

Alternative factors in differential diagnosis of infantile fibrosarcoma

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ABSTRACT

The aim of this study was to determine the role of nm23 expression, percentage of Ki-67 labelling, vascularisation, mitosis and histologic features such as pattern, cellularity and density of inflammatory cell infiltrate in differential diagnosis of fibrous tumors. Specimens of 16 fibrous tumor cases were studied: 6 infantile fibrosarcoma (IFS), 3 adult fibrosarcoma (AFS) and 7 benign fibrous proliferations. All findings were examined and correlated statistically. In conclusion, number of inflammatory cells, histologic pattern and age of the patient are helpful in differential diagnosis of IFS and AFS. [Turk J Cancer 2004;34(4):150-155]

KEY WORDS:

fibrous tumors, nm23, Ki-67, angiogenesis, mitosis

INTRODUCTION

Fibrous proliferative lesions of infancy and childhood are considered as a separate category of fibrous tumors. They have characteristic structure and behaviour differing from those found in older children and adults (1). There are several investigated factors, which have helped to define the prognosis of pediatric solid tumors with multidisciplinary teams and care (2). IFS have a favourable outcome, although local recurrence and metastases can occur (3-5). Despite the present histologic parameters, new markers in differential diagnosis of fibrous tumors are necessary.

The evaluation of the degree of cellular proliferation in tumor tissue is one of the most useful methods to examine the behaviour of tumor (6). The older but still widely used method is mitotic count in routinely processed sections. Cell proliferation can also be investigated by immunohistochemical staining for nuclear antigens related to cell growth and division such as Ki-67, Ki-S1 and PCNA (6,7). Similarly, evaluation of the vessel number and density of tumor is widely searched in tumors. A huge literature has appeared in the past few years concerning the potential importance of determination of vascularisation in tumor tissue (8,9).

Nm23 gene family is well known to be putative metastasis suppresser genes and is related with differentiation. There are 4 defined types. Nm23-H1, nm23-H2, Dr-nm23 and nm23-H4 genes encode nucleoside diphosphate (NDP)

kinase (10). These genes are expressed in different tumor types where their levels are alternatively associated with reduced or increased metastatic progressive potential such as neuroblastoma, rectal cancer, nasopharyngeal carcinoma, serous ovarian carcinoma, thyroid carcinoma, lung cancer, breast cancer and retinoblastoma (7,8,10-16).

The aim of our research is to determine the histopathologic properties of IFS and the role of nm23 expression, percentage of Ki-67 labelling, vascularisation and mitosis in the differential diagnosis of fibrous tumors.

MATERIALS AND METHODS

This study included 6 IFS, 3 AFS and 7 benign fibrous tumors of childhood (fibrous hamartoma, fibromatosis and myofibromatosis). Patients with IFS and BFT were diagnosed, treated and followed in Children's Education and Research Hospital by Oncology Study Group between 1991 and 2001 and AFS cases in Dokuz Eylül University Faculty of Medicine. Formalin-fixed and paraffin-embedded, well-preserved tissue blocks were used for immunohistochemical study. Nm23-NDP kinase Ab-1 (diluted, Neomarkers, USA), Ki-67 (DAKO, USA) and CD34-Ab1 (Neomarkers, USA) were applied as primary antibodies. The evaluation was made being unaware of any of the clinical features. Nm23 expression was graded as negative and positive. The immune staining for Ki-67 was scored according to quantity. For each case, 5000 tumor cells were observed and positive cells were counted. The degree of positive cells was classified as low and high according to the calculated cut-point value. This value was found 150/5000 for Ki-67 on SPSS program.

The CD34 labelling slides were investigated for the evaluation of vascularisation status. The most vascularised areas were chosen for assessment at x40 magnification. An ocular square lattice with 121 points composed of 11 horizontal and 11 vertical test lines with known total test line length ($LR = 2.6875 \mu\text{m}$) was superimposed on the test fields. The number of intersections (In) between the test lines and the labelled vessel walls were counted. The area of each measuring field was 0.36 mm^2 . The points without vessel touch were counted (Istr). Volume portion of stroma V_v (str) was computed according to V_v (str) = $Istr/121$ and VSD was computed according to $VSD = (In$

$\times 2 / LR \times V_v$ (str)). The number of vessel (N) within measuring field was counted and the number of vessels per mm^2 stroma (NVES) was computed according to $NVES = N/V_v$. The VSD and NVES values of each patient were calculated by this method (17,18) and the cut-point values were analyzed using SPSS. The values found were $11.2/\text{mm}^3$ for VSD and $8.4/\text{mm}^2$ for NVES.

Mitotic figures were investigated in sections stained with haematoxylin-eosin. The number of mitosis in 10 consecutive high-power fields was counted. The cut-point value for mitosis was 10/10 high power field (x400). Cellularity was evaluated qualitatively and histologic pattern was recorded. Inflammatory cells were counted per 5000 cells in leukocyte common antigen stained (immunohistochemically) slides. The mean of each group were correlated.

Descriptives and frequencies of the parameters were evaluated and compared across disease groups. P values less than 0.05 were considered to be statistically significant.

RESULTS

Six cases were IFS, 3 were AFS and 7 were benign fibrous tumors of childhood. The properties of cases are shown at table 1. Desmin and S100 were negative in all cases, while vimentin was positive in all of them. Ten patients were male and 6 were female. All three groups included male and female patients. There was no sex dominance in any group. IFS cases were located in the extremities in 4 cases. One case was sacral and the other one was cervical. BFTs included fibrous hamartoma, fibromatosis, myofibromatosis in different localisation. AFSs located at lower extremity, abdominal wall and infraumbilical region. IFSs showed moderate or high cellularity, AFSs were all high cellular, BFTs showed low or moderate cellularity. Nm23 expression was observed in 83% of IFSs, in 71% of BFTs and in 66% of AFSs. IFSs and AFSs did not contain abundant collagen in the stromal matrix compared with BFTs. The histologic pattern in IFSs was mainly fasciculated or intermixed, while it was widely herringbone like in AFSs and varying in BFTs. The mean age in IFSs was 13 months (1-24), 55 month in BFTs and 44 years in AFSs. The mean number of Ki67 per 5000 cell (Figure 1) was 240 in IFSs, 26 in BFTs and 300 in AFSs. The mean

number of mitosis per 10 hpf was 15 in IFSs, 2.7 in BFTs and 25 in AFSs. The mean number of inflammatory cells per 5000 cells (Figure 2) was 115 in IFSs, 131 in BFTs and 20 in AFSs. IFS and AFS cases all had high VSD (mean 16.8, 15.8) and NVES (mean 10.64, 9.98) while BFP cases all had low VSD and NVES (mean 8.6, 7.4) degree.

In statistical analysis; there was difference for cellularity, degree of Ki67 labelling, mitotic index and status of VSD-NVES between all fibrosarcomas (IFS+AFS) and BFPs

($p < 0.05$), but not between IFS and AFS ($p > 0.05$). Histologic patterns were fasciculated or intermixed in IFS but regularly herringbone like in AFSs. Inflammatory cells were more in number in IFS than AFS and BFP. Not only collagen amount, nm23 expression, sex and localisation were not found important in differential diagnosis but these parameters were also not independent diagnostic parameters in regression analyses.

Table 1
The properties of the cases

Patient no	Diagnosis	Age (months)	Sex	Localisation	Cellularity	Histologic Pattern	Mitosis	Ki67 (Per 5000 cells)	Nm23	Collagen	Inflammatory cells
1	IFS	2	F	Leg	High	Intermix	12	200	+	-	12
2	IFS	10	F	Scapula	High	Intermix	3	430	+	-	20
3	IFS	24	M	Leg	Low	Intermix	3	230	+	-	50
4	IFS	1	M	Neck	High	Intermix	3	300	+	-	15
5	IFS	16	M	Arm	High	Intermix	22	300	-	-	15
6	IFS	24	M	Sacrum	Medium	Intermix	2	10	+	High	20
7	AFS	564	M	Abdomen	High	Herringbone	53	300	+	-	10
8	AFS	576	M	Umblicus	High	Herringbone	30	150	+	-	30
9	AFS	300	M	Knee	High	Herringbone	25	250	-	Low	20
10	Myxo fibrom	16	M	Mandible	Low	Storiform	2	20	-	High	30
11	Myxo fibrom	84	F	Thorax	Low	Diffuse	3	18	+	Low	20
12	Myxo fibrom	12	M	Neck	Medium	Storiform	3	100	+	Low	50
13	Fibrom	72	M	Hand	Medium	Storiform	3	22	+	Low	10
14	Digital Fibromatosis	12	M	Hand	Low	Storiform	2	10	-	-	30
15	Fibromatosis	60	F	Periumblical	Medium	Diffuse	4	6	+	-	50
16	Infantile Fibromatosis	24	M	Wrist	Low	Intermixed	1	10	+	-	10

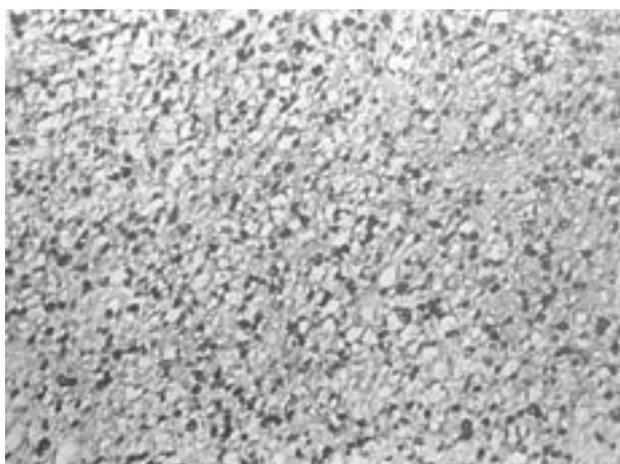


Fig 1. High Ki67 nuclear activity in infantile fibrosarcoma (Ki67, DAB x100)

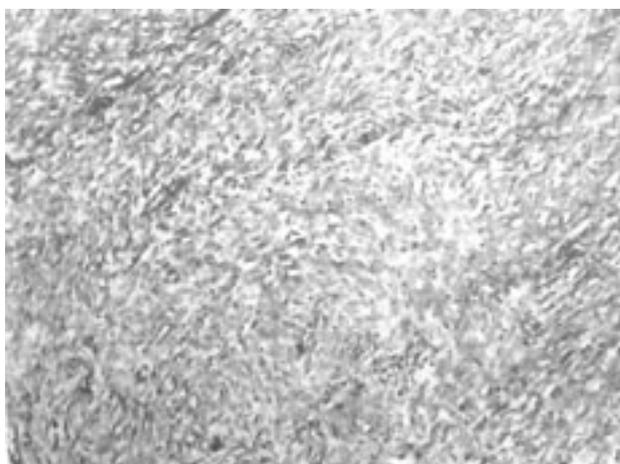


Fig 2. Increased inflammatory cells in infantile fibrosarcoma (LCA, DAB x100)

DISCUSSION

Fibrous tumors of infancy and childhood are considered in different categories and can be divided into two groups: one including benign lesions such as fibrous hamartoma of infancy, fibromatosis, and myofibromatosis; the other IFSs (1). IFSs are considered to be a rare pediatric tumor group and are composed of fusiform cells with a fasciculated architecture (3). It is often difficult to distinguish fibrosarcoma from other spindle cell tumors. Differential diagnosis also include sarcomas such as malignant peripheral nerve sheath tumor, malignant fibrous histiocytoma, monophasic fibrous synovial sarcoma, spindle cell rhabdomyosarcoma and leiomyosarcoma, as well as the aggressive fibromatosis (1,3).

IFS (under 1 year of age) have a favourable outcome, although local recurrence and metastases may occur (3). Complete excision at diagnosis is the treatment of choice and is related to the best outcome (4). Microscopic residuals are difficult to treat with chemo-radiotherapy in both forms of fibrosarcomas. Although IFS have low potential for metastatic spread and surgical extirpation alone results in excellent prognosis, amputation rate is high to provide complete excision. Recently preoperative chemotherapy is given to avoid amputation in a few cases with dramatic response results (19).

BFTs, especially cellular fibromatosis cases are difficult to differentiate from IFS. Although age is a very helpful criteria, sometimes IFS are observed at ages of 10 and AFS might be observed at any age. The clinical behaviour and therapy models of these three tumor groups are different.

The genetic rearrangement detected in patients with IFS is t(12;15)(p13;q25) resulting in ETV6-NTRK3 gene fusion similar with congenital mesoblastic nephroma (5,20,21). ETV6-NTRK3 is claimed to be a reliable and specific modality for the diagnosis of IFS; differentiating from other benign and malignant infantile pediatric spindle cell tumors of the soft tissue, like infantile fibromatosis, myofibromatosis (22).

Nm23 gene family is also known to associate with metastasis and differentiation. Recently prognostic importance of nm23 expression is investigated for several neoplasms (7-16). It is suggested that biological significance of nm23 expression might be different in different tissues and neoplasms. For example, Dr-nm23 is the third member of human nm23 gene family found to be associated with differentiation in neuroblastoma cells (10). Immunohistochemical expression of nm23 was found more intensive in patients with nonrecurrent disease and alive patients among 50 ovarian cancer patients (12). In primary, non-small cell lung cancer nm23 is found to be a suppresser of systemic but not lymphatic metastasis (15). In breast cancer, the expression of NDP kinase/nm23 has been reported to correlate with good prognosis and a lack of nodal metastasis (7). But in retinoblastoma, nm23 staining was observed to indicate a tendency to metastases (16). In thyroid follicular carcinoma, a significant inverse association was observed between metastatic disease and nm23 H1 expression (14). Nm23 H1 expression was related with tumor progression

in nasopharyngeal carcinoma (8). In rectal cancer nm23 expression failed to correlate with distant metastasis (11). In vitro transfection experiments show that the nm23 gene suppresses metastasis, although the evidence from clinical studies is contradictory. Metastatic behaviour of IFS is rare but there is no found parameter to guess this behaviour. Although nm23 expression is thought to be a metastasis suppresser gene, we did not observe a difference in the expression between IFS and AFS cases for nm23 expression.

Vascularisation and its contribution on tumor growth have been widely studied in different neoplasms. A huge literature has appeared in the past few years concerning the potential importance of determination of vascularisation in tumor tissue (9,17,18). In some of these studies, intratumoral microvessel density was found to have a prognostic value for survival. Growth of solid tumors requires the new capillary network. The term angiogenesis was coined in 1935 to describe the formation of new blood vessels in the placenta (9). The reason why we choose stereologic assessment of vascularisation by VSD and NVES was to avoid methodological disadvantages of quantification of vascularisation caused by two dimensional sections or errors that might be caused by observer bias. This study suggested that quantitative assessment of vascularisation is not predictive to distinguish IFS from AFS; however FSs (AFS+IFS) show higher vascularisation than BFPs.

Both nuclear morphometry and evaluation of the cell proliferative activity have been reported to be useful tools in predicting prognosis in malignant tumors. These features especially DNA ploidy and S-phase fraction can be evaluated with flow cytometry and there is no question that they are correlated with prognosis. In addition, several other methods are available for the degree of cellular proliferation in tumor tissue. The older and still widely used method is mitotic count in routinely processed sections. Cell proliferation can also be investigated with immunohistochemical staining for nuclear antigens related to cell growth and division such as Ki-67, Ki-S1 and PCNA. As well as Nuclear Organizer Region (NOR), evaluation with AgNOR staining is another indicator of cell proliferation (6). Present study also suggested that mitotic degree and Ki-67 expression are predictive for fibrosarcomas but not helpful for differential diagnosis of IFS from AFS.

IFS affect chiefly the distal portions of the extremities while adult fibrosarcomas are common in the thigh (23,24). In our series localisation of the tumor does not have a distinct role in differential diagnosis.

In conclusion, many parameters are helpful in differential diagnosis of IFS and BFP. However number of inflammatory cells, histologic pattern and age of the patient is helpful in differential diagnosis of IFS and AFS.

References

1. Weiss SW, Goldblum JR, editors. Fibrous tumors of infancy and childhood. In: *Soft Tissue Tumors*. 4th ed. London: Mosby, 2001;347-401.
2. Herrera JM, Krebs A, Harris P, et al. Childhood tumors. *Surg Clin North Am* 2000;80:747-60.
3. Voute PA. Rare Tumors. In: Voute PA, Kalifa C, Barrett A, editors. *Cancer in children: clinical management*. 4th ed. Oxford University Press, 1999;339-47.
4. Cecchetto G, Carli M, Alaggio R, et al. Fibrosarcoma in pediatric patients: results of the Italian Co-operative Group Studies (1979-1995). *J Surg Oncol* 2001;78:225-31.
5. Meis-Kindblom JM. Congenital Infantile Fibrosarcoma. A clinicopathologic study of 10 cases and molecular detection of the ETV6-NTRK3 fusion transcripts using paraffin embedded tissues. *Am J Clin Pathol* 2001;115:348-55.
6. Rosai J. Special techniques in surgical pathology. In: Ackerman's surgical pathology. 8th ed. St. Louis: Mosby, 1996;29-62.
7. Terasaki M, Fukuzawo Y, Kijima H, et al. Decreased nm23 expression, but not Ki-67 labelling index, is significantly correlated with lymph node metastasis of breast invasive ductal carcinoma. *Int J Mol Med* 2002;9:25-9.
8. Huang GW, Mo WN, Kuang GQ, et al. Expression of p16, nm23-H1, E-cadherin, and CD44 gene products and their significance in nasopharyngeal carcinoma. *Laryngoscope* 2001;111:1465-71.
9. Folkman J, Klagsbrun M. Angiogenetic factors. *Science* 1987;235:442-7.
10. Negroni A, Venturelli D, Tanno B, et al. Neuroblastoma specific effects of DR-nm23 and its mutants forms on differentiation and apoptosis. *Cell Death Differ* 2000;7:843-50.
11. Gunther K, Dworak O, Remke S, et al. Prediction of distant metastases after curative surgery for rectal cancer. *J Surg Res* 2002;103:68-78.
12. Simone G, Falco G, Caponio MA, et al. Nm23 expression in malignant ascitic effusion of serous ovarian adenocarcinoma. *Int J Oncol* 2001;19:885-90.
13. Tas F, Tuzlali S, Aydiner A, et al. Prognostic role of nm23 gene expression in patients with ovarian cancer. *Am J Clin Oncol* 2002;25:164-7.
14. Zafon C, Obiols G, Castellvi J, et al. Nm23-H1 immunoreactivity as a prognostic factor in differentiated thyroid carcinoma. *J Clin Endocrinol Metab* 2001;86:3975-80.
15. Tomita M, Ayabe T, Matsuzaki Y, et al. Expression of nm23-H1 gene product in mediastinal lymph nodes from lung cancer patients. *Eur J Cardiothorac Surg* 2001;19:904-7.
16. Bardak Y, Cekic O, Ayhan A, et al. Nucleotide diphosphate kinase (nm23 Protein) expression in retinoblastoma. *Ophthalmic Res* 2000;32:73-8.
17. Barth PJ, Weingartner K, Köhler HH, et al. Assessment of the vascularisation in prostatic carcinoma: A morphometric investigation. *Hum Pathol* 1996;27:1306-10.
18. Köhler HH, Barth PJ, Siebel A, et al. Quantitative assessment of vascular surface density in renal cell carcinomas. *Br J Urol* 1996;77:650-9.
19. Kurkchubasche AG, Halvorson EG, Forman EN, et al. The role of preoperative chemotherapy in the treatment of infantile fibrosarcoma. *J Pediatr Surg* 2000;35:880-3.
20. Adem C, Gisselsson D, Cin PD, et al. ETV6 rearrangements in patients with infantile fibrosarcomas and congenital mesoblastic nephromas by florescence in situ hybridisation. *Mod Pathol* 2001;14:1246-51.
21. Argani P, Fritsch M, Kadkol SS, et al. Detection of the ETV6-NTRK3 chimerical RNA of infantile fibrosarcoma/cellular congenital mesoblastic nephroma in paraffin-embedded tissue: application to challenging pediatric renal stromal tumors. *Mod Pathol* 2000;13:29-36.
22. Bourgeois LM, Knezevich SR, Mathers JA, et al. Molecular detection of the ETV6- NTRK3 gene fusion differentiates congenital fibrosarcoma from other childhood spindle cell tumors. *Am J Surg Pathol* 2000;24:937-46.
23. Sah SP, Agrawal CS, Rani S. Congenital infantile fibrosarcoma of the upper extremity. *Indian J Pathol Microbiol* 2000;43:347-9.
24. Yalcın B, Leblebicioglu G, Guler E, et al. Congenital infantile fibrosarcoma of the thigh in a newborn. *Tumori* 2001;87:436-8.