New Method to Predict Postoperative Complications: Protein Electrophoresis by Geometrical Electrofocusing

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Purpose: To evaluate drainage liquid protein electrophoresis as a predictive marker for postoperative complications.
Assumption: Postoperative drainage liquid has variable protein concentrations which decrease progressively in the absence of local complications, therefore the postoperative rising of protein fractions could be a signal for a future complication.
Material and method: Drainage liquid samples collected at 24 h, 72 h and 5 days after surgery from patients operated in the 1st Surgery Clinic of the County Emergency Clinical Hospital of Tîrgu Mureș, were analyzed regarding the protein concentration and compared against a sample of human serum provided by the I.C. Cantacuzino institute, București. Quantitative variations in protein concentrations were assessed by 280 nm spectrophotometry and qualitative variations were evaluated by colorimetric comparison of the protein fractions obtained at electrophoresis by geometrical electrofocusing. Results were computer analyzed and graphically transposed.
Results: Electrophoresis identified, in all cases, protein fractions similar with those from the reference serum. All protein fractions tended to decrease in samples obtained from operated patients with normal, uncomplicated postoperative evolution. In case of a complicated evolution (local peritonitis after splenopancreatectomy) protein levels were constantly elevated in all three samples (at 24 h, 72 h and 5 days).
Conclusions: Geometrical electrofocusing of serum protein proved to be qualitatively efficient, low cost and very sensible, detecting protein concentrations over 10 mg/dl; normal serum protein concentration is over 7000 mg/dl. This method could be a predictive factor for a local postoperative complication and also a trigger for protein replacements in cases with large amount of drainage liquid losses.

Keywords: postoperative complications, geometrical electrofocusing

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Introduction

During the course of early postoperative evolution of patients who have undergone surgery, a number of infectious complications with effects on postoperative morbidity and mortality can occur. For these patients, dynamic changes of electrophoreses resulting from the analysis of drain fluid may reveal the occurrence of an infectious process [1,2,3].

The quantity of liquid collected through the drain tubes gradually diminishes, hence making it difficult to determine protein concentration by conventional methods [4].

Geometric electrofocusing is a method which can ensure the analysis of liquids with low protein levels, without the necessary prior concentration of samples [5].

The aim of our study was to assess the usefulness of protein electrophoresis by geometrical electrofocusing, as a prognostic factor of postoperative evolution.

Material and method

The drain fluid was collected at intervals of 24 hours, 72 hours and 5 days after surgery from patients operated in the 1st Surgery Clinic of the County Emergency Clinical Hospital of Tîrgu Mureș between 2009 and 2012. The drainage tubes used had the same chemical composition and comparative dimensions and samples were obtained in similar conditions. The principle of geometric electrofocusing is that the method enhances electrophoreses in flat-plane gels and is based on the principle of protein concentration from the liquids analysed during electrophoresis, around the areas of focus of the electric field lines of force which cross the gel migration [5].

Using the above mentioned method, applied to the classic procedure, the electric field lines of force shall pass through the tangents of contact between the gel migration and the cylinders containing previously jellified samples.

Focusing the electric field lines of force at the level of the separating gel is ensured by cross-sectioning the gel migration and inserting a cylinder containing a jellified sample between the sectioned surfaces.

The device, with dimensions of 76/24 mm, is 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 cylinder containing the sample tangential to the section

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

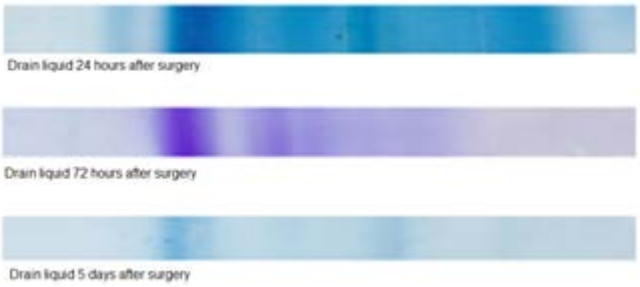

Fig. 2. Electrophoretic image of proteins in drain fluid collected at variable time intervals, from an operated patient, with favorable postoperative evolution

surfaces of the gel migration, no matter what the diameter of the cylinder or the height of the gel migration may be.

Results

From the fluids we analysed, we obtained variable protein concentrations. In all cases, electrophoretic separation showed the presence of protein fractions similar to those of the reference serum.

Figures 1–2 present the images of electrophoreses obtained from the analysis of drain fluid from an operated patient with uncomplicated postoperative evolution.

Figures 3–4 show the electrophoretic aspects obtained using the same method in a case of postoperative evolution complicated with splenic fossa abscess after splenopancreatectomy. The previous decrease of proteic fractions in time are not present in this case.

Protein electrophoreses obtained by geometrical electrofocusing were scanned and colorimetrically compared with those from patient serum and the results were processed using a proprietary graphical software named CROMOS.

Discussions

Using electrophoretic analysis of the proteins from the drain fluid constitutes an absolute novelty, as the technique

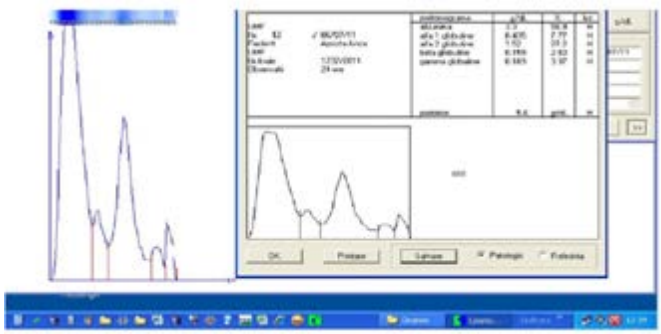

Fig. 4. Graphical representation of proteic fractions from the drain liquid 24 hrs postoperatively

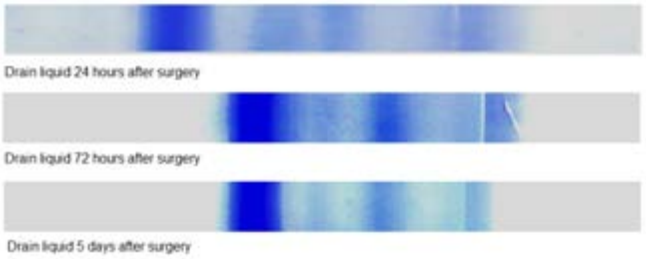

Fig. 3. Electrophoretic image of proteins in drain fluid collected at variable time intervals, from an operated patient, with complicated postoperative evolution

has never been applied before. As the quantity of proteins eliminated through the drain is variable, there can be no question of reference liquid. Comparation of results can only be performed in evolution, the reference serum being useful for identifying the electrophoretically separated fractions [6,7].

The quantity of liquid obtained from the drain tubes diminishes during the postoperative evolution, making the determination of protein concentration by classical methods rather difficult [8,9,10].

Geometric electrofocusing is a process developed in 1994 and patented in 1998 [5], which can ensure the analysis of liquids with low protein levels, without the necessary prior concentration of samples, allowing the increase of the volume of samples introduced for separation, approximately 10 times compared to traditional methods [10], without altering the geometry of the separation system, thus eliminating all technological disadvantages related to concentration processes [5].

Conclusions

1. Geometric electrofocusing used to detect proteins from drain liquid is particularly useful for the evaluation of local postoperative evolution.
2. The sensitivity of the method permits the detection of a quantity of proteins of over 10 mg/dl.
3. 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.
4. 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.
5. From the fluids we analysed, we obtained variable protein concentrations. In all cases, electrophoretic separation showed the presence of protein fractions similar to those of reference serum.

References

1. Gu Z, Pan J, Bankowski MJ, Hayden RT. Quantitative Real-Time Polymerase Chain Reaction Detection of BK Virus Using Labeled Primers. Archives of Pathology & Laboratory Medicine. 2010;134(3):444-448.
2. Matsui H, Shimokawa O, Kaneko T, Nagano Y. The pathophysiology of non-steroidal anti-inflammatory drug (NSAID)-induced mucosal injuries

in stomach and small intestine. Journal of Clinical Biochemistry and Nutrition. 2011;48(2):107-111.
3. Takahashi T, Otaka M, Odashima M, Tamaki K. Correlation of Heat Shock Protein Expression to Gender Difference in Development of Stress-Induced Gastric Mucosal Injury in Rats. Journal of Clinical Biochemistry and Nutrition. 2010;47(1):64-73.
4. Popescu OV. Electroforeza proteinelor in geluri de poliacrilamida. Bucuresti. Ed. Tehnica, 1990, pp:17-18.
5. Schiopu A, Schiopu A Jr. Separarea electroforetica a proteinelor prin electrofocusare geometrica. University Press, Târgu Mures 2002, 973-85602-8-4
6. Borghini S, Fiore M, Di Duca M. Candidate Genes in Patients with Autoinflammatory Syndrome Resembling Tumor Necrosis Factor Receptor-associated Periodic Syndrome Without Mutations in the TNFRSF1A Gene. J Rheumatol. 2011;38(7):1378-84.
7. Yazisiz V, Ongut G, Terzioglu E, Karayalcin U. Clinical importance of antineutrophil cytoplasmic antibody positivity during propylthiouracil treatment. Int J Clin Pract. 2010;64:19-24.
8. Keren DF. Procedures for the Evaluation of Monoclonal Immunoglobulins. Archives of Pathology & Laboratory Medicine. 1999;123(2):126-132.
9. Ranganathan P, Harsha HC, Pandey A. Molecular Alterations in Exocrine Neoplasms of the Pancreas. Archives of Pathology & Laboratory Medicine. 2009;133(3):405-412.
10. Wiśniewski JR. Mass Spectrometry-Based Proteomics: Principles, Perspectives, Challenges. Archives of Pathology & Laboratory Medicine. 2008;132(10):1566-1569.