

Comparison of methods for the quantification of albumin in serum and total protein in cerebrospinal fluid.

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Abstract

Introduction. Albumin is the protein that marks the passage of cerebrospinal fluid proteins into the serum.

Materials and methods. Serum albumin was quantified by bromocresol green dye technique and by using the modified microalbuminuria method and for protein in cerebrospinal fluid, the pyrogallol molybdate red complex technique and the bromocresol green method were used.

Results. Compared methods in serum, the modified ELISA method had a sensitivity of 100%, a specificity of 80% and the cut-off point was less than or equal to 58 g / L. In both fluids the bromocresol green method had a sensitivity of 72.7%, specificity of 60% and the cut-off point was greater than 34g / L. The areas under the ROC curves of the two methods did not show significant differences for $p = 0.074$. In the methods for protein in cerebrospinal fluid the pyrogallol molybdate red complex had a sensitivity of 88.9% and a specificity of 90.5 and the cut-off point was less than or equal to 960mg / L and the bromocresol green method had a sensitivity of 66.7, a specificity of 85.7 and a cut-off

point less than or equal to 700mg / L. The comparison between the ROC curves of the two methods studied was significant with $p=0.138$.

Conclusions. The modified ELISA method is better than the bromocresol green method for protein in terms of specificity and sensitivity and in methods for quantifying serum albumin both methods do not show significant differences and have lower specificity and sensitivity than methods for quantifying protein in cerebrospinal fluid.

INTRODUCTION

Albumin is a protein that is used as a marker molecule to evaluate the passage of cerebrospinal fluid (CSF) proteins into the blood due to its properties. Its synthesis is exclusively hepatic, which is why all the albumin that is dosed in the CSF comes from the bloodstream. It is not catabolized in this biological liquid and its structure and molecular weight are known.

That is why this protein is one of the main anabolites that are measured in a Neuroimmunology laboratory.

There are numerous methods for quantification, from the simplest to the most sophisticated with their respective values of sensitivity and specificity.

Albumin can be used for the rapid diagnosis of Guillain Barré syndrome⁽¹⁾ and this requires that the results be obtained in the shortest possible time. However for other determinations for other diagnoses a rapid result is not required but a higher sensitivity. That is why they should be available to a group of methods that can be used in accordance with the scope that is expected of them.

The objective of this work is to establish a comparison between different laboratory methods to quantify albumin⁽²⁾ in serum and protein in CSF and to select the appropriate method that has the sensitivity required for the purposes of a laboratory of this type and that are suitable to the sensitivity and specificity requirements and that the costs are low and the result can be obtained quickly and reliably.

METHODS

We used 32 serum samples and 30 CSF samples from the LABCEL sero-library. The samples were processed in duplicate and the following methods were used to quantify albumin.

The methods used for the quantification of serum albumin were the colorimetric method of bromocresol green⁽³⁾, the modified microalbuminuria method for the quantification of albumin⁽⁴⁾ and for the determination in LCR, the bromocresol and pyrogallol method was used molybdate red complex technique.

The bromocresol green method is based on the fact that the albumin present in the sample, in a reaction medium buffered at pH 3.6 and with adequate ionic strength, binds specifically with the dye. The change in the optical properties of the albumin complex translated into an increase in the absorbance measured at 625 nm with respect to the solution of the free dye allows photometric quantification of albumin proportionally to its concentration in the sample.

The other method used for the quantification of serum albumin and CSF is the method for the quantification of modified microalbuminuria that is based on a heterogeneous "sandwich" immunoenzymatic assay that uses ultramicroELISA strips (10 μ L per well) coated as a solid phase. with monoclonal antibodies against human albumin, obtained in mice of the Balb / c line⁽⁴⁾

The sensitivity of the red pyrogallol-molybdate complex makes it suitable for use in cerebrospinal fluid. This method produces a colored complex that is quantified spectrophotometrically at 600 nm

RESULTS

Compared methods in serum, the modified ELISA method had a sensitivity of 100%, a specificity of 80% and the cut-off point was less than or equal to 58 g / L. In both fluids the bromocresol green method had a sensitivity of 72.7%, specificity of 60% and the cut-off point was greater than 34g / L. The areas under the ROC curves of the two methods did not show significant differences for $p = 0.074$.

In the methods for protein in cerebrospinal fluid the pyrogallol molybdate red complex had a sensitivity of 88.9% and a specificity of 90.5 and the cut-off point was less than or equal to 960mg / L and the bromocresol green method had a sensitivity of 66.7, a

specificity of 85.7 and a cut-off point less than or equal to 700mg / L. The comparison between the ROC curves of the two methods studied was significant with $p=0.138$.

Table 1. Comparison between albumin methods. ROC curves areas.

	ROC curve for VBC-CSF protein	ROC curve for PG-CSF protein	ROC curve for VBC-Serum	ROC curve for ME-Serum
N	30	30	32	25
Area under the ROC curve	0.794	0.876	0.652	0.861
Standard error	0.083	0.063	0.112	0.073
Pairwise comparison of ROC curves	Difference between areas	0.082	Difference between areas	0.209
	Standard error	0.055	Standard error	0.117
	Significance P	0.138	Significance P	0.074

There are no difference in area under the curve between the two methods using for quantify protein in CSF according to Table 1.

In spite of there are significant difference between the microELISA test and the VBC method as we expected taking into account that the ELISA methods are always superior in comparison with the colorimetric methods. You may observed the difference between areas and the p value in Table 1.

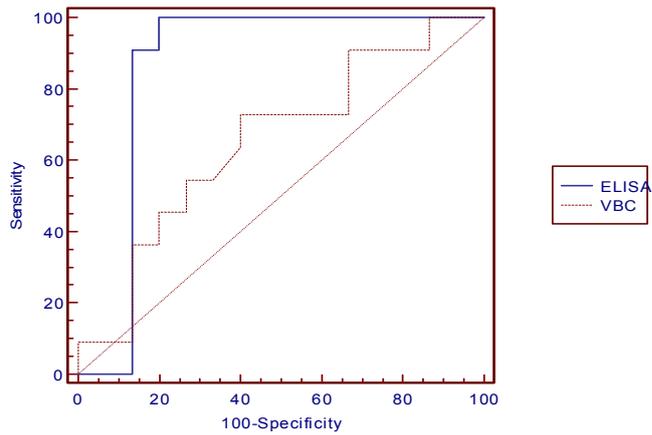


Figure 1. Comparison between ROC curves between ELISA and VBC for serum albumin and the methods for protein content in CSF.

The comparison between ROC curves between the methods for quantify total protein in CSF was observed in Figure 1.

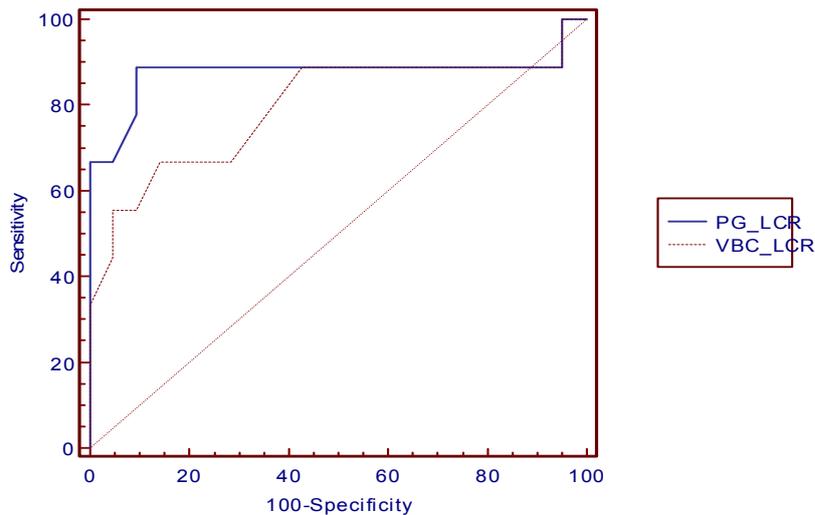


Figure 2. Comparison between ROC curves between PG and VBC for protein in cerebrospinal fluid.

The comparison of the two different methods for albumin quantification was shown in Figure 2

DISCUSSION

The comparison of the two different methods for protein quantification in cerebrospinal fluid there are no difference between both. It is important to look for other methods capable to increase the sensibility and specificity for this purposes. This methods employed could be use with limitations in such kind of biological fluids.

The method more adequate for albumin quantification was the microELISA technique. This method was employed originally for quantification albumin in urine for microalbuminuria diagnosis and was modify in order to be useful for albumin quantification in CSF ⁽⁴⁾

The selected method for quantify albumin in CSF is the microELISA test modified.

Because this one has more sensitivity and specificity demonstrated in the ROC curve analysis in spite that the selected method is more expensive than the colorimetric method. For quantification of protein content both analized methods had similar parameters and was not expensive at all and the results could be obtained in a short period of time but for cerebrospinal fluid analysis taking into account the requirements of such kind of lab it will advisable to look for a better method.

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