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Postmortem Evaluation of Serum and Urine Neopterin Concentrations

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ABSTRACT: Cellular immune response is accompanied by the release of neopterin. Increased neopterin levels in urine and serum are observed in patients during viral infections, autoimmune diseases, and allograft rejections and certain malignant diseases. We investigated postmortem neopterin concentrations in urine and serum samples taken from 32 corpses 3 to 69 h (mean 19.3 h) after death. Urine neopterin concentrations in corpses are similar to those of healthy live controls and are independent of the time after death. In contrast, serum neopterin concentrations are frequently greatly increased in corpses, and the levels are higher in sera collected more than 10 h after death in comparison with samples obtained earlier.

Neopterin measurement in urine and serum samples of corpses is feasible. It appears likely that urine neopterin concentrations could aid the diagnosis of inflammatory diseases in corpses.

KEYWORDS: pathology and biology, neopterin, cause of death, postmortem neopterin levels, time after death, cellular immune system activation

Postmortem biochemical analyses of body fluids provide additional indications of the cause of death and a better understanding of the related pathophysiological facts. Furthermore, biochemical findings may allow an estimate of the time of death in a late postmortal phase [*1,2*].

Large amounts of *D*-erythro-neopterin, a pteridine derivative, are produced and secreted by macrophages after stimulation with T-cell-derived interferon-gamma. Therefore, neopterin concentrations in serum and urine reflect the activation status of the cell-mediated immune system [*3,4*].

In recent years, immunoassays to quantify neopterin have become commercially available. Urine and serum neopterin levels are frequently used in transplantation surgery for the diagnosis of immunological complications such as graft rejections or infections [*5*]. Neopterin concentrations reflect the extent and activity of autoimmune diseases [*6*], and they also reveal predictive information in certain malignant diseases [*7*] and in human immunodeficiency virus infections [*4,8*]. It is of interest, that neopterin concentrations increase in cases of infections by viruses, intracellular bacteria, and parasites before clinical symptoms are apparent and antibody seroconversion occurs [*4*].

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By neopterin analyses, various inflammatory disorders can be distinguished from non-inflammatory disorders [*3,6*]. Thus, postmortal neopterin analysis may be helpful in aiding differential diagnoses.

In the present study, the feasibility of postmortal neopterin analyses is investigated. Neopterin concentrations in serum and urine are correlated to the time after death when the samples were collected.

Material and Methods

We investigated 32 corpses [23 males, 9 females; all adults; mean age, 58 years; standard deviation (SD), 20.2] who died of various causes of death. After death, 30 serum and 26 urine samples were taken (3 to 69 h; mean, 19.3). Only those corpses were included in the study for whom the time of death could be satisfactorily estimated. In all investigated cases, a sudden death was diagnosed, in which acute myocardial infarction (N = 20), pulmonary embolism (N = 2), bursting of an aneurysm (N = 2), a shotgun wound (N = 1), strangulation (N = 1), intoxication (N = 4) (2 alcohol, 1 carbon monoxide, and 1 methaqualon), and trauma (N = 2) (craniocerebral trauma) were ascertained. Inflammatory disorders as the cause of death were excluded by autopsy and histological evaluation. The blood samples were taken by puncturing the vena subclavia, vena axillaris, vena femoralis, or vena cava inferior, and the urine samples by puncturing the bladder.

In addition, a follow-up study was performed on a man who died by suicidal gunshot. Sequential urine (N = 6) and serum (N = 6) samples were collected 4 to 21 h after death.

Because neopterin is sensitive to light, the samples were covered by tin foil. Specimens were stored at minus 20°C until measured. Neopterin and creatinine concentrations in the urine and serum were determined by automated high-pressure liquid chromatography (HPLC) techniques on reversed-phase columns as described [*3*]. Briefly, 100 μL of urine was diluted with 1 mL of potassium phosphate buffer (15 mmol/L, pH 6.4), and 10 μL of this mixture was injected onto 125 by 4-mm C_{18} reversed-phase columns (LiChroCart, Merck, Darmstadt, Germany) at 25°C. The compounds were eluted at a flow rate of 0.8 mL/min. Neopterin was monitored by its native fluorescence (353-nm excitation, 438-nm emission). To account for physiological variations in urine excretion, we related neopterin concentrations to urinary creatinine concentrations, which were quantified in the same chromatographic run by measuring the absorbance at 235 nm. Serum samples were acidified but not deproteinized and applied to a 4-propylbenzene sulfonic acid/modified silica sorbent cartridge, which quantitatively retains the analytes but not the serum proteins. The retained analytes are then eluted from the cartridge directly onto the liquid chromatography column and analyzed as described above.

Grouped data were compared using the nonparametric Wilcoxon Mann Whitney test. For correlation analyses we used Spearman's rank correlation statistic.

Results

Postmortem mean urine neopterin levels were similar to the concentration ranges observed in vivo, while serum neopterin was extremely high in a large number of samples (Table 1). Urine and serum neopterin levels were significantly higher compared to healthy live controls ($P < 0.01$).

Urine neopterin per creatinine levels did not vary with the time after death. Samples obtained before 10 h since death did not differ from samples obtained later (Z = 1.32, P = n.s., Wilcoxon test). In contrast, serum neopterin levels were dramatically higher in some samples obtained at later times after death (Z = 1.89, P = 0.06) reaching a maximum level of 216 nmol/L (Table 2). In parallel to neopterin changes, serum creatinine levels were higher in samples obtained more than 10 h after death than in earlier collected

TABLE 1—*Postmortem neopterin concentrations in serum and urine of 32 cases.*

	N	Median	25th-75th Percentile	Normal Range
Urine neopterin, μmol/mol creatinine	26	228	192–301	≤250
Serum neopterin, nmol/L	32	39.2	20.0–78.1	≤8.7
Serum creatinine, μmol/L	22	228	158–358	<100
Serum neopterin, μmol/mol creatinine	22	122	80.8–319	<90

TABLE 2—*Serum and urine neopterin concentrations in samples obtained <10 and ≥10 h after death;* N *and median (25th–75th percentile) are shown, respectively.*

	<10 H after Death			≥10 H after Death		
	N	Median	25th–75th Percentile	*N*	Median	25th–75th Percentile
Urine neopterin, μmol/mol creatinine	9	216	188–237	17	236	197–329
Serum neopterin, nmol/L	9	16.4	10.9–68.0	23	52.5	22.1–88.6
Serum creatinine, μmol/L	8	157	116–197	14	309	234–421
Serum neopterin, μmol/mol creatinine	8	122	99–445	14	116	53.7–224
Age	9	52	37–68	23	67	55–76

sera ($Z = 2.97$, $P = 0.003$). The serum neopterin per creatinine ratio was not different between the two groups ($Z = 0.78$, P = n.s.).

Serum neopterin ($r_s = 0.417$, $P = 0.020$; Spearman's rank correlation) and serum creatinine ($r_s = 0.537$, $P = 0.014$) correlated positively to the time after death. In contrast, no such correlation was obtained with the urine neopterin per creatinine ratio ($r_s = 0.258$, P = n.s.) and the serum neopterin per creatinine ratio ($r_s = 0.065$, P = n.s.).

Follow-up samples obtained 4 to 21 h after death from one case who died from a suicidal chest shot showed that urine neopterin levels were normal and remained nearly unchanged with time (Fig. 1, Panel A). In contrast, serum neopterin and creatinine levels were low at 4 h after death but increased slightly even from the beginning of the follow-up. Later on, serum neopterin and creatinine concentrations increased prominently, reaching extremely high concentrations (Fig. 1, Panels B and D). Comparing the quotients between serum neopterin and creatinine only partially decreased the variation with time (Fig. 1, Panel C).

Discussion

The data show that the analysis of postmortem neopterin concentrations is feasible. Whereas urine neopterin levels in corpses are independent of the time after death and appear to remain stable during several hours, serum neopterin levels in corpses increase significantly with time. These cross-sectional data are confirmed by the follow-up of a

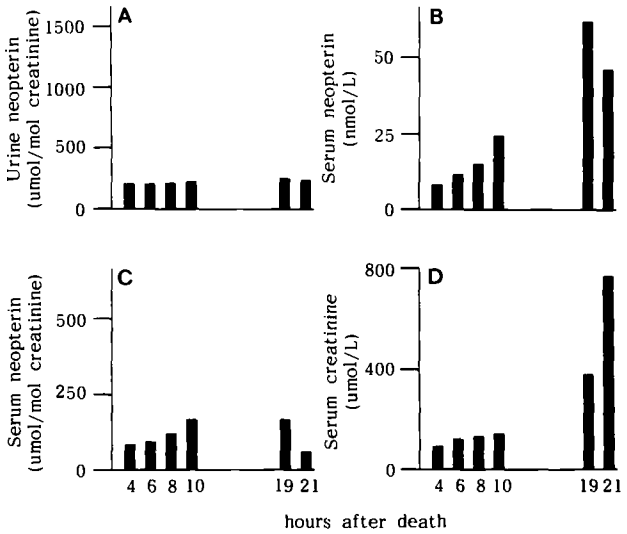

FIG. 1—*Changes of urine neopterin per creatinine* (A), *serum neopterin* (B), *serum neopterin per creatinine* (C), *and serum creatinine* (D) *during the follow-up of one case who died after suicidal gunshot.*

case of a victim who died from a gunshot wound. Sequential samples were taken 4 to 21 h after death. The urine neopterin concentration was within the normal range of healthy live controls and remained unchanged during the time of observation. In contrast, serum neopterin and creatinine concentrations were in the normal range only during the first few hours after death and then rapidly increased, reaching a concentration range which is seldom observed in patients [3]. In some serum samples which were obtained from corpses more than 10 h after death, neopterin concentrations were above 200 nmol/L. In samples from live controls, levels around 200 nmol/L were almost exclusively seen in hemodialysis patients [9]. The reason for the increase of serum neopterin after death is not yet clear and makes further investigations necessary.

Conclusions

As known from in-vivo studies, neopterin concentrations provide an aid for differential diagnoses between inflammatory disorders and non-inflammatory conditions. In corpses, urine neopterin concentrations appear to reflect the in-vivo urine status for several hours after death and can therefore be considered as a potential diagnostic criterion. In our study, approximately half of the corpses had urine neopterin concentrations slightly above the normal range of healthy live controls. It appears likely that inflammatory conditions are the reason for this observation. Preliminary data indicate that neopterin levels are higher in individuals who died and had had inflammatory diseases (data not shown). Further studies are under way to clarify this point.

In contrast to urine concentrations, serum neopterin levels increase with time. Neopterin per creatinine ratios could partially account for this effect. To use serum creatinine for the adjustment of neopterin concentrations may account to some degree for the time-dependent increase of serum neopterin. This might allow investigators to estimate neopterin concentrations before death from samples obtained later after death. With this approach, the serum neopterin per creatinine ratio may be used in serum specimens similar to the use of urine neopterin levels. However, the large variation between different

samples may limit its routine application. Further studies will show the relevance of this proposal.

Serum neopterin concentrations correlated significantly with the time of death. However, the correlation was not very strong; it was less expressed than that of serum creatinine. A similar correlation between creatinine concentrations and the time after death was shown earlier to occur, for example, in bird muscle [*10*]. It remains to be shown whether quantitation of serum neopterin could aid in the estimation of the time of death.

References

[*1*] Staak, M., Madea, B., and Henssge, C., "Determination of the Time Since Death—New Developments," *Japanese Journal of Legal Medicine*, Vol. 43, 1989, pp. 76–77.

[*2*] Balasooriya, B. A., St. Wilson, C. A., and Williams, A. R., "The Biochemical Changes in Pericardial Fluid after Death. An Investigation of the Relationship Between the Time Since Death and the Rise or Fall in Electrolyte and Enzyme Concentrations and Their Possible Usefulness in Determining the Time of Death," *Forensic Science International*, Vol. 26, Oct. 1984, pp. 93–102.

[*3*] Wachter, H., Fuchs, D., Hausen, A., Reibnegger, G., and Werner, E. R., "Neopterin as Marker for Activation of Cellular Immunity: Immunologic Basis and Clinical Application," *Advances in Clinical Chemistry*, Vol. 27, 1989, pp. 81–141.

[*4*] Fuchs, D., Hausen, A., Reibnegger, G., Werner, E. R., Dierich, M. P., and Wachter, H., "Neopterin as a Marker for Activated Cell-Mediated Immunity," *Immunology Today*, Vol. 9, No. 5, May 1988, pp. 150–153.

[*5*] Margreiter, R., Fuchs, D., Hausen, A., Huber, C., Reibnegger, G., et al., "Neopterin as a New Biochemical Marker for Diagnosis of Allograft Rejection," *Transplantation*, Vol. 36, No. 6, Dec. 1983, pp. 650–653.

[*6*] Reibnegger, G., Egg, D., Fuchs, D., Günther, G., Hausen, A., et al., "Urinary Neopterin Reflects Clinical Activity in Patients With Rheumatoid Arthritis," *Arthritis and Rheumatism*, Vol. 29, No. 9, Sept. 1986, pp. 1063–1070.

[*7*] Reibnegger, G., Bichler, A. H., Dapunt, O., Fuchs, D. N., Fuith, L. C., et al., "Neopterin as a Prognostic Indicator in Patients with Carcinoma of the Uterine Cervix," *Cancer Research*, Vol. 46, Feb. 1986, pp. 950–955.

[*8*] Fahey, J. L., Taylor, J. M. G., Detels, R., Hofman, B., Melmed, R., et al., "The Prognostic Value of Cellular and Serologic Markers in Infections with Human Immumodeficiency Virus Type 1," *The New England Journal of Medicine*, Vol. 322, Jan. 1990, pp. 166–172.

[*9*] Fuchs, D., Hausen, A., Reibnegger, G., Werner, E. R., Dittrich, P., et al., "Neopterin Levels in Long-Term Hemodialysis," *Clinical Nephrology*, Vol. 30, No. 4, April 1988, pp. 220–224.

[*10*] Gallois-Montbrun, F. G., Barres, D. R., and Durigon, M., "Postmortem Interval Estimation by Biochemical Determination in Birds Muscle," *Forensic Science International*, Vol. 37, No. 3, May 1988, pp. 189–192.

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