Eosinophil degranulation as an allergy activation marker
Admiraal, C.J.

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Chapter 2

Effect of clotting temperature and blood eosinophil concentration on the eosinophil cationic protein concentration in serum

C.J. Pronk-Admiraal and P.C.M. Bartels
Department of Clinical Chemistry, Haematology and Immunology, Medical Centre Alkmaar, The Netherlands

Effects of clotting temperature on serum ECP levels

Abstract

Earlier investigations have indicated the need for defined incubation conditions of blood samples before establishment of eosinophil cationic protein concentration in serum. Therefore, the effect of different clotting temperatures and blood eosinophil concentrations on the serum eosinophil cationic protein concentration was quantified in 40 individuals. Our results showed that serum eosinophil cationic protein concentrations strongly depend on the clotting temperature. Blood samples clotted for one hour at 37°C had 5 - 10 times higher serum eosinophil cationic protein concentrations than blood samples of the same person clotted for one hour at 0°C. Higher blood eosinophil counts resulted also in increased serum eosinophil cationic protein concentrations.
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Introduction

The eosinophilic granulocyte is associated with helminthic infections and also takes part in different mechanisms of inflammatory processes. The eosinophil secretory granules contain several specific cationic proteins. Some of these proteins have been further characterized, including eosinophil cationic protein (ECP), major basic protein, eosinophil protein X or eosinophil-derived neurotoxin (1). Several studies have indicated that the levels of eosinophil granule proteins in serum, for example, ECP, are closely correlated with indices of asthma severity (2). Therefore, determination of ECP concentrations in serum may provide an additional tool for monitoring the activity grade of asthmatic inflammation and the efficacy of anti-inflammatory therapy. ECP concentrations in serum can now be measured by use of a radio-immuno-assay kit. It is important to study in particular the effect of handling blood samples, because the level of ECP in the samples is known to be affected by the method of treatment (3). Probably, besides the effect of clotting temperature, also an effect of blood eosinophil concentration on the serum ECP concentration exists.

Materials and Methods

Blood samples were taken from 40 outclinic patients. From every patient two serum samples (Vacutainer*-system, ref. 367783, with addition of SST gel and clot activator, Becton Dickinson, Plymouth, UK) and one plasma sample (Vacutainer*-system, ref. 367652, with addition of K3EDTA as an anti-coagulant, Becton Dickinson, Plymouth, UK) were obtained. Serum ECP concentrations were established with a radio-immuno-assay kit (Kabi Pharmacia, Uppsala, Sweden). After venepuncture, blood samples should be allowed to clot, according to the manufacturer's instructions, at room temperature for 60 ± 10 minutes. To investigate the temperature dependency during
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clotting, from every individual one blood sample was clotted for 60 ± 10 minutes in ice-water (0°C) while the other one was clotted in a warmed bath of 37°C for the same time. After clotting, serum samples were centrifuged at room temperature during 10 minutes at 1350 x g. Subsequently, serum samples were stored at -20°C until the serum ECP concentration was assayed. Eosinophil concentrations in blood samples were determined with a Sysmex NE-8000 hematology analyzer (Charles Goffin Medical Systems BV, Tiel, The Netherlands).

Results

Serum ECP concentrations from samples clotted at 0°C and 37°C, respectively, are plotted against the blood eosinophil counts in figure 1. At a certain eosinophil concentration, a strong difference was demonstrated between the serum ECP concentrations resulting after clotting at 0°C and 37°C, respectively. Serum ECP concentrations in samples clotted at 37°C were established to be approximately 5 - 10 times higher than in samples clotted at 0°C. In figure 1 the effect of the blood eosinophil counts on the serum ECP concentration is also shown. Samples with higher blood eosinophil counts usually yielded increased values for serum ECP concentration. The effect of temperature on the serum ECP concentration is more obviously shown in figure 2. ECP concentrations of samples clotted at 37°C are plotted against samples clotted at 0°C of the same subject. It is shown that a higher temperature during clotting definitely gives rise to a higher serum ECP concentration. Serum ECP concentrations resulting from clotting at 37°C are 5 - 10 times higher in comparison with samples clotted at 0°C.
Figure 1: ECP concentrations in serum from samples clotted at 0°C (open triangles) and 37°C (black circles), respectively, versus blood eosinophil counts in 40 individuals. Results of statistical analysis gave: 

\[ y = 84.7x + 22.4, \quad r = 0.67 \quad (37°C) \]

\[ y = 7.3x + 2.9, \quad r = 0.51 \quad (0°C) \]
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Figure 2: ECP concentrations in serum from samples clotted at 37°C (y axis) versus ECP concentrations in serum from samples clotted at 0°C (x axis), in 40 individuals. Statistical analysis of results: $y = 4.6x + 22.1$, $r = 0.52$. 
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Discussion and conclusion

It should be emphasized that the intracellular amount of ECP in eosinophils is about 100 - 1000 times as high as the amount of ECP released in serum (4). As a consequence, the amount of actually in vitro released ECP is only a very small part of the total content of ECP in eosinophils. The mechanism of ECP release has not yet been elucidated. Further details concerning the interaction between clotting and ECP release after blood sampling are not available.

Because of the striking effect of clotting temperature on in vitro release of ECP, blood samples should be clotted in our opinion at a precisely fixed temperature. This conclusion is in contrast to the less strictly described manufacturer's instructions. For choosing conditions with respect to an optimal clotting temperature, empirical findings should be considered. Clotting at 0°C induces such a low serum ECP concentration that it even does not exceed in many cases the minimum detection limit of the radio-immuno-assay kit. Therefore, we prefer to clot the blood samples at 37°C. This temperature will yield in different individuals a more exaggerated range of ECP concentrations that are still within the detection range of the kit. An additional advantage in practice is that this temperature can be simply and accurately standardized in a water bath. Temperature dependency during clotting has been shown previously in samples from atopic patients. However, in this study (5), serum ECP concentrations in samples clotted at 37°C were only 2 times higher than after clotting at 4°C. In the cases just mentioned serum ECP concentrations from atopic patients (5, 6) and from asthmatic patients (6) clotted at 4°C are above the minimum detection limit of the kit. The latter findings are not in agreement with our observations.

Clinical interpretation of the serum ECP concentration alone in individuals with atopic allergy and asthma is not conclusive for an unambiguous diagnosis. Healthy people with a normal or high blood eosinophil count may have a rather high serum ECP concentration (5). In experiments of the latter study also a correlation between these
Parameters was observed in healthy persons but not in asthmatic patients. If it is true that eosinophils of allergic patients excrete ECP more easily than do eosinophils of healthy persons (4), it will be of clinical importance to calculate the ratio of serum ECP concentration and the blood eosinophil count. A discrepancy in the ratios of serum ECP and blood eosinophil concentrations might yield useful information with respect to the primed state of the eosinophil. Therefore, blood eosinophil counts should also be considered in the clinical interpretation of ECP levels.

Probably, other conditions in the test environment should be investigated in more detail to yield insight in the effect of clotting on the primed state of the eosinophil.
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References


