Use of Sialic Acid as a Complementary Method in the Cytological Diagnosis: Preliminary Study

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Abstract

Introduction: Cellular morphology does not allow, in many cases, to safely establish a diagnosis of malignancy or benignity. Sialic acid is found in the membranes of well-differentiated mature cells, normally located in the alpha-2,3 position. During tumor progression, changes occur in glycosylation of proteins and lipids, including alterations in the sialylation patterns of tumor cells. Objective: To confirm the overexpression of alpha-2,6 sialinization in exfoliated cells of body fluids and bronchoalveolar lavage (BAL) as a malignant indicator mechanism, using glycan-binding lectins. Materials and Methods: Thirty samples (20 effusion liquids and 10 BAL) diagnosed by Giemsa and Papanicolaou staining as negative and positive for malignancy, were studied. They were then stained with fluorescein-labeled Sambucus nigra lectin (Sigma Chemicals, USA), which specifically recognizes sialic acid in alpha-2,6 position. The fluorescence obtained at 515 nm evidenced the presence of sialic acid in the 2,6 position. Results: Negative body fluids for malignancy showed a fine and homogeneous fluorescence pattern for reactive mesothelial cells. Neoplastic cells revealed a thick, heterogeneous pattern. In BAL, benign hyperplastic cells showed a homogeneous fine pattern while neoplastic cells showed a thick and heterogeneous fluorescence pattern. The pattern described was observed in all cases in the cell membrane. Conclusion: It was observed that the change in sialic acid conformation detected through Sambucus nigra Lectin could be used as a complementary method for the diagnosis of malignancy in different cytological samples.

Keywords: Lectins, mesothelial cells, neoplastic cells, sialic acid
There are numerous cell populations whose maturation and survival depend on the stability of the enzyme sialyltransferase alpha-2,6 (myeloid, thymic, intestinal and oligodendrocyte cells) and the consequent expression of its product.\(^\text{[4,5]}\)

It is known that changes in the glycosylation of proteins and lipids occur during tumor progression, including alterations in the sialylation patterns of tumor cells. Although it is true that alterations in sialylation are not a consequence and not a cause of neoplastic transformation, they have been proposed as an important event in the induction of invasion and metastasis.

Among the alterations in sialic acid expression patterns in cancer it can be found the expression of sialylated antigens of the family Thomsen–Freidenreich and sialyl Lewis, as well as the increase of sialic acid in alpha-2,6 bond attached to the disaccharide GaLβ1,4-GlcNAc (lactosamine). These changes are seen in different types of cancer such as breast, colorectal, hepatocarcinoma and cervical cancer.\(^\text{[6,7]}\)

Tumor cells through the cell surface glycans can couple to a variety of endogenous lectins both at the primary site of the tumor and in the circulation. Lectins are a family of carbohydrate-binding proteins that specifically recognize glycans. Fundamental processes such as cell–cell recognition, cell adhesion, mobility and pathogen–host interaction are facilitated by lectins in healthy organisms. The common expression of lectins on endothelial cells, immune cells, in the extracellular matrix or soluble adhesion molecules allow them to bind to glycans of tumor cells and therefore affect the progression of such cells.\(^\text{[8]}\)

**Materials and Methods**

The remaining material of the assistance work (the report of the biochemical parameters for the clinical diagnosis) is the one that is used to carry out our research work. Samples were processed to determine the presence of benign or neoplastic cells within 15 minutes of reception. Fluids (pleural, ascitic and BAL) were centrifuged at 1500 g for 10 minutes and the pellets were deposited on slides, fixed in 96° ethyl alcohol for Papanicolaou and AgNOR staining, and air-dried for Giemsa staining.

Thirty samples were analyzed: 20 effusion liquids (12 pleural liquids and 8 ascites) and 10 BAL. For each case (BAL and effusions), four smears were made (one for Giemsa, one for PAP, one for AgNOR and one for the measurement of sialic acid expression). The diagnosis of malignancy or benignity was made using Giemsa, Papanicolaou and AgNOR stains. Adenocarcinomas were the most frequent tumors in our series. The final cytological diagnosis was confirmed by histological examination, radiology, images studies and follow up. The AgNOR technique was developed according to the references.\(^\text{[9,10]}\)

For the study of sialic acid, the cytological smears were fixed for 30 minutes in 4% paraformaldehyde and maintained at 4°C until use, then were stained with fluorescein-labeled Sambucus nigra lectin (Sigma Chemicals, USA), which specifically recognized sialic acid in alpha-2,6 position. The expression of sialic acid was observed in a fluorescence microscope after 30 minutes of incubation with the lectin Sambucus nigra. The initial concentration of the reagent was 2 mg/ml. Different dilutions of the lectin were made, determining a dilution of $4 \times 10^{-3}$ mg/ml as the optimum to decrease the background.

The samples were incubated for 30 minutes at 4°C in the dark with the fluorescently labeled lectins, at the optimum concentration they were washed two times with phosphate-buffered saline (PBS) and observed by fluorescence microscopy at 515 nm. The presence of sialic acid in alpha position 2,6 was detected by the green fluorescence.

Our study was carried out according to the principles of Declaration of Helsinki for research in human subjects.\(^\text{[11]}\)

**Results**

The fluorescence patterns described below were observed in cell membranes.

Reactive mesothelial cells, whose expression of sialic acid α-2,6 bound is low, showed a fine and homogeneous fluorescence pattern [Figure 1].

Neoplastic cells revealed a coarse and heterogeneous pattern, contrary to the previous case, indicating a high expression of sialic acid in the bound studied [Figure 2]. The sensitivity (S) and specificity (Sp) of the AgNOR technique were 85% and 95%, respectively. The S and Sp of sialic acid in the differentiation between reactive mesothelial cells and neoplastic cells were 90% and 100%, respectively. Table 1 shows the results obtained with the different staining and complementary methods.

In BAL, cells with benign hyperplasia showed a fine homogeneous pattern [Figure 3] while adenocarcinomatous cells presented a heterogeneous thick fluorescence pattern [Figure 4]. The S and Sp in the expression of sialic

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**Figure 1:** Ascitic fluid: Reactive mesothelial cells – (x400)
acid to differentiate between bronchial hyperplasia and adenocarcinoma was 90% for both parameters. There were no cases of squamous cell carcinoma in our series. Table 2 shows the results obtained with the different methods.

Table 3 shows S and Sp to differentiate benign and malignant cells in BAL and body fluids using sialic acid assay.

**Discussion**

The accurate cytological diagnosis of tumors leads to a rapid treatment of the patient, which translates into better quality of life. The greater the number of markers available in the laboratory to arrive to a definitive diagnosis, the faster and more effective the treatment.

Taking into account that there are changes in the glycosylation of proteins and lipids during the progression of a tumor, including alterations in the sialylation patterns of tumor cells, in the present paper we have studied the expression patterns of sialic acid in cells obtained from body fluids and BAL.

Sialyltransferase alpha-2,6 is known to be overexpressed in several types of cancer, such as breast, colorectal, hepatocarcinoma and cervical cancer and tumorogenesis and...
metastasis are frequently associated with structural alterations and oligosaccharide expression of cell surface glycoproteins and glycolipids.\[^{12}\]

In a study conducted in patients with hepatocellular carcinoma, an increase in tumor reactivity was observed with the Sambucus nigra lectin, which specifically binds alpha-2,6-linked sialic acid, while in patients with cirrhosis, no abnormality was detected in the expression of alpha-2,6-sialylation.\[^{13}\]

The thin and homogeneous pattern was observed in the reactive or benign cells, indicating low expression of sialic acid in the alpha-2,6 position. On the other hand, the thick and heterogeneous pattern was present in the neoplastic cells, due to the increased expression of the sialic acid in the studied position.

According to our results, high S and SP values were observed for the sialic acid test as a marker to differentiate between benign and malignant cells, both in body fluids and in BAL. The values of S and Sp in body fluids are higher than those obtained with the AgNOR technique. The difference in the expression of sialic acid between neoplastic and benign cells could be used as a good parameter of differentiation of diverse populations, both in fluids from serous cavities and in BAL. However, although the results are encouraging, it has been planned to increase the number of cases to reach conclusions that are more definitive.

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### Conflicts of interest
There are no conflicts of interest.

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