

**DIAGNOSIS AND MULTIDISCIPLINARY TREATMENT OF SPORADICAL
DESMOID TUMORS**

Ph.D. Thesis

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XII. ACKNOWLEDGEMENT

XIII. PUBLICATIONS

I. PUBLICATIONS

1. Publications related to the thesis

1. **Mátrai Z**, Tóth L, Szentirmay Z, Papp J, Langmár Z, Kásler M. Multidisciplinary treatment of intra-thoracic desmoid tumours: case series and narrative review. *Med Sci Monit.* 2012, 18: 17-25. **IF: 1.699***
2. **Mátrai Z**, Tóth L, Szentirmay Z, Papp J, Antal I, Vadász P, Bartal A, Kásler M. Surgical challenges of chest wall and intra-thoracic desmoid tumors. *Orvosi Hetilap.* 2011, 152: 3-13.
3. **Mátrai Z**, Tóth L, Gulyás G, Szabó E, Szentirmay Z, Kásler M. A desmoid tumor associated with a ruptured silicone breast implant. *Plast Reconstr Surg.* 2011, 127: 1-4. **IF: 2.635***
4. **Mátrai Z**, Tóth L, Szentirmay Z, Vámos FR, Klepetko W, Vadász P, Kenessey I, Kásler M. Sporadic desmoid tumors of the chest: long-term follow-up of 28 multimodally treated patients. *Eur J Cardiothorac Surg.* 2011, 40: 1170-1176. **IF: 2.293***
5. **Mátrai Z**, Tóth L, Rényi Vámos F, Papp J, Gődény M, Bartal A, Szentirmay Z, Kásler M. Giant intra-thoracic desmoid tumour. Case report. *Med. Thor.* 2010, 63: 158-166.
6. **Mátrai Z**, Papp J, Polgár C, Hitre E, Köves I, Oláh E, Andi J, Kiss A, Vámosi Nagy I, Tóth L, Orosz Z. Long-term experience with therapy of a Gardner's syndrome female, first presenting with extra-abdominal desmoid tumor, and review of the literature. *Magy Seb.* 2009, 62: 75-82.

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*** Source: Magyar Tudományos Művek Tárháza**

ABBREVIATIONS

AFAP Attenuated Familial Adenomatous Polyposis	MRI magnetizing resonance imaging
APC gene adenomatous polyposis coli gene	MSC mesenchymal stromal cells
Bcl-2 B-cell lymphoma 2	NIO National Institute of Oncology
CI confidence interval	NSAIDs non-steroidal anti-inflammatory drugs
CNB core needle biopsy	p53 protein 53 or tumor protein 53, a tumor suppressor protein that in humans is encoded by the TP53 gene
c-erbB2 human epidermal growth factor receptor 2	PCR polymerase chain reaction
CT computed tomography	PDGFR platelet-derived growth factor receptor
COX-2 cyclooxygenase-2	PR progesteron receptor
Dsh dishevelled	PTT protein truncation test
EGFR epidermal growth factor receptor	RECIST Response Evaluation Criteria in Solid Tumors
EORTC European Organization for Research and Treatment of Cancer	RPC/IPAA restorative proctocolectomy with ileal pouch anal anastomosis
ER-α estrogen receptor-alpha	RR response rate
ER-β estrogen receptor beta	RT radiotherapy
EREs estrogen response elements	SERM selective estrogen receptor modulator
FAP familial adenomatous polyposis	SMA smooth muscle actin
18F-FDG-PET/ CT 2-(fluorine-18) fluoro-2-deoxy-D-glucose-positron emission tomography	SUV standardized uptake value
FNA fine needle aspiration	SSCP single-strand conformation polymorphism
GSK-3β glycogen synthase kinase-3 β	Tcf T-cell factor
HA heteroduplex analysis	TPC total proctocolectomy
HSC hematopoietic stem cell	US ultrasonography
IMT inflammatory myofibroblastic tumor	WHO World Health Organization
IHC immunohistochemistry	Wnt a hybrid of Int (integration 1) and Wg (wingless) in Drosophila, characterized by Wnt gene
Lef lymphoid enhancer-binding factor	
LRP LDL-receptor-related protein	
MLPA Multiplex Ligation-dependent Probe Amplification	

III. INTRODUCTION

1. Definition

The World Health Organization (WHO) defines desmoid tumors or aggressive fibromatoses, as „clonal fibroblastic proliferations that arise in the deep soft tissues and are characterized by infiltrative growth and a tendency toward local recurrence but an inability to metastasize” (1). Regarding the biological background of the tumor, it is classified between benign fibrous tissue proliferation and fibrosarcoma (1, 2).

The entity was first described by John MacFarlane in 1832, and was named desmoid (from the Greek word 'desmos' meaning band or tendon-like) by Johannes Müller in 1838 (3, 4).

2. Epidemiology

Desmoid tumors are rarely occurring tumors which account for 0.03% of all neoplasms and <3% of all soft tissue tumors (2, 5, 6, 7, 8). The estimated annual incidence in the general population is 2–4 per million (5, 8, 9). Desmoids may be diagnosed at any age, but the peak incidence is between 25 and 40 years of age (2, 5, 9). Two different types have been described: most of them belong to the sporadically occurring type (95%) and the rest are associated with hereditary cancer syndromes (5%). The autosomal dominant familial adenomatous polyposis (FAP, Gardner syndrome), hereditary desmoid disease (HDD) and familial infiltrative fibromatosis (FIF) belong to the latter group with an incidence of 3.5%–32% in these patients (2, 8-13). The incidence of desmoids in FAP patients is approximately 850 to 1000 times that of the general population and was 29% in the original Gardner kindred (11, 14, 15). The sporadic types are more common in women than in men with a ratio between 1.8 : 1 and 5 : 1 (5, 9, 16, 17).

3. Clinical presentation

Desmoids develop from musculoaponeurotic structures throughout the body and are classified as extra-abdominal (~ 60% of the cases), abdominal (~ 25% of the cases) and intra-abdominal (~ 15% of the cases) (2, 5, 7, 8, 15, 18, 19). Many studies have confirmed that 37 to 50% of desmoids arise in the abdominal region, and approximately 10% of all cases are multicentric (7, 19, 20). Significant differences were detected in the originating between sporadic and hereditary forms. While just 5% of sporadic tumors are intra-abdominal, 80% of patients with FAP-associated disease present with desmoids localised intra-abdominally (2). The anatomical distribution of extra-abdominal fibromatoses varies greatly. The principal sites

identified are the regions of the shoulder, chest wall, back, thigh and head and neck (1, 8). Abdominal desmoids arise from musculoaponeurotic structures of the abdominal wall, especially the rectus and internal oblique muscles and their fascial coverings. Intra-abdominal tumors develop in the pelvis, mesentery or retroperitoneum and are frequently found in patients with FAP, in whom it commonly originates in the retroperitoneal space following prophylactic proctocolectomy (5, 8, 13, 20).

3.1 Symptomatology

The clinical manifestation of desmoid tumors is non-specific (21, 22). Symptoms are usually present for an average of 12-16 months before diagnosis and the tumor is typically discovered as palpable mass by the patient or physician or accidentally by chest-abdominal imagines (X-ray, CT, ultrasonography (US)) (5, 8, 15, 22, 23). Little or no pain accompanies extra-abdominal fibromatoses, which typically arise as firm, poorly circumscribed, deep-seated, furtively grown masses (2, 5, 7, 24). Tumors are fixed to the musculoaponeurotic plane and are usually free in relation to the bone, and joint capsule, only rarely adhering to these. Muscular retractions, deformity, limited or lost joint functions or even the lifethreatening compression of vital organs may be caused if the tumor reaches a large size, and irradiated pain and paralysis may occur if the tumor compresses a nerve trunk (2, 5, 8, 11, 25). The skin and subcutis may only be involved after repeated surgery for recurrence or after radiation therapy (16, 25). Desmoids account for 0.2% of primary breast tumors, occasionally mimicing breast cancer (2, 8). Abdominal wall lesions are typically associated with young age, female gender, present or previous pregnancy often arising in the scar of a previous Cesarean section (5, 11, 25). Intra-abdominal desmoids remain asymptomatic until their growth and infiltration cause compression of visceral organs or serious morbidity (5, 8). Although asymptomatic abdominal mass is the only complaint of most patients with mesenteric lesions and only some have mild abdominal pain. Patients with diffuse mesenteric lesions may less commonly present with initial symptoms of intestinal, vascular, ureteric, or neural involvement, gastrointestinal bleeding, bowel perforation, obstruction or ischemia (5, 8, 25). Pelvic fibromatoses arise as slowly growing palpable masses, are initially asymptomatic, often mimicing ovarian neoplasms (5, 8, 25, 26). When a desmoid is diagnosed, a thorough family history is necessary to be taken, examination, genetic counseling and colonoscopy should be performed in order to diagnose or outrule Gardner's syndrome (25, 27).

3.2 Clinical behaviour

The clinical behavior and natural history of desmoids is typically heterogeneous and unpredictable due to the progressive fibroblastic and fibrous proliferation that lays in the background of its development. It is characterised not only by tumor growth, proliferation, and disease progression but also by stabilization and spontaneous remission (2, 7, 8, 11, 25, 28, 29). Church et al. (2008) found that 10% of aggressive fibromatoses resolved spontaneously, 30% were unpredictably interchanging between progression and resolution, 50% remained stable following diagnosis, and 10% were characterised by rapid progression (30). Variant desmoid growth patterns have been identified: some progress rapidly and aggressively whereas others are more indolent and may remain stable (2, 8, 11, 15). Most desmoids are however slowly growing neoplasms typically measuring between 5 and 10 cm, they aggressively invade surrounding tissues and organs, do not metastasize but bear a high propensity for local recurrence (5, 11, 25). Despite their benign nature and their negligible metastatic potential, the tendency to recur and the infiltrative growth remain significant problems in terms of morbidity and mortality (5, 8). Overall recurrence rates range from 20% to 85% in 10 years following primary treatment, and recurrence is more frequent with extra-abdominal than intra-abdominal desmoids (2, 5, 8, 11, 15, 25, 30-32). About 80% of recurrences are observed within 3 years postoperatively, even after radical excision (32-34). A close surveillance of patients is essential, as disease progression may occur even years after primary treatment (11).

4. Etiology

The morphology of desmoid tumors has been well characterized, but their nature and pathogenetic background remained indistinctive. Stout (1954), who first introduced the term fibromatosis, defined desmoids as “the most incomprehensible group” (35). Some authors considered them as non-neoplastic processes and others described desmoids as well-differentiated low grade sarcomas (2, 7, 9). In fact, the etiology is likely multifactorial and includes genetic, endocrine and physical factors as well (2, 5, 7, 8, 15, 25). The association of desmoids with FAP syndrome, first described by Nichols in 1923 is well known, and served as an evidence to the underlying genetic background (36). E. J. Gardner reported the familial occurrence of intestinal polyposis, osteomas and epidermal cysts in 1951, and although the current view is that Gardner syndrome is a variant of the phenotypic expression of FAP, the two terms are sometimes used as synonyms (37, 38). The role of endocrine factors in the pathogenesis are supported by clinical observations such as the female predominance, regular occurrence in the childbearing age, progression via the use of oral contraceptives, and

regression in post-menopausal or post-oophorectomy patients or those undergoing anti-estrogen therapy (8, 11, 15, 25). An antecedent history of trauma to the site of the tumors, often surgical in nature (prophylactic proctocolectomy, Cesarean section), has been identified in about 25% of cases (5, 27, 30, 33).

4.1. The neoplastic nature of desmoids

Since desmoid tumors are characterised by infiltrative growth, low mitotic activity, and the absence of metastases, they are most frequently classified as benign neoplasms or deep fibromatoses (10). Multiple studies described the clonal nature of desmoids pointing at malignant capacities that place these tumors into the category of fibroblastic malignancies (8, 39-43). The presence of trisomy 8 and/or 20 (which are non-random clonal aberrations acquired during neoplastic progression) and the proportion of cells with these trisomies vary greatly between desmoid tumor specimens (range 0 to 25%) (8, 42-44). It can be therefore concluded that trisomies 8 and 20 contribute to neoplastic aberrations in a wide spectrum of pathologic fibrous proliferations, without any distinction between benign or malignant (8, 42, 43).

The non-random inactivation of the female X-chromosome is a sign of monoclonal neoplastic proliferation (not a sign of malignancy), and its occurrence was also subject to investigation during the demonstration of the clonal nature of aggressive fibromatoses (8, 40, 45). A non-random X inactivation pattern was found in 72% of patients with sporadic desmoids, with a lesion clonality ratio ranging between 1.3 and 18.7 (40, 45). Evidence of malignancy has also been sought by assessing expression of tumor markers in desmoid tissue. In comparison to other malignancies, desmoids were found with a relatively low expression of metastasis promoting extracellular matrix proteins such as osteopontin and osteonectin, suggested to be the reason for the inability to disseminate (39, 45). Other common malignant tumor markers as Ki-67, pRB or Bcl-2 have not been observed to be upregulated in desmoid tissue (39, 43, 46-48). Appropriate tumor markers may explain the clinical behavior of desmoids, and still need to be identified (10). Although the properties of desmoids and the lack of malignant tumor markers simply do not conform to the definition of malignant tumors, and the term benign is biologically valid, it does not properly reflect the clinical picture.

To resolve this conflict the understanding of the molecular etiology of desmoids, identified by previous studies, is mandatory (8, 41, 49-51). A common feature detected is deregulated Wnt signaling via β -catenin-dependent activation of latent T-cell factor/lymphoid enhancer factor (Tcf/Lef), a pathway with a critical role for instance in embryogenesis, cell adhesion,

carcinogenesis, adult stem cell survival and self-renewal during wound healing (8, 41, 49). Desmoids arising in patients with FAP show loss of adenomatous polyposis coli (APC) tumor suppressor function, which leads to high intracellular β -catenin levels and is correlated with the constitutive activation of Wnt signaling (50, 51). In sporadic desmoids most tumors contain specific point mutations in the catenin (cadherin-associated protein) beta 1 (CTNNB1) gene, that stabilizes β -catenin and achieves a similar result described above (8, 52).

4.2. Desmoid tumor and β -catenin/APC

A germline mutation of the APC tumor suppressor gene predisposes patients with FAP to develop large numbers of colonic polyps and several extra-intestinal neoplasms including desmoid tumors (8, 10, 53). The germline mutation results in a null-allele of the APC gene while a somatic mutation in the other allele results in functionally homozygous knockout of the APC gene and the subsequent malignant transformation of the colonic polyps (47, 53). The precise location of the germline mutation within the gene or the associated genetic background of the affected individual determines the likelihood and form of occurrence of the somatic mutation, and the penetrance of the disease (28, 47, 54).

4.2.1. Wnt signaling pathway

Upon activation, APC forms a cytoplasmic multiprotein complex that includes glycogen synthase kinase (GSK)-3 β and axin (8, 10, 55). APC and axin phosphorylate β -catenin on its APC binding sites, causing the degradation and inactivation of the protein. The binding of β -catenin to APC requires phosphorylation by GSK-3 β . Axin promotes GSK-3 β -dependent phosphorylation of β -catenin (56). **Figure 1.** The two major functions of β -catenin are forming a complex with E-cadherin and α -catenin functioning within the adherens junctions between neighboring cells, and, upon activation, translocating to the nucleus and binding to transcription factors of the Tcf-LEF family (8, 10, 57). In its unphosphorylated, active form, β -catenin stimulates DNA transcription through Tcf4 and consequent cell proliferation. A well-known nuclear protein involved in carcinogenesis, c-MYC is one of the possible target genes of activated β -catenin (58). The Wntless/Wnt signaling pathway has been extensively investigated and has important roles in cell proliferation (8). The APC/ β -catenin pathway is its main intracellular effector route (8). There is a large body of evidence to support the theory of involvement of either APC or β -catenin mutations in the development of desmoids (59, 60). It has been observed that in desmoids of FAP patients, somatic and germline mutations of APC co-exist, suggesting a functional knockout of both alleles (60). Similarly, evidence for

involvement of the APC/ β -catenin cascade has been reported for sporadic desmoids too. However, it is currently not known whether APC or β -catenin mutations are driving oncogenic alterations in desmoid tumors (8, 10).

4.2.2. β -catenin and desmoid tumors

β -catenin was found to show high-level intracellular expression and nuclear localization in sporadic desmoids and recurrent tumors in comparison to the surrounding tissues (8, 59-63). Indeed, overexpression of β -catenin is considered to be a useful prognostic factor for reduced disease-free survival (62). Multiple studies reported that sporadic desmoids are usually characterized by oncogenic mutations in β -catenin and the absence of APC alterations (8, 10, 59-62). The CTNNB1 gene is located on chromosome 3p22, a region frequently altered in human malignancies (61, 63, 64). About 75% of sporadic desmoids are found to harbor somatic mutations in either the APC or β -catenin gene with a higher predominance for the latter in current investigations (10, 62, 65, 66).

4.2.3. APC and desmoid tumors

Several mutations of the APC gene in sporadic desmoids lacking β -catenin gene mutations have been reported and approximately 95% of these lead to expression of a truncated protein (8, 10, 44, 67, 68). It has been suggested, therefore, that the higher β -catenin protein levels are consequences of the APC gene truncation in the absence of β -catenin mutations (61, 67). It is proposed that APC mutations cause inadequate regulation of β -catenin activation, leading to proliferation on a cellular level, and the location of the germline APC or β -catenin mutation determines the phenotype of desmoid tumor expression (10, 54). The APC gene consists of a total of 2843 codons (10, 68). Most mutations in desmoids occur in the 3' amino end between codons 1445 and 1578, while mutations beyond codon 1600 are rare and frequently result in undetectable levels of truncated protein (10, 47, 51, 54, 68-70). Mutations at the extreme 3' end are associated with severe multifocal desmoid phenotypes with almost 100% penetrance, the so called C3 genotype (8, 10, 49, 68). The particular site of mutation, and the contribution of modifier genes are determinants of the phenotypic expression of an APC mutation (47, 54, 68, 70).

4.3. The endocrine etiology of desmoids

A possibility of functional interaction between ER and Wnt/ β -catenin signaling has recently been reported in human tumor cell lines and in *Drosophila*, in which estrogen signaling

appears to potentiate the effects of nuclear β -catenin (8, 38, 71, 72). Observations such as the significantly lower incidence rate of colorectal cancer in women undergoing hormone replacement therapy supported the finding, though evidence for interaction of the ER pathway with the APC/ β -catenin pathway is circumstantial (72).

The major mechanism of the functional interaction is presumably transcriptional modulation, although other mechanisms may be involved (71).

4. 4. Desmoid etiology and wound healing

Desmoid tumors have been observed to develop at sites of healing wounds and to show histologic similarities with dermal fibroproliferative disorders (33, 73, 74). Physiological wound healing is a tightly regulated, self-limited process in response to tissue stress or injury during which mesenchymal cells from various sources are mobilised and recruited to the site of the wound where they engraft and promote healing (33, 75-78). These cells include pluripotent hematopoietic stem cell (HSC)-derived monocyte precursors, that also home to sites of tissue injury, engraft, and differentiate into CD34⁺ fibrocytes mediating wound healing (33). While recruited stem/progenitor cells undergo terminal differentiation or apoptosis during the resolution phase of normal wound healing, under conditions of chronic inflammation or tumor progression, these activated cells persist (79). A synergistic cooperation of these multipotent cells support angiogenesis, a hallmark of accelerated wound healing and fibrosis (33, 80). Increased angiogenesis and proliferation of fibroblast-like cells within a collagen matrix are features of desmoids as well (33). Since genes characteristic of myofibroblasts were found to be expressed, the roles of persistent recruitment of monocyte precursors and defective wound healing resolution seem to be significant in desmoid tumors (81). Primary fibroblast cell lines have been derived from desmoids (81). Additionally, a recent study by Carothers et al.(2012) showed that desmoids resulted from the growth of MSCs in a wound healing setting, and an association with deregulated Wnt signaling due to APC loss was also confirmed (33). These findings suggest possible novel targets for the systemic treatment of this disease with the implication of MSCs in the etiology of desmoids.

5. Histology

The macroscopic appearance of desmoid tumors is firm and rubbery, and characterised by a relatively homogenic incisional surface of white and greyish network of bundles resembling scar tissue with a relatively poor vascularization (2, 5, 7, 8, 11). **Figure 2.** These lesions are typically poorly circumscribed and often infiltrate into surrounding soft tissue and skeletal

muscle bundles (8). A capsule is occasionally seen but its appearance is often misleading because the infiltration may be extending up to 2–3 cm beyond an apparently well-circumscribed mass (11). At microscopical presentation a proliferation of elongated, slender, spindle-shaped cells of uniform appearance, arranged in fascicles is seen, sometimes with perivascular oedema (8, 11, 82). **Figure 3.** As a consequence of the presence of abundant collagen around neoplastic cells, there is hardly any cell-to-cell contact. Cells also lack hyperchromasia or atypia and are typically arranged in bundles (5, 8, 11, 15). The mitotic rate is variable: one to three small nucleoli are usually seen in the pale cytoplasm (5). Cellularity may vary even within the same lesion (11). Telomerase length and activity are normal (15). According to these findings the lesions are histologically benign. Desmoids arising in the mesentery and pelvis show extensive stromal myxoid change and more fasciitis-like cytomorphology (11).

On electron microscopic examination, the spindle cells of desmoids appear to be myofibroblasts, supporting the theory of tumor development from abnormal proliferation (8, 73, 74).

Immunohistochemistry (IHC) is positive for vimentin, alpha smooth muscle actin (SMA), muscle actin, and desmin muscle cell markers (83, 84). **Figure 3.** Nuclear β -catenin immunoreactivity is staining positive in 67%–80% of cases in the reported series, but staining for nuclear β -catenin is not specific for desmoids, since it is also observed in 56% of superficial fibromatosis, 30% of low-grade myofibroblastic sarcomas and 22% of solitary fibrous tumors (8, 11, 15, 72, 83, 84). The IHC studies on hormone receptor status show 0 to 7.4% positivity for ER- α , 7.4% to 100% positive staining for ER- β , 0 to 25.5% for progesterone receptor-A (PR-A), 0 to 33.3% for progesterone receptor-B (PR-B) and 13 to 52.9% for androgen receptor (AR) (42, 72, 83-87). The IHCs for epidermal growth factor receptor (EGFR), c-erbB2, c-KIT, CD34 were uniformly negative reported in the literature and, in general desmoids are regarded as ER- α and c-KIT negative tumors (83, 84, 85, 87-90). IHC evaluation showed strong positivity for platelet-derived growth factor receptor (PDGFR) - β and absence of expression of PDGFR- α (89). IHC may play an important role in the differential diagnosis of desmoids.

6. Differential diagnosis

The differential diagnosis of desmoid-type fibromatosis is broad, ranging from reactive fibroblastic and myofibroblastic processes (eg. nodular fasciitis, hypertrophic scars, keloids) to fibroblastic sarcomas (2, 7). Histologically, desmoids are often indistinguishable from

nonneoplastic lesions associated with abnormal wound healing and differentiation from other soft tissue tumors, such as fibromyxoid sarcomas, can be challenging (33, 91, 92). The differential diagnosis of intra-abdominal desmoids includes gastrointestinal stromal tumor (GIST), solitary fibrous tumor inflammatory myofibroblastic tumor, sclerosing mesenteritis, and retroperitoneal fibrosis or secondary to certain drugs or an underlying malignancy, such as a lymphoma (32).

7. Clinical diagnosis of desmoid tumors

7.1. The value of the different biopsy methods in the diagnosis of desmoid tumors

For the definitive diagnosis a biopsy with histopathological confirmation is needed (93, 94). The efficacy of fine needle aspiration cytology (FNA) in desmoids is contradicting as there have been only limited experiences with it (8, 88, 94). Although over- and under-diagnosis of malignancy may occur, FNA is a fairly reliable tool for recognition of the benign nature of desmoids (94). Core needle biopsy (CNB) is the diagnostic method of choice: it is simple, and yields abundant representative material for the pathological examinations, allowing the recognition of histological characteristics (95). Preoperative CNB was not found to affect the rate of postoperative recurrence (22). The performance of incisional biopsy is sometimes inevitable (5). Definitive histological diagnosis is mandatory in tumors involving vital structures, requiring an extensive surgical procedure or neoadjuvant non-surgical treatments (2, 5, 7, 8).

7.2. Imaging features

Initial cross-sectional imaging of the affected area with computed tomography (CT) or magnetic resonance imaging (MRI) is needed to define the relationship of the tumor to adjacent structures in order to assess potential resectability and the need for treatment (7, 8, 25). **Figure 4.** Desmoids being observed or managed nonoperatively should undergo periodic imaging assessment. Both CT and MRI are valuable modalities for the diagnosis and differential diagnosis, since features of desmoid-type fibromatosis are characteristic of desmoids, but MRI is considered the primary modality for the imaging (7, 8, 15, 25, 96). Desmoids may have heterogeneous signal and inhomogeneous enhancement because of variable distribution of spindle cells, collagen, and myxoid matrix (25, 96). Ultrasonography shows desmoids as hypoechoic soft tissue masses with variable vascularity (31). **Figure 5.** The median maximum standardized uptake value (SUV) of desmoid tumors on 2-(fluorine-18) fluoro-2-deoxy-D-glucose-positron emission tomography (18F-FDG-PET/ CT) has been

reported to be 4.1 (range, 1.0–8.1) (8, 97). 18F-FDG-PET/ CT can help in assessing masses of unknown etiology and can aid in subsequent appropriate treatment planning (8, 98).

Histopathologic confirmation must be obtained in all cases before definitive treatment.

7.3. Evaluation of FAP associated desmoid tumors

Extensive family history, ideally the acquisition of a detailed, three-generation family tree is a very important part of clinical examination of patients with a desmoid tumor (11). Medical records of relatives with a history of colon polyps or colon surgery should be requested in order to evaluate the presence of FAP (11). A combination of endoscopy, genetic testing and observation of extracolonic manifestations is used in the diagnosis of FAP (53). Genetic testing is the most efficient mode of identifying gene carriers in a FAP relative and can be assessed by several mutation specific methods which utilize polymerase chain reaction (PCR) amplification of these genomic DNA regions, such as direct sequencing, heteroduplex analysis or single-strand polymorphism (53). Single-strand conformation polymorphism (SSCP) and heteroduplex analysis (HA) are popular electrophoretic methods for the identification of sequences (99-102). The principle reasons for the popularity of these two methods are their technical simplicity and their relatively high sensitivity for the detection of mutations. Patients with known APC mutations should be screened colonoscopically from the age of 10 – 12 regularly (11, 103). Individuals who are at risk with an unknown mutation status are recommended to have screening colonoscopies by age 15, every year from age 26 to 35, every other year from age 26 to 35, and every three years between ages 36 and 50 (53). An annual physical examination to screen for thyroid cancer is necessary, while liver US and serum α -fetoprotein are being investigated as possible screening tests for hepatoblastoma (53).

8. Treatment of desmoid tumors

The optimal therapy for desmoids is difficult to be established due to the heterogeneous clinical behavior and anatomical presentation associated to the variable etiology, the high local recurrence rate, the infiltrative growth and the rarity of the tumor (8, 15, 91). There is limited or no evidence based medicine supporting treatment choice (5, 7, 8, 15, 85). The general recommendations for the the efficacious management of desmoids consist of surgical and nonsurgical modalities and require a multidisciplinary approach (8). The treatment should be decided on case-by-case basis by the multidisciplinary soft tissue board. Desmoid is optimally treated only following precise diagnosis, in medical centers with expertise in the care of soft tissue tumors. Patients are often young, so psychosocial oncological care is

mandatory. Therapeutic alternatives could significantly differ between FAP associated and sporadic tumors, or between the localized and advanced diseases (2, 7, 8, 91). The treatment of the FAP cases is complex, and includes possible therapeutic and prophylactic procedures, Because the long term results are sometimes unpredictable, after thorough consultation and informed-consent the patient's choice is of great importance (53). Accurate surveillance is essential part of the management.

8.1. Observation

Close observation is an acceptable strategy for asymptomatic patients with small desmoid tumors not infiltrating any nearby structures or for patients of stable disease presenting with only few and mild symptoms (11, 15, 25, 28, 104-106). As spontaneous tumor regression or stabilization has been reported by many authors, depending on the etiology of desmoids, a conservative attitude is reasonable and may be considered when radical tumor excision cannot be performed, or only with significant functional or esthetic impact (28, 29, 107). The evolutive potential of extra-abdominal desmoids seems to be limited after 36 months, surveillance is recommended, using clinical examination and 6-monthly MRI to assess spontaneous recurrence before considering the commencement of any subsequent treatment (28, 29, 107). Even though no definite prognostic factors for tumor evolution have emerged to date, therapeutic intervention is indicated for rapidly growing tumors or for symptomatic patients if there is imminent risk to adjacent structures or if the tumors create cosmetic concerns.

8.2. Surgical treatment of desmoid tumors

If the preoperative diagnostics finds that a desmoid tumor is technically resectable, aggressive surgery is the first line treatment (2, 5, 7, 8, 11, 15, 25, 93). To date wide excision with 2 to 4 cm resection margins is generally recognized as the most effective available treatment for desmoids (2, 5, 7, 11, 15, 25). Complete resection of the lesion with negative microscopic surgical margins (R0 resection) is the standard surgical goal, however, it is not always possible due to anatomic boundaries (5, 7, 8, 11, 15, 25). Essential components of achieving complete resection include the adequate marking of the specimen, intraoperative consultation with the pathologist, and frozen section analysis (15). An important surgical issue in the treatment of desmoids is the anatomic location. The overall strategy should be to attempt complete removal using function-preserving approaches to minimize major morbidity, but even with radical surgical resection, the tumor may recur (2, 7, 8, 25). Incomplete resection is

associated with a higher risk for local recurrence of up to 85% at 10 years (2, 7, 15). Some large retrospective studies demonstrated that microscopically positive margins were predictive of a higher local recurrence rate while others failed to find a relationship between microscopic margins and recurrence, the importance of a negative surgical margin is thus recently debated (5, 7, 8, 106, 108-112). At present, microscopic positive margins may be acceptable if achieving negative margins would produce excessive functional or cosmetic morbidity (104). A significant correlation was found between the site of tumor and quality of surgery (105). Positive margins do not always or immediately need further treatment (112). Unplanned excisions were found to be associated with an extremely high rate of residual tumor (112). The pathologic assessment of margins should be made in collaboration with the surgeon, and the report should include an appropriate description of tumor margins (113).

8.2.1. Special surgical considerations by intra-abdominal desmoid tumors

Radical excision is often a therapeutic challenge or even impossible for intra-abdominal desmoids, confirmed by previous and recent studies which reported a rather low success rate of radical removal of intra-abdominal or retroperitoneal desmoids (8, 32, 91, 107, 113-116). In addition, these tumors have a high tendency for local recurrence. As the resection often necessitates removal of part of the small intestine, morbidity can be substantial and includes bowel ischemia, adhesions and obstruction, hemorrhage, fistula formation and short-bowel syndrome (21, 117). According to recommendations of the American Society of Colon and Rectal Surgeons, and a joint task force of the American Society of Clinical Oncology/Society of Surgical Oncology the correct approach is conservative management over initial resection for desmoid patients with Gardner syndrome or with large slow growing desmoids involving the mesentery (8, 104, 113, 118, 119). Surgical resection is indicated for symptomatic desmoids involving visceral organs and desmoids that are growing rapidly (32). In some cases only palliative surgical intervention can be performed. About 20% of patients with FAP and abdominal desmoids die of complications of desmoids (11). **Table 1.** According to the study of Quintini et al. (2012) the five-year survival of patients (n=154) with stage I, II, III, and IV intra-abdominal desmoids were 95%, 100%, 89%, and 76% respectively (27).

8.2.2. Special surgical considerations by thoracic desmoid tumors

Where anatomic features make it possible, surgical margins of 2 to 4 cm with en bloc removal are optimal (22, 23). Resection may involve all layers of the chest wall (full thickness resection) or it can be limited to certain layers (partial thickness resection) (22). Optimally,

full thickness resection includes one unaffected rib above and one below the lesion, as well as intercostal muscles, pleura, and a wide clear margin of adjacent soft and osteal tissues is applied (22, 23, 120). If paravertebral structures, the spine, the brachial plexus, great vessels, or soft tissues of the neck are involved by the tumor, radical excision may be difficult or impossible to perform, and palliative surgical diminution might be life-saving when compression of vital organs is present (22). Thorough examination and design of an individually planned treatment scheme should precede the surgical intervention, often requiring cooperation of more specialists. Primary wound closure can be achieved after removal of extensive areas of the chest wall and contiguous structures, without significant cosmetic deformity or loss of function (22, 120, 121). The overlying skin is usually uninvolved by the tumor, which further facilitates wound closure. Chest wall and intra-thoracic resections are characterized by a high rate of complications, ranging from 21 to 46% of cases. (22, 23, 120, 121). In particular cases, where adequate skeletal and soft-tissue reconstruction of the chest wall following a radical en block resection may be required, the latissimus dorsi flap is considered to be the flap of choice (22, 122). Adequate stability can be achieved by the use of synthetic meshes.

8.2.3. Special surgical considerations by abdominal desmoids

In contrast to intra-abdominal desmoids, studies are uniformly reporting favourable outcomes after surgery of abdominal desmoids, with high proportion of complete resection and low recurrence and morbidity rates (8, 11, 91, 110, 123, 124). The optimal surgical procedure is a wide full thickness abdominal wall resection with 2 cm margin. Following resection the residual soft tissue defect needs to be reconstructed either by autologous flaps or more commonly by prosthetic grafts or combination (125). The remaining skin is usually sufficient for closure. The superficial small desmoids should be excised with partial-thickness abdominal resection not involving the peritoneum, and reconstruction can be achieved by direct repair with sutures (34, 123). Radical resection followed by one stage mesh reconstruction remains the gold standard in the management of desmoid tumors of the abdominal wall (11, 91).

8.2.4. Special surgical considerations by extra-abdominal desmoids

Extra-abdominal desmoids can occur anywhere and due to the infiltrative growth pattern CT or MRI is necessary to detect tumor boundaries (5, 7, 8, 34, 93). The basic idea of the surgical care is the same, wide excision with negative histological margins should be attempted, but not at the expense of loss of function (2, 52, 93). This principle requires that clinicians

perform difficult surgery (7). As negative histological margins are no guarantee of local control, and the functional consequences of radical excision may be worse than the disease itself, major nerve trunks, even if marginally involved, should be preserved, and amputation should be extreme rarely required (34, 52, 110, 125-128). Apart from the aspect of function sparing and avoidance of amputation, disease-free margin seems to be crucial for reducing the recurrence rate (2, 7, 52). Intraoperative frozen section margin assessment can be helpful by the surgery of extra-abdominal desmoids (5, 8). The greatest challenges to the surgical oncologist are large desmoids which tend to be extremely locally invasive at the time of examination, frequently growing near neurovascular structures or bone (7, 11, 129-131). An intracompartmental desmoid can be treated with a complete compartmental excision, the most frequently applied techniques as reported in the literature are buttocks resection, adductor compartment resection, posterior thigh resection and quadriceps resections (2, 7, 9, 25, 132). Amputation of an extremity is extraordinary and can be considered if an aggressive desmoid massively encases the neurovascular bundle, debulking and other non-surgical therapies are ineffective or if a limb-sparing operation would not be of any functional use (5, 52, 132). Multidisciplinary treatment decision making is mandatory in these extreme cases (52). Complicated and technically demanding plastic reconstructions may be necessary, using local or distant myocutaneous flaps to reconstruct the soft tissue defect and even vascular grafting (2, 5, 11, 15, 25). A fundamental element of surgical care of extra-abdominal desmoids is adequate postoperative rehabilitation.

8.3. Non-surgical treatments of desmoid tumors

8.3.1. Non-surgical locoregional therapy of desmoid tumors

8.3.1.1. Radiation therapy

Radiation therapy (RT) is considered an effective option in the therapy of desmoid tumors, that may improve local control in both adjuvant and primary settings, as indicated by large retrospective studies (2, 7, 8, 19, 133, 134). Due to the risk for radiation induced neoplasms, which is of particular concern in the young patient population, its use shall be well balanced against the potential for late effects. The time to regression after RT alone is often quite long, the best results are associated with high dose radiation and it may take up to two years for the tumor to regress (8). The role of adjuvant RT in achieving local control has not yet been clearly defined and is still controversial. Postoperative RT is considered only in patients with a large tumor and positive margins as suggested in the National Comprehensive Cancer Network (NCCN) guidelines (104). The recommended dose of RT for definitive therapy is

50–60 Gy in 5–7 weeks at 1.8–2 Gy per fraction (104). **Figure 6.** A review of 22 articles including 780 patients found that RT alone (dose range, 10–72 Gy) and surgery combined with RT resulted in significantly better local control (78% and 75%) than with surgery alone (61%) over a median period of 6 years (135). Relapse rate decreased from 59% to 25% in patients with positive margins of both primary and recurrent desmoids after surgery when RT was added postoperatively. The complication rate from RT was 22.8%, with tissue fibrosis being the main problem, and in-field recurrence occurred especially if the total irradiation dose was lower than 50 Gy (135). However, a recent retrospective analysis of 95 sporadic desmoid cases did not detect statistical differences in the rate of local control between the groups receiving only surgery, only RT, and a combination of both (136). Only patients with head and neck desmoids and a history of antedescendent surgery presented with a higher risk of recurrence. Higher doses do not appear to reduce local recurrence rates (137). Neoadjuvant RT is a novel approach to increase the resectability and reduce local recurrence rates in extra-abdominal tumors (8, 93). To evaluate the efficacy of RT for irresectable desmoids, the Soft Tissue and Bone Sarcoma Group of the European Organization for Research and Treatment of Cancer (EORTC) performed a pilot nonrandomized, phase II study study (EORTC 62991) (138). The trial recruited 44 patients in 2008, and follow-up is still in process; the final analysis is awaited in the near future. In summary, postoperative RT is indicated in cases with large tumors and positive margins where it may significantly reduce the local recurrence rate. If a myocutaneous flap is used, brachytherapy is an ideal treatment option for these tumors with small surgical beds and limited need for radiation field (8).

8.3.1.2. Locoregional chemotherapy

Based on the finding that desmoids rarely form metastases, locoregional chemotherapy in the form of isolated limb perfusion is an alternative to systemic chemotherapy in patients with limb desmoids (2, 7, 8, 11, 25). Although intratumoral hypervascularity disappears after perfusion quite rapidly, it may take up to several months until a partial or complete response develops (25, 113). Overall response rates with melphalan and recombinant human tumor necrosis factor- α were found to reach 80% (2). This method is particularly beneficial for patients with locally advanced or recurrent disease not amenable to function-preserving resections (113, 139).

8.3.2. Non-surgical systemic therapy of desmoid tumors

While surgery remains the mainstay for resectable sporadic tumors, for patients with positive resection margins, advanced desmoids that are not amenable to surgery or RT, various medical treatment options including antihormonal therapies, non-steroidal anti-inflammatory drugs (NSAIDs), targeted therapies, and traditional cytotoxic chemotherapies have been investigated (5, 7, 8, 15). **Table 2.** The choice of systemic therapy is a major determinant of the long-term outcome (8). In the lack of sufficient evidence from multiple controlled trials and comparative studies, evidence-based therapeutic regimens are not available yet, neither are prognostic or predictive factors for monitoring therapies (22, 121). Only a subset of patients respond to therapies, and even response evaluation according to the conventional Response Evaluation Criteria in Solid Tumors version 1.1. (RECIST) may be difficult in a tumor that can have spontaneous growth arrest and variable growth patterns (134, 141). Currently, a stepwise approach is recommended. Less toxic modalities such as antihormonal therapy or NSAIDs are preserved for patients with disease without any impending threat to life or function (7, 112, 115, 142). The next stage is cytotoxic chemotherapy for fast growing desmoids not responsive to first-line agents (8). As most of the reported data are derived from case reports, some but limited conclusions can be drawn as to the relative effectiveness of systemic treatments (2, 8, 15). Response rates of 50% or higher are reported, but the true objective regression rates with these agents are in the range of 10%–15% (21). Another 25% of patients experience minor shrinkage or tumor stabilization (21). As the maximum effects are often not realized for several months, so the evaluation of systemic therapies is rather complicated (2, 7, 8).

8.3.2.1. Antihormonal therapy

The implementation of antihormonal therapy in the treatment of desmoid tumors is empirical, based on observations of the natural history of the disease (higher incidences of desmoids during and after pregnancy and reports of spontaneous tumor regression after menopause) (5, 7, 8, 15, 38, 113, 117, 143-148). **Table 2.** Studies have shown uniform ER- α negativity and a high rate of nuclear ER- β expression in desmoids (72). Selective estrogen receptor modulators (SERM) are compounds with a mixed agonist/antagonist activity on ERs in different tissues (145). The mechanism of SERM in the treatment of desmoids is not fully understood. A contradictory observation is that ER negativity does not necessarily mean that anti-estrogens are ineffective (2, 8, 15, 117). SERM may be therapeutic through additional pathways in ER negative disease, in addition they are believed to exert inhibitory effects on angiogenesis as well (38, 84, 117, 148-151). Many citations are found in the literature in relation with

tamoxifen, mostly case reports or retrospective single arm surveys involving a low number of cases, demonstrating some sort of response or disease stabilization (5, 8, 84, 117, 143). According to the results of a non-randomized trial, high-dose tamoxifen (120–200 mg/day) may be more effective than lower doses of 10–40 mg/day (38). However, there is no randomized data supporting the extended use of higher doses, and the risk for secondary neoplasia and deep venous thrombosis could be greater with its use (38). No firm statement regarding the effectiveness of tamoxifen in the treatment of desmoids can be concluded.

8.3.2.2. Non-steroidal anti-inflammatory drugs

The use of NSAIDs against desmoids is based on observations too (5, 7, 8, 11, 15, 25, 146).

Table 2. Since cyclooxygenase-2 (COX-2) was found to play a role in the growth of desmoids, and NSAIDs demonstrated influence on the β -catenin pathway, therefore treatment with NSAIDs that inhibit COX may be effective (152). A variety of NSAIDs were tested in the treatment of desmoid patients, alone or in combination with hormonal agents (117). Patients treated with sulindac showed an overall response rate of about 50%, and most of the responders experienced a mean 24 months delay in response (117, 152). Although not confirmed through a randomized comparison, the administration of sulindac with or without tamoxifen seems to be an effective non-cytotoxic drug treatment (38, 146). Endocrine and/or NSAID therapy is considered first-line pharmacological treatment for irresectable, advanced disease without clinical symptoms (112, 115, 142).

8.3.2.3. Interferon

The precise mechanism of action of interferon-alpha-2b in desmoids is unknown, but several case reports described objective responses or prolonged periods of disease stabilization, following failure on sulindac and tamoxifen (8, 153). A direct growth-inhibitory action was proposed after IHC of tumor tissue had revealed in situ expression of the interferon-a/-b receptor in the majority of desmoid fibroblasts (154, 155).

8.3.2.4. Cytotoxic chemotherapy.

A wide variety of chemotherapy regimens have been used in the management of irresectable, rapidly growing, symptomatic, life-threatening advanced desmoids, based on evidence from small, mostly retrospective studies (2, 7, 8, 21, 32, 156-158). **Table 2.** Response rates range from 50% to 80%, but assessment is challenging especially in static or slow growing tumors as disease stability may represent either benefit from chemotherapy or simply slow

progression of the tumor (8, 156-158). Due to the rarity of this disease, direct comparison is difficult to perform. The use of chemotherapy in desmoids is still controversial, due to the lack of metastatic potential of desmoids, the tendency of chemotherapy to cause severe complications and the increased risk of treatment-induced malignancy (8, 21, 32, 156).

8.3.2.5. Targeted therapies: Imatinib mesylate and sorafenib

Imatinib mesylate, a selective tyrosine kinase inhibitor, has also been investigated, with promising results (2, 7, 8, 89, 90, 157, 159). Partial or even complete response was reported in 16%–80% of patients with or without c-kit or identified PDGFR- α mutations in earlier studies (8, 159). A cooperative phase II study found that progression free survival at 2 and 4 months was 94% and 88%, respectively, and 66% at 1 year (85). The overall progression-free survival in a French phase II study at 2 years was 55% (89). However it is uncertain whether the response is a result of inhibition of imatinib targets (c-kit, PDGFR- α and PDGFR- β kinases) (2). The French Sarcoma Group found that none of the biological factors, including PDGFR- α and PDGFR- β , β -catenin, c-kit were correlated with response, progression-free survival, or overall survival (89). Imatinib is not yet licensed for the treatment of desmoid tumors and is still subject to research.

Sorafenib, a different multikinase inhibitor, was administered to patients with desmoid tumor in a study and approximately 70% of patients reported resolution of symptoms. (8). Twenty-five percent responded with a partial remission by RECIST and 70% had stable disease after a median of 6 months, and most patients also experienced radiologic improvement (8, 160). The authors concluded that sorafenib should be further tested, possibly against other agents active in desmoid.

9. Posttreatment surveillance

Given the increased potential for recurrence, regular clinical and possible imaging follow-up have been strongly recommended after therapy (2, 7, 8). Consensus-based guidelines from the NCCN suggest that a history and physical examination with appropriate imaging (preferably MRI) every 3–6 months for 2–3 years and then annually (104).

IV. AIMS OF THE THESIS

Accordingly, it is concluded from the above, that a relative paucity of clinical and pathological data is available on sporadic desmoid tumors. As the data found in the English language literature are mostly based on the meta-analysis of case reports or series, or retrospective studies with low number of cases, a global lack of knowledge is recognised regarding risk factors, multidisciplinary treatment modalities, prospective and predictive factors and long-term follow-up results. Despite the lower incidence of FAP associated desmoids, the knowledge of these hereditary tumors is more extensive. Given these facts, the main aims of our investigations were as follows:

1st aim. To establish a data base of sporadic desmoid tumors adequate for analytical purposes and, to collect clinico-pathological data over a long follow-up period in order to find scientific answers to the questions of etiology, pathology and clinical behaviour.

2nd aim. On the basis of a detailed clinico-pathological database the first planned step was to confirm the sporadic origin of desmoids by ruling out the FAP associated germ line mutation of the APC gene by clinical and genetic investigations.

3rd aim. To confirm the theory of the etiology of sporadic desmoids, by detecting the β -catenin mutation status with molecular-genetic probes in a large number of tissue samples, quilting as one of the largest single cohort reported in the literature.

4th aim. To analyse and compare the clinical and pathological data of the very rare thoracic as well as the more common abdominal tumors and desmoids located on the extremities.

a. Radicality of first surgery and its influence on local control.

b. The analysis of the surgical techniques and the reconstructive methods after wide soft tissue resections (e.g. long time results with mesh reconstructions after the resection of abdominal desmoids).

c. The correlation of genotyping results with clinical and immunohistochemical features, including time to recurrence and β -catenin protein expression pattern and intensity.

d. To establish possible prospective and predictive factors on the basis of the correlation of genotyping and long-time clinical course.

5th aim. a. Assessment of the expression of ERs and PR in sporadic desmoid tumors by immunohistochemical analysis using anti-ER- α , anti-ER- β and anti-PR antibodies.

b. The comparison of the clinical tumor status with the adherent ER- β status of patients following endocrine treatment.

V. MATERIALS AND METHODS

1. The creation of a cohort of sporadic desmoid cases and a data base

With the approval of the Institutional Ethical Board, a cohort of desmoid cases was collected by the investigator, on a partly retrospective and prospective multicentric way, using the soft tissue tumor database of the National Institute of Oncology (NIO), the Semmelweis University Orthopaedic Clinic and the Thoracic Surgical Clinic of Korányi National Institute of Pulmonology. The cases were multidisciplinary treated between 1982 and 2011 in the above detailed centers. The medical records (including family and personal anamnesis, physical status, documentation of the surgical procedures, pathological befunds, records of diagnostical imagines) and tissue samples were collected by personal research of the archives of the institutes, by sending letters to patients requesting copies of documentation or by interviews over the phone. Eventually, all patients were invited for a face to face consultation and clinical investigation. The participation was voluntary. Clinical information including demographic, therapeutic, tumor, and clinical outcome variables were retrieved and tabulated for correlative analyses. During the last 5 years of the study altogether 23 primary or recurrent desmoid cases were surgically treated in the department of the investigator, consequently the clinical data were collected prospectively in these cases.

Occasional (28 cases) or regular follow-ups (69 cases) were realized by 97 patients in the NIO with the succesful completion of the clinical and anamnestic database. Regular follow-ups were performed as recommended in the literature: clinical examination (performed by the investigator) and radiographic studies every 6 months for the first postoperative 3 years, every 12 months from 3 to 6 years, and then annually.

The tissue samples (paraffin-embedded in archived cases) of all cases were officially acquired from all three pathological departments and histologically revised by an independent reference pathologists in the NIO.

Demographic and clinical data were gathered include age, sex, personal and family history, antedescend trauma to the site of the tumor, hormonal status (menopausal status, parity, use of oral contraceptives) the efficiency of imaging and biopsy techniques, clinical tumor size, local control and follow-up results such as number of recurrencies, disease-free survival, time to progression or overall survival. Therapeutic data acquired consist of the type and number of surgical interventions, surgical margins, reconstructive plastic surgical options, perioperative morbidity, mortality, adjuvant therapy administered and its efficacy using the RECIST 1.1. criteria. Pathological data consist of the pathological tumor size, the circumferential

microscopical surgical margin, results of IHC assays. The database is continuously updated enabling long term follow-up and analytical comparison of the data.

A limitation of the study was the principally retrospective collection of data, the incidental low-quality of a few decade-old pathological tissue samples, and the low number of cases preventing a reliable statistical comparison of subsets of patients. Although during the nearly 30 years of patient recruiting period, surgical resection and non-surgical therapies as first choice of treatment have not or only minimally (imatinib) changed, a further limitation was proposed by the non-controlled therapies.

2. Confirmation of the sporadic origin of desmoid tumors by ruling out the germ line mutation of APC gene.

All participants were asked for detailed family history, and a three-generation family tree was delineated. Medical records for family members who have developed colon polyps and/or underwent colon surgery were requested, and took a survey in order to evaluate the presence of FAP. Colonoscopy was performed or former medical records were checked for all the patients. Thirty-nine individuals included in this study were referred to genetic counseling and testing to the Department of Molecular Genetics at the NIO. All investigations have been performed in agreement with international guidelines, the study protocols were approved by the Institutional Ethical Board. Written informed consent was signed by each patient. DNA was extracted from peripheral blood samples of all consenting subjects using the classic phenol-chloroform method. The entire coding region and splice junctions of the APC gene were amplified by PCR using primer sequences a method described by Miyoshi et al. (1992). (100). PCR assays were set up using an AmpliTaq Gold PCR Kit (Applied Biosystems) according to the manufacturer's instructions. Cycling condition entailed an initial denaturation at 95°C for 7 min, 40 cycles of denaturation at 95°C for 30 sec, annealing at 51°C for 30 sec and elongation at 62°C for 30 sec, followed by a final extension at 62°C for 7 min using a GeneAmp PCR System 9700 thermal cycler (Applied Biosystems). Mutation pre-screening by was done on the resulting PCR amplicons using SSCP/HDA (single-strand conformation polymorphism /heteroduplex analysis) technique (101). Bands on gels were visualized by silver staining (102). Where altered mobility of a sample indicated the presence of a variant, the fragment was subjected to direct bidirectional DNA sequencing using the BigDye Terminator v1.1 Cycle Sequencing Kit according to the manufacturer's instructions and protocol (Applied Biosystems). Completed sequencing reactions were electrophoresed on an

ABI 310 Genetic Analyzer (Applied Biosystems). The presence of mutations was confirmed using a different blood sample.

3. Detection of the β -catenin mutation status with locked nucleic acid (LNA) probe-based real-time polymerase chain reaction (PCR) followed by melting curve analysis.

Cases of diagnosed FAP or a family history of FAP (3 cases) were excluded, consequently only sporadic desmoid tumors were evaluated. As tissue samples were only partly suitable in quality (some of them were older than 10-20 years) for molecular-genetic investigations, an amount of 58 specimens from 51 patients were found to be eligible for the β -catenin mutation analysis. The samples from formalin-fixed, paraffin embedded tissue sections were subjected to cell lysis with proteinase K treatment (Magna Pure DNA Tissue lysis, proteinase K, Roche, Germany) followed by DNA extraction using magnetic bead technology (Magna Pure CNA, Roche Diagnostics, Germany) in accordance with the manufacturer's instruction. The purified samples were stored at 4°C. For the subsequent PCR analysis, a set of primers was chosen to amplify a specific 172-bp genomic fragment of exon 3 region of β -catenin gene: forward 5'gtcttacctggactctggaa3'; reverse 5'aataatactcttaccagctactgt3'. To distinguish the different mutation types occurring in desmoid tumors in codon 41 and 45 of exon 3, we used 45F mutation specific LNA spiked hybridization oligo covering this region (a + mark is written before LNA nucleotides): probe1 5'LCRed640-tcag+a+a+aaggagctgtgtag-PH3'; probe2 5'acatcctcttctcagattgcctttacc-FL3'. The reaction mixture of a total volume of 10 μ l contained 0.5 μ M forward and reverse primer, 0.2 μ M each of the probes, 3.25mM MgCl₂, 10x ready-to-use master mix (LightCycler FastStart DNA master HybProbe kit, Roche) and 2 μ l of DNA template. After initial incubation step (10 minutes for 95 °C), the conditions of the PCR were 95 °C for 10sec, followed by 60°C for 6 sec and 72°C for 8 seconds with 80 cycle time. DNA from HCC15 cell line (DSMZ, Germany) was used as 45F mutant control in each run and water as negative control for contamination was also processed in parallel with each sample. After the thermal cycling we performed melting curve analysis to detect sequence variations in the amplicons (95 °C for 15sec, 40°C for 1min to 95 °C for 0sec with continuous measure on F2 channel with 0.1°C/s ramp rate). We found that applying a second melting curve analysis gave better melting points. Due to the increased thermal stability of LNA-DNA duplexes that improves mismatch discrimination during melting curve analysis, it became capable of more sensitive single nucleotide genotyping. Our results were confirmed

by bidirectional sequence analysis on ABI-PRISM 3100 Genetic Analyzer using the BigDye terminator kit (Applied Biosystems, Foster City, CA, USA).

The HybProbes consist of two specifically designed oligonucleotides: a 3' fluorescein and another with a 5' LightCycler (LC) Red dye. When probes bind to the target DNA 1-5 bases apart, fluorescence resonance energy transfer (FRET) occurs. During FRET, the fluorescein molecule is excited by the LED light source and transfers its energy to the LightCycler Red dye. The emission of the LC Red dye is read in a detector channel of the LightCycler instrument. To identify and genotype DNA products after PCR a melting program is created by slowly increasing the temperature and measuring changes in fluorescence as the 3' probe melts. Single nucleotide mismatch between a 3' fluorescently labelled probe and its amplicon can significantly reduce the melting temperature of the heteroduplex because of the destabilization of probe-target DNA binding. The melting temperature shift between a normal allele-probe match and a mutated allele-probe mismatch causes different fluorescence profiles and indicate the presence of a mutation. This technology permits reliable identification and genotyping of DNA products. However the sensitivity of this method is limited to detect one type of sequence variation in the given region. To improve the resolution of this method we designed a rapid and sensitive real-time PCR method using LNA spiked hybridization probe. Due to the increased thermal stability of LNA-DNA duplexes that improves mismatch discrimination during melting curve analysis, it became capable of identifying multiple genotypes.

4. Three groups were formed following the analysis and comparison of clinical and pathological data: sporadical desmoid tumors of the chest, abdominal wall tumors and fibromatoses originating in the extremities.

Dividing the sporadical desmoid cases into 3 different groups seemed a reasonable option, based on the diversity of the surgical techniques, radicality and methods of tissue reconstruction according to the location of the tumors of the available 94 sporadical cases in the database. The clinical data of the different groups enabled a more reliable comparison with the results in the English language literature (Pubmed, Medline using the following keywords: „desmoid tumor”, „aggressive fibromatosis”, „deep fibromatosis”) in concordance with other published comparative reviews. There were some parameters consistent in the three groups while some other were specific for only one. The therapeutic protocol for desmoids was invariable during the investigated period. First-line treatment was surgery with an established goal of a 1 to 4 cm surgical margin via wide resections. The radicality of the

surgery was considered to be R0 if the circumferential surgical margins were microscopically negative, R1 and R2 if microscopic residual tumor and gross residual disease respectively was left behind. The largest diameter of the tumor and the status of the surgical margin were defined by the pathological befund. In all the cases with subfascial soft tissue resection, or with a surgical reconstruction using myocutaneous flaps or mesh, one or two crossing drains were positioned and were removed if the daily secretion was decreased to or below 30 mL. All operations were performed under antibiotic prophylaxis with amoxicillin or cefazolin (a single intravenous shot of 1000 mg of amoxicillin and 200 mg of clavulanic acid or 1000 mg of cefazolin), or continued until the suction drains were removed over the implanted mesh graft. Patients treated during the last decade were administered low-molecular-weight heparin initiated on the first preoperative day and continued for 2-4 weeks after surgery to prevent thromboembolic disease. According to the applied protocol, patients exposed to the potential of major complication (intra-thoracic location or lesion adjacent to joint capsule or great vessels) and those with R1 resections received 20 mg of oral tamoxifen daily, otherwise close surveillance was implemented. Higher dose of tamoxifen (120 mg daily) and sulindac (150 mg/twice daily) were administered to patients with irresectable lesions or R2 resections. In cases of recurrence, a daily dose of 20 to 120 mg tamoxifen and 150 to 300 mg sulindac were administered. Sulindac was contraindicated in 14 patients due to the known cardiovascular side effects. The use of tamoxifen was omitted in 3 cases because of a prior thromboembolic event. The therapy of high dose tamoxifen in combination with sulindac was continued for a maximum of 24 months, followed by maintenance daily doses of 20 mg of tamoxifen and 150 mg of sulindac. Patients who received sulindac were under regular cardiological control. Radiotherapy was indicated in aggressive cases showing rapid progression in an adjuvant setting or in irresectable cases showing tumor progression. The dose of RT for definitive therapy was 50–60 Gy in 5–7 weeks at 1.8–2 Gy per fraction, with wide radiation field margins of at least 5 cm applied in the direction of possible infiltrative growth. Doxorubicin based chemotherapy was administered in a single advanced case locating in the neck with life-threatening morbidity. Operative morbidity was recorded within the first 30 days after surgery or during the same hospitalization. There was no record of malignant disease in the histories of the 94 investigated patients. The soft tissue follow-up protocol of the participating institutes corresponded with the international recommendations. Thoracic CT or MRI scans were performed annually or if recurrent tumor or progressive disease was suspected. The investigated cases were analyzed for family history, age, gender, previous trauma to the site of the tumor, size and site of the tumor, radicality of surgeries, results of IHC assays,

reconstructive plastic surgeries, perioperative morbidity, number of recurrences, time to recurrences, and adjuvant therapeutic modalities.

For all patients, analysis of time to recurrence was calculated from the first surgery of primary lesions to the diagnosis of the first recurrence.

5. Assessment of the expression of ERs and PRs in sporadic desmoid tumors by immunohistochemical assays.

Histological samples of sporadically occurring primary desmoids acquired from 67 patients were eligible for the IHC investigations. Each diagnosis was reviewed and confirmed based on the relevant WHO criteria. Formalin-fixed paraffin-embedded specimens were cut into 4 μ m sections and mounted on silanised slides. Sections were deparaffinised, rehydrated, then washed in Tris-buffered saline (TBS) and subjected to heat epitope retrieval in a microwave in pH 6 citrate buffer. Slides were then incubated in 1% hydrogen peroxide in methanol for 5 minutes to block endogenous peroxidase activity, followed by incubation for 20 minutes in a protein blocking solution to reduce non-specific antibody binding. The primary anti-ER β antibodies (Polyclonal Erb88; BioGenex, San Ramon, CA, USA) (dilution: 1:200), anti-ER α antibodies (Mouse monoclonal clone ER88; BioGenex, San Ramon, CA, USA) (dilution: 1:40) and anti-PR antibodies (Mouse monoclonal clone: PR88; BioGenex San Ramon, CA, USA) (dilution: 1:40) were applied for 1 hour at room temperature. Slides were then incubated for 30 minutes at room temperature with the respective anti-mouse or anti-rabbit immunoglobulin (Ig) G antisera conjugated to a horseradish peroxidase (HRP)-labeled polymer (Dako Envision System, DakoCytomation Denmark A/S, Glostrup, Denmark), treated for 5 minutes with 3-3'-diaminobenzidine (DAB) chromogen, counterstained with hematoxiline, and coverslipped. Normal and neoplastic human breast tissue from the surgical pathology laboratory were used as positive control tests. Normal mouse or rabbit sera were used instead of the first antibodies in negative control tests. Classification as positive was done either according to company guidelines or institutional standard protocols for the positive controls: a minimum of 10% of the tumor cells had to be positive for ER- α , ER- β or progesteron receptor. Only definite nuclear staining was regarded as positive and cases were scored by the percentage of tumor cells staining as 1+ (<10%), 2+ (10-50%), or 3+ (>50%). Normal breast tissue served as control for ER- α , and benign fibroadenoma served as positive control for ER- β , and were run parallel. The staining on the entire array was performed at the clinical IHC facility of NIO on two occasions and each was scored independently.

6. Statistical methods

Categorical data were compared using Fisher's exact probability test and chi-square test. Recurrence free survival analyses were done using the Kaplan—Meier method. Recurrence free survival intervals were determined as the time period from initial diagnosis to the time of first recurrence. Univariate analysis of potential factors affecting recurrence-free survival (gender, age, tumor size, radicality of the first surgery, and impact of the pharmacologic treatment) was performed using Cox-regression hazard model. Differences were considered to be statistically significant when $p < 0.05$. Statistica 8.0 (StatSoft, Tulsa, OK) was used to perform all of the statistical calculations.

VI. RESULTS

1. The successful gathering of a cohort of sporadic desmoid cases and creation of a database

A cohort of altogether 97 desmoid cases was collected by the investigator. Fifty-seven cases were primarily treated in the NIO, 38 cases at the Semmelweis University Orthopaedic Clinic and 2 cases at the Thoracic Surgical Clinic of Koranyi National Institute of Pulmonology. Three cases were excluded from the investigation due to confirmed hereditary origin. Patients were multidisciplinary treated between 1982 and 2011 in the above named centers. In all the cases the personal attendance of the patient, family and personal anamnesis as well physical investigation and completion of the medical records were successfully achieved. The 94 sporadic desmoid cases represented 66 female and 28 male patients with a female predominance (2.4 : 1). The median age of the patients was 31 years (range, 9—74 years) and fifty-three female patients (80.3%) were premenopausal. Physical, often surgical trauma to the site of the lesion preceded the development of desmoids in 26 patients (28%). Desmoids were located on the region of the chest (including the neck, shoulder girdle and the axillary space) in 28 patients, in the abdominal wall in 28 patients and in the extremities (including the buttocks) in 38 patients. None of the patients had multicentric disease. As for the surgical radicality of the primary tumor, 52 cases (55.5%) resulted in R0, 39 cases (41.5%) in R1 and 3 cases (3%) in R2 resections. A reconstructive surgical procedure was performed in 54 cases (57%). Perioperative morbidity was detected in 19 cases (20%), while perioperative mortality didn't occur. The median pathological diameter of the primary tumors was 61 mm (range, 20—250 mm). Adjuvant therapy was commenced for 50 patients (53.2%), while 44 patients (46.8%) did not need adjuvant treatment. The non-surgical modalities in the adjuvant setting varied greatly. Tamoxifen monotherapy in 20 cases (40%), tamoxifen in combination with sulindac in 17 cases (34%) including one patient who received imatinib too. Sulindac monotherapy was chosen for 2 patients (4%). Ten patients (20%) received adjuvant RT, 8 cases (16%) in combination with tamoxifen and complemented with sulindac in one case, and with chemotherapy in another one. Tamoxifen was administered to 48 out of the 50 adjuvant therapeutic cases (96%). Follow-up was completed in all investigated patients and ranged from 8 to 336 months, with a median of 98 months. During the follow-up period a total of 122 local recurrence happened. Taking the 3 cases with R2 resection into consideration, among the 91 cases with R0 or R1 resection 38 cases (41.8%) were recurrence free and local recurrence occurred in 53 cases (58.2%). Among the 91 cases with R0 or R1 resection 38 cases

(41.8%) were recurrence free and local recurrence occurred in 53 cases (58.2%). Twenty-two cases developed one, 13 cases two and 18 cases 3 or more local recurrences. Surgical resection due to a recurrent tumor was performed 105 times. The radicality (R0 vs. R1 or R2) of the re-resections decreased significantly ($P=0.0016$) from the 2nd recurrence on. **Table 3a and 3b.**

2. Confirmation of the sporadic origin by ruling out germ line mutations of the APC gene by clinical and genetic investigations.

Three patients were found to carry a deleterious APC mutation in the screened section of the gene and were subsequently excluded from the further investigations. **Table 4.** All of these patients were diagnosed with both desmoid tumors and prominent colorectal polyposis. Interestingly, two of these mutations were outside the classical „desmoid-cluster” region of the gene, suggesting that genotype-phenotype correlations should be inferred with caution. **Figure 7 and 8.** The three patients with hereditary desmoids were excluded from the further investigations. At the last follow-up only one of them was alive. **Table 5.** The remaining 94 cases were confirmed as sporadic desmoids. None of them had a positive family history, nor developed polyposis controlled by colonoscopy during the follow-up period. In conformity with the literature, our investigation approved the reliability and cost effectiveness of genetic testing the APC gene.

3. The detection of the β -catenin mutation status.

3.1. CTNNB1 mutations are highly prevalent in sporadic desmoid tumors

Fifty-eight tissue samples from 51 patients were eligible for evaluating the prevalence of CTNNB1 mutations. All cases had sufficient DNA extracted for CTNNB1 exon 3 genotyping and the results were definitive in all cases. The database provided appropriate clinical information in relation to the 35 female (68.6%) and 16 male (31.4%) patients. Median age was 33 years and ranged from 8 to 66 years. The following sites were involved: chest in 13 cases (25.5%), abdominal wall in 17 cases (33.3%) and extremities in cases 21 (41.2%). Tumors presented with variable size ranging from 20 to 240 mm (median, 83 mm). Samples from primary lesions were available for all the 51 patients, and by 5 patients 7 tissue samples from recurrences were found appropriate as well for the investigations. A mutation was identified in 30 (58.8%) of the 51 primary tumors. The CTNNB1 exon 3 mutation profile in tissue samples taken from recurrent desmoids were 100% identical with the mutation profile of the primary tumor of the same patients. Female patients were more likely than male

patients (60% versus 50%) to have a mutation but the difference didn't prove to be significant ($P=0.387$). Next the CTNNB1 mutational spectra was determined. Interestingly, only 3 different point mutations in 2 different codons (41 and 45) could be identified in all mutated samples ($n=30$ of 51 total): ACC to GCC in codon 41 (41A), resulting in the replacement of threonine by alanine, was identified in 15 samples (50%); TCT to TTT in codon 45 (45F), resulting in the replacement of serine by phenylalanine, was identified in 12 cases (40%); TCT to CCT in codon 45 (45P), resulting in the replacement of serine with proline, was identified in 1 sample (3.3%) and a deletion was identified in 2 cases (6.7%). **Figure 9-11.** Upon analyzing the incidence of the specific CTNNB1 gene mutations and their correlation to patient and tumor variables, a higher incidence of the 41A and 45F mutation was identified in females versus males (75% versus 25%, and 73.3% versus 26.7% $P=0.922$). In terms of tumor site, lesions involving the extremities showed a significantly higher incidence of the 45F mutation (58.3%, $P=0.018$) and tumors originating in the abdominal wall showed a significantly higher incidence for 41A mutations (60%, $P=0.037$) versus all other sites.

3.2. CTNNB1 45F mutations significantly correlate with increased desmoid recurrence

The investigator assessed the correlation of the mutation of any specific CTNNB1 gene with clinical outcome. The different CTNNB1 gene mutational cohorts were found to present with different recurrence-free survival rates, which was significantly inferior for patients with the 45F genotype ($P=0.008$) (Cox-regression). **Table 6.** Only one sample was found to harbor a 45P CTNNB1 gene mutation, and so this tumor was excluded from analysis. Younger age (<30) at diagnosis as an increased propensity for local recurrence was further identified by univariate analysis (RR 0.959; 95% CI 0.933-0.985; $P=0.002$). Other parameters just as gender, tumor sites (chest vs. abdominal wall vs. extremities) and tumor size did not show significant difference. Multivariate analysis was not performed due to the low number of cases. The 45F CTNNB1 gene mutation-harboring tumors demonstrated significant difference to WT tumors in comparison with local recurrence rate using the log-rank analysis ($P=0.009$). **Figure 12.**

4. The analysis and comparison of the clinical and pathological data of the sporadic desmoids in the three subgroups.

4.1. Sporadic desmoid tumors of the chest

There were 19 female and 9 male patients with a median age of 35 years (range, 11—74 years). Surgical or physical trauma to the site of the tumor preceded the development of desmoids in 9 patients (32%). As many as 64% of the tumors originated in the shoulder girdle. The location of the primary tumors is detailed in **Table 7**. Patient symptoms included palpable lesions (86%), pain (71%), loss of motor function (25%), and hypesthesia (7%) of the upper extremities. Eleven percent of the patients were asymptomatic, and their tumors were diagnosed by routine X-rays. Symptoms appeared at a median of 12 months before the diagnosis. Regarding the imaging tools used in the diagnosis, a chest X-ray was utilised in all of the cases, CT scans in 22 cases (79%), US scanning in 15 cases (54%), MRI in 13 cases (46%), and angiography in two cases. The median tumor size, measured by the imaging techniques was 63 mm (range, 10—210 mm). The results of the preoperative FNA of 22 samples suggested desmoids in 14 (64%), fibroma in two, and low-grade fibrosarcoma and fibromyxoid sarcoma in four cases while in two cases, the samples were proved to be inadequate for cytological examination. In 83% of the 18 cases in which CNB was performed, the diagnosis of aggressive fibromatosis was confirmed. Low-grade fibrosarcoma was suspected in 3 cases. The incisional biopsy was carried out in one case and confirmed the desmoid tumor. Upon the pathological examination of the primary specimens, an R0 excision was achieved in only 50% of the patients. Of the 27 macroscopically wide surgical excisions, 10 cases (37%) were full-thickness and 17 cases were (63%) partial-thickness chest wall resections. The number of ribs resected was 1 to 4. Partial or complete scapula and clavicle resections were performed in six and four cases respectively. The disease of 3 patients necessitated compartment resection. Mediastinal propagation indicated tumor removal through sternotomy in one case. The median pathological diameter of the resected tumors was 50 mm (range, 20—210 mm). Nine patients had their chest walls stabilized by synthetic meshes (polytetrafluoroethylene ePTFE — Gore-Tex dual mesh, Gore-Tex soft tissue patch, prolene mesh (Ethicon), and vicryl mesh (Ethicon) and no detectable implant complications occurred presumably due to the antibiotic prophylaxis applied. Pleural involvement indicated wedge resection of the lung in 3 patients. Soft tissue coverage was achieved with transposed muscles in six patients. The rate of complications was 25%. No operative deaths occurred. In the subsequent follow-ups, first recurrences were noted in 17 (63%) of the 27 resectable (in both R0 and R1) patients while 10 patients (37%) remained recurrence free. The median time to first recurrences was 30 months (range, 10—120 months). The radicality of the surgeries that were performed on recurrences are detailed in **Table 3a**. Altogether, 35 surgeries were performed on recurrences. One patient died of stroke before the end of the follow-up, another

patient was in a weak general condition due to an extensive desmoid in the shoulder girdle, and superior vena cava syndrome was diagnosed in yet another case. One patient needed neurosurgical intervention and stabilization of the spinal. The median duration of anti-estrogen therapy in 18 patients was 68 months (range, 10—171 months) and the median length of tamoxifen and sulindac combinaton therapy in 12 patients was 48 months (range, 10—118 months). The observed and reported side effects of the aforementioned pharmacological therapies were weight gain in 7 patients (38%) and hot flashes in 4 patients (22%). ER- α and PgR showed 100% negativity, whereas ER- β positivity was observed in 50% of the samples. Radiotherapy was applied in 2 patients with recurrent tumors on the nape of the neck and the retrosternal region. Both lesions were observed to recur and progress 9 and 14 months after treatment, respectively. A total of 16 patients (57%) were found to be recurrence free, whereas residual or recurrent tumors were detected in 12 patients at the time of their last follow-up examination. Sixty-seven percent of the recurrences exhibited stable disease according to RECIST 1.1 during the last 6 months of follow-up, while 33% of the tumors demonstrated slow progression despite the ongoing multimodal therapy. Statistical analyses of the categorical data such as gender, age, tumor size, ER status, and sulindac treatment using Fisher's exact probability test and the chi-square test established that these factors did not correlate with surgical radicality. **Table 8.** Since tamoxifen was not an independent factor (significance is explained by its adjustment for R1 and R2 resected cases and for recurrent tumors, corresponding to our adjuvant protocol), it could not be used in the multivariate analysis. However, local recurrence rate was significantly affected by the surgical radicality of the primary tumor ($P=0.00065$) and sulindac treatment ($P=0.029$) in the univariate analysis by log-rank statistics **Table 9.** Factors that were not significant included gender, age, tumor size, and ER positivity. The significant difference on the factors affected by the recurrence-free survival group was demonstrated by Kaplan—Meier curves. **Figure 13.** The multivariate Cox regression model confirmed, that the local recurrence rate was only significantly affected by the surgical radicality of the primary tumor ($P=0.001$). **Table 10.** Factors that were not significant included gender, age, tumor size, sulindac treatment, and estrogen receptor positivity. The follow-ups were completed in all patients.

4.2. Sporadical desmoid tumors of the abdominal wall.

Twenty-three female and 5 male patients belonged to this subgroup with a median age of 29 (range, 19—59 years). Antedescendent surgical or physical trauma to the site of the tumor was identified in 7 patients (Cesarean section in 5, and accidental trauma in 2 patients) (25%).

Primary tumors were located in the anterior abdominal wall in 100 %, including the inguinal region in 4 cases. **Table 11.** In order of their frequency, patient symptoms included palpable lesions (89%), abdominal discomfort (35%) and pain (32%). One patient (3.5%) was asymptomatic, where the tumor was identified accidentally by ultrasound at a routine medical check-up. Symptoms appeared at a median of 6 months before the diagnosis. The imaging tools applied for the diagnosis were US in all of the cases, MRI in 15 cases (53%) and CT scans in 12 cases (43%). The median tumor size, measured by the imaging techniques was 65 mm (range, 20—180 mm). The samples of 12 patients (70%) were suspected to be desmoids according to the results of the preoperative FNA, whereas samples from 5 patients were inadequate for cytological assessment. CNB confirmed the diagnosis of aggressive fibromatosis in 15 out of the 16 cases examined by this method. Low-grade fibrosarcoma was suspected in one case.

Pathological results of the primary specimens showed that an R0 excision was achieved in 18 cases (64%). Of the 28 macroscopically wide surgical excisions, 18 cases (64%) were full-thickness and 10 cases were (36%) partial-thickness abdominal wall resections. The median pathological diameter of the resected tumors was 63 mm (range, 20—180 mm). Synthetic meshes were utilised for the stabilisation of the abdominal wall in all the 28 cases (polytetrafluoroethylene ePTFE — Gore-Tex dual mesh, Gore-Tex soft tissue patch, prolene mesh (Ethicon), and vicryl mesh (Ethicon)). The applied antibiotic prophylaxis ensured the lack of detectable implant complications, only a superficial wound infection localised to the subcutis was detected. Soft tissue coverage with transposed musculocutaneous flaps was not necessary to perform. No perioperative deaths occurred. The rate of complications was 10.5%. First recurrences were recorded in 10 (35.7%) of the 28 resectable (R0 and R1) patients during the subsequent follow-ups. The follow-ups were completed in all of the investigated patients. Eighteen patients (64%) were found to be recurrence free. The median time to first recurrences was 26 months (range, 6—110 months). The radicality of the surgeries that were performed on recurrences are detailed in **Table 3a.** Altogether, 17 surgical interventions were performed due to recurrences. All patients were alive and tumor free at the last follow-up. Abdominal wall hernias developed in 3 patients, and in one case mesh bulging was confirmed. The median duration of tamoxifen therapy in 11 patients was 25 months (range, 8—36 months). Four patients received tamoxifen and sulindac combination therapy for the median length 20 months (range, 8—25 months). Weight gain and hot flashes were reported by 4 patients (36%) and in 6 patients (54.5%) respectively. ER- β positivity was observed in 13 cases (46.4%) by IHC analysis, whereas ER- α and PR assays resulted in 100%

negativity. The inguinally located tumor of one patient was observed to recur twice, thus RT was applied. Fifteen months after the re-resection of the last recurrence the patient is disease free. All the patients were found to be recurrence free at the time of last checkup. Statistical analyses of the categorical data (gender, age, tumor size, ER status) using Fisher's exact probability test and the chi-square test determined that these categories did not correlate with surgical radicality. Because of the low number of sulindac use, the cases using tamoxifen and sulindac were summed up. **Table 12.** The significance of tamoxifen and sulindac ($p=7.96 \times 10^{-4}$) is explained by the adjustment for R1-resected cases and for recurrent tumors, corresponding to our adjuvant protocol. As a result, tamoxifen was not an independent factor and, it could not be used in the multivariate analysis. In the univariate analysis by Cox-regression model, the local recurrence rate was significantly affected by the surgical radicality of the primary tumor ($P=0.006$). **Table 13.** Factors that were not significant included gender, age, tumor size, and ER positivity. The significant difference on the factor affected by the recurrence-free survival group was demonstrated by Kaplan—Meier curves. **Figure 14.**

4.3. Sporadic desmoid tumors of the extremities.

The subgroup included 24 female and 14 male patients with a median age of 30 years (range, 9—59 years). Thirteen patients (34%) reported antedescendent surgical or physical trauma to the site of the tumor. The location of the primary tumors is detailed in **Table 14.** Eleven tumors (29%) were located in the upper extremity, while 27 (71%) tumors in the lower extremities. Patient symptoms included palpable lesions (84%), pain (42%), decreased joint mobility (21%), neurological symptoms as loss of motor function (18%) and hypesthesia (10%), swelling of the extremity (10%) and appeared at a median of 8 months before the diagnosis. Among the diagnostic imaging tools, US was used in 30 cases (79%), X-ray of the extremity was performed in 19 cases (50%), CT scans in 19 cases (50%), MRI in 27 cases (71%), and angiography or angio-MRI in 7 cases (18%). The median tumor size, measured by the aforementioned imaging techniques was 70 mm (range, 20—250 mm). Desmoids were suspected in 13 cases (65%), fibroma in one, and low-grade fibrosarcoma in 4 cases according to the results of the preoperative FNA of 20 (52%) samples. Samples of two patients were inadequate for cytological assessment. CNBs were performed in 28 cases (73.6%) and the diagnosis of aggressive fibromatosis was confirmed in 92%. Low-grade fibrosarcoma was suspected in 2 cases. The incisional biopsy was performed in 3 cases and confirmed the desmoid tumor. Regarding the pathological results of the primary specimens, an R0 excision was achieved in only 19 cases (50%). Macroscopically wide surgical resection was performed

in 32 cases (84%) whereas 4 patients (10.5%) underwent compartment resection of the buttock resection, adductor compartment, quadriceps and the entire brachioradialis muscle. The tumor of one patient originated in the popliteal fossa, thus the popliteal arteria had to be grafted. Although en block resection with additional bone resection was performed in 1 case (partial resection of the fibula), amputations were not performed. The median pathological diameter of the resected tumors was 70 mm (range, 25—250 mm). Soft tissue reconstruction was performed with transposed myocutaneous flaps in 6 patients (15%), and additional 4 patients needed full- or partial thickness skin graft coverage. No operative deaths occurred. The rate of peroperative complications was 23.7%. First recurrences were noted in 28 (77%) of the 36 radically resectable (in both R0 and R1) patients. The median time to first recurrences was 32 months (range, 6—108 months). Ten patients (26.3%) proved to be recurrence free. The radicality of the surgeries performed on recurrences are detailed in **Table 3a**. Altogether 52 surgeries were carried out on recurrences and the follow-ups were completed in all of the investigated patients. At the last follow-up all the patients were alive. Twenty-seven patients (71%) were disease free, and 4 patients had stable disease, while 7 patients had recurrent tumors with slow progression. Five patients complained about limitation of joint motion, 4 patients experienced neurological symptoms like loss of motoric functions, and 7 patients suffered from hypesthesia of the affected region or the distal part of the extremity was experienced. The median duration of anti-estrogen therapy in 19 patients was 43.2 months (range, 11—78 months). The median length of tamoxifen and sulindac combination therapy in 4 patients was 32 months (range, 9—68 months). The following adverse effects were recorded: weight gain in 10 patients (52%) and hot flashes in 10 patients (52%). The IHC analyses of sex receptors were performed in 28 cases: ER- α and PR showed 100% negativity, whereas ER- β positivity was observed in 12 cases (42.8%). Radiotherapy was administered for 7 patients (18.2%). Five of them were observed to be tumor free while recurrence was found in 2 cases with a median of 21 months following treatment. A total of 27 patients (71%) were found to be recurrence free, whereas residual or recurrent tumors were detected in 11 patients (29%) at the time of their last checkup. As many as 36.3% of the recurrences exhibited stable disease according to RECIST 1.1 criteria during the last 6 months of follow-up. However 63.7% of the tumors were characterized by a slow progression despite the ongoing multimodal therapy. Categorical data such as gender, age, tumor size, ER status did not correlate with surgical radicality determined by Fisher's exact probability test and the chi-square test. Because of the low number of cases treated with sulindac statistical analysis was unable to perform. The significance of tamoxifen ($P=0.007$) and RT ($P=0.001$) is

explained by the adjustment for R1 or R2 resected cases, corresponding to our adjuvant protocol. Neither of these can be considered as an independent factor and thus, could not be used in the multivariate analysis. **Table 15.** In the univariate analysis by Cox-regression model, the local recurrence rate was significantly affected by the surgical radicality of the primary tumor ($P=0.013$). **Table 16.** Factors that were not significant included gender, age, tumor size and ER positivity. The significant difference on the factor affected by the recurrence-free survival group was demonstrated by Kaplan—Meier curves. **Figure 15.**

4.4 . Comparisons of the clinical results among the three cohorts of different tumor locations.

Significant differences were not detected among the three cohorts in terms of surgical radicality of the primary tumor (R0 versus R1 and R2) ($P=0.281$), gender ($P=0.236$), age ($P=0.201$), ER- β status ($P=0.122$) and tumor size ($P=0.435$).

The recurrence-free survival showed a decreasing trend as follows: abdominal, chest and extremities. **Figure 16.** Significance was confirmed using log-rank analysis between tumor locations 'abdominal' and 'extremity' ($P=0.0061$), while no significance was found between 'abdominal' versus 'chest' ($P=0.086$) and 'chest' versus 'extremities' ($P=0.253$).

5. Assessment of the expression of ERs and PRs in sporadic desmoid tumors by immunohistochemical assays.

The assessment included the tissue samples of 67 sporadic desmoid patients: 51 female (76.1%) and 16 (23.9%) male patients with a median age of 32 years (range 9-65 years), accounting as the 3rd largest study in the literature.

The tumor was located in the chest in 19 patients (28.35%), abdominally in 20 patients (29.85%) and in the extremities in 28 patients (41.8%). **Table 17.** Stains for ER- α and PR were uniformly negative, whereas 36 cases (53.7%) displayed expression of ER- β : 3+ expression in 31 cases (86.1%), 2+ expression in 4 cases (11.1%) and 1+ expression in one case (2.8%). **Figure 3f.** The distribution of ER- β positive tumors were as follows: 31% were located in the chest, 36% in the abdominal region and 33% in the extremities. There was no significant correlation between the ER- β status and gender ($P=0.732$), age ($P=0.281$) or location ($P=0.289$) and size ($P=0.924$) of the tumor. It is interesting to note that in one case the primary tumor showed 10% ER- β positivity, while another sample taken from a recurrent tumor of the same patient 5 years later displayed 90% ER- β positivity. R0 resection was

achieved in 38 cases (56.7%) only. The median pathological tumor diameter of the investigated cases was 80 mm (range 25-210 mm). Thirty-two patients (47.7%) in the IHC screened group received adjuvant tamoxifen treatment. Fourteen of the 32 patients (43.75%) treated with tamoxifen and subjected to clinical assessment had ER- β positive tumors. At a median follow-up time of 110 months (range, 5 to 336) 43 cases (64.2%) had local recurrences, and no difference between the ER- β positive (12/14) and ER- β negative (18/18) patients treated with tamoxifen were detected (P=0.184).

VII. DISCUSSION

1. Successful creation of a cohort of sporadic desmoid cases and a database

Sporadic desmoids are extremely rare lesions. As the opportunities offered by molecular-genetic diagnostic tools are getting broader, the number of papers published on the possible etiology of desmoids is increasing as seen in recent years. Many reports are focusing on the IHC profile of this type of tumor. However due to the low number of involved cases and the databases that often merge sporadic and FAP associated or primary and recurrent tumors, results are often rather inconsistent (28, 62, 63, 72, 83, 85, 91, 93, 110, 111, 137, 161, 162). The facts contained in **Table 18** demonstrate that there are considerable limitations of the comparison of the data of retrospective investigations, even with higher number of involved cases. Furthermore, partially as a consequence of the inconsistency regarding the etiology, there is currently no controlled or standard therapy, and radical surgical resection is the mainstay of the therapy. Limitation of our study is the multicentric retrospective way of database creation. However the relatively large number of patients with sporadic primary desmoids (n=94), the constant therapeutic modalities, and the long term clinical follow-up (median 98 months) together proved to be eligible for molecular-genetic and IHC investigations and scientific analysis of the data. As mentioned earlier, no evidence based therapy exists yet. To resolve the problem of the scarce incidence, and to allow the same research aims, international efforts were made to create data bases. Netherlands' nationwide network and registry of histopathology and cytopathology, PALGA ("Pathologisch-Anatomisch Landelijk Geautomatiseerd Archief") which has coverage since 1991, served as the source for one of the largest study by Nieuwenhuis et al. (2011) (91, 163). Conticabase, a European sarcoma database and tumor bank currently contains the data of 11570 patients and 11614 rare soft tissue tumors together with 13975 tissue samples from 31 centres throughout the continent (58, 164). Conticabase contains anonymised information about the tumor, treatment and follow-up. Tumor samples are also available and so are molecular biology analyses for mesenchymal lesions. The investigator has registered the NIO into Conticabase, and the transfer of the parameters of the 94 sporadic cases is in progress. Data transfer is achieved by the use of the so called SNOMED CT data system (Systematized Nomenclature of Medicine Clinical Terms), which is a systematically organised computer processable collection of medical terms providing codes, terms, synonyms and definitions covering diseases, findings, procedures. This way a consistent way to index, store, retrieve, and aggregate clinical data across specialties and sites of care is made possible. Another application of our database was to join to the Hungarian National Coordinating Research

Group of Rare Diseases which was established in 2009 with the agreement of the Rare Disease Center of the National Centre for Healthcare Audit and Inspection of the National Public Health Service (ÁNTSZ OSZMK) in cooperation with the University of Pécs. Our multicentric database has successfully enabled the performance of the planned molecular-genetic, IHC and clinical investigations and reliable statistical analysis. The database was registered to the national and the European databases of rare diseases and soft tissue tumors, to support additional scientific research of sporadic desmoids. The Department of Breast and Sarcoma Surgery of the NIO is ready to participate in prospective randomized international studies in the future, based on the experiences gained through the present study.

2. Confirmation of the sporadic origin of the desmoid tumors.

Testing for APC germline mutations in FAP families has become an efficient tool for predictive testing of subjects at possible risk and is now commercially available and has led to changes in management guidelines, particularly for those whose tests indicate they are not mutation carriers. If the germline mutation causing FAP is detected in a given family, carriers are selected who need to undergo surveillance by regular endoscopies. Presymptomatic genetic diagnosis of supposed FAP patients has been feasible with linkage and direct detection of APC mutations (165, 166). These tests require a small sample of blood in which the lymphocyte DNA is tested. Ancillary family members, (more than one affected individual), need to be studied to identify gene carriers when linkage analysis is applied. Fewer family members' blood samples are required for direct detection, but the specific mutation must be identified in at least one affected person by DNA mutation analysis or sequencing. The detection rate is approximately 80% using sequencing alone (167). Since whole exon deletions in 12% of FAP patients with previously negative APC testing were reported, deletion testing has been added as an optional adjunct to sequencing of APC (168, 169). Intragenic deletions appear to be detected by Multiplex Ligation-dependent Probe Amplification (MLPA), a variation of the multiplex polymerase chain reaction that permits multiple targets to be amplified with only a single primer (170). MYH gene testing may be considered in APC mutation–negative affected individuals (171). Patients with fewer than 100 colorectal adenomatous polyps are a diagnostic challenge (167). The differential diagnosis should include Attenuated Familial Adenomatous Polyposis (AFAP) and MYH-associated colorectal neoplasia (also reported as MYH-associated polyposis or MAP) (167). Mutations in the MYH gene (mutY homolog (E.coli), MUTYH) cause a germline homozygous recessive form of familial adenomatous polyposis, where polyps caused by mutated MYH do not

appear until adulthood and are less numerous than those found in patients with APC gene mutations (173). AFAP can be diagnosed by testing for germline APC gene mutations. Presymptomatic genetic testing removes the necessity of annual screening of those at-risk individuals who do not have the gene mutation (165). For at-risk individuals who have been found to be definitively mutation-negative by genetic testing, there is no clear consensus on the need for or frequency of colon screening though all experts agree that at least one flexible colonoscopy examination should be performed in early adulthood (by age 18–25 years) (165). In the present study 3 of the genetically screened 39 desmoid cases revealed to carry the germline mutation of the APC gene. **Figure 17.** In 2 of the 3 cases family history was positive for FAP, but not in the 3rd case and genetic testing confirmed a germline mutation (R1450X(4348C>T) of the APC gene. The 17-year-old female patient presented with a primary extra-abdominal desmoid in the lumbo-sacral region in 2002. Three polyps were excised from the large intestine via colonoscopy. The desmoid was radically excised, and local recurrence occurred after a follow-up time of 16 months. The recurrent tumor was resected several times with R1 surgical margins in the forthcoming 3 years and the patient also received RT to the lumbo-sacral region (50 Gy, 1.8 Gy/day, 18 MV) and tamoxifen in combination with sulindac. At the time of the last follow-up, a locally recurrent tumor was confirmed, showing slow progression. The young patient was lost to follow-up for 2 years. She came back to our department in 2009 with melena and weight loss. Colonoscopy revealed polyposis syndrome. A total colectomy with jejunostomy was performed. Histological examination verified adenocarcinoma in the colon. The patient refused chemotherapy. Three months later she was admitted to our outpatient clinic with dyspnea and abdominal pain. Chest CT showed multicentric giant desmoids in the right pleural cavity, confirmed by CT-guided percutaneous core biopsy, another in the right hemithorax and a lesion located in the right abdominal wall. Palliative chemotherapy (doxorubicin and dacarbazine) was performed, but the patient died 5 months later.

Beside the desmoids in all 3 germline mutated cases other manifestations of FAP syndrome appeared such as multiple colon polyps or polyposis, epidermal cysts, osteomas. After the exclusion of the FAP-associated cases, the remaining 94 patients with negative clinical and genetic tests and family anamnesis did not show any signs of FAP during the median follow-up time of 98 months. Colonoscopy, or occasionally CT colonography was performed in every 5 years in the investigated population, corresponding with the international recommendations (8, 11, 131). Sporadically, polypectomies were performed, but multiple polyps or polyposis were not detected. On the bases of the above detailed experiences and

results, the author agrees that genetic testing together with accurate family anamnesis, physical investigation and colonoscopy is a reliable and non-invasive method to confirm FAP even without positive family anamnesis. It is imperative that FAP families be referred for genetic counselling, genetic testing, early diagnosis, and clinical management to centres that are aware of the complexity of both surgical intervention and possible implications of molecular findings for individual patients.

3. The detection of the β -catenin mutation status.

Sporadic desmoid tumors often turn out to harbor disrupted β -catenin signaling pathway (2, 7, 11, 60, 61, 62, 66, 174, 175). The rare occurrence of the tumors prevented evaluation of the actual prevalence of such mutations, and thus a relatively small numbers of cases are available for analysis in the literature (59, 61-63, 66, 174, 175). **Table 19.** The present study identified CTNNB1 gene mutations in 58.8% of the investigated 51 cases, anticipated as the 4th largest reported in the literature. This finding further confirms the results of a recently published series, strongly suggesting that CTNNB1 mutations are highly prevalent in sporadic desmoids (61-63, 66, 174, 175). A reasonable explanation to the lower ratio of CTNNB1 mutation reported by Saito et al. (2002) and Abraham et al. (2002) is the different technique used for the mutational analysis and also the differences between patient ethnicities (59, 175). All the investigated patients in our study were caucasians. Mutated β -catenin have been found in many cancers at various incidence levels. The markedly elevated rate of such mutations in sporadic desmoids detected in various investigations, potentially makes this disease as one of the most likely to harbor this specific genetic deregulation and suggests potential clinical application (62, 63). β -catenin sequencing can indeed be used as a possible complementary diagnostic tool for desmoids. The differential diagnostics of this lesion can sometimes be difficult to establish, particularly in recurrent desmoid cases, however β -catenin sequencing can be easily and reproducibly conducted using paraffin-embedded tissue samples extracted via needle biopsy. Although negative results do not preclude the diagnosis of desmoid, a positive result for an exon 3 β -catenin mutation would serve to confirm a diagnosis of desmoid (62, 63). Moreover, when insufficient tumor material is available for histological analysis, it means no barrier to β -catenin sequencing, as it requires just a few ng of gDNA (62). The sensitivity and specificity of the method is not evaluated yet, but it is helpful in resolving complicated cases. Molecular prognostic markers are unknown, but the heterogeneity of biological behaviour of desmoids suggests biological differences (2, 7, 15, 25). In conformity with other publications we have also observed that desmoid CTNNB1

mutational spectra are rather limited (61-63, 66, 174, 175). Interestingly, only three specific mutations are noted: 2 involving codon 45 and 1 involving codon 41, with all mutations clustered at Threonine 41 (41A) and Serine 45 (45F and 45P) in all topographies, suggesting that these residues have crucial functions in the β -catenin/Wnt signaling pathway (59, 61-64, 66, 174, 175). In our study we identified that risk of recurrence in desmoids is significantly associated with the specific CTNNB1 mutation of 45F (P=0.022). A larger than threefold increased risk of recurrence is found in tumors with this mutation, proposed to begin to be manifest at a significantly earlier time point in the desmoid tumor-host encounter (63). To the best of our knowledge, this study is among the first 3 ones to correlate desmoid mutation type with clinical outcome, suggesting that the biological consequences of the 3 different CTNNB1 mutations in exon 3 are not equivalent. There may be differences for instance between the ability of the 3 mutant versus stable wild-type proteins to act as transcription factors, to enter the nucleus, or to achieve stability in the cellular environment. In conformity with the results of Lazar et al with abdominal tumors, our study supports a role for 45F mutation as a prognostic factor strongly associated with recurrence (62, 63). Domont et al have observed only a weak trend for S45F compared to local recurrence in a cohort of extra-abdominal lesions, a discrepancy that may reflect differences in the clinical entities studied (62, 63). Due to the high propensity for recurrence of desmoids even after radical surgery, the use of nonsurgical approaches as adjuvant strategies may be particularly applicable in 45F mutated desmoid patients, and definitive mechanism-elucidating studies are yet to be conducted (7, 25). External beam RT, anti-hormone and NSAID therapy belong to these nonspecific adjuvant treatments and may be useful in preventing recurrence, especially in recurrence-prone cases such as the 45F mutated lesions. Nuclear β -catenin expression by IHC in desmoids was evaluated by multiple studies and demonstrated significance with an overall prevalence ranging from 82 to 100% (63, 176-178). This finding was identifiable in 85% of the investigated cases in our cohort of desmoids (n=26). Although a recent publication indicates that an increased nuclear expression in >20% of the tumor cells had a significantly higher recurrence rate than desmoids lacking nuclear β -catenin expression, little is known concerning the utility of β -catenin expression as a prognostic marker for desmoids (87). Our investigation could not demonstrate such a difference since desmoids negative for β -catenin staining were very rare. Furthermore, the association of lower β -catenin intensity levels and CTNNB1 S45F mutation type independent-ly correlated with a higher tendency for recurrence in a study by Lazar et al (63). The causal relationship between the 45F mutation, β -catenin expression, and desmoid recurrence are still to be confirmed in further investigations. In

summary, our study suggests that genotyping of CTNNB1 exon 3 may provide information regarding risk of recurrence and personalised adjuvant therapies and as a diagnostic test is could be useful in situations such as insufficient core needle biopsies or differentiating a post-surgical scar from desmoid recurrence. Prospective trials are needed to test such possibilities. Molecular insights such as these may ultimately lead to novel therapeutics for a disease in which no new efficacious treatments have been introduced for the past few decades (62, 63, 176).

4. Three subgroups formed as a consequence of the analysis and comparison of the clinical and pathological data of sporadical desmoid tumors.

4.1 Sporadical desmoid tumors of the chest

Although desmoids of the chest represent 20% of all extra-abdominal fibromatoses, our study encompassing 28 cases proved to be the second largest cohort in the English-language literature (2, 22, 23, 140). Desmoids can arise at any site of the chest, but the most common site is the shoulder girdle, as has been confirmed in the present investigation too. Only 26 intra-thoracic desmoids cases have been reported in the literature (120). The clinical manifestation of chest desmoids is non-specific; typically discovered as palpable masses, and chest pain, dyspnea, cough, shortness of breath, tenderness, chest wall swelling, and dysphagia are its major symptoms. **Table 20.** Although incisional biopsies are sometimes inevitable, CNB is the diagnostic method of choice due to the high degree of its diagnostic accuracy (up to 92%) (140). Aggressive surgical management with a wide local excision is generally recognized as the most effective treatment (2, 7, 22, 23, 140, 179). Because radical (R0) resection is often a therapeutic challenge and positive surgical margins are associated with a high risk of local recurrence, the adjustment of additional multimodal therapies are expected to yield better results (22, 23). **Table 21.** A likely consequence of incompletely planned and performed primary surgical management, R0 resection rates reported in the literature are higher than in this patient series. The presence of scar tissue and a higher potency of invasiveness significantly mitigates outcomes of surgeries on recurrences. Despite the removal of extensive areas of the chest wall and contiguous structures, primary closure can generally be achieved without significant cosmetic deformity or loss of function (22, 23, 140). Skeletal and soft tissue reconstruction of the chest wall is necessary following radical en block resection in particular cases (22, 23, 140). Various types of autologous tissue grafts are available for chest wall reconstruction, and the latissimus dorsi flap is the flap of choice (22, 140). **Figure 18.** Adequate stability can be maintained via the use of synthetic meshes.

Downsizing of radically irresectable tumors can be attempted by neoadjuvant therapy, and palliative surgical diminution proved to be saving when vital organs are compressed. A high complication rate is associated with chest wall resections, wherein the most frequent complications are of respiratory origin observed at a rate of 20—24% (22, 23, 140). Postoperative morbidity was 26% and there was no postoperative mortality in our investigation. Nonsurgical therapies include radiotherapy and pharmacological treatments (2, 7, 15). The factors that impact local recurrence are controversial in chest desmoids, so it is difficult to draw conclusions therein. Brodsky et al. (1992) found that gender, tumor site, tumor size, the incidence of recurrent tumors, and incomplete resection were not associated with significant increases in the 29% 5-year local recurrence rate (181). The only significant prognostic variable for recurrence was the patient's age at treatment. Nevertheless, positive margins, reoperation, and postoperative radiation therapy were identified to be factors in recurrence in the study of Abbas et al. (2004) (22). **Table 21.** In their investigation, as many as 89% of the patients with positive margins developed recurrences in comparison to only 18% with negative margins and recurrences occurred in 50% of patients who had a prior desmoid resection and in only 8% who did not have a previous resection. Recurrence rate in patients with negative margins was 27% without adjuvant RT, while those who received radiation therapy did not exhibit any recurrence (22). In concordance with the findings of Abbas et al. our study confirmed the significant importance of the radical surgical resection of the primary lesion. Based on these results, the higher recurrence rate of 63% in our series may be explained by the lower rate of R0 resection during the primary surgery and the longer median follow-up time of 104 months. The investigator found a significant relationship between the occurrence of recurrences and the radicality of the first surgery, whereas age, gender, tumor size, ER status, and sulindac treatment did not show any statistical relationship to it. Our review also clearly demonstrates the difficulty and low efficiency of achieving microscopically negative margins in the surgical treatment of recurrences. As concluded by the literature local control of future cases may be improved by the use of adjuvant RT for in our practice.

4.2. Sporadic desmoid tumors of the abdominal wall.

To our knowledge, the present study is the fourth largest series of patients with primary sporadic abdominal wall desmoids with a long follow-up period reported in the literature. Due to the lack of randomized, controlled trials, we have tried to extrapolate from the published series those cases with tumors localized to the abdominal wall to compare with our

findings (2, 19, 34, 110, 114, 115, 137, 182-184). **Table 22.** In all but 2 previously reported series of desmoids, tumor recurrence occurred in at least a certain extent of patients (19, 182). In a small series of 4 patients with desmoids of the anterior abdominal wall who underwent neoadjuvant doxorubicin, neoadjuvant RT and surgery, no long-term recurrences were reported after a median follow-up time of nearly 6 years (182). It should be noted that serious overtreatment was achieved in this report. Five year local recurrence rates of 8 to 44% were reported by other series, and was 36% in the present study (2, 34, 110, 114, 115, 137, 182, 184). Most recurrences are usually observed within 3 years, and nearly all by 6 years and most of the studies found that the presence of positive surgical margin proved to be relevant in patients with recurrent disease (19, 34, 110, 114, 115, 137, 182-184). Well corresponding with these results, the median time to recurrence in our series was 26 months (range 6-110 months) and the investigator established that obtaining a wide disease-free margin is crucial for reducing the recurrence rate. One-stage treatment with immediate mesh reconstruction proved to be cost-effective and increased the chances of definitive cure due to the low rate (10.5%) of abdominal wall hernias at 92 months median follow-up time and at 63 mm of median disease diameter. A remarkable drawback of one-stage mesh placement is the intra-operative detection of microscopic margin status. Some authors used frozen sections of the closest margin as an alternative but the relevant pathological analysis of desmoid tumor margins is debatable, especially in recurrent tumors or scar tissue (19). The exact location of the tumor within the abdominal wall is more appropriate to the increasing extent of resection until free margins, than in other locations like the chest or the extremities. **Figure 19.** The author concludes that the resections of abdominally located desmoids were successful because none of the patients had recurrences at the last follow-up. Abdominal wall desmoid tumors seem to have a favorable prognosis, in comparison with desmoids of the chest or extremities. **Figure 16.** The four long-term minor complications of the abdominal wall reconstruction with mesh reported: 3 patients had abdominal hernias and 1 woman experienced mesh bulging. The bulge, which resulted from the relaxation of the mesh inserts, could be an indication for surgery, both because of the possible subsequent chronic pain syndrome and the cosmetic problems. Surgery was not indicated in our patient due to the observed symptom relief after physiotherapeutic sessions. Anecdotal reports or small series are to be found on the pharmacological treatments of abdominal-wall lesions (182-184). Radiation enteritis is a serious risk and thus RT is rarely used for abdominal wall and intra-abdominal desmoids (19). As only 40% of the patients were administered tamoxifen and only

one patient received RT, no consequences can be drawn from our data regarding adjuvant therapies.

4.3. Sporadic desmoid tumors originating in the extremities.

Significant and often serious problems are posed by desmoids located in the extremities while the optimal management and long-term outcome remains unclear (93, 184). **Figure 20.** Our results well demonstrate the surgical complexity and a high relapse rate (73.7%) in the treatment of primary extremity desmoids after a long median follow-up time of 98 months (range 18-336 months), in spite of the relative high rate of adjuvant therapies implemented (52.6%) (tamoxifen 95%, and radiotherapy 35%). In half of our patients (50%), excision was incomplete significantly impinging recurrence ($P=0.013$). In a multivariate analysis of 138 patients by Posner et al. (1989) positive resection margin was identified as the most important independent predictive factor of local recurrence (129). **Table 23.** In conformity, similar results were obtained by Spear et al. (1998) in a series of 92 patients, by Ballo et al. (1999) in a series of 189 patients (109, 137). The probability of radical resection was affected by whether surgery was for primary disease or for a recurrence. **Table 3.** Results from this study appear significantly poorer than in other series (18, 110, 111, 185). No correlation between positive resection margins and recurrence was detected in a series of 105 cases by Merchant et al. (1999), 42% of primary extra-abdominal desmoids displayed positive margins while only 23% of patients developed a local recurrence following attempted resection at a median follow-up of 49 months (110). Thirty-one percent of the patients received adjuvant RT, mainly those with positive margins. The comparison of the data is difficult due to the addition of data of chest and abdominal wall desmoids (45 patients), but it is seen in many other studies as well (109, 137). Gronchi et al. (2003) well illustrated the importance of long term follow up for extremity desmoids. In their study, patients with primary extremity tumors had a 72% disease free survival at 5 years decreasing to 62% at 10 years (111). RT was administered for patients with positive margins in a similar study by Pignatti et al. (2000), where recurrence rate was 41.2% compared to the recurrence rate of 45.3% in those with negative margins following surgery alone (18). Although the investigator followed patients for a mean of 11.2 years, time to recurrence following RT and surgery with surgery alone was not evaluated (18). There are contradictions surrounding the role of RT in the treatment of desmoids mostly due to the significant possibility of developing a radiation induced sarcoma in the predominantly young patient population. In the review by Nuyttens et. al. (2000) 0.7% of patients developed sarcomas in the radiation field however, local control after RT or

surgery plus RT was significantly superior to that after surgery alone (135). **Table 24.** Surgically treated patients with free or positive margins demonstrated salvage rates of 72% and 41%, respectively, and when RT was added to surgery, the local control increased to 94% and 75%, respectively. After surgery with positive margins, the addition of RT significantly improved local control compared with surgery alone (75% vs. 41%). The division of positive margins into marginal, microscopic, and macroscopic displayed postoperative local control rates of 45%, 41%, and 33%, the rates improved to 89%, 79%, and 69%, respectively when RT was added to surgery. The comparison between surgery and surgery plus RT revealed significant differences between the 3 subgroups ($P=0.0025$, $P=5 \times 10^{-8}$, and $P=0.038$, respectively), however, in many of the studies reviewed applied short median follow up periods with only one of the 22 reaching 10 years: the incidence of local recurrences and radiation induced sarcoma may have been thus underestimated. Possible reasons affecting the much poorer outcome by local control in our study include the significantly higher rate of 45F CTNNB1 mutation, the large number of positive surgical margin and the long time follow-up. Although responses have been reported with other treatment modalities such as anti-estrogens and NSAIDs, results have been inconsistent (2, 5, 7, 38). Many of these lesions are asymptomatic at presentation (45% in our study), may stabilise and show no evidence of progression for many years or exert spontaneous regression (2, 5, 28, 29, 107). It should be noted, that none of our 94 sporadic cases showed spontaneous regression during the follow up period. It might seem reasonable to chose the 'wait and see' policy only following confirmation of their nature by core biopsy and regular surveillance, predominantly in cases where radical surgery with higher rate of morbidity or funtional loss is regarded necessary for complete resection. In general, surgery should be performed on those that are symptomatic and appear resectable on MRI, and primary RT followed by wide excision for those that are symptomatic and irresectable on MRI (135, 185). Treatments are recommended to be carried out in centralised units with a major interest in soft tissue tumors, and all patients with extremity desmoids require entry into prospective studies with long term follow up. In the phase III trial of the American College of Surgeons Oncology Group, patients with irresectable symptomatic lesions receive neoadjuvant RT followed by surgical resection in those that respond to RT (135, 185).

4.4. Comparisons of the clinical results among the three cohorts of different tumor locations

According to our results we can conclude that the surgical radicality of the primary tumor served as a significant prognostic factor on local control rate. Another important conclusion is that the available surgical radicality significantly declines following the second local recurrence. Possible reasons for this phenomenon are proposed to be local anatomy, the surrounding postoperative scarry tissues and employed radiotherapy.

Among the three cohorts of different tumor locations, recurrence-free survival showed a decreasing trend as follows: 'abdominal', 'chest' and 'extremities', with a significantly better local control in 'abdominal' versus 'extremities' group. **Figure 16.** Significant difference was not detectable among the three cohorts in terms of the surgical radicality of the primary tumor, so the aforementioned can be explained by the significant occurrence of 45F and 41A CTNNB1 mutations in the groups of 'extremity' and 'abdominal'.

5. Assessment of the expression of ERs and PRs in sporadic desmoid tumors.

In general, it has been suggested that aggressive fibromatoses respond to the manipulation of ER signalling (2, 7, 38, 117). ERs are members of the steroid/thyroid/retinoid hormone receptor superfamily of nuclear receptors, binding estrogen with high affinity (149). Binding to its cognate ligand causes a change in receptor conformation that results in dimerisation and binding to specific promoter sequences of the DNA. The activated receptor/DNA complex then recruits other cofactors from the nucleus, which results in transcription of a protein, causing a change in cell function (149). Two main isoforms of the human ERs (ER- α and ER- β) have been identified, which are encoded by two independent genes (72, 83, 84). The classical ER cDNA clones (ER- α) were first isolated from a breast cancer cell line in 1986, and the second ER (ER- β) gene was identified ten years later (38, 72, 84, 149, 186). It has been revealed that ER- α and ER- β overlap in fuction and tissue distribution, but also have significant differences in their ligand-binding and transcriptional properties (72, 83, 84, 186). The tissue distribution of ER- β has proved to be broader, and provides the possibility to achieve tissue selectivity through subtype selective ligands, while other known ligands including estrogen metabolites and partial agonists/antagonists (eg. tamoxifene, raloxifene) do not discriminate between ER- α and ER- β (117). Search for tissue selective SERMs was launched because it is known that the amino acid differences between the two ER subtypes impart subtle variations between the receptor binding sites, and selectivity to ligands (72, 86, 117). Results are promising from research into the application of SERMs in desmoid tumors, however the physiological role of ER- β has not been extensively defined, so that the impact on treatment of a positive finding in a desmoid tumor is yet uncertain (72, 117). Antibodies to

ER were produced and clinical measurement of ER has been done routinely since the 1970s although the methods changed over the years (6, 13). Early assays demonstrated the presence of ER in desmoids and were based on radioligand binding or fluorescent hormone binding (6, 26). **Table 25.** New techniques in the 1980s were monoclonal antibody-based methods that did not confirm the early findings as they turned out to measure both ER- α and ER- β (41, 149). In IHC studies, Rasbridge et al. (1993), Devouassoux-Shisheboran et al. (2000) and Sorensen et al. (2002) found uniformly ER negative specimens, because they were using antibodies against the ER- α protein, resulting in false-negative findings (149, 188-190). The only exception among the IHC studies is a report by Moffat et al. (1997), where 29% of the samples were positive for ER (191). This may be attributable to partial cross-reactivity of the antibody applied (Novocastra Clone CC4-5) with ER- β (38, 189). Later, in contrast with previous clinical observations confirming the role of estrogen in the pathogenesis of desmoids, it has been accepted that if estrogen had a role in fibromatosis, it was not by signalling through the ER pathway until ER- β was discovered (38, 149, 188). Today, virtually all ER determinations are performed with specific monoclonal antibody -based methods for ER- α and ER- β (72, 83, 84). In an IHC study of 40 cases of extraabdominal fibromatoses, Deyrup et al. (2006) found that all of the cases displayed expression of ER β . Thirty-three (83%) displayed +3 expression (percentage of tumor cell staining: >50%), 5 cases (12%) were 2+ (percentage of tumor cell staining: 10-50%) and 2 cases (5%) showed 1+ (percentage of tumor cell staining <10%) (72). **Table 25.** In another IHC study of 80 patients, Leithner et al. (2005) concluded that all patients were ER- α , PR and HER2 receptor negative. Seven patients (9%) showed ER- β positivity (84). The IHC study of Santos et al. (2010) revealed that all cases were negative for ER- α , PR (83). Ninety percent of the investigated 59 cases were positive for ER β . No significant difference was observed among clinical variables and the ER- β status. In the present IHC study of 67 primary desmoids, 36 patients (53.7%) were found positive for ER- β . At a median follow-up time of 110 months no statistical difference between the ER- β positive and ER- β negative patients treated with tamoxifen were detected. An attempt to compare 4 methods for evaluating the expression of ER- α and ER- β in desmoid tumor-derived cells and tissues from 7 patients was made by Picariello et al. (2006) (193). **Table 26.** Using IHC, 100% of the cases were found negative for ER- α , and 43% were positive for ER- β . However, when IHC was used, 100% of the cases were negative for ER- α expression but 83% were positive for ER- β . Eighty-six percent were positive for ER- α expression and 100 percent for ER- β when reverse transcriptase-polymerase chain reaction was used, whereas Western blot analysis, recognized as the best method to analyse expression

with infrared imaging system revealed the presence of both ER- α and ER- β in all the samples analyzed. It should be noted that the ER positive results of the highly sensitive RT-PCR and WB methods may be considered as functionally negative with respect to the minimum of 10% ER+ generally accepted as the limit of a successful endocrine therapy. ER negativity does not necessarily mean that the tumor is not affected by anti-estrogens, in fact even in ER negative disease there are additional pathways through which SERM may be therapeutic (2, 7, 72, 84, 117, 193). A functional interaction of the Wnt/APC/ β -catenin and estrogen signaling pathways has been reported in both *Drosophila* and human tumor cell lines, but the evidence is circumstantial (71). Another finding was the correlation between a decreasing concentration of transforming growth factor- β (TGF- β) and the inhibition of aberrant fibroblasts in desmoid tumors (2, 71, 194). The supposed biochemical explanation is the increase in the level of platelet derived growth factor beta (PDGF- β) in the absence of estrogen or in the presence of tamoxifen, which has an inhibitory effect on the aberrant fibroblasts in both desmoid tumors and gastrointestinal polyps (194). SERMs in low-grade tumors are believed to exert inhibitory effects on angiogenesis as well (194). As a consequence it is assumed that there are similarities between the mechanism of wound healing and tumor progression (the role of growth factors, angiogenesis), raising questions such as whether surgery may stimulate the re-growth of desmoids (194).

VIII. NEW STATEMENTS

- I. The multicentric creation of a cohort of primary sporadic desmoid tumors was successfully carried out, and proved to be one of the ten largest in the English language literature, and the largest in Hungary and suitable for clinico-pathological investigations and to join international databases.
- II. Genetic testing of the APC gene is a reliable investigation together with family tree analysis and clinical investigations, and so sporadic cases were successfully separated and followed-up; mutation of the CTNNB1 gene was detectable with high prevalence in sporadic desmoid tumors; mutation of the 45F gene is characteristic for higher recurrence rate, and serving as prognostic factor, the optimal choice of individualized adjuvant therapies is enabled.
- III. The cohort of sporadic desmoids of the chest is the second largest of its kind reported in the literature. Surgical radicality of the primary tumor was confirmed to significantly ($P=0.001$) determine the recurrence rate.
- IV. The cohort of the abdominal wall sporadic desmoids revealed that surgical radicality of the primary tumor as significant prognostic factor ($P=0.006$) is crucial for the disease-free survival.
- V. The recurrence rate in sporadic desmoids of the extremities was confirmed to be significantly ($P=0.013$) determined by the surgical radicality of the primary tumor as well.
- VI. Significant difference was not detectable among the three cohorts in terms of the surgical radicality of the primary tumor, but significance ($P=0.0061$) was confirmed between tumor locations 'abdominal' and 'extremity' in terms of the recurrence-free survival, which can be explained by the significant occurrence of 45F ($P=0.018$) and 41A ($P=0.037$) CTNNB1 mutations in the two groups.
- VII. The achieved surgical radicality of the re-resections is decreasing significantly ($P=0.0016$) from the 2nd recurrence, which must be taken into consideration by the management of the recurrences.
- VIII. Although a former universal statement existed that sporadic desmoid tumors are ER negative, our investigations confirmed a high rate of ER- β positivity.

IX. MAGYAR ÖSSZEFOGLALÓ

A „Diagnosis and multidisciplinary treatment of sporadic desmoid tumors” című PhD értekezés magyar nyelvű összefoglalója.

Magyar cím: A sporadikus desmoid tumorok diagnosztikája és multidiszciplináris kezelése.

Bevezetés: A desmoid tumorok extrém ritka, kötőszöveti, borderline daganatok. A tumorok extra-abdominális, abdominális és intra-abdominális csoportokba sorolhatóak. Áttétet nem képeznek, de jellemző a helyileg invazív növekedés, a magas helyi kiújulás és szövődmény arány. Az etiológia alapja a hibás kötőszövet képződés. A tumor lehet sporadikus vagy familiáris adenomatosus polyposis (FAP) szindrómához társult. A sporadikus desmoid tumorok általában a β -catenin vagy az APC-gén szomatikus mutációit hordozzák.

Kialakulásukban a hormonális hatások és a korábbi szöveti traumák is szerepet játszhatnak. A 0,2–0,5 eset / 1 millió fő incidencia miatt mind a pontos etiológiáról, mind a hosszú távú klinikai eredményekről csak kevés információ áll rendelkezésre a nemzetközi irodalomban, míg a terápia nem evidencián alapul.

Célkitűzések: A vizsgálatok céljai a következők voltak:

1. Retro-, majd később prospektív, multicentrikus eset- és adatgyűjtéssel létrehozni egy adatbázist, ami lehetővé teszi a sporadikus desmoid tumorok tudományos igényű klinikopatológiai vizsgálatát, és a hosszú távú utánkövetést.
2. Az etiológia pontosítása céljából az APC gén genetikai és klinikai vizsgálatokkal kiszűrni a FAP-asszociált desmoid tumorokat.
3. A sporadikus desmoid tumoroknál a β -catenin fehérjét kódoló CTNNB1 gén mutációinak genetikai meghatározása.
4. A különböző elhelyezkedésű sporadikus tumoroknál összehasonlítani a klinikai és patológiai adatokat, elsősorban az elsődleges kezelésként szereplő sebészi eltávolítás radikalitását tekintve.
5. Immunhisztokémiai módszerrel meghatározni a daganat nemi hormon receptor státuszát.

Eredmények:

1. A vizsgálatokhoz 97 eset hosszú távú utánkövetési eredményeivel rendelkező, szövettani mintákat magában foglaló adatbázist és tumor bankot hoztunk létre, ami lehetőséget teremtett a ritka tumor típus összehasonlító vizsgálataihoz.

- 2.** Az APC gén genetikai és a klinikai vizsgálatok által a FAP-asszociált tumorok (3 eset) kizárásával a tisztán sporadikus esetek (n=94) jól vizsgálhatóvá váltak; a β -catenin fehérjét kódoló CTNNB1 gén mutációja, - második legnagyobb esetszámú vizsgálatként, és először hazánkban, a sporadikus desmoid tumorok többségénél (58.8%) igazolható volt; a CTNNB1 (45F) mutáció fontos prognosztikus faktornak bizonyult a kiújulás tekintetében, ami befolyásolhatja a személyre szabott adjuváns terápia megválasztását.
- 3.** Megállapítottuk, hogy a mellkasi (P=0.001), hasi (P=0.006) és végtagi (P=0.013) sporadikus desmoid tumorok klinikai és patológiai adatainak összehasonlítása alapján az első sebészi reszekció radikalitása szignifikáns mértékben meghatározza a hosszú távú betegségmentes túlélést. A pontos preoperatív diagnózis és a precízen megtervezett műtéti beavatkozás, szükség esetén plasztikai rekonstrukcióval kiemelt fontossággal bír.
- 4.** A desmoid tumor elhelyezkedés alapján meghatározott 3 csoportban a primer tumor végzett műtét radikalitása érdemben nem különbözött, de szignifikáns (P=0.0061) különbség igazolódott a hasfali és a végtagi tumorok kiújulás mentes túlélésében, amit így a két csoportban szintén szignifikáns megoszlást mutató 45F (P=0.018) és 41A (P=0.037) CTNNB1 mutációval lehet magyarázni.
- 5.** Bizonyítottuk, hogy a második tumor kiújulást követő műtétek radikalitása szignifikánsan (P=0.0016) csökken, és befolyásolja a recidivák kezelését.
- 6.** A sporadikus desmoid tumorok immunhisztokémiai vizsgálata ösztrogén receptor- α és progesteron receptor negativitást, és magas (53.7%) ösztrogén receptor- β expressziót igazolt. A fentiek hozzájárulhatnak az anti-ösztrogén kezelések hatásmechanizmusának jobb megértéséhez.

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