

## Immunofluorometrical Exploring of Cortisol Hormone Levels in the Serum of Patients with Histologically Verified Microcellular Lung Cancer

Ivan Milosevic  
Nikole Pasica 35/35, 34000 Kragujevac, Yugoslavia

**Abstract:** It is already known in the science that the cells of microcellular lung cancer can produce ACTH. By following the levels of Cortisol as one consequence of ACTH producing, in the serum of microcellular lung cancer patients, we could make some statistical conclusions how significant these levels would be in eventual future early diagnostic procedures, besides already existing tumormarkers etc. This work would connect the Oncology, Endocrinology and Immunology fields containing very interesting immunofluorometrical procedures, hormonal theories and statistical estimates.

**Key words:** Cortisol hormone, serum, microcellular lung cancer

### INTRODUCTION

Generally, the Lung Cancers are malignant tumors of epithelial tissue, fast propagating and giving the bad prognosis. Microcellular lung cancer is characterized by nondiferented miniature, compactly packed, unequal cells, containing one atypical rotund nucleus each. Mitoses are very frequent and that is why the proliferation of those cells is very fast and this histological type rapidly gives the metastases. There are more histological types of microcellular cancer: intermediar, combined, oatcell etc. Microcellular type takes 20% of all Lung Cancers and its cells can secrete the ACTH. Adrenocorticotrophic Hormone is a complexed protein erected of 39 amino acids and its molecule weight is approximately 5000. The anterior part of pituitary gland-adenohipophise, secretes the ACTH and its role is to activate adrenocortical hormones, aldosterone and cortisol. Cortisol is a corticosteroid produced in reticular zone of adrenal gland as a consequence of ACTH influence. Cortisol stimulates the gluconeogenesis process, mobilizes the fat acids from the fat tissue, has an anti-inflammatory activity etc. On the basis of above presented we can make the following connection: Microcellular lung cancer – ACTH – Cortisol.

### MATERIALS AND METHODS

As much as possible number of blood samples from microcellular lung cancer patients should be collected by venipuncture and preserved in refrigerator in small test tubes under properly conditions. Put the samples under already known time-resolved fluoroimmunoassay procedure, based on the competitive reaction between europium labeled cortisol and sample cortisol for a limited amount of binding sites on cortisol specific, biotinylated

monoclonal antibodies, derived from mice. The use of danazol and neutralized trichloroacetic acid in the Cortisol Assay Buffer facilitates the release of cortisol from the binding proteins. Thus the assay measures the total amount of cortisol in the test specimen. Streptavidin, produced by the bacterium *Streptomyces avidinii*, is coated on the solid phase and binds the biotinylated antibody, giving convenient separation of the antibody bound and free antigen. Enhancement Solution dissociates europium ions, where they from highly fluorescent chelates with components of the Enhancement Solution. The fluorescence in each well is then measured. The fluorescence is inversely proportional to the quantity of cortisol (Fig. 1).

**Required materials:** Time-resolved fluorometer plus printer and (optional) computer, Automatic washer, Automatic shaker, Micropipette, Distilled water, Centrifuge.

**Statistical estimate:** In this article will be presented already known, one of the simplest of possible statistical estimates, the t-test for the big depended samples. This test considers the studies between the sampled group and the control one. Its formula is:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{SE}; SE = \sqrt{\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2} - 2r_{1,2} \times SE_1 \times SE_2}; SD^2 = \frac{\sum(x - \bar{x})^2}{N}$$

$\bar{x}_1$  – arithmetical middle of the sampled group

$\bar{x}_2$  – arithmetical middle of the control group

SE- standard mistake

$SD_1^2$ -variance of the sampled group

$SD_2^2$ -variance of the control group

SD- standard deviation

$r_{1,2}$ - coefficient of corelacy

DF- degree of freedom; DF=n-1

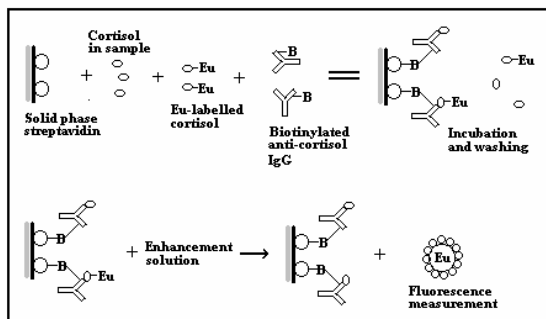


Fig. 1: The time-resolved fluoroimmunoassay. The two phases of it are presented

Table 1: Levels of Cortisol estimated apparently healthy men and women

Fraction	Reference limit	Confidence interval ( $\mu\text{g dL}^{-1}$ )
Morning sample	2.5%	244 nmol L <sup>-1</sup> (8.85 $\mu\text{g dL}^{-1}$ )
	97.5%	727 nmol L <sup>-1</sup> (26.4 $\mu\text{g dL}^{-1}$ )
Afternoon sample	2.5%	110 nmol L <sup>-1</sup> (4.00 $\mu\text{g dL}^{-1}$ )
	97.5%	418 nmol L <sup>-1</sup> (15.2 $\mu\text{g dL}^{-1}$ )

It is recommended that the laboratories establish their own reference values

The numeral size of t-test presents us could we or not take the alternative hypothesis (throw away or not the zero hypothesis) by which we affirm is there the statistically important difference between sampled and control groups. We can do it by comparing the numeral size of t-test with the probabilities of zero hypothesis given in for it especially created tables.

### CONCLUSION

By following of cortisol levels in the serum of microcellular lung cancer patients and making determinate statistical conclusions about its importance, that procedure could be eventually used as one of early or advanced diagnostic methods concerning mentioned disease. What would we get with that?

The histological verification is very slow and painful, especially when the microcellular lung cancer is in the question. This histological type gives the metastases fastest then all others and it needs to be diagonize in fastest possible way in order to be prevented by adequate reaction and therapy.

At the same time the biopsy as a very invasive method would be avoided.

When the tumor markers (CA-50, NSE etc.) are in the question as a diagnostic methods, this procedure is still in progress and developing, often unsound, but in a combination with the cortisol levels exploring, could be more reliable.

Even the syndromes that appear as a consequence of high levels of cortisol, such Cushing syndrome is, or similar ones, in a combination with classical clinical picture of lung cancer, could direct us to the microcellular type and in that case, the exploring of cortisol levels in the serum of those patients would be necessary and useful too.

Of course, using this presented procedure we should pay attention on sex, growth, age, day period because of variability of cortisol levels depended of those factors, (Table 1), as to make a difference between the morning and afternoon samples.

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