducing environment (thus converting NO_2^- and NO_3^- to NO) and then detecting the resulting NO concentration by chemiluminescence analyzer (CLD 77 AM, ECO PHYSICS Switzerland) (Hampl et al, 1996). The blood samples were centrifuged and the incurred serum was stored at -60°C . Serum samples were processed in less than 2 weeks. Each sample (100 μl) was thawed and injected into a purge vessel containing vanadium (III) chloride (7 ml 0.1

M VCl_3 in 2 M HCl) at 90°C . The purge vessel was under constant bubbling with a stream of Helium that carried NO into the analyzer. To prevent damage to the chemiluminescence analyzer by the hydrochloric acid vapor, a gas bubbler filled with aqueous potassium hydroxide was installed between the purge vessel and the NO analyzer. Inside the reaction chamber of the NO analyzer NO reacts very quickly with ozone (produced in the analyzer by electric discharge). The NO_2 produced by this reaction is in the excited state. As its unstable electrons return to their original ground state, they dissipate energy in the form of photons. Light emission is linearly related to the NO content of the sample. NOx levels are expressed as parts per billion (ppb) of NO in the gaseous phase.

The results were analyzed statistically using the one-factor ANOVA followed by Fisher's post hoc test. The differences were considered significant when $p < 0.05$. The results are presented as the mean values with the standard errors of the mean.

Results

There was no statistically significant difference in serum NOx concentration at the time of admission in the group of patients with minimal injury when compared to the control group of healthy volunteers (30.9 ± 2.9 vs 27.5 ± 2.8 ppb) (Fig 1). In the group of polytrauma patients with moderate injuries, the serum NOx concentration on admission was significantly higher than in the group of healthy volunteers (42.4 ± 5.4 vs 27.5 ± 2.8 ppb). In the group of polytrauma patients with severe injuries, the serum concentration of NOx at time of admission was doubled compared to healthy volunteers (67.9 ± 7.7 vs 27.5 ± 2.8 ppb, $p < 0.05$). NOx concentrations in the group with severe polytrauma were significantly higher than those in the group classified as moderate polytrauma.

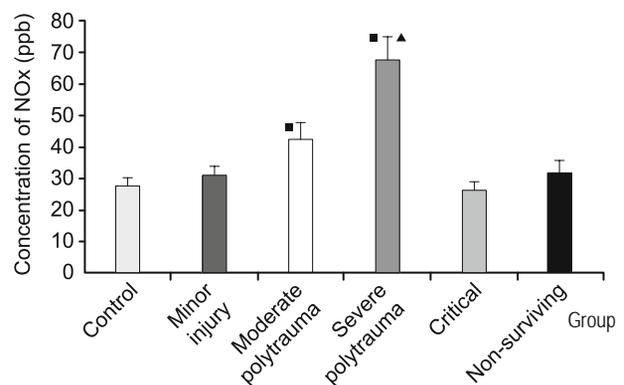


Fig. 1. Severe and moderate polytrauma significantly increases serum NOx concentration, while NOx is normal in patients that require cardiopulmonary resuscitation or die within 48 hours after admission. Control - healthy volunteers, minor injury - patients with ISS <16, moderate polytrauma - patients with a mild course (ISS score of between 16–25), severe polytrauma - patients with a severe course (ISS score of > 25), critical - patients with critical injuries necessitating cardiopulmonary resuscitation and/or high volume blood substitutions, and non-surviving v patients who died within 48 hours after admission. ■ $p < 0.05$ vs control group, ▲ $p < 0.05$ moderate vs severe group.

Tab. 1. Basic characteristic of the patients groups.

Group	n	Age	% females
minimal injury	15	42±4	20
moderate polytrauma	30	39±3	16.7
severe polytrauma	40	38±2	12
critical	12	39±5	25
non surviving	11	44±7	36
controls	20	34±3	67

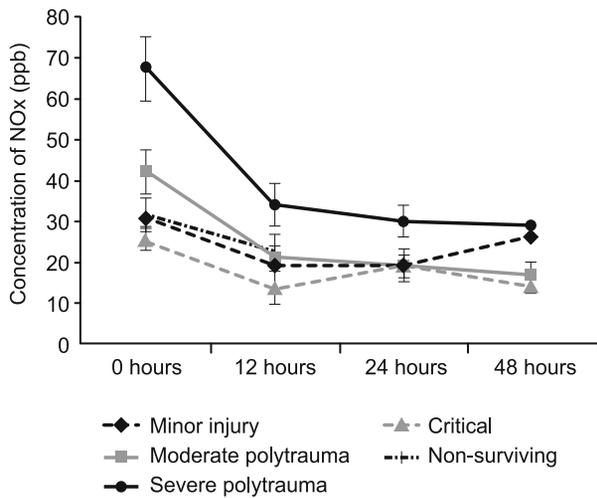


Fig. 2. Serum NOx normalizes within 12 hours after admission in all groups of injured patients. Legend as in Figure 1. The statistical differences among the groups at 0 hours are shown in Fig. 1. At the remaining time points, there were no statistically significant differences among the groups.

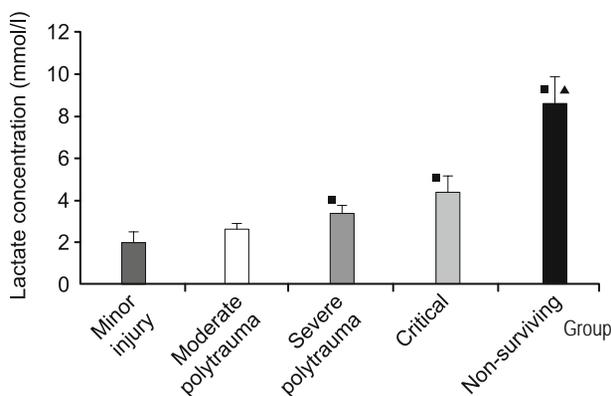


Fig. 3. Serum lactate levels on admission are increased in proportion to the severity of polytrauma. Legend as in Figure 1. ■ $p < 0.05$ vs minor injury, ▲ $p < 0.05$ vs all groups.

Surprisingly, on admission, there was no statistically significant difference in serum NOx concentrations between the group of patients with a critical course and the control group (25.9 ± 3.0 vs 27.5 ± 2.8 ppb). The same was true for the group of patients who eventually died (31.6 ± 4.1 vs 27.5 ± 2.8 ppb).

In all groups with elevated serum NOx concentrations on admission, the values reached normal (i.e. they were not statistically different from the control group) at the measurement 12 hours after admission. Thereafter, the NOx values remained essentially stable (Fig. 2). There were no significant differences among the groups after the initial measurement.

There were no notable differences in CVP and MAP during the study. Both parameters confirmed the adequacy of therapy and blood transfusions. The values of hemoglobin concentration (Hb) conveyed similar information. It is well known that even a massive

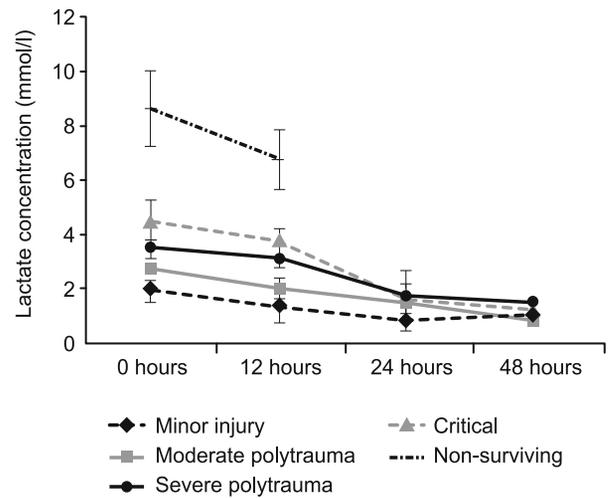


Fig. 4. The lactate concentrations decreased in all groups of injured patients during the first day after the injury. Non-surviving vs critical, $p=0.0236$. Legend as in Fig. 1.

hemorrhage is initially only slightly reflected in blood cell count due to the symmetric loss of plasma and cellular elements. Probably due to this phenomenon, only slight differences in Hb were seen among the groups at the time of admission. During all subsequent time intervals, the Hb concentrations reflected the substitution of the patients with blood derivatives. It was maintained within the range of 8.5–10.8 g/dL.

The values of international normalized ratio (INR), that were measured to inform about the initial state of coagulation, brought no notable information apart from the fact that they were radically deviated from the norm (which is 0.9–1.3) in the groups of patients with critical injury and in those who died shortly after admission. The INR was 3.0 in the non-surviving group and 1.8 in the group with critical injuries.

Serum lactate concentration and glycaemia at the time of admission showed similar differences among the groups as seen with the serum NOx concentrations with one principal difference – the highest levels were found in the group of patients, who were critically injured and especially in those, who did not survive. The initial concentration of serum lactate in the group of patients with severe polytrauma was more than two-fold higher than in the control group; in the group of critically injured patients with lethal course, this concentration was six-fold that of the control group (Fig. 3). The lactate concentrations decreased in all groups of injured patients during the first day after the injury (Fig. 4).

Discussion

Our working hypothesis that NOx serum levels on admission would correlate with prognosis of patients with polytrauma was only partially confirmed by our data. While serum NOx markedly increased with the severity of polytrauma up to the category of severe polytrauma, it was surprisingly normal in patients with the most critical and lethal course. This latter aspect of our results is

hard to explain on the basis of what is known about the biology of NO in tissue injury. Hypothetically, marked energy depletion in severe shock might lead to relative inactivity of NO synthase, but we have no solid data to support such an interpretation.

A rapid decline in the values for NOx concentration between time = 0 and time = 12 hours in all groups, where NOx was elevated at admission, is also not entirely clear, but a similar situation was also seen with values of both glycaemia and lactate. At least a partial effect of therapy (with improved blood/plasma volume) on this finding seems quite likely. This trend conflicts with the published results from Huang et al (2003). In the study of traumatic shock in rats they demonstrated an elevation in the concentration of NOx with a subsequent plateau within 24 hours after trauma, or rather, after resuscitation. Differences in plasma volume management between rats and human patients could be suspected as a potential explanation for this discrepancy.

Considering all other measured parameters, the trends of INR, glycaemia, and especially the lactate levels are not without interest. Unfortunately, INR and glycaemia cannot be used as markers due to the simple fact that important details of the relevant personal history in patients with polytrauma are often unavailable or misleading (elevated glycaemia in diabetic patients, or increased INR in patients on anticoagulants). However, our data on lactate concentration are promising because the groups of patients with more severe polytrauma had higher lactate levels. Thus, lactate concentration might serve as a marker of polytrauma severity and prognosis. The rate of lactate clearance was previously linked to survival (Abramson et al, 1993). In the study of Abramson et al (Abramson et al, 1993), all patients whose lactate level normalized in 24 hours survived. If lactate levels cleared to normal between 24 and 48 hours, the survival rate was 75 %. Therefore, the time needed to normalize serum lactate levels is an important prognostic factor for the survival in severely injured patients. In concordance with this finding, our Figure 4 shows that patients, who did not survive beyond 24 hours from admission had lactate levels at 12 hours after admission that were several times normal.

The data of this study lead to the possibility to interpret serum NOx values as an early marker of the severity of injury after poly-

trauma with a prognostic value. Its increase could attest for quite a severe injury with the possibility of a number of other complications. On the other hand, little or no increase in the concentration of NO metabolites upon an initial examination in a patient with a clinically obvious serious state may indicate a high mortality risk. Even more intriguing is the possibility to use the combination of the initial levels of serum NOx and lactate in prognosis. Elevated levels of NOx and lactate, with a high likelihood, may alert for a potentially critical course. The combined finding of nearly normal levels of NOx and an excessively high level of lactate in a polytraumatic patient should alert to the possibility of the most critical course.

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