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## Introduction

Chromogranin A (CgA) is an acid hydrophilic 439 amino acids protein (48kDa), localized in the dense core secretory granules of many normal and neoplastic neuroendocrine cells of the diffuse neuroendocrine system (DES), and endocrine cells of the endocrine glands (eg. pancreatic islets). CgA plays important role in intracellular and extracellular function of endocrine and neuroendocrine cells. CgA became a main, nonspecific marker of neuroendocrine tumours (NET), because it is secreted by most NETs, particularly GEP-NET (gastroenteropancreatic neuroendocrine tumors), metastatic midgut carcinoids, pancreatic tumours and pheochromocytoma.

There are various commercial assays for the measurement of CgA concentration in serum or plasma. These assays have different analytical techniques (radioimmunoassay, ELISA, CLIA), have different standardization, and use different antibodies which recognize different epitopes of CgA molecule. Using mainly IRMA method we found markedly higher CgA levels in plasma than in serum in a large group of patients with neuroendocrine tumours (1,2,3).

## Aim of the study

Our study was designed to confirm the noted earlier differences in CgA levels measured in serum and plasma, and to establish the respective reference ranges in a group of healthy males.

## Material and method

145 male blood donors (age mean  $\pm$  SD 35,7  $\pm$  9,4; range 19 - 61 years) were investigated. The following exclusion criteria were settled: treatment with proton pump inhibitors, histamine H<sub>2</sub>-receptors blockers, corticosteroids, presence of some chronic diseases such as impaired renal or hepatic function, inflammatory bowel diseases, prostate cancer. At each collection, blood was withdrawn into two tubes: one with EDTA<sub>2</sub>K (plasma) and one with clot activator (serum). After blood collection, venous blood was centrifuged (10 minutes, 3500 rpm) and plasma (EDTA<sub>2</sub>K) and serum samples were frozen at - 30°C and stored until assayed.

Chromogranin A was measured by immunoradiometric method (IRMA) (CGA-RIA CT, CIS bio International, Gif-sur-Ivette cedex, France). Analytical sensitivity was 1,5 ng/ml.

Data were expressed as median (and the range) and mean  $\pm$  SD. The t-Student test and Wilcoxon test were performed to estimate differences between groups. The relationship between the compared results was expressed using Spearman's rank correlation analysis and Pearson linear correlation analysis. The reference ranges were expressed as 2,5 to 97,5 percentiles. A p-value of < 0,05 was considered to be significant and p < 0,01 highly significant. All statistical analysis were performed using statistical software (PQStat ver. 1.4.2.324).

## Results

In blood donors, the median (and the range) of CgA concentration determined for serum samples was 42,0 ng/ml (16-108 ng/ml) and for plasma samples was 58,0 ng/ml (23-153 ng/ml). The differences between serum and plasma ranged 15%–79% (median 26%) (Table I). Plasma CgA levels were significantly higher in relation to serum CgA levels (p<0,0001) (Fig. 1).

Correlation of CgA in serum and plasma was  $r = 0,9099$ ;  $r^2 = 0,8493$ ;  $p < 0,01$  (Fig 2). The determined reference ranges for CgA measured in serum and plasma in males expressed as 2,5 to 97,5 percentile were: 21,0 – 108,0 ng/ml and 31,0 – 153,0 ng/ml respectively (Table II).

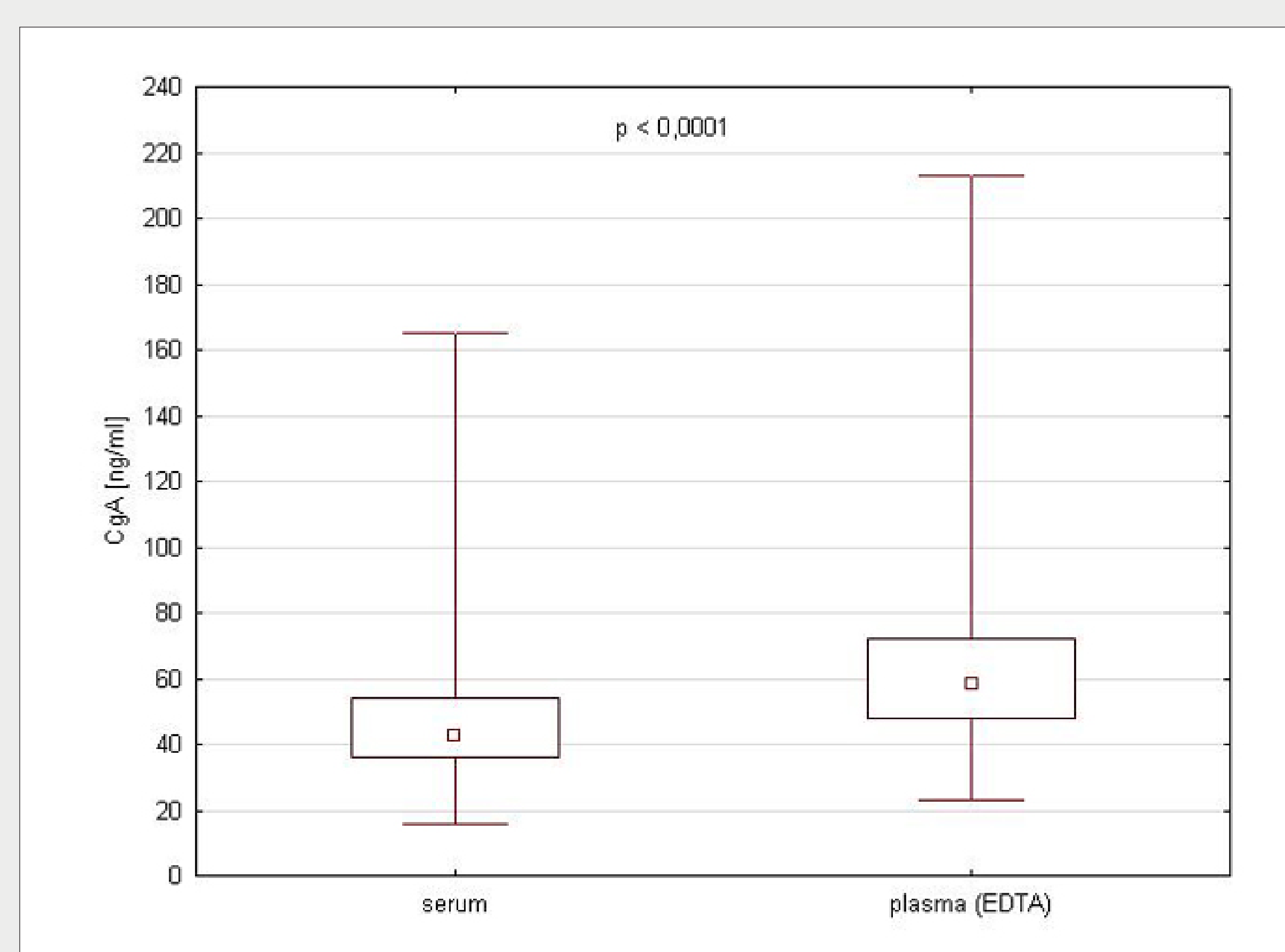


Fig. 1. CgA levels in serum and plasma (EDTA<sub>2</sub>K).

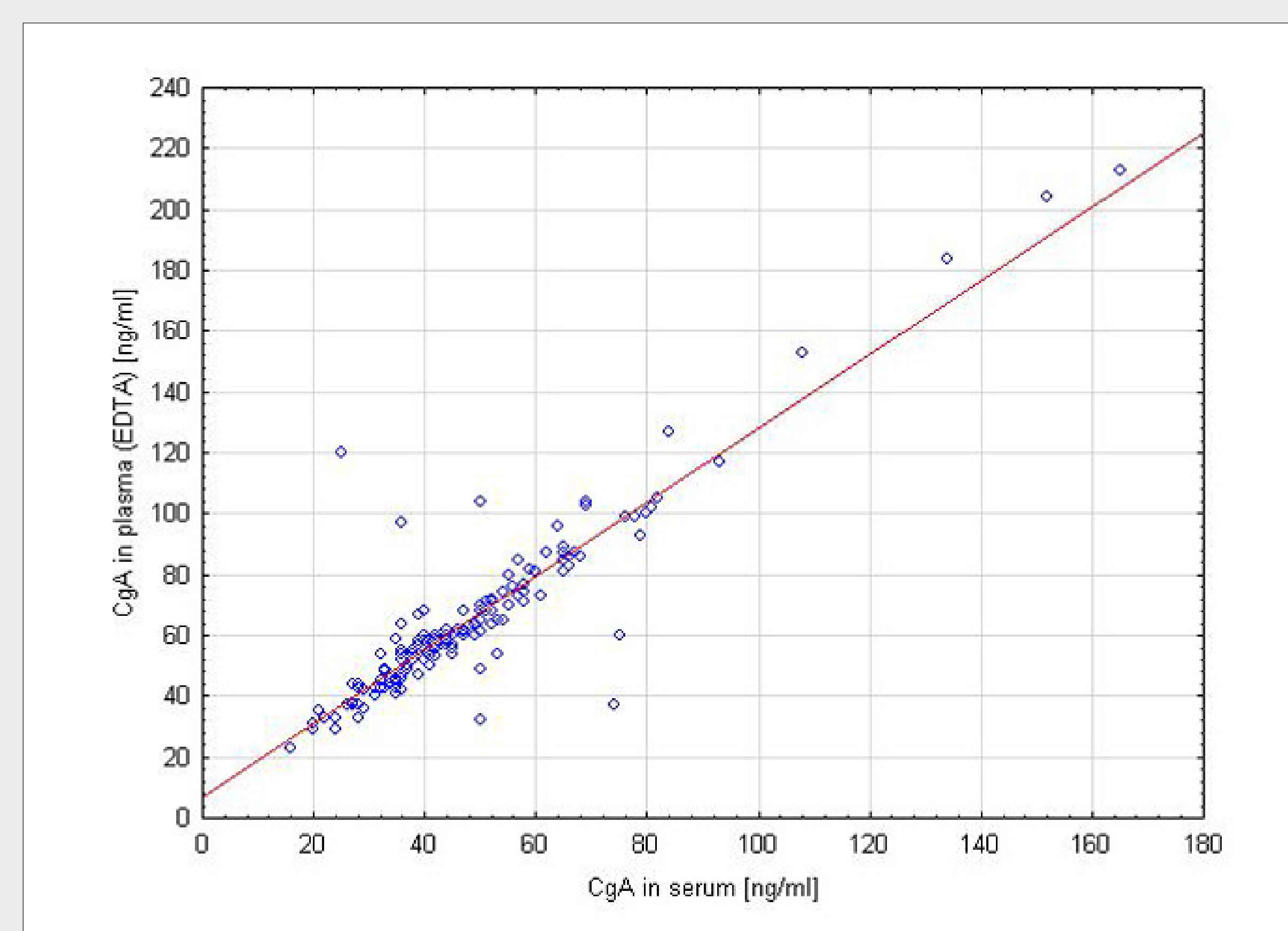


Fig. 2. Correlation of CgA in serum and plasma (EDTA<sub>2</sub>K).

## Table I.

Comparison of the CgA median (and the range) and mean  $\pm$  SD in serum and plasma (EDTA<sub>2</sub>K) in healthy males.

Serum [ng/ml] (n = 145)			Plasma (EDTA <sub>2</sub> K) [ng/ml] (n = 145)		
median	range	mean $\pm$ SD	median	range	mean $\pm$ SD
42,0	16-108	45,7 $\pm$ 15,8	58,0	23-153	61,9 $\pm$ 21,3
Difference 15 – 79% (median 26%) p < 0,0001					

## Table II.

Percentile distribution of CgA reference range in serum and plasma (EDTA<sub>2</sub>K).

CgA	Percentiles								
	2,5 <sup>th</sup>	5 <sup>th</sup>	10 <sup>th</sup>	25 <sup>th</sup>	50 <sup>th</sup>	75 <sup>th</sup>	90 <sup>th</sup>	95 <sup>th</sup>	97,5 <sup>th</sup>
serum	21	25	28	36	42,5	54	69	81	108
plasma (EDTA <sub>2</sub> K)	31	33	39	48	58,5	72	98	105	153

## Conclusion

Significant differences in the concentrations of CgA measured in plasma and in serum demand application of separate reference ranges adjusted to the sort of the investigated material.

## References

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