

## Nestin and caveolin-1 in the diagnosis of GISTs

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### Abstract

Gastrointestinal stromal tumors (GISTs) are the most common primary mesenchymal neoplasias of the gastrointestinal tract, typically expressing c-kit (CD117) and CD34. Recently, it was reported that nestin and caveolin-1 are also expressed in some human sarcomas, GISTs included. We performed a retrospective study on formalin fixed, paraffin embedded samples from 81 cases of confirmed GISTs, aiming to characterize their immunohistochemical profile, including nestin and caveolin-1 expressions. Tissue samples were evaluated immunohistochemically for CD117, CD34, nestin and caveolin-1. The patients (M:F 36:45), aged 46 to 84 years, had spindle cell type GISTs in 56.7% of cases, epithelioid in 30.8% and mixed pattern in 12.3%. Immunohistochemically, CD117 was positive in 88.9% of GISTs, CD34 in 85.1%, nestin in 77.7% and caveolin-1 in 71.6% of the tumors. Of nine c-kit negative GISTs, 66.7% expressed nestin, the same as caveolin-1 and 44.5% expressed both nestin and caveolin-1. Statistical analysis using Kendall's and Spearman's tests revealed significant correlations between nestin and caveolin-1 expressions ( $p=0.024$ ). Our results suggest that nestin and caveolin-1 could be considered sensitive markers in GISTs, together with CD117 and CD34, for diagnostic purposes. Their significant expression in CD117 negative GISTs could represent an immunohistochemical alternative in establishing the diagnosis of these tumors.

**Keywords:** GIST, nestin, caveolin-1, c-kit negative, diagnosis.

### Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasias of the gastrointestinal tract (80%) and typically express CD117 (c-kit protein) [1–3]. GISTs predominantly occur in middle aged and elderly patients, with no significant difference in sex. They may arise throughout the gut, but the commonest sites are the stomach (60–70%), the small intestine (20–30%), the colorectum (5%), the esophagus (up to 5%). Rarely, GISTs develop in the retroperitoneum, the omentum and the mesentery [3]. Their origin seems to be the interstitial Cajal cell of the gut (ICC) [4, 5]. Normally, ICCs are located in and around the myenteric plexus and are thought to function as intestinal pacemaker cells [4, 5].

In the past, GISTs were often misclassified as leiomyomas or leiomyosarcomas. Subsequently, it has been determined that they have distinct ultrastructural features and immunophenotypical markers if compared to smooth muscle tumors [6].

Morphologically, GISTs are characterized as spindle-cell type, epithelioid type or, rarely, mixed type, when both features are present. The prognostic significance of cell type was proved in several studies, which showed that the epithelioid and mixed cell type GISTs are associated with a poorer prognosis, in contrast with a longer survival rate in the spindle cell type tumors. [6, 7] The most notable immunohistological marker for GISTs is c-kit (CD 117) [1–3, 6]. The discovery of c-kit proto-oncogene mutations in the pathogenesis of these

tumors, and the development of imatinib mesylate (Glivec<sup>®</sup>), a specific inhibitor of c-kit tyrosine kinase function, have improved the treatment of GISTs, mostly the metastatic or unresectable ones [8, 9]. Considering that certain GISTs do not express C-kit and, since the availability and the proven efficiency of specific therapies are increasing, novel markers have been tested recently in order to improve the diagnostic accuracy.

Nestin, an intermediate filament protein that belongs to the sixth class of intermediate filaments, is expressed in proliferating progenitor cells of developing and regenerating tissues, and is considered as a marker for multipotential neuroepithelial stem cells. In addition, nestin is shown to be predominantly expressed in tumors that are thought to arise from immature cells, such as primitive neuroectodermal tumors, medulloblastomas, pediatric rhabdomyosarcomas, melanoma, testicular stromal tumors and GISTs [10, 11]. Nestin is also detected in proliferating endothelial cells (ECs) and is involved in the early stages of lineage commitment, proliferation and differentiation [12]. Nestin expression is correlated with proangiogenic chemokines (CXCL12 and its receptor CXCR4) and growth factors (VEGF, PDGF-B and its receptor PDGFRbeta). Moreover, it was demonstrated that patients with GISTs who respond to Glivec<sup>®</sup> show a marked decline in the circulating levels of VEGF, and some studies suggest that the VEGF pathway is involved in tumor growth and differentiation [13]. Our paper intended to assess the contribution of nestin in the diagnosis of GISTs. Except for the known role of C-kit in GISTs pathogenesis, these tumors may

also show a number of cytogenetic anomalies that correlate with disease progression [14, 15]. One such anomaly refers to the family of caveolins [16].

Caveolins are a group of highly conserved 20–25 kD integral membrane proteins, and constitute the principal proteic components of caveolae [16]. The caveolin family consists of four proteins, namely caveolins-1 $\alpha$ , -1 $\beta$ , -2 and -3, and these are encoded by three genes (*CAV-1*, *CAV-2*, and *CAV-3*) [17]. Caveolin-1 (also known as caveolin, Cav-1 or VIP21) was the first member of the caveolin family to be identified, and it has been confirmed as a structural component of caveolae and of transport vesicles derived from the trans-Golgi network [18–20]. Caveolin-1 expression is lost or reduced during cell transformation *via* the activated oncogenes, and so it could be considered a putative tumor suppressor gene [21]. The expression of caveolin-1 has been demonstrated in several types of sarcomas [22] and carcinomas [23–27]. It appears to have different functions, depending on the tumor type, and these findings have led some investigators to hypothesize that it may play a role in various stages of carcinogenesis [28, 29]. A study of Cho WJ and Daniel EE demonstrated caveolin-1 expression in all classes of ICC (ICC of myenteric plexus, deep muscle plexus, serosa and intramucosal), using double-immunofluorescent labeling with primary antibodies for c-kit and caveolin-1 [30]. Furthermore, it is well known that caveolae are notably abundant in smooth muscle cells [20, 31]. These findings suggest that pluripotent mesenchymal precursor cells could differentiate in both ICCs and smooth muscle cells and also that they could have caveolar structures. Starting from this data, we studied caveolin-1 immunohistochemical expression in GISTs.

## Materials and Methods

### Tissue samples

Eighty-one formalin-fixed paraffin-embedded samples of confirmed GISTs were selected from the archive of “Victor Babeș” National Institute. Paraffin blocks were cut at 5  $\mu$ m and stained with Hematoxylin–Eosin. The series included patients ranging in age from 47 to 84 years (mean 59.28, standard deviation 11.976), with a women/men ratio of 45/36. Among the 81 GISTs, 38 were located in the stomach (46.9%), 39 in the small intestine (48.1%) and four in the colorectum (4.9%). GISTs distribution according to sex and site is illustrated in Figure 1.

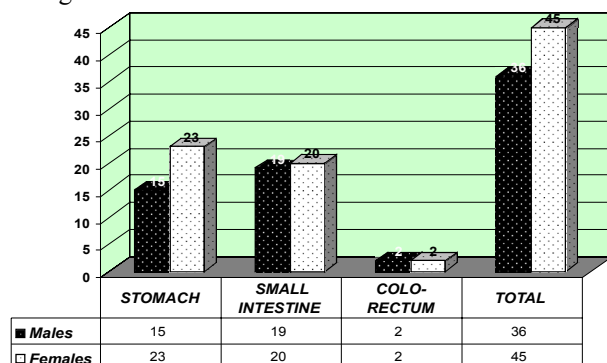


Figure 1 – GISTs distribution according to sex and site.

## Immunohistochemistry

For immunohistochemistry (IHC), paraffin sections were deparaffinized, rehydrated and rinsed in PBS pH 7.4. Staining was performed using a Dako Envision<sup>TM</sup> + Dual Link System-HRP, (Dako, Carpinteria, USA) according to the manufacturer's instructions, and counterstained with Meyer's Hematoxylin. The cases were tested using antibodies against: nestin, caveolin-1, CD117 and CD34. Details regarding the antibodies are given in Table 1.

Table 1 – Details regarding the antibodies used in the studied GISTs

Antibody	Company	Dilution	Clone	Positive control
Nestin	Santa Cruz Biotechnology, Inc., USA	1:50	10c2	human brain tissue
Caveolin-1	Santa Cruz Biotechnology, Inc., USA	1:50	polyclonal rabbit	smooth muscle cells or endothelium
CD117	DakoCytomation, Glostrup, Denmark	1:400	polyclonal rabbit	intestinal interstitial Cajal cell
CD34	DakoCytomation, Glostrup, Denmark	1:50	QBEnd-10	capillary vessel walls

For negative control, an identical procedure was performed omitting the primary antibody. Both cytoplasmic and membrane staining were considered positive. For all these antibodies, the proportion of positive stained cells was considered as 0 – no cells stained, 1 – between 0 and 10% stained positive, 2 – between 10 and 50% stained positive, 3 – if more than 50% stained positive. The percentage was established by calculating the average value on a minimum of 10 high power fields (40 $\times$ ). All specimens were examined and photographed with a Nikon Eclipse 600 microscope (Nikon, Japan).

## Statistical analysis

Statistical analysis was performed using SPSS software (SPSS 15.0, Chicago, IL, USA). Correlation between parameters was analyzed using Kendall's and Spearman's tests. A *p*-value of less than 0.05 was considered statistically significant.

## Results

We noted a number of 25 GISTs with epithelioid cells (30.8%), 46 cases (56.7%) with spindle cells and 10 cases (12.3%) with mixed pattern. GISTs distribution according to sex, location and cell type is illustrated in Figure 2.

Nestin was positive in 63 cases (77.7%), 32 located in the stomach (59%), 28 in the small intestine (30%) and three in the colon (11%), in patients aged 30 to 80-year-old (mean 59.73; standard deviation 11.165) with a women/men ratio of 33/30.

According to cell type, nestin expression was observed in 36 spindle cell type GISTs (57.2%), in 20 epithelioid cell type tumors (31.8%) and in seven GISTs with mixed pattern (11%).



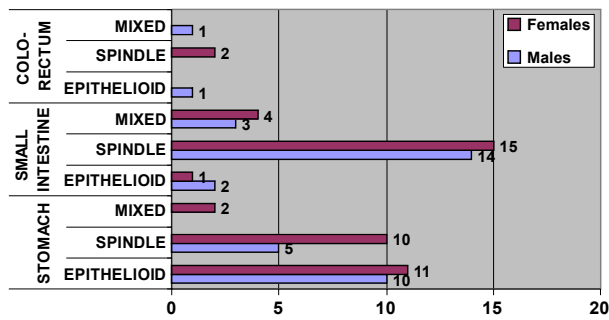


Figure 2 – GISTs distribution according to sex, site and cell type.

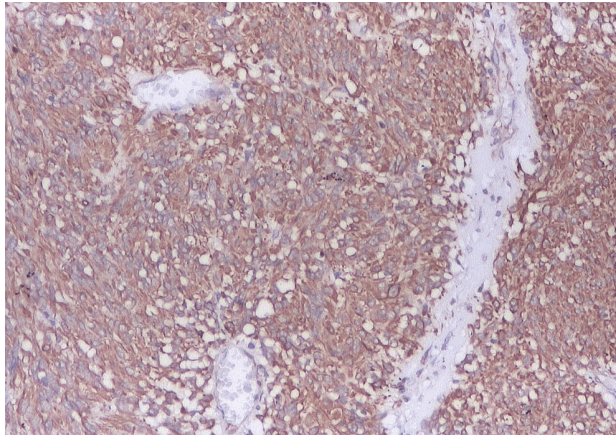


Figure 3 – Nestin expression in the epithelioid cell type GIST, ob. 10x.

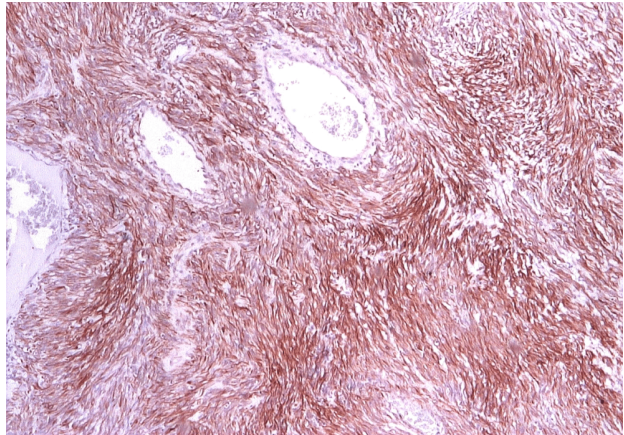


Figure 4 – Nestin expression in the spindle cell type GIST, ob. 10x.

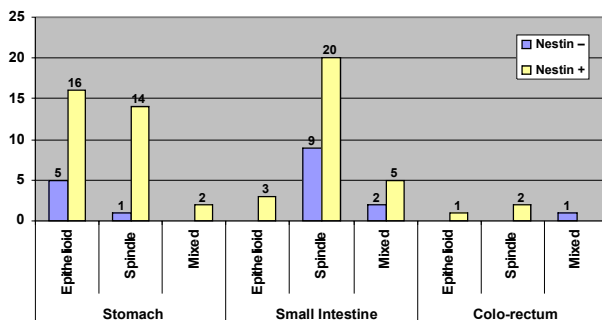


Figure 5 – Nestin expression in GISTs, according to site and cell type.

Caveolin-1 positivity was observed in 58 GISTs (71.6%), 24 located in the stomach (41.3%), 32 in the small intestine (55.1%) and two in the colon (3.44%), in

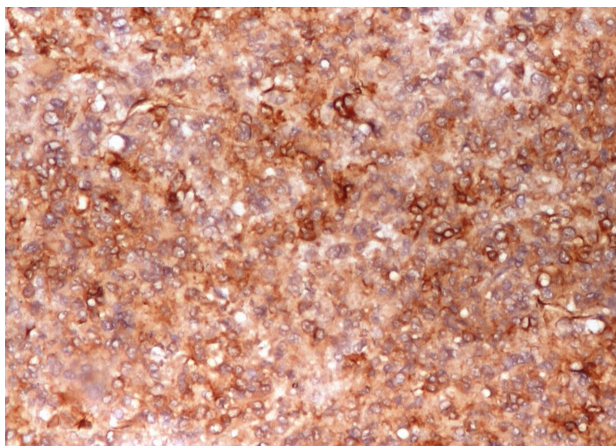


Figure 6 – Caveolin-1 expression in the epithelioid cell type GIST, ob. 10x.

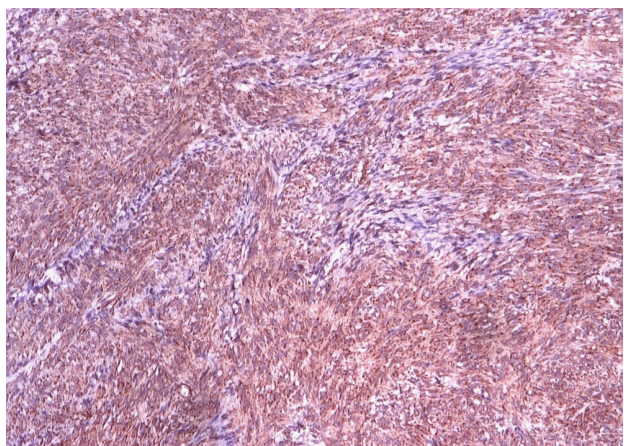


Figure 7 – Caveolin-1 expression in the spindle cell type GIST, ob. 10x.

In the stomach, nestin was positive in: 16 epithelioid cell type GISTs (50%) (Figure 3), 14 spindle cell type GISTs (43.75%) and two mixed type GISTs (6.25%). In the small intestine, nestin was positive in: three epithelioid cell type GISTs (10.7%), 20 spindle cell type GISTs (71.4%) (Figure 4) and five mixed type GISTs (17.8%).

At colorectal level, nestin expression was noted in: one case of all epithelioid cell type GISTs tested, two spindle cell type GISTs and no case of mixed type. All these data are illustrated in Figure 5.

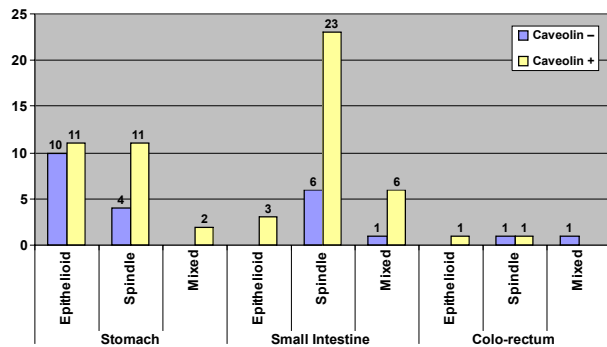
patients ranging from 36 to 84-year-old (mean 60.41; standard deviation 11.806) with a women/men ratio of 30/28.

Caveolin-1 expression according to cell type was observed in 35 spindle cell type GISTs (60%), in 15 epithelioid cell type tumors (26%) and in eight GISTs with mixed pattern (14%).

In the stomach, caveolin-1 was positive in 11 epithelioid cell type GISTs (45.8%) (Figure 6), in 11 spindle cell type GISTs (45.8%), and in two mixed type GISTs (8.3%). In the small intestine, caveolin-1 was positive in three epithelioid cell type GISTs (9.3%), 23 spindle cell type GISTs (71.8%) (Figure 7) and six mixed type GISTs (18.7%).

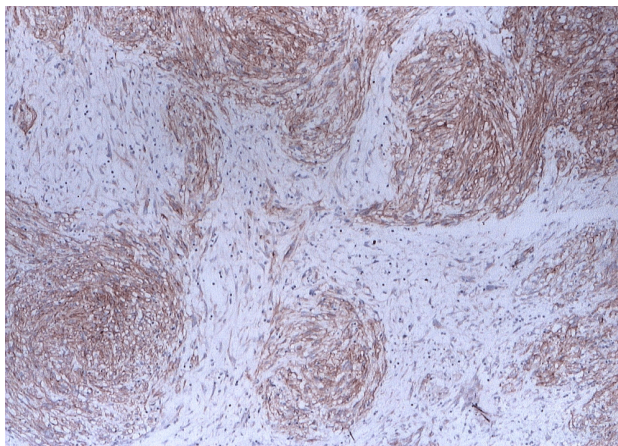


At colorectal level, caveolin-1 expression was noted in: one case of all epithelioid cell type GISTs; one spindle cell type GIST; and no case of mixed type. These results are illustrated in Figure 8.



**Figure 8 – Caveolin-1 expression in GISTs, according to site and cell type.**

A comparative analysis of nestin positive cases/nestin negative cases ratio with caveolin-1 positive/caveolin-1 negative cases ratio showed a 5.33 nestin ratio and a 1.71 caveolin-1 ratio in the stomach, while in the small intestine the caveolin-1 ratio is 4.57, compared to 2.54 nestin ratio (Table 2).



**Figure 9 – CD117 expression in the spindle cell type GIST, ob. 10x.**

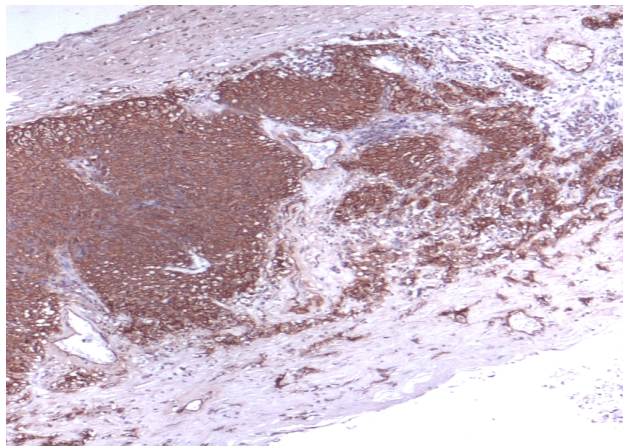
Nine CD117 negative GISTs were observed (11.1%); eight were situated in the stomach and one in the small intestine. According to cell type, seven were epithelioid cell type GISTs and two cases with both types of cells. Within c-kit negative GISTs, six expressed nestin (66.7%), all situated in the stomach and six were also caveolin-1 positive (five cases in the stomach and in one in the small intestine); in detail, of the nine c-kit negative cases, four expressed both nestin and caveolin (44.5%), all located in the stomach; two cases (22.3%) expressed only caveolin-1 (one in the small intestine and one in the stomach) and also two cases expressed only nestin (both situated in the stomach). According to cell type, nestin expression was encountered in five epithelioid cell type negative GISTs and in one mixed type, while caveolin-1 expression was observed in five epithelioid cell type c-kit negative GISTs, and in one spindle cell type tumor. There was a single CD117 negative case with no nestin or caveolin-1 staining, situated in the stomach and showing an epithelioid cell type.

CD34 was positive in 68 of GISTs (83.91%), 35 from

**Table 2 – A comparative analysis of nestin positive cases/nestin negative cases ratio with caveolin-1 positive/caveolin-1 negative cases ratio in the studied GISTs**

Location/cell type	Nestin+/Nestin- ratio	Caveolin-1+/Caveolin-1- ratio
Stomach	5.33	1.71
Small intestine	2.54	4.57
Colorectum	3	1
Epithelioid cell type	4	1.36
Spindle cell type	3.6	3.5
Mixed cell type	2.33	4
Total no. of cases	3.5	2.52

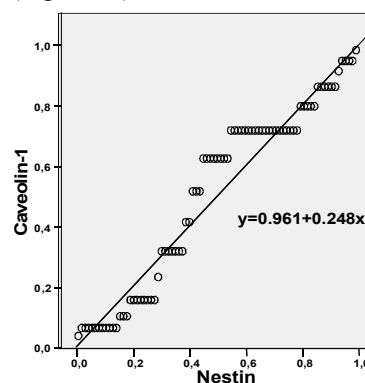
A number of 72 out of 81 GISTs (88.9%) were positive for CD117, 30 situated in the stomach (41.6%), 38 in the small intestine (52.7%) and four in the colon (5.5%), in patients aged between 30 and 84 (mean 60.69; standard deviation 11.733) with a women/men ratio of 38/34. From the 72 CD117 positive cases, 47 showed spindle cells (65.2%) (Figure 9), 18 were epithelioid tumors (25%) (Figure 10) and seven had mixed pattern (9.8%).



**Figure 10 – CD117 expression in the epithelioid cell type GIST, ob. 10x.**

the stomach (51.4%), 30 situated in the small intestine (44.1%) and three in the colorectum (4.5%). Among CD34 positive gastric GISTs, 20 were with epithelioid cells (57.1%), while, in the small intestine, 22 out of 30 GISTs had spindle cells (73.3%).

Statistical analysis using Kendall's and Spearman's tests revealed significant correlation between nestin and caveolin-1 expressions (Kendall/Spearman:  $p=0.024$ ). (Figure 11).



**Figure 11 – Normal P-P plot of regression standardized residual.**

## Discussion

In our series, we found a slight female predominance (women/men ratio of 45/36) and a median age of 59.28 (standard deviation 11.976). The most frequent location was the small intestine (48.1%), followed by the stomach (46.9%); and the spindle cells pattern was predominant (56.7%). These findings agree with known literature data [3, 5].

GISTs incidence in women is higher than in men in the stomach, approximately the same in the small intestine and equally distributed in the colorectum. According to sex, cell type and location, women have a higher incidence of spindle cell type GISTs in the stomach and colorectum, while, in the the small intestine spindle cell type and in the gastric epithelioid cell type GISTs, the incidence was approximately the same both in women and men.

Regarding nestin, some studies suggest that it could be a useful molecular marker in characterizing tumors originated from immature cells, such as stem or progenitor cells [10]. It was also proven that nestin is expressed in some human sarcomas including GIST [10, 11]; our findings showed 77.7% positivity in GISTs, almost equally distributed in men and women (men/women ratio of 33/30). Its expression in GISTs was more frequently encountered in the stomach (59%), followed by the small intestine (30%) and colorectum (11%). According to cell type, nestin expression was more frequently observed in the spindle cell type (57.2%), compared to 31.8% in the epithelioid cell type and 11% in the mixed type GISTs.

According to tumor site and cell type, nestin expression was higher in spindle cell tumors from the small intestine (71.4%), followed by epithelioid cell type tumors in the stomach (50%) and mixed cell type GISTs from the small intestine (17.8%). Therefore, our data confirmed nestin a good marker in establishing the diagnosis of GISTs. These results are similar to other findings [19, 20] and support the stem cell–Cajal cell GIST origin.

Caveolin-1 proved to be positive in 71.6% of cases, more frequently in women (women/men ratio of 30/28). Its expression was more frequently encountered in the small intestine (55.1%), followed by the stomach (41.3%) and, very rare, the colorectum (3.44%). According to cell type, caveolin-1 was more frequently observed in the spindle cell type (60%), the same as nestin, and only 26% in the epithelioid cell type and 14% in the mixed cell type tumors. According to site and cell type, its expression was higher in the spindle cell type GISTs from the small intestine (71.8%), followed at significant distance by the epithelioid and spindle cell types from the stomach (45.8% each). Thus, we can observe that *nestin and caveolin-1 expressions are more frequently encountered in the small intestine spindle cell type GISTs*.

According to the direct comparison ratios on caveolin-1 and nestin reactivity, we may conclude that *nestin is highly positive in GISTs located in the stomach, while caveolin-1 positivity is more frequent in GISTs located in the small intestine*; nestin expression is also more frequent in the epithelioid and spindle cell

*type tumors, while caveolin-1 in the spindle and mixed cell type ones.*

These results are confirmed by the statistical correlation between nestin and caveolin-1 expressions ( $p=0.024$ ).

Our finding of 71.6% caveolin-1 positive GISTs is different from a previous study performed by Kim EJ *et al.*, in 2005, when, on 108 tested GISTs, 14% caveolin-1 positive cases were observed [32]. The explanation would be the geographical diversity in frequency and distribution of GISTs.

CD117 positivity in 88.9% of cases is lower compared to some findings [3, 5, 33] and almost similar with other publications that made a complex study of GISTs, including pathology and immunohistochemistry [34–36]. Within CD117 positive cases, the most frequent location was the small intestine (52.7%). According to cell type, the most frequent CD117 positive cases were encountered in spindle cells GISTs (62.5%). Our percentage of 5.5% c-kit positive colorectum GISTs is in the boundaries of 4–10% found in literature [35, 36].

Of nine CD117 negative GISTs, the predominant location was the stomach (88.9%) and the most frequent cell type was epithelioid (77.8%). More often, CD117 negative tumors express both nestin and caveolin-1 (44.5%), rather than either of the two markers alone (22.3%). Nestin was positive in all CD117 negative GISTs from the stomach, whereas caveolin-1 also was encountered in one GIST from the small intestine. There was a single case where no nestin or caveolin-1 expression could be detected. *These findings indicated that nestin and caveolin-1 could represent a reliable immunohistochemical alternative in the diagnosis of CD117 negative GISTs.*

## Conclusions

Our results suggest that nestin and caveolin-1 could be sensitive markers in the diagnosis of GISTs. Their significant expression in CD117 negative GISTs could represent an immunohistochemical alternative in establishing the diagnosis of these tumors.

## Acknowledgments

This work was supported by Excellency National Research Program CEEX 42/2005.

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Received: November 21<sup>st</sup>, 2011

Accepted: January 25<sup>th</sup>, 2012