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Short communication

Comparison of the influence of EDTA-K3 and sodium citrate on haematology analysis in healthy dogs

M. Żmigrodzka, A. Winnicka, M. Guzera

Department of Pathology and Veterinary Diagnostics, Faculty of Veterinary Medicine,
Warsaw University of Life Sciences, Nowoursynowska 159c, 02-776 Warsaw, Poland

Abstract

The study was carried out on 30 clinically healthy dogs of various breeds. Haemoglobin concentration, haematocrit, platelet count and platelet haematocrit were significantly lower in citrate blood than in tripotassium ethylenediaminetetraacetic acid (EDTA-K3) blood. The study confirmed the limited usage of sodium citrate in haematology analysis, unless canine EDTA-dependent thrombocytopenia is suspected.

Key words: anticoagulants, haematology analysis, canine

Introduction

Tripotassium ethylenediaminetetraacetic acid (EDTA-K3) is the most commonly used anticoagulant in veterinary laboratory diagnostics for routine haematology analysis. The optimal EDTA concentration in animal blood samples is 1.5 mg/ml. Too low concentration causes clot formation, whereas too high concentration causes erythrocyte shrinking because of hypertonicity of the plasma. Neutrophils and monocytes appeared to be the most sensitive to high EDTA concentrations. In contrast, lymphocytes are more stable. EDTA can also cause anticoagulant-induced pseudothrombocytopenia, and platelet clumping would be visible in blood smears. These effects are not triggered in the whole blood samples by other anticoagulants. Platelet aggregates are created by the cold antibodies to the glycoprotein IIb/IIIa of the platelet membrane.

In patients with Glanzmann's thrombasthenia, in which gpIIb/IIIa is not present on the surface of the platelets, agglutination in blood samples with EDTA or citrate has not been observed, what confirms the cold antibodies hypothesis (Van der Meer et al. 2002). In many cases pseudo-leucocytosis is associated with EDTA-dependent pseudothrombocytopenia, as a spurious leucocytes measurement. Haematology analyser erroneously counts platelet microaggregates over the 35 fl as lymphocytes. In 2008, EDTA-dependent pseudothrombocytopenia was described for the first time in veterinary medicine (Wills et al. 2008).

Sodium citrate is used for coagulation analysis, erythrocyte sedimentation rate evaluation and for haematology analysis in humans with suspected pseudothrombocytopenia due to EDTA. The aim of this study was to compare the influence of EDTA-K3 and sodium citrate on haematology analysis in healthy dogs.

Table 1. Haematological parameters of healthy dogs (n=30) in EDTA-K3 and sodium citrate blood samples.

Examined parameters	Whole blood		
	reference values	mean \pm SD	
		EDTA-K3	sodium citrate
Erythrocytes ($\times 10^{12}/l$)	5.50-8.50	7.18 \pm 0.80	6.59 \pm 0.83
Haemoglobin (g/dl)	12.0-18.0	16,43 \pm 1.37	15.08 \pm 1.60*
Haematocrit (l/l)	0.37-0.55	0.49 \pm 0.46	0.43 \pm 0.51***
MCV (fl)	60.0-77.0	66.76 \pm 2.51	66.93 \pm 2.35
Leucocytes ($\times 10^9/l$)	6.0-16.50	8.86 \pm 2.76	9.88 \pm 2.60
Platelets ($\times 10^9/l$)	200.0-580.0	282.0 \pm 68.70	155.96 \pm 87.0*
MPV(fl)	6.0-9.0	8.77 \pm 0.64	8.60 \pm 0.85
PCT (fl/ μ l)	1.0-3.40	2.50 \pm 0.50	1.30 \pm 0.60*

Significant differences: * $p < 0.05$, *** $p < 0.001$ in comparison to EDTA-K3 blood.

Materials and Methods

The study was carried out on 30 clinically healthy dogs of various breeds aged from seven months to 12 years, which had not been treated nor vaccinated for 3 weeks before the blood collection. Peripheral blood was collected from the cephalic vein into tubes with: EDTA-K3, 3.2% sodium citrate and into biochemical tubes (Medlab, Poland). Haematological analyses were performed using impedance analyser (Abacus, France); blood films were stained in accordance with the May-Grünwald and Giemsa techniques and evaluated under a light microscope. The activity of aspartate and alanine transaminase, as well as the urea and magnesium concentration were calculated in serum by colorimetric methods. Statistical analysis was performed in Statistica 6.0 using Wilcoxon's test ($p < 0.05$).

Results and Discussion

The biochemistry results confirmed good liver and kidneys condition of the patients, and eliminated hypomagnesaemia (which suppresses thrombocyte activity) as a factor. Mean values of haematology parameters were within the reference range (Table 1). The haemoglobin concentration (HGB), haematocrit (HCT), number of platelets (PLT) and platelet haematocrit (PCT) in citrate blood samples were significantly lower than these values in EDTA-K3 blood samples. Haematology impedance analyser used in this study to determined HGB used the cyanomethaemoglobin method. Significantly lower values of HGB in citrate whole blood were the result of lower ability of HGB to oxidate in that anticoagulant.

McShine et al (1990) proved that both human platelet count and mean volume (MPV) in citrate blood is significantly lower than in EDTA. Similar observations were made in 1996 by O'Malley et al. who used a combination of EDTA and citrate. Acid citrate dextrose was the only anticoagulant which did not have any influence on platelet volume. The studies conduc-

ted on dogs with neoplastic diseases showed lower platelet count and mean platelet volume in citrate than in EDTA (Stokol et al. 2008). Authors think that citrate is a stronger platelet activator in sick animals and it therefore causes formation of platelet microaggregates. In our experiment, the platelet count in healthy dogs was lower in sodium citrate samples. Blood smears (EDTA and citrate) were made in every dog used in the experiment, and in citrate blood platelet microaggregates were not observed. PCT, which is a product of PLT and MPV, was also lower in citric blood in examined dogs.

The study showed that there is limited possibility of using sodium citrate in routine haematology analysis as it influences on HGB and PLT parameters. In higher concentration it also has an influence on erythrocyte and leucocyte morphology. In patients with low PLT count in EDTA, without clinical symptoms of coagulation disorders, control morphology in citrate blood should be carried out to rule out EDTA-dependent thrombocytopenia.

References

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