

Changes in serum ECP levels after storage at room temperature

Wantke F, Demmer CM, Götz M, Jarisch R. Changes in serum ECP levels after storage at room temperature.
Allergy 1994; 49: 483–484. © Munksgaard 1994.

F. Wantke¹, C. M. Demmer¹, M. Götz^{1,2},
R. Jarisch¹

¹ Dermatologic and Pediatric Allergy Clinic, Vienna; ² Department of Pediatrics, University of Vienna, Austria

Key words: ECP levels; eosinophil cationic protein; screening test; storage.

Felix Wantke, MD
Dermatologic and Pediatric Allergy Clinic
Franz Jonas Platz 8/6
A-1210 Vienna
Austria

Accepted for publication 30 August 1993

Eosinophil cationic protein (ECP) level is a new variable in monitoring asthma and wheezy bronchitis (1, 2, 4, 5). As assessment of ECP requires expensive equipment and laboratory handling of radioactive substances, only a few centers are prepared for this procedure (4). In addition, sera for ECP determinations are supposed to be processed rapidly.

Therefore, as general practitioners seldom assess ECP, patients from most parts of Austria have to be sent to a distant hospital or laboratory. This time-consuming journey is seldom made; thus, patients do not receive adequate, up-to-date treatment.

The aim of the present study was to evaluate the stability over time of ECP samples at room temperature. It is hoped that mailing of serum ECP samples from general practitioners to a laboratory within 24 h might obviate the need of patients to travel.

Material and methods

Blood samples

Whole blood was drawn from 20 patients (10 men, 10 women, mean age 42.7 years, range 19–73 years) with suspected wheezy bronchitis who were not taking steroids and from 10 healthy controls (five men, five women, mean age 36.6 years, range 27–48 years), 6-ml SST tubes (Vacutainer, Rutherford, USA) being used. After storage for 70 min at room temperature, as recommended by the manufacturer (4),

blood was centrifuged at 4°C for 10 min at 2500 rpm (1100 g). A volume of 500 µl supernatant of each patient was pipetted into two polypropylene tubes. The first tube was frozen immediately at –20°C; the second tube was rested for 24 h at room temperature (about 20°C) and then frozen at –20°C, too.

Assessment of eosinophil cationic protein

ECP assessment was done by radioimmunoassay (Pharmacia, Uppsala, Sweden) (4). The normal range of ECP is 2.3–16.0 µg/l; total variation coefficients of the kit vary from 6.6 to 12.0%.

Statistical analysis

For statistical analysis, the sign test was used.

Results

After 24-h storage at room temperature, ECP levels of patients showed a minimal and insignificant mean decrease of 13%. In contrast, controls showed a mean increase of ECP levels of 5.7% (Table 1).

Pathologic ECP values did not drop to normal after 24-h storage at room temperature, and normal ECP values did not reach the pathologic range after 24-h storage (Table 1).

Moderately elevated ECP levels were found in three patients. However, in 17 patients, serum ECP

Table 1. Mean ECP levels in 20 patients and 10 controls

Eosinophil cationic protein levels in $\mu\text{g/l}^*$	Manufacturers' requirements	After storage for 24 h	P value
Patients ($n = 20$)	9.7 ± 6.2	8.4 ± 7.3	0.2
Range	4.0–24.9	1.9–24.8	
Elevated levels ($n = 3$)	23.1 ± 1.4	23.9 ± 0.7	NS
Range	21.5–24.9	23.0–24.8	
Controls ($n = 10$)	9.2 ± 5.6	9.7 ± 5.5	NS
Range	2.0–17.6	4.0–19.4	
Elevated levels ($n = 2$)	17.5 ± 0.1	18.3 ± 1.2	NS
Range	17.4–17.6	17.1–19.4	

* ECP: normal range 2.3–16.0 $\mu\text{g/l}$.

values were in the normal range. In addition, two controls had slightly elevated ECP levels.

Discussion

The eosinophilic cell has been reported to release ECP in whole blood according to temperature and length of time (3, 5, 6). In our investigations, serum ECP levels after storage for 24 h at room temperature were almost the same as when done according to the instructions of the manufacturer. As only minimal differences were detected in elevated ECP levels of patients and controls, and no pathologic ECP value dropped to normal range, these findings indicate only slight catabolism of ECP in serum. Although our patients did not have elevated ECP levels in most cases, they showed a stronger catabolism than controls, as ECP levels decreased in 14/20 patients but in only 3/10 controls. The biggest differences in ECP levels after 24-h storage were seen in the low normal range at levels of about 5 $\mu\text{g/l}$.

These differences, being of no diagnostic importance, may also be attributed to the sensitivity of the ECP kit.

According to these findings, ECP assessment is possible with a 24-h delay. Laboratory handling of ECP blood samples by the general practitioner is reduced to centrifuging the tubes, collecting the supernatant, and sending the ECP samples by express mail to a laboratory.

According to these data, serum ECP was not significantly catabolized despite storage at room temperature for 24 h. Thus, even when determination can be done only after a 24-h delay, the information gained from ECP assessment is still reliable and serves as a valid screening index.

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