ORIGINAL ARTICLE

# Is serum protein electrophoresis useful in separating the "high risk group" in patients with colonic polyps?

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## Abstract

**BACKGROUND**: Adenomas have the highest potential or clinical value from among colonic polyps of developing into adenocarcinoma. The aims of this paper are: to establish criteria to identify the high risk group of patients in a group of patients with colonic polyps, to work out a simple scheme for follow-up care after endoscopic polypectomy, and to establish indications for surgery. The usefulness of determination of electrophoresis of serum proteins has been specially analysed to detect early development of malignant growths in patients with colonic polyps regarding alfa-1/alfa-2 and alfa/beta. 67 cases - 21 women, 46 men were tested. Follow-up endoscopy with the electrophoresis was performed after 6 weeks, 6 and 12 months after polypectomy. 97 polyps were resected with endoscopy in 67 patients. 38 patients (39.17%), those constituting the high risk group, were selected. Included were all polyps with grade II and III of cellular differentiation.

**CONCLUSIONS**: 1) alfa-1/alfa-2 and alfa/beta is a helpful test in identifying the high risk group among patients with colonic polyps and it can be used as a screening test, 2) the determination of beta-2-macroglobuline is not useful in the diagnosis of this group of patients, 3) the electrophoresis of proteins should be the first test to perform on patients with colonic polyps. The relation of electrophoresis to endoscopic polypectomy aids evaluations of patients specially predisposed to malignant.

## INTRODUCTION

In the last few years there have been an increasing number of immunologic, molecular biological and genetic studies carried out. They have dealt with the understanding of mechanisms controlling the processes of the proliferation and differentiation of cells and those mechanisms having an effect on malignant transformation.

Colonic polyps occur in about 5% of the human adult population, and isolated or multiple polyps can be found in 1/3 of patients aged 55 and more. Adenomas have the most clinical research value from among colonic polyps - as well as being of frequent occurrence there is a possibility that they may transform into adenocarcinoma [1,2,3]. Retrospectives studies revealed that, among 3–5% of patients with adenomas of colon, cancer occurs in the next 10 years [4,5].

**The main aims of this paper are:**

1. to establish the criteria useful in identifying the high risk group of patients in patients with polyps of the colon
2. to work out a simple scheme for the follow-up care of patients after endoscopic polypectomy
3. to establish indications for surgery

The usefulness of determination the electrophoresis of serum proteins has been specially analysed to detect early malignant transformation in patients with colonic polyps regarding alfa-1/alfa-2 and alfa/beta.

## PATIENTS AND METHOD

Patients with polyps of the colon diagnosed by endoscopic examination were tested. Patients were treated in Colorectal Outpatients Surgery in St. Barbara Hospital in Sosnowiec, Poland. 67 cases - 21 women (31.3%) aged 27–74 (average age: 57.1) and 46 men (68.7) aged 35–73 (average age: 52.7) were tested. Patients with enteropathies (colitis ulcerosa), chronic renopathies and a chronic liver illness (nephrotic syndrome, glomerulonephritis, cirrhosis and viral inflammation of the liver), rheumatic disease, chronic and acute inflammations, or past and present neoplastic disease were excluded from the sample.

Blood samples were collected before polypectomy to determine concentrations of the following neoplastic markers: CEA, CA 19-9, CA 72-4 (using Abbott's kits - USA). 5 $cm^3$ of blood were collected to a dry test-tube from the cubital vein and stored in -20 °C after centrifugation.

Polyps from polypectomies were tested at the University of Silesia Medical Academy Department of Pathology in Katowice, Poland.

Follow-up endoscopy with the simultaneous electrophoresis of serum proteins was performed at time intervals of 6 weeks, 6 months, and 12 months after polypectomy.

Similar tests were been performed on 11 patients with diagnosed cancer of the colon in different stages of progression of disease and 10 healthy volunteers from among hospital workers.

The electrophoresis of serum proteins was performed with the use of Beckman's apparatus with agar gel.

The results of a selection of parameters, according to the histopathological type of polyps, were evaluated by numeric group analysis of coefficients of Pearson's correlation and Euclidean distances. The statistical analysis was performed with Systat for Windows 5.0.

## RESULTS

97 polyps, histopathologically tested, were resected with endoscopy in 67 patients. Histopathological results are shown in Table 1. Hyperplastic polyps were the most numerous: 40 (41.23%). One case of cancer was revealed (1.03%).

Polyps differ in size. Results of mean sizes according to the histopathological diagnosis are shown in Table 2. Polyps up to 0.5 cm in diameter, of hyperplastic type, were the most numerous: 22 of 97 ( 22.68%). Polyps with cancerous cells had a diameter of over 2 cm.

According to the results of the tests, patients constituting the high risk group (38 patients - 39.17%) were selected from the group of patients with colonic polyps. All polyps with grade II and III of cellular differentiation

**Table 1.** Results of histopathological type.

| Histopathological type | cases | % |
|---|---|---|
| Hyperplastic polyps | 40 | 41.23% |
| Adenoma tubulare | 29 | 29.89% |
| Adenoma tubulovillosum | 22 | 22.68% |
| Adenoma villosum | 5 | 5.15% |
| Cancer | 1 | 1.03% |

**Table 2.** Results of mean sizes according to the histopathological diagnosis.

| Size [cm] | Hyperplastic polyps | Adenoma tubulare | Adenoma tubulovillosum | Adenoma villosum | Cancer |
|---|---|---|---|---|---|
| <0.5 | 22 (22.68%) | 14 (14.43%) | 4 (4.12%) | | |
| 0.5–1.0 | 13 (13.40%) | 9 (9.27%) | 5 (5.15%) | 2 (2.06%) | |
| 1.0–2.0 | 4 (4.12%) | 3 (3.09%) | 7 (7.20%) | 2 (2.06%) | |
| >2.0 | 1 (1.03%) | 3 (3.09%) | 6 (6.18%) | 1 (1.03%) | 1 (1.03%) |

**Table 3.** Histopathological type in "high risk" group.

| Histopathological type | cases | % |
|---|---|---|
| Hyperplastic polyps | 9 | 23.68 % |
| Adenoma tubulare | 13 | 34.21 % |
| Adenoma tubulovillosum | 10 | 26.31 % |
| Adenoma villosum | 5 | 13.15 % |
| Cancer | 1 | 2.63 % |

were included in this group. The histopathological classification of this group is shown in Table 3.

The results of determination of the electrophoresis of serum proteins according to histopathological diagnosis are shown in Tables 4–8.

Concentrations of alfa-1/alfa-2 in the group of healthy patients were in the normal range (0.20–0.28) and concentrations of alfa/beta were 1.52–1.65.

**Table 4.** Results in group Polypus (glandularis, fibrosus, mucosus).

| | Normal range | B [%] | N [%] | A [%] |
|---|---|---|---|---|
| **Alfa-1/alfa-2** | 0.20–0.28 | 11.1 | 88.9 | 0.0 |
| **Alfa/beta** | 1.52–1.65 | 28.6 | 28.5 | 42.9 |

B – below normal range, N – normal range, A – above normal range

**Table 5.** Results in group Adenoma tubulare.

| | Normal range | B [%] | N [%] | A [%] |
|---|---|---|---|---|
| **Alfa-1/alfa-2** | 0.20–0.28 | 16.7 | 58.3 | 25.0 |
| **Alfa/beta** | 1.52–1.65 | 45.5 | 18.1 | 36.4 |

B – below normal range, N – normal range, A – above normal range

**Table 6.** Results in group Adenoma tubulovillosum.

| | Normal range | B [%] | N [%] | A [%] |
|---|---|---|---|---|
| **Alfa-1/alfa-2** | 0.20–0.28 | 28.6 | 42.8 | 28.6 |
| **Alfa/beta** | 1.52–1.65 | 71.4 | 0.0 | 28.6 |

B – below normal range, N – normal range, A – above normal range

**Table 7.** Results in group Adenoma villosum.

| | Normal range | B [%] | N [%] | A [%] |
|---|---|---|---|---|
| **Alfa-1/alfa-2** | 0.20–0.28 | 28.6 | 14.3 | 57.1 |
| **Alfa/beta** | 1.52–1.65 | 57.1 | 28.6 | 14.3 |

B – below normal range, N – normal range, A – above normal range

**Table 8.** Results in group Adenocarcinoma.

| | Normal range | B [%] | N [%] | A [%] |
|---|---|---|---|---|
| **Alfa-1/alfa-2** | 0.20–0.28 | 0.0 | 27.3 | 72.7 |
| **Alfa/beta** | 1.52–1.65 | 54.5 | 18.2 | 27.3 |

B – below normal range, N – normal range, A – above normal range

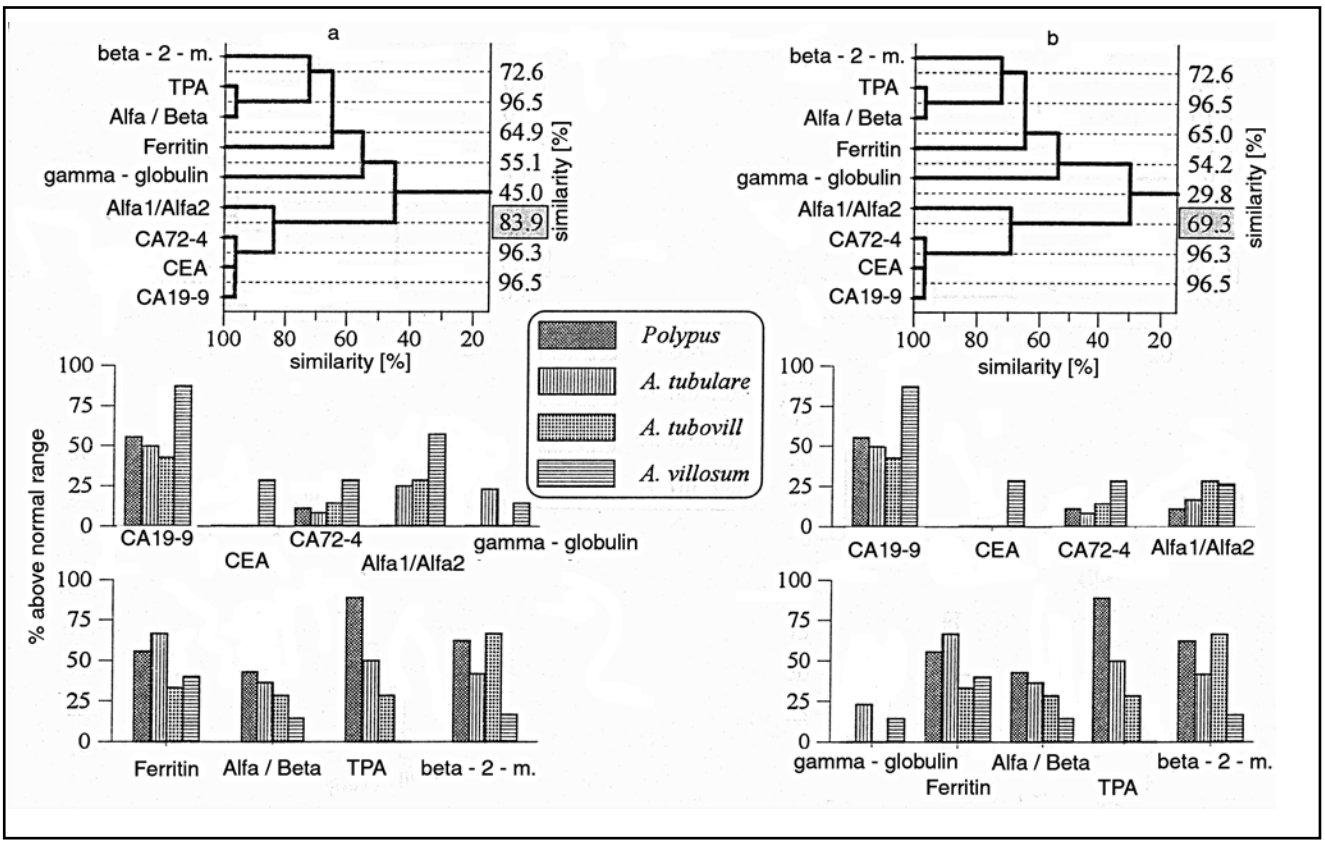


**Figure 1.** A numeric group analysis of parameters of groups : Polypus, A. Tubulare, A. Tubulovillosum, A. villosum with coefficients of Pearson's correlation. Parameters of grouping: a - Alfa-1/Alfa-2 > 0.28 Alfa/Beta > 1.65, other parameters > normal range
b - Alfa-1/Alfa-2 < 0.20 Alfa/Beta > 1.65, other parameters > normal range

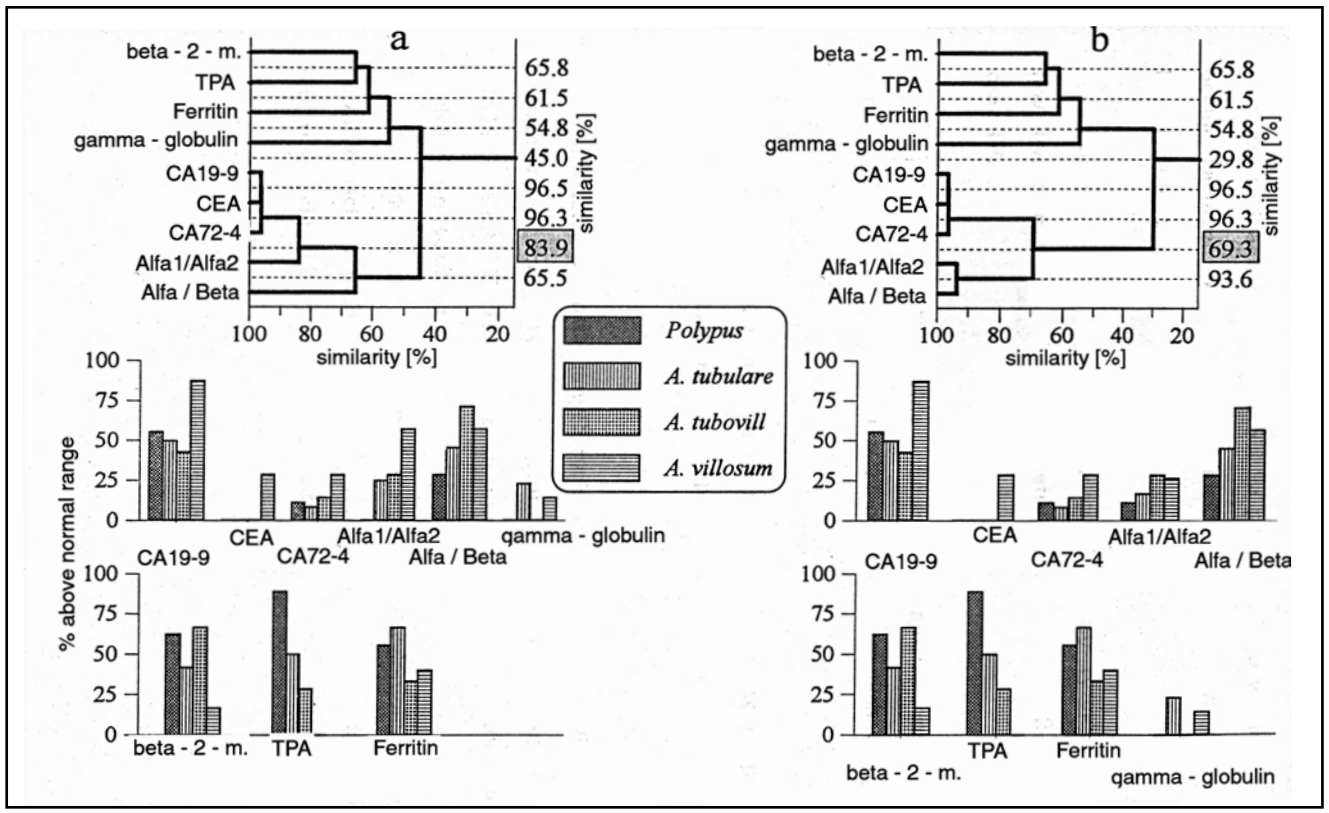


**Figure 2.** A numeric group analysis of parameters of groups : Polypus, A. Tubulare, A. Tubulovillosum, A. villosum with coefficients of Pearson's correlation. Parameters of grouping: a - Alfa-1/Alfa-2 > 0.28 Alfa/Beta < 1.52, other parameters > normal range
b - Alfa-1/Alfa-2 < 0.20 Alfa/Beta < 1.52, other parameters > normal range

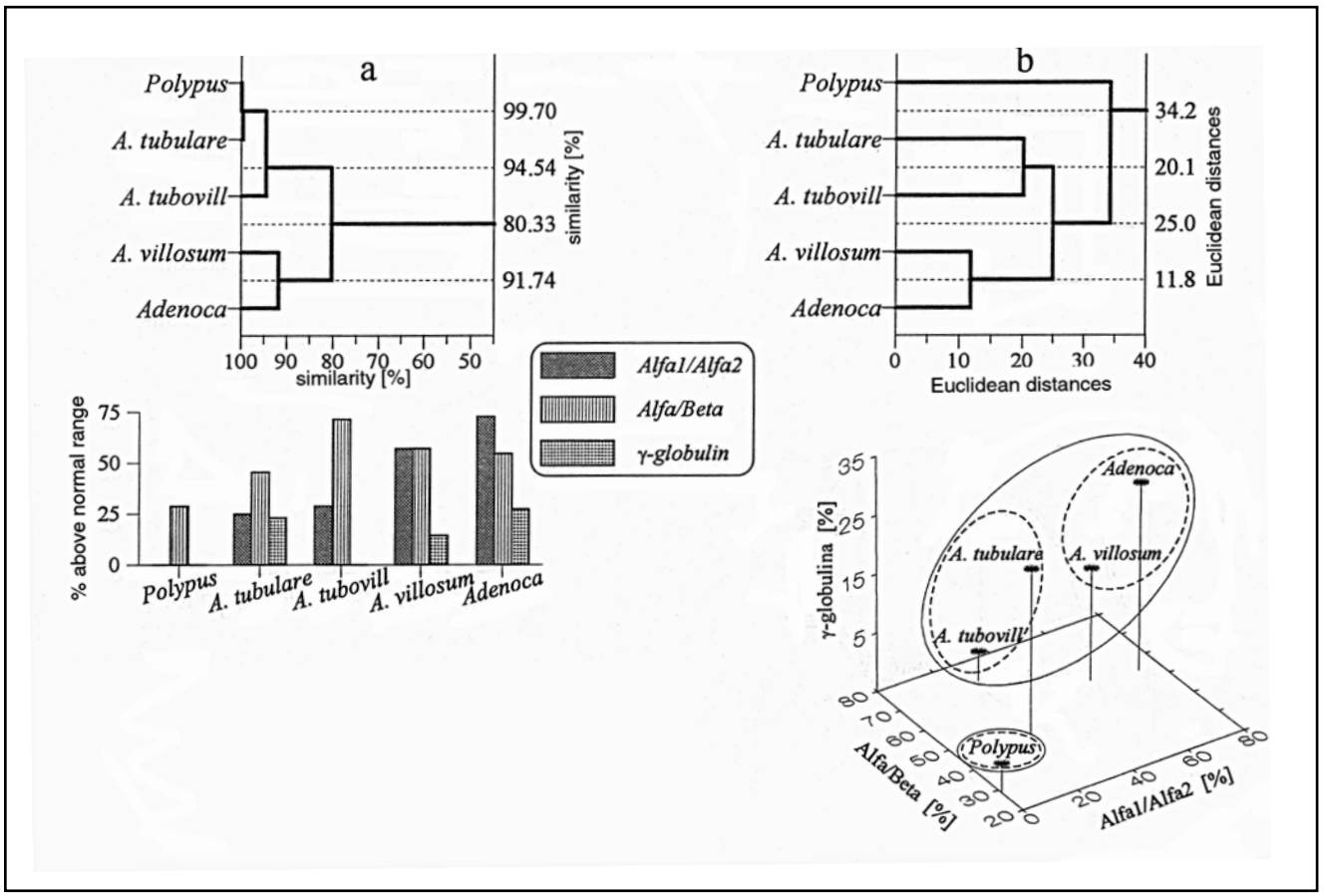


**Figure 3.** A numeric group analysis of risk of neoplastic transformation for parameters : Alfa-1/Alfa-2 > 0.28, Alfa/Beta < 1.52 and gamma - globulin with: a - coefficients of Pearson's correlation, b- Euclidean distances.

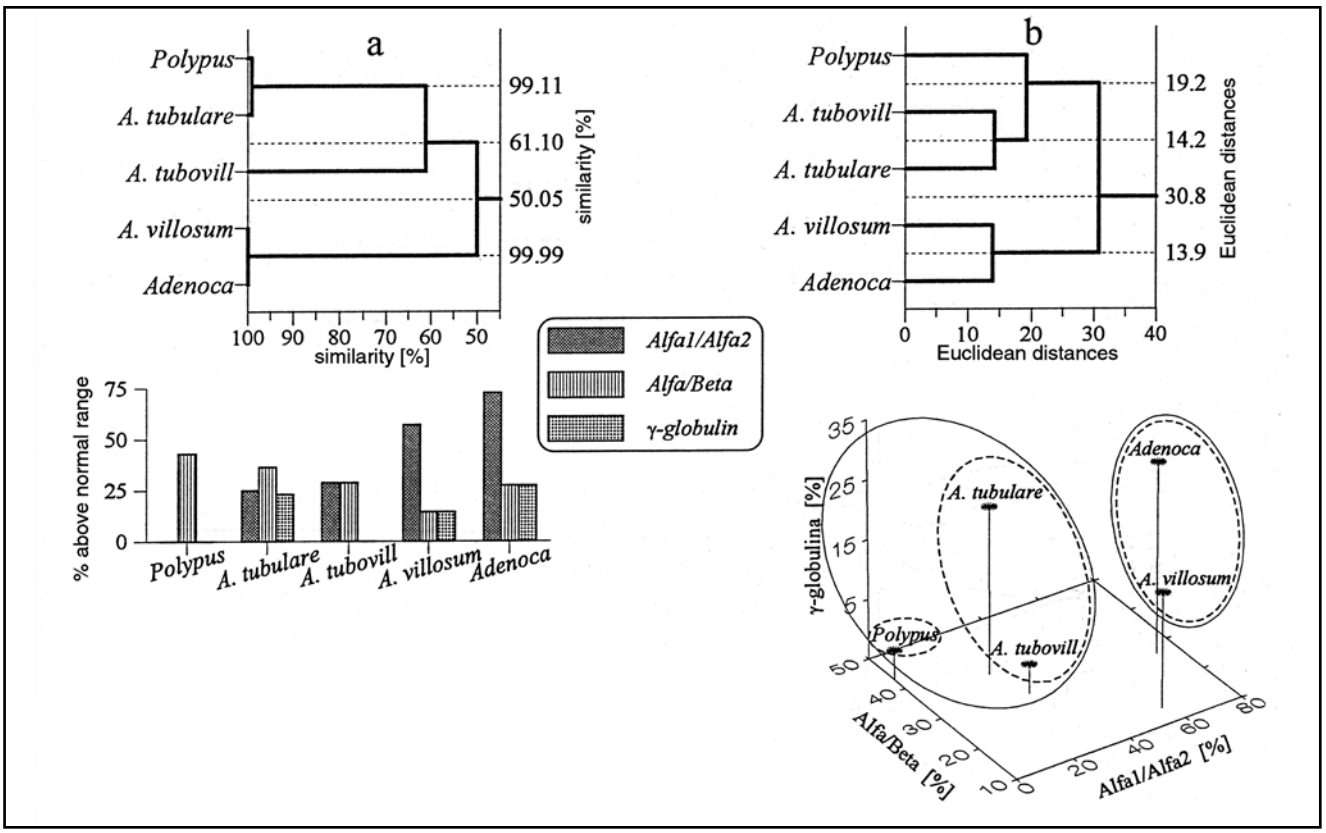


**Figure 4.** A numeric group analysis of risk of neoplastic transformation for parameters : Alfa-1/Alfa-2 > 0.28, Alfa/Beta < 1.65 and gamma-globulin with: a - coefficients of Pearson's correlation, b- Euclidean distances.

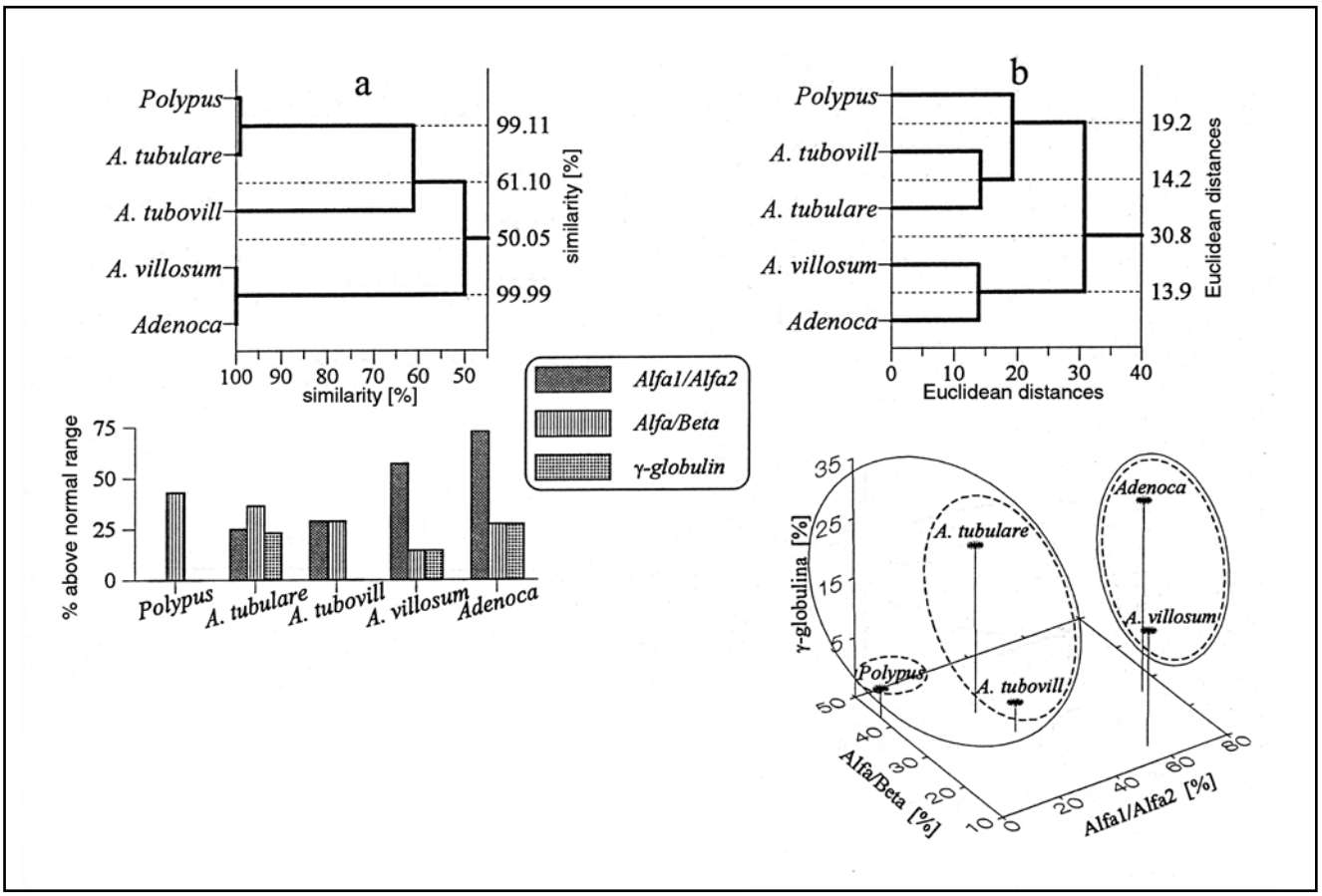


**Figure 5.** A numeric group analysis of risk of neoplastic transformation for parameters : Alfa-1/Alfa-2 > 0.20, Alfa/Beta < 1.52 and gamma-globulin with: a - coefficients of Pearson's correlation, b- Euclidean distances.

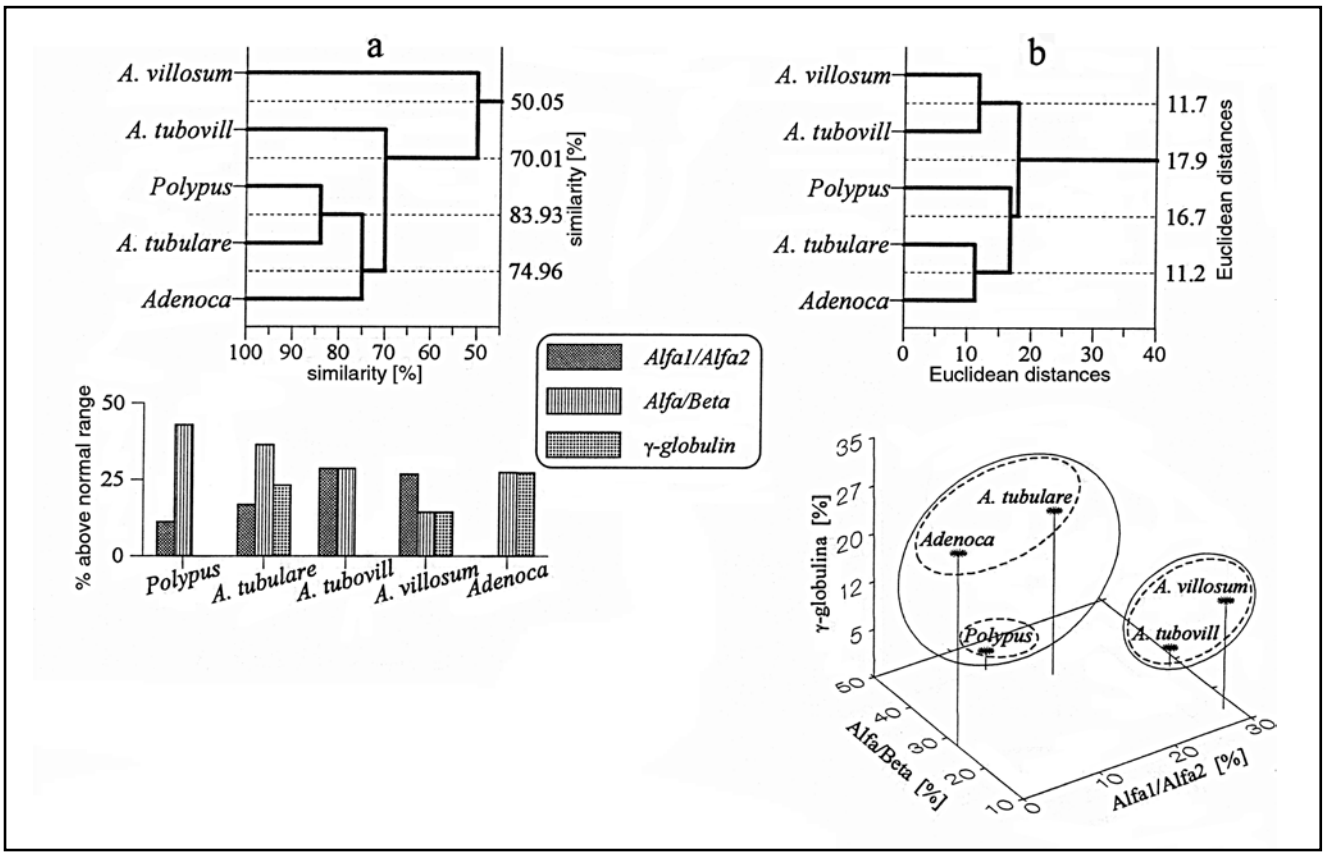


**Figure 6.** A numeric group analysis of risk of neoplastic transformation for parameters : Alfa-1/Alfa-2 > 0.20, Alfa/Beta < 1.65 and gamma-globulin with: a - coefficients of Pearson's correlation, b- Euclidean distances.

## DISCUSSION

Colonic polyps pose a diagnostic problem to clinicians. Morson [2] has divided colonic polyps into 4 groups: neoplastic, hamartomatic, inflammatory, and-metaplastic. Familial intestinal polyposis is a particular lesion that causes colonic cancer. Neoplastic polyps rise from the mucosa of the colon as well as nonmucous parts of the colon wall: muscular, fatty, nervous and vascular tissue. Adenoma has the greatest clinical research importance because of both its' frequent occurrence and the possibility that it might develop into adenocarcinoma [1,2,3]. There are 3 types of adenomas: tubular, villous, and tubulovillous. Common features of all adenomas are: a disturbance of the regulation mechanism of divisions of the regeneration and differentiation processes of colon adenous cells [6] and, as mentioned, a possibility to transform into the adenocarcinoma in the case of a genetic disturbance [1,2,3,7,8,9,10,11,12,13,14]. Despite different theories of malignant transformation, there is no clear scheme for the treatment of patients with colonic polyps as a group specially threatened with malignant transformation [1].

The proteasal-antiproteasal balance of serum is strictly connected with both the dynamics of malignant transformation in the phase of induction and the later period [15], therefore serum proteins were tested in our study. The concentrations of proteins like alfa-1, alfa-2 globulin are increased in patients with colonic cancer. Alfa-1, alfa-2 globulin have features of protease inhibitors such as: alfa-1-antitrypsin, alfa-1-antichymotrypsin, alfa-2-macroglobulin [16,17]. This correlates with our findings. Neoplastic cells contain much more hydrolytic enzyms (plasminogen, plasmin, collegenase, cathepsins G, B, H, D, trypsin, chymotrypsin) – with an ability to destroy connective tissue – than healthy cells and they are able to secrete enzymes. The intensity of the proteolysis is regulated by the antiproteolytic activity of serum protease inhibitors in physiological conditions [18].

The analysis of our results revealed that the relationship of alfa-1/alfa-2 concentrations is elevated above 0.28. It proves that alfa-2 macroglobulin dominates other inhibitors. There is not any increase in the relationship of alfa-1/alfa-2 in group Polypus, in groups: A. villosum - 57.1%, A. tubulovillosum - 28.6%, A. tubulare - 25% and 72.7% in the case of cancer (Tables 4–8). Statistical analysis revealed that only results above the upper limit are significant (Figures 1 and 2).

There is no usefulness in detecting gamma - glubulin. It is probably because of the fact that there are no

marked immunological disturbances in colonic polyps (Figure 3–6). Our results are similar to those presented by other authors [10,12,17,19,20].

The proteasal-antiproteasal balance is disturbed with a predominance of the proteolysis that causes the removal of a stroma with the connective tissue from the surroundings of the tumor. The antiproteasal ineffectiveness causes the penetration of neoplasmal cells into the circulation and infiltration to the connective tissue of the target organ.

Serum inhibitors reduce the invasiveness of neoplasm by having a direct effect on proteases as well as by stimulation of immunological reactions of the host. Active proteases of blood (cathepsin) can cause hypogammaglobulinemia because they cause the deterioration of immunoglobulins after the penetration to the circulation.

The appearance of lysosomal enzymes (secreted by leucocytes) is a result of the inflammatory process: Alfa-1 protease's inhibitor (alfa-1-PI), alfa-2-macroglobuline (alfa-2-m.) inhibiting the elastase and alfa-1-antichymotrypsin (alfa-2-ACT) inhibiting cathepsin G.

Alfa-2-m is the most important of serum inhibitors [19] because it is the main serum cytotoxic inhibitor against neoplasmal cells [21], as the cells of adenoma. It has the most extensive antiproteasal activity in serum and it controls the unspecific proteolysis by inactivating all lysosomal proteases. Alfa-2-m is not the acute phase protein in the case of acute inflammations or necrosis of tissues, therefore the concetration decreases, contrary to alfa-1 PI or alfa-1-ACT [19]. The concentration of alfa-2-m decreases in the case of advanced cancer or dissemination [19].

An increased concentration of alfa-1-PI occurs in tissues refractory to proteases secreted by neoplasmal tissues, and, most of all, in walls of vessels and cartilages. Complexes of cathepsin G and alfa-1-ACT are captured by macrophages and fibroblasts stimulated to secrete cytokins and IL-6 IL-1 TNF inhibits the local spread of neoplasm and SIRS.

The activity of antithrombin III (AT III) is also very important in the neoplasmal processes. AT III inhibits the spread of neoplasm and metastasises [19].

Dysproteinemia is specific to colonic cancer: hypoalbuminemia and hyperglobulinemia of alfa-1 and alfa-2 is strictly connected to the antiproteolytic activity of serum. Our findings confirm this: we observed an increase in the activity of protease's inhibitors that differs in terms of the frequency of occurence according to the localisation of the tumor- almost a 100% increase of alfa-1 and alfa-2 in patients with colonic cancer and a 50% increase in rectal cancer. Similar results were revealed by Bogdanikowa *et al*. [17].

## CONCLUSIONS

1. The electrophoresis of proteins, especially the relationship of alfa-1/alfa-2 to alfa/beta, is a helpful test in identifying the high risk groups among patients with colonic polyps and it can be used as a screening test.
2. The determination of beta-2-macroglobuline is not useful the diagnosis of this group of patients.
3. The electrophoresis of proteins should be the first test that is performed on patients with colonic polyps. The connection between electrophoresis and endoscopic polypectomy aide in the evaluation of patients that are particulary predisposed to development of malignant transformation of polyps.

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