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Postmortem neopterin concentrations: comparison of diagnoses with and without cellular immunological background

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Summary. Increased neopterin levels in urine and serum of living humans indicate an activation of the cellular immune system. We investigated 119 urine and 48 serum samples from 129 corpses taken at necropsy; 29 cases with a background of cellular immune activation were compared to 100 corpses with no such indication. Our investigations show the feasibility of postmortem neopterin measurements. However, different kinetics of serum and urine concentrations after death were observed. In addition, the data show that urine and serum neopterin concentrations were significantly higher when cellular immunological abnormalities were present when compared to the control group and to living healthy controls. The findings suggest, that increased postmortem urine neopterin concentrations in necropsy indicate pathological processes linked with cellular immune activation.

Key words: Postmortem neopterin levels – Cause of death – Cellular immune system activation

Zusammenfassung. Erhöhte Neopterinspiegel in Serum und Harn Lebender sind ein Hinweis auf eine gesteigerte Aktivität des zellulären Immunsystems. Wir untersuchten 119 Harn- und 48 Serumproben von insgesamt 129 Leichen; bei 29 Fällen (Gruppe I) konnten Erkrankungen, die bekannterweise mit zellulären immunologischen Veränderungen einhergehen, festgestellt werden, bei den restlichen 100 Fällen (Gruppe II) lieferte die Obduktion keinen Anhalt auf derartige Erkrankungen. Die Auswertung unserer Ergebnisse ergab eine signifikante Erhöhung der Neopterinkonzentrationen in Serum und Harn in Gruppe I, sowohl gegenüber der Kontrollgruppe (Gruppe II) als auch gegenüber gesunden Personen. Da Neopterin im Harn zumindest über 24 Stunden post mortem stabil ist, könnte ein erhöhter Neopterinwert einen diagnostischen Hinweis auf eine Erkrankung, die mit zellulärer Immunaktivierung einhergeht, liefern.

Schlüsselwörter: Postmortale Neopterinkonzentrationen – Todesursachen – Zellvermittelte Immunaktivierung

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Introduction

The main objective of a forensic necropsy is to ascertain the cause of death and estimation of the time of death. The diagnosis of the cause of death needs further diagnostical criteria, such as histological, toxicological or even biochemical findings [1].

Neopterin release has been demonstrated to be a specific indicator for stimulation of the T-lymphocyte-macrophage system. In vitro, large amounts of neopterin are produced by human macrophages upon stimulation with interferon-gamma [2, 3]. As clinical studies show, conditions with known association to cellular immune activation such as allograft rejections, viral infections, infections by intracellular protozoa and bacteria, autoimmune and certain malignant diseases are indicated by high neopterin levels [3].

Preliminary investigations confirmed the feasibility of postmortem neopterin evaluation in serum and urine. Urine neopterin levels were similar to in vivo concentrations, whereas serum neopterin concentrations were higher in the majority of cases and showed a time-dependent increase [4].

Similarly higher neopterin concentrations were assumed in corpses with organic disorders which are known to be associated with cellular immune activation when compared to disorders in which no activation of the cellular immune system is apparent. It is of particular interest to sensitively recognize immunological abnormalities in corpses when the cause of death with unknown pathogenetic background is suspected: e.g., there is an ongoing discussion whether acute virus infections or immune regulatory abnormalities are involved in the etiology of the sudden infant death syndrome (SIDS). In these cases samples taken prior to death are extremely rare. Thus, the feasiblity of postmortem evaluation of the cellular immune system would be an important factor to help solve the puzzling questions around the pathogenesis of SIDS and possibly other unclarified conditions.

In this study, we compared urine and serum specimens taken from corpses with various causes of death in order to evaluate whether post mortem neopterin levels

Table 1. Causes of death in 129 cases (n; sex; range of age; and median are shown)

Group I (n = 29)	Group II (n = 100)
20 males, 9 females	77 males, 23 females
Age: 34–97 yrs (median 76)	Age: 11–86 yrs (median 59)
11 cardiac death	45 cardiac death
10 pneumonia	11 fatal loss of blood
5 pulmonary embolism	9 intoxication
1 sepsis	6 craniocerebral trauma
1 myocarditis	6 hanging
1 suffocation	4 aorta-rupture and hemopericardium
	2 apoplexy
	3 venous air embolism
	3 suffocation
	2 pulmonary embolism
	2 asthma
	2 refrigeration
	1 cerebral aneurysm-rupture
	1 drowning
	1 anaphylaxis
	1 gun-shot

Table 2. Diseases with cellular immunological background diagnosed at necropsy in group I of Table 1 (n = 29)

Pneumonia (18)	Gastric carcinoma (2)
Pulmonary tuberculosis (2)	Bronchial carcinoma (1)
Myocarditis (1)	Renal carcinoma (1)
Peritonitis (1)	Malignant lymphoma (1)
Hepatocirrhosis (1)	
Sepsis (1)	

are associated with similar pathological conditions as known from patients.

Material and methods

From 129 corpses, 119 urine and 48 serum specimens (97 males, 32 females; age range: 11–97 years; median: 61 years) were investigated. The samples were taken by puncturing the urinary bladder and the Vena subclavia after a median time of 16 hours after death (interquartile range: 9.5–28 hours after death). Urine and serum samples taken at necropsy were protected from sunlight by tin-foil and stored at −20°C until measurement. Neopterin and creatinine levels in urine and serum were quantified by automated high pressure liquid chromatography (HPLC) techniques on reversed phase [4, 5, 6].

All individuals had died unexpectedly and according to necropsy and histological findings could be classified into 2 groups; group I (n = 29) where disorders were diagnosed which were known from in vivo observations to be associated with activation of the cellular immune system (e.g. infections with viruses or intracellular bacteria, malignancies) and with a high incidence of increased neopterin levels, and group II (n = 100) wihtout such diagnoses. The causes of death in these cases are listed in Table 1. Table 2 shows the immunological disorders diagnosed in the first group of patients (note: the disorder listed is not necessarily identical with the cause of death rather as an additional observation. Therefore, the number of events shown in Table 2 may differ from Table 1 in some aspects).

For statistical comparison of data the non-parametric Wilcoxon Mann Whitney test was used. Correlation analyses were performed with Spearman's rank correlation statistic. Frequencies were compared using Pearson's Chi-Square test.

Results

Mean postmortem urine and serum neopterin and creatinine concentrations were higher than the upper normal limits of living individuals (Table 3).

Comparing group I and group II we found no statistically significant differences between the sexes ($z = 0.78$, $P > 0.05$) and in the time after death ($z = 1.22$, P = n.s.). There was no influence of protracted resuscitation on neopterin and creatinine levels. In contrast, in group I the age was significantly higher than in group II ($z = 2.60$, $P < 0.01$).

Similar to living individuals, specimens taken from corpses with diagnoses indicating cellular immunological background (group I) showed significantly higher urine neopterin levels than group II ($z = 5.57$, $P < 0.0001$) (Fig. 1): median urine neopterin concentrations were about 4-fold increased in these corpses (817 µmol neopterin/mol creatinine) compared to levels in corpses of group II (192 µmol neopterin/mol creatinine). Serum neopterin levels were twice as high in group I compared to group II ($P > 0.05$). The variance of serum neopterin was much larger than that of urine concentrations in group II.

There were no significant differences between serum creatinine ($z = 0.51$, P = n.s.) and the serum neopterin creatinine ratio (data not shown) between the two groups.

Urinary neopterin levels in both groups did not increase with the time after death. Serum neopterin and creatinine concentrations correlated positively with the time after death in group II, but not in group I.

Urinary neopterin concentrations showed a positive correlation with age in group II ($r_s = 0.32$, $P = 0.002$)

Table 3. Postmortem urine neopterin : creatinine, serum neopterin and creatinine concentrations in all examinations

	Number of cases	Mean	S.D.	Median	25th–75th percentile	Normal range[b]
Urine neopterin : creatinine (µmol/mol)	119	418[a]	520	216	148–366	≤ 250
Serum neopterin (nmol/l)	48	119[a]	194	54	21–115	≤ 8.7
Serum creatinine (µmol/l)	15	508[a]	477	298	158–686	< 100

[a] Significantly higher than concentrations in healthy living controls ($P < 0.001$)
[b] Normal range in healthy alive controls

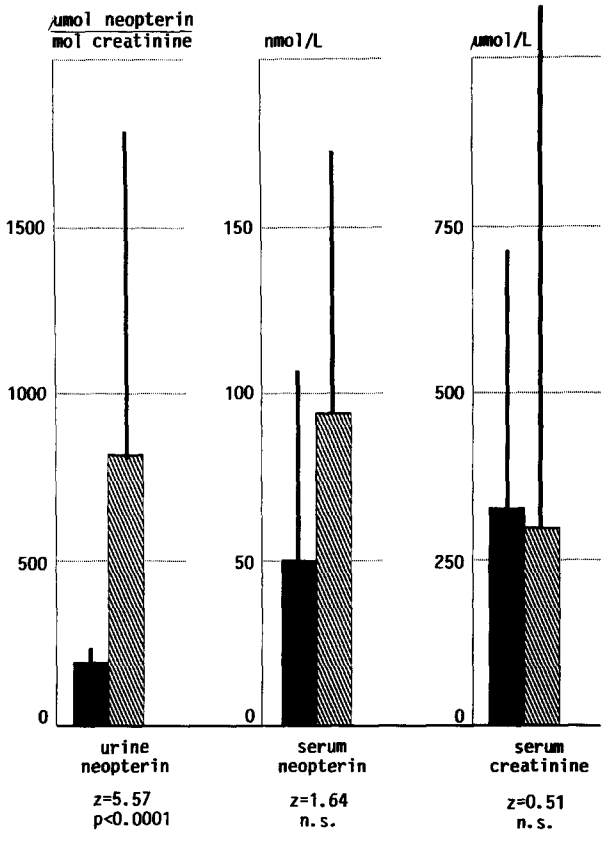

Fig. 1. Comparison of postmortem neopterin and creatinine concentrations in cases belonging to group I (diagnoses of disorders with cellular immunological background; hatched bars), and group II (filled bars) (medians and 75th percentiles are shown; P values from unpaired Wilcoxon Mann Whitney rank test)

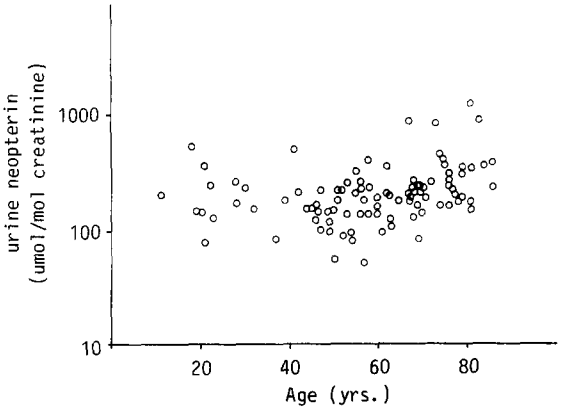

Fig. 2. Age dependence of urinary neopterin concentration in corpses without signs of disorders with cellular immunological background ($r_s = 0.32$, $P < 0.01$)

(Fig. 2), which was absent in group I). No such correlation was observed concerning serum neopterin and serum creatinine.

Serum neopterin and serum creatinine levels varied with the time after death, whereas urine neopterin levels remained stable (data not shown).

Discussion

Clinical studies have demonstrated that elevated neopterin levels in urine and serum are associated with conditions involving activation of cellular immunity. As a consequence high serum and urine neopterin concentrations were particularly observed in patients with infectious or malignant diseases.

Similarly higher neopterin concentrations were found in corpses where organic disorders linked to cellular immune activation were known. The differences were only significant for urinary neopterin values. No difference was seen in serum neopterin concentrations, although mean values were almost twice as high in group I compared to group II. A larger variance of serum neopterin levels may be the reason for this observation.

Postmortem examinations of serum and urine neopterin specimens indicated a different kinetic behaviour in relation to the time after death [4]. Urine neopterin levels remained stable for at least 24 hours, therefore, postmortem urine neopterin concentrations seem to reflect in vivo concentrations. Urinary neopterin levels remained nearly unchanged with time and even 20 days after death a urinary neopterin level within the normal neopterin range was found in one corpse in group II. In contrast, serum concentrations were dependent on the time after death [4]. Serum neopterin in our study also showed a time dependent increase confirming our earlier results (not shown) [4]. Therefore, the feasibility as a diagnostic criterion is reduced compared to urine measurements. However, different times of sampling may be the reason for this variation and it is possible that a controlled sample collection with respect to the time after death may improve the statistical significance of serum neopterin concentrations.

In normal healthy individuals urinary neopterin concentrations increase with age, especially above 65 years of age. This age-dependent increase is much smaller compared with the change of urinary neopterin levels in corpses. Therefore, age can only marginally contribute to the significant difference of urine neopterin levels between the two groups of corpses.

The reason for the postmortem increase of serum neopterin concentrations is not yet known. Perhaps immunological processes are involved. Elevation in body temperature in the early postmortem period has been described [7] and it is conceivable that immunological processes and the release of cytokines could cause neopterin secretion from macrophages and the initial postmortem elevation in body temperature. The phenomenon is probably caused by the continuing metabolic activity of body tissue or by bacterial metabolic processes taking place in the intestine.

Our data show that urine neopterin concentrations provide an aid for differential diagnoses between disorders with and without involvement of cell-mediated immune activation. In living individuals neopterin changes were observed very early during the course of virus infections. Neopterin increases are probably the first sign of infection before antibody seroconversion becomes detectable. Immediately after the infectious agent is de-

stroyed neopterin concentrations normalize [2, 3]. Thus the increased neopterin concentrations found in a pilot study of children who died form SIDS [8], may indeed indicate abnormal immunopathology which is concordant with the view that the syndrome is related to acute infection.

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