Electrophoretic Separation of Proteins from the Drain Fluid by Geometric Electrofocusing in Conjunction with Local Complications in the Surgical Patient

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Introduction: Local postoperative complications are affecting the evolution of surgical patients, which is the main reason why early diagnosis is a priority concern. Our objective was to
Objective: To study the opportunity of protein analysis of the proteins from the drain fluid, as evolution markers of the surgical patient.
Material and method: We have analyzed drain fluid collected after 24 h, 72 h and 5 days after surgery. We have used the following: determination of protein concentration by spectrophotometric analysis at 280 nm and protein separation by geometric electrofocusing (patent no. 109585C1/30.03.1995)
Results: From the analyzed liquids, we obtained variable protein concentrations. In all cases, electrophoretic separation showed the presence of protein fractions similar to those of reference serum.
Conclusion: The analysis protocol allows precise quantitative determination of the proteins from the drain fluid. Geometric electrofocusing, approached for the first time for this specific type of analysis, has proved to be highly effective in terms of quality and affordable due to the low cost.

Keywords: electrophoresis, geometrical electrofocusing, drain liquid

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Introduction

During the post-operative evolution of patients undergoing surgery, a number of local complications affecting postoperative morbidity and mortality may develop [1,2,3]. Early diagnosis in those cases and appropriate therapy for each particular case may influence the future evolution of patients. In this article, we propose a quick and economical method to detect local postoperative complications, which consists in the dynamic evaluation of proteins from drained fluids, using the geometrical electrofocusing [4,5]. We presume as work hypothesis that this method is able to predict early local postoperative complications even before their occurence.

Our objective is to study the opportunity of protein analysis from the drained fluids, as markers of postoperative evolution, using protein electrophoresis by geometrical electrofocusing.

Material and method

The samples of drained fluids were collected at intervals of 24 hours, 72 hours and 5 days after surgery, from patients who underwent all types of surgery requiring drainage. As reference electrophoretic sample we used a human serum obtained from the I.C. Cantacuzino Institute, Bucharest. The samples have been processed the same day as they were collected, completing the following protocol:

- We examined the macroscopic appearance of the liquid and removed the samples which contained traces of blood.
- A quantity of 0.5 ml of liquid was transferred into azidated test tubes (with deposits of Na azide) and sealed.
- We centrifuged the samples at 5000 rpm in a centrifuge with bucket dumps, drawing the supernatant afterwards.
- We determined the proteinemia from the supernatant by ultraviolet spectrophotometry, at 210 nm, using quartz glass vats with a thickness of 10 mm, with reference to isotonic NaCl.
- We introduced 200 ml from each sample in thermostat test tubes at 52°C and then a quantity of 400 ml of gelling agent preheated to 52°C was poured over each sample.
- We stirred the contents of the test tubes and then we repositioned them in the thermostat bath for 20 minutes.
- We aspirated quantities of 200 ml in each gelling tube.
- Microscope slides measuring 76/24mm were put on a plane surface.
- On each slide we poured 3 ml of agarose gel at boiling temperature.
- After cooling at room temperature for 40 minutes, we sectioned the gel perpendicular in the longitudinal direction, and then we distanced the two resulting blocks by sliding them, the microscope slides being thus prepared for insertion tubes with jellified samples.
- After 30 minutes from the time of aspiration in the test tubes, the cylinders containing the samples were removed and placed between the sections of the gel migration.

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Fig. 1. Electrophoretic image of a human reference serum from the I.C. Cantacuzino Institute, Bucharest, processed by geometric electrofocusing

- After the insertion of the cylinder containing the jellified sample, the two agarose gel blocks were brought together until they made perfect contact.
- The device was inserted into the migration chamber, which was connected to a DC power source; the contact between the gels and buffer tanks was ensured by paper decks soaked in buffer solution.
- The separation lasted 50 minutes, using 15 V/slide.
- After separation, the plates were fixed with 10% ATA for 30 minutes, flushed with running water, covered with filter paper and dried at room temperature overnight.
- The dried slides were flushed with water and colored.

We performed dosing of the protein concentration from the drained fluids through spectrophotometry at 280 nm. Light radiation with a wavelength of 280nm, from the ultraviolet range, is proportionally absorbed by tryptophan, tyrosine and phenyl-alanine, aminoacids which are present in the protein structure.

In order to assess the linearity and to calculate the slope coefficient, we established a calibration curve using bovine serum albumin (BSA). To obtain the extinctions of the experimental standard curve, we started with an initial stock albumin concentration of 100 mg/dl, in NaCI 9 g/l, buffered with barbiturate buffer with a pH of 7.4, which is the level that ensures the maximum protein absorption.

The measurements were performed using a UV spectrophotometer, placed inside quartz glass vats, having a thickness of 10 mm. To draw the calibration curve, we made dilutions, beginning from an initial volume of 2 ml of NaCI, adding gradually 20 ml of standard albumin solution, the mixture being added directly into the vats. The standard curve, obtained through the additional procedure presented above, has a linear portion between 37.5 mg/dl and 155 mg/dl, which can be used for measurements and which defines the sensitivity field in linear method. Calculation of the protein concentration can also be performed by computation, consisting of multiplying the extinction value at 280 nm by a certain slope coefficient. When we analyze serum or plasma, we can use an average slope coefficient, since both serum and plasma are protein mixed in various proportions. The average value of the experimentally determined slope coefficient is 10.5.

Concluding, the calculation of the results can be performed either by interpolation on the calibration curve, or pursuant to the following equation:

Fig. 2. Electrophoretic image of proteins in drain fluid taken at variable time intervals, from an operated patient

Protein concentration (g/dl) = E / a × d × final vol. / serum vol. = E × 10.5, where a = serum slope coefficient, d = thickness of the vat and E = extinction.

Geometrical electrofocusing improved electrophoresis in plane gels and is based on protein concentrations in focusing areas, induced by electric fields of force which bypass the gels. The focusing areas are obtained by cross-section of the gels and inserting cylinders with jellified samples into those sections.

The microscope slides, with dimensions of 76/24mm, have to be installed inside the classic electrophoresis devices, thus increasing the performance of the traditional procedure, without the need of additional installations. An increase in the diameter of the cylinder containing the jellified sample, allows an increase of the sample volume used for separation, about 10 times compared to traditional methods, without modifying the geometry of the separation system. The required condition of the process is to maintain the tangentiality of the cylinder containing the sample to the section surfaces of the gel migration, no matter what the diameter of the cylinder or the height of the gel migration may be.

Results

Variable protein concentrations have being obtained from the analyzed fluids. In all cases, electrophoretic separation showed the presence of protein fractions similar to those of reference serum. Figures 1 and 2 present the images of electrophoreses obtained from the analysis of drained fluids from a patient operated with the diagnosis of antral gastric neoplasm, who has undergone the following surgical procedure: subtotal gastrectomy with gastrojejunal anastomosis, sub hepatic and Douglas drainage, with uncomplicated postoperative evolution.

Discussion

Using electrophoretic analysis of the proteins from the drain fluid represents an absolute novelty, as the technique has never been applied before.

As the quantity of proteins eliminated through the drain is absolutely variable, there can be no question of reference liquid [4,6], comparation of the results can only be made in evolution, the reference serum being useful for identifying the electrophoretically separated fractions [7,8].

The quantity of liquid obtained from the drain tubes diminishes during postoperative evolution, making the de-

termination of protein concentration by classical methods rather difficult [9,10].

Geometric electrofocusing is a process developed in 1994 (Şchiopu A., Şchiopu A. Jr.), which can ensure the analysis of liquids with low protein levels, without the necessary prior concentrating of samples, allowing the increase of the volume of samples introduced for separation, approximately 10 times compared to traditional methods, without altering the geometry of the separation system, thus eliminating all technological disadvantages related to concentrating processes [6].

Conclusion

- The sensitivity of the method permits the detection of a quantity of proteins of over 10 mg/dl.
- Extending the method to different cases can provide important data regarding the local post-operatory evolution, as well as the opportunity of substituting proteins lost through the drain.
- Geometrical electrofocusing, approached for the first time for this specific type of analysis, has proved to be highly effective in terms of quality and affordable due to the low cost.
- Geometrical electrofocusing, used to detect proteins from drained liquids, could be useful for the evaluation of local postoperative evolution, but requires thoroughgoing studies.

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