Murine double minute 2 (MDM2) expression by immunohistochemistry in lipomatous tumours and a clinical cohort

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Helsinki 7.11.2016
Thesis
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INTRODUCTION: Differentiating atypical lipomatous tumours (ALT) and well-differentiated liposarcomas (WDLS), also known as grade 1 liposarcomas (G1LS), from benign lipomas is a common diagnostic problem for pathologists. ALT/WDLS are characterised by amplification of the murine double minute 2 (MDM2) gene and our aim was to investigate if immunohistochemistry for MDM2 can be used to diagnose ALT/WDLS. We also reported treatment and calculated survival rates for patients with grade 1 liposarcomas (G1LS) at Helsinki University Central Hospital (HUCH).

METHODS: 25 lipomas, 17 ALT and 21 G1LS were reviewed and initiated for MDM2 immunohistochemistry. For the clinical cohort we included 53 patients with a final diagnosis of G1LS during 1995-2007.

RESULTS: The sensitivity of MDM2 immunohistochemistry for ALT and G1LS was 35% and 86%, respectively. The specificity in distinguishing ALT and G1LS from lipomas was 100%. The 5-year local-recurrence free survival (LRFS) for G1LS was 80%, and the 10-year LRFS was 68%. Local recurrence occurred in 16/53 patients. Retroperitoneal and intra-abdominal tumours had a 4.224 times greater risk of local recurrence compared to tumours in the extremities or trunk wall. Only one patient died due to G1LS.

DISCUSSION: MDM2 immunohistochemistry is a good supportive method when diagnosing lipomatous tumours. It was highly specific in differentiating ALT and G1LS from lipomas and also relatively sensitive in recognising ALT and G1LS. We reported that G1LS are recurring tumours with tumour site strongly affecting the risk of local recurrence. Despite the high risk of local recurrence, the mortality of G1LS is low.
# Introduction and review of the literature

1. **General** .................................................................................................................. 1
2. **Atypical lipomatous tumour / well-differentiated liposarcoma** ..................... 1
3. **Histopathology** ..................................................................................................... 3
4. **Cytogenetics** ......................................................................................................... 5
5. **MDM2 immunohistochemistry** ........................................................................... 6
6. **Clinical management** .......................................................................................... 7
7. **Prognosis** ............................................................................................................... 9

## Objectives

10

## Materials and methods

10

1. **MDM2 immunohistochemistry** ........................................................................... 10
2. **Clinical review** ..................................................................................................... 12

## Results

13

1. **Immunohistochemical staining** ............................................................................ 13
2. **Clinical review** ..................................................................................................... 15

## Discussion

17

References ...................................................................................................................... 21
1 Introduction and review of the literature

1.1 General

Soft tissue sarcomas originate from mesenchymal stem cells and are fairly rare malignancies, accounting only for approximately 1% of all cancers in human adult life (1). In Finland, the Finnish Cancer Registry records approximately 200 new cases of soft tissue sarcomas each year, with an incidence of 3:100 000. Liposarcomas represent the most common type of soft tissue sarcomas, accounting for 20% of all soft tissue malignancies. (2,3)

Among liposarcomas, the most prevalent subgroup is atypical lipomatous tumour/ well-differentiated liposarcoma (ALT/WDLS), which accounts for 40-45% of all liposarcomas. Characteristic for ALT/WDLS is amplification of the murine double minute 2 (MDM2) gene, which plays an important role in the tumourigenesis of ALT/WDLS. (2,4)

Atypical lipomatous tumours/ well-differentiated liposarcomas consist mainly of mature adipocytes, and tumours may contain only focal cellular changes visible microscopically. If biopsies are limited, ALT/WDLS may easily be mistaken for benign lipomas, due to the fact that lipomas may have cellular changes that mimic those of ALT/WDLS. (5) These features have made distinguishing ALT/WDLS from lipomas one of the most common diagnostic problems for pathologists when it comes to lipomatous tumours (6).

1.2 Atypical lipomatous tumour / well-differentiated liposarcoma

Lipomatous tumours are classified into three major groups: benign, intermediate, and malignant. However, only the intermediate and malignant types are recognised as
liposarcoma (7). Soft tissue sarcomas are also graded based on cell differentiation, with grade 1 and grade 2 being well-differentiated cancers, while grade 3 and grade 4 are poorly differentiated or dedifferentiated lesions (8). Malignant lipomatous tumours consist of dedifferentiated liposarcoma, myxoid liposarcoma and pleomorphic liposarcoma. These are aggressive high-grade cancers that have a tendency to metastasise. (9)

The intermediate group of lipomatous tumours include atypical lipomatous tumour/ well-differentiated liposarcoma (ALT/WDLS), which accounts for 40-45% of all liposarcomas, making it the most common subtype of liposarcoma (10,11). It should be noted that atypical lipomatous tumour (ALT) is a synonym for well-differentiated liposarcoma (WDLS) and used particularly to describe tumours in the limbs and trunk that are manageable with complete resection (12). The term WDLS, on the other hand, is used for tumours located in the retroperitoneum and mediastinum and other sites where clean surgical margins are more difficult to achieve (7). Pathologists at HUSLAB usually classify these tumours as grade 1 liposarcomas (G1LS).

ALT/WDLS are locally aggressive low-grade tumours that develop in the extremities in approximately 75% of the cases. They may however also occur in less common locations such as the retroperitoneum, mediastinum, and the paratesticular/inguinal region. Liposarcoma most often occur in middle-aged individuals, with the highest incidence between 40 to 60 years. (13) ALT/WDLS are slow growing tumours, and a painless palpable mass in the extremities or abdomen may be the only symptom (14).

Although intermediate malignant tumours, ALT/WDLS rarely metastasise. They do however have the ability to dedifferentiate, thereby gaining the potential of seeding distant metastases. (15) Dedifferentiation occurs almost exclusively for lesions in the retroperitoneum or mediastinum and is a rare event for tumours in the extremities (6,16).

The only curative treatment for ALT/WDLS is surgery with a clean margin (1,10). Adequate margins are important when removing ALT/WDLS, as there is a risk of local recurrence if margins are insufficient (17). Tumours located in the retroperitoneum,
mediastinum, and paratesticular region are more likely to recur compared to tumours located in the deep soft tissue of the limbs. This is due to the fact that it is more difficult to achieve clear margins when surgically removing these tumours. (5,18)

### 1.3 Histopathology

ALT/WDLS can histologically be divided into four subgroups: adipocytic or lipoma-like, sclerosing, inflammatory, and spindle cell (19). Lipoma-like liposarcomas are composed mainly of mature adipocytes with a wide variation in shape and size. Focal nuclear atypia and hyperchromasia is characteristic for ALT/WDLS, along with hyperchromatic stromal cells dispersed within fibrous septa. Monovacuolated and multivacuolated lipoblasts may also be present. However, they do not make, nor are they required for a diagnosis of liposarcoma. It is not uncommon for lipoblasts to also be present in many benign lipomatous tumours. (20)

Sclerosing liposarcomas most commonly develop in the retroperitoneum or paratesticular region. These tumours are characterised by scattered eccentric stromal cells and multivacuolated lipoblasts embedded in a fibrillary collagenous stroma. (7) Inflammatory liposarcoma, on the other hand, is distinguished by the presence of a chronic inflammatory infiltrate. This infiltrate usually consists of polyphenotypical lymphoplasmacytic aggregates, although T-cells may be the most prominent inflammatory component in some tumours. (12)

Spindle cell liposarcoma, a rare type of liposarcoma, is composed of spindle cells that resemble neural cells. These cells are set in a fibrous and/or myxoid background combined with a lipomatous component that most often includes lipoblasts. (21) Cytogenetically spindle cell liposarcoma differ from other ALT types, which suggests that these tumours represent a unique type of liposarcoma (22).
One of the more challenging diagnostic problems for practicing pathologists regarding lipomatous tumours is distinguishing ALT/WDLS from benign lipomas. This is due to the similar histologic features of lipomas and ALT/WDLS, especially the lipoma-like subtype (Figure 1). (15,23) The cellular changes characteristic for ALT/WDLS might occur only focally, and if biopsies are limited, these changes might be overlooked (5,14). On the other hand, liponecrosis and multinucleated macrophages may be found in lipomas, and these changes could be misjudged as lipoblasts and atypical cells, respectively (24).

![Figure 1. Hematoxylin and eosin (H&E) staining of lipoma (A) and atypical lipomatous tumour (B) showing the similar histology of the tumours.](image)

Because of the fact that ALT/WDLS have the risk of recurring locally, it is important to be able to distinguish these tumours from benign lipomas, which recur more seldom (23,24). Researchers have therefore turned their attention to investigate the benefits of using immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) when differentiating ALT/WDLS from lipomas (25,26). These methods are possible to utilise because of chromosomal changes characteristic for ALT/WDLS.
1.4 Cytogenetics

The molecular alterations in ALT/WDLS have long been acknowledged, and it has improved our understanding of these tumours. ALT/WDLS are characterised by supernumerary ring and/or giant marker chromosomes containing amplicons of the 12q13-15 chromosome region (18,27). These cytogenetical features can also be found in low-grade osteosarcomas. (28) Amplification usually involves the murine double minute 2 (MDM2) gene, which is often co-amplified with the cyclin-dependent kinases (CDK4) gene. Amplification of MDM2 independent of CDK4 may however also occur. Other genes located in this region include high-mobility group AT-hook 2 (HMGA2), carboxypeptidase M (CPM), and sarcoma amplified sequence (SAS). (29)

Besides amplification, another chromosomal feature typical for ALT/WDLS is the presence of a neocentromere lacking alpha-satellite sequences. This feature seems to be recurring reliably only in ALT/WDLS tumours. (27) However, amplification of the MDM2 gene is considered the true molecular trademark of ALT/WDLS and one of the most significant factors in the tumourigenesis of these tumours. Amplification leads to overexpression of the MDM2 protein, which in turn functions as a negative regulator of p53. (30)

The p53 protein is a tumour suppressor and regulates several cellular events including cell cycle arrest, metabolism, apoptosis and aging. During cellular stress and DNA damage p53 is activated, bringing the cell cycle to a halt or inducing programmed cell death. This makes p53 an important protective protein in the development of cancer, and p53 is in fact the most commonly mutated or dysfunctional tumour suppressor in human cancers. (31)

MDM2 is the main regulator of p53 activity, and its transcription is in fact activated by p53 itself (32). MDM2 suppresses the features of p53 by two mechanisms. First, it functions as an E3 ubiquitin ligase targeting p53 for ubiquitination. After being targeted, p53 is transported out of the nucleus and undergoes proteasomal degradation. (33) Second, MDM2 binds to the N-terminal domain of p53 and masks its transcriptional activity, although it is unclear if binding alone is sufficient to suppress p53 (34).
Although amplification of the \textit{MDM2} gene is the most common reason for MDM2 protein overexpression, studies have shown that MDM2 can be overexpressed without gene amplification. This indicates the existence of alternative mechanisms for overexpression of MDM2. \((35)\)

The overexpression of MDM2 being an important cause of the development of ALT/WDLS has made MDM2-inhibition an interesting target for cancer therapy. Nutlin-3A is a small molecule that inhibits MDM2 from binding p53 and thereby enhances p53 stability. This allows p53 to induce apoptosis and cell cycle arrest. \((30)\) One of the downsides however is the toxicity of Nutlin-3A, and other antagonists are currently being assessed \((24,34)\).

### 1.5 MDM2 immunohistochemistry

Amplification of the \textit{MDM2} gene characteristic for ALT/WDLS is not present in benign lipomatous tumours \((15)\). This fact makes immunohistochemistry a possible method to utilise when differentiating ALT/WDLS from lipomas.

In a large-scale study, Binh et al. used immunohistochemical staining for both MDM2 and CDK4 on 559 soft tissue tumours including benign and malignant lipomatous tumours and other sarcomas. MDM2 was positive in 5% while CDK4 was positive in 2% of benign adipocytic tumours. In ALT/WDLS however, MDM2 and CDK4 were positive in 100% and 91%, respectively. The specificity of MDM2 in distinguishing ALT/WDLS from benign adipose tumours was 96%, slightly lower than the specificity of CDK4 at 98%. \((25)\)

In a paper by Sirvent et al. 200 soft tissue tumours were analysed using MDM2 and CDK4 immunohistochemistry. The report showed promising results in differentiating ALT/WDLS from lipomas and other non-malignant tumours using MDM2 markers with a sensitivity and specificity of 80% and 93%, respectively. \((36)\)
Thway et al. assessed the positivity of three different immunohistochemical markers. In addition to using MDM2 and CDK4, they implemented p16 in their report consisting of 216 lipomatous tumours, both benign and malignant. Of 31 ALT/WDLS, 25 expressed MDM2. Nearly half of these however, were only weakly positive. They also showed that 28% of lipomas were positive for MDM2. Both the sensitivity and specificity were better with markers CDK4 and p16. (24)

In small-scale reports, the sensitivity of MDM2 markers in identifying ALT/WDLS ranged from 24% to 100%, and the specificity in differentiating between ALT/WDLS and lipomas ranged from 58.8% to 100% (2,26,31,32,37).

Although immunohistochemistry seems to be a usable method in detecting MDM2 overexpression in ALT/WDLS, using it alone might not be adequate in distinguishing ALT/WDLS from benign lipomatous tumours (36). Immunohistochemistry is however a basic method and more suitable on a day-to-day basis compared to other methods available such as in situ hybridisation or polymerase chain reaction (15).

In addition to being a simple technique, immunohistochemistry has also been shown to have high reproducibility when it comes to identifying MDM2 overexpression in ALT/WDLS. Binh et al. compared the stains from two laboratories and demonstrated that the reproducibility of MDM2 immunohistochemistry is even better than the reproducibility when staining estrogen receptors in breast cancer. (38) Interestingly, MDM2 immunopositivity has also been found in low-grade osteosarcomas and chondrosarcomas (39,40).

1.6 Clinical management

ALT/WDLS are slow growing tumours that can become very large in size and be either asymptomatic or show nonspecific symptoms. Liposarcomas in the extremities tend to be painless tumours, and an enlarging mass may be the only symptom. Retroperitoneal
liposarcomas may however cause abdominal pain or less commonly gastrointestinal bleeding, incomplete bowel obstruction or neurologic symptoms due to invasion or pressure on neurovascular structures. (16)

Imaging is usually the first step in diagnosing soft tissue tumours. For tumours located in the extremities, magnetic resonance imaging (MRI) is usually recommended, while a computerised tomography (CT) scan of the chest, abdomen and pelvis is used for lesions in the abdomen or retroperitoneum. ALT/WDLS is one of the few histologic subtypes of soft tissue tumours that can be diagnosed based on CT or MRI findings. (41)

Although imaging techniques may be helpful adjuncts when dealing with soft tissue tumours, the final diagnosis is always based on a histologic sample. These samples may be acquired from a needle or open biopsy or as a sample from the removed tumour. (8) The golden standard for diagnosing lesions in the extremities and trunk is multiple core needle biopsies (42). No general agreement on preoperative biopsies of retroperitoneal lipomatous tumours currently exists in the literature. Tumours are more likely to be liposarcomas than benign lipomas and are best removed surgically without pre-operative biopsies. If tumours are likely to be incompletely resected, a core needle biopsy should be acquired. (16,41,43) Biopsies should be obtained without contaminating the peritoneal cavity.

Surgery is the only curative form of therapy when it comes to ALT/WDLS. In order to reduce the risk of local recurrence, the tumour should be removed with a margin of normal tissue. (16,44) Liposarcomas should be removed as an en bloc resection. Removal of retroperitoneal liposarcoma commonly involves removal of nearby organs in order to achieve negative margins (43). Lesions in the extremities formerly required amputation of the limb, but nowadays these tumours can be treated with limb-sparing resection (45). The excision of soft tissue tumours in both the retroperitoneum and extremities are extensive operations and may require reconstructive surgery as well (17).

Adjuvant therapy is only of limited importance when treating ALT/WDLS. Radiation therapy is not used when treating ALT/WDLS, as they consist mainly of mature fat cells
that are not sensitive to radiation therapy. (41) Chemotherapy has no role when treating ALT/WDLS (10) as the proliferation rate is usually low.

1.7 Prognosis

The prognosis of ALT/WDLS is affected by several factors. Adequate surgical margins are considered to be one of the most important prognostic factors (43). Patients with microscopically positive and negative resection margins have been reported to have a 10-year local recurrence-free survival of 53% and 100%, respectively (46). Location of the primary lesion also affects the risk for local recurrence. Difficulty in achieving clear margins for retroperitoneal and mediastinal tumours make them more likely to recur compared to tumours at other sites (10,44).

Tumours in non-visceral regions have a mortality rate close to 0%, compared to the mortality rate of tumours in the retroperitoneum or other visceral sites, which can be almost 80%. The high mortality rate is due to the high probability of multiple relapses in these sites. (12)

The histologic grade is also a critical factor, and tumours are graded based on histologic findings including mitosis, cellularity, necrosis, differentiation and stromal content. In one report, the 5-year survival rate for low-grade ALT/WDLS tumours was 90%. (16)

ALT/WDLS have a low potential of metastasising with an estimated risk of less than 1% (41). The metastatic potential does grow however if ALT/WDLS undergo dedifferentiation and develop a high-grade sarcoma component (4). Tumours in the retroperitoneum and inguinal regions are more likely to dedifferentiate compared to lesions in the extremities, thereby making them more prone to metastasising (18).
Tumour size usually loses its prognostic significance when stratified for margin. Tumours in the retroperitoneum are more likely to reach a large size, making them more difficult to control locally compared to tumours in the extremity (15,47).

2 Objectives

Two main objectives were set for this thesis. The first objective was to evaluate the usability of MDM2 immunohistochemistry when dealing with lipomatous tumours. We investigated the sensitivity of this method in positively staining atypical lipomatous tumours and grade 1 liposarcomas (G1LS) and assessed the specificity of using MDM2 immunohistochemistry when distinguishing these tumours from benign lipomas. We also wanted to demonstrate that immunohistochemical staining for MDM2 could be used on a daily basis by pathologists at HUSLAB as a supportive adjunct when analysing samples from adipocytic lesions.

The second objective of this thesis was to review journals of patients who had a final diagnosis of primary G1LS at Helsinki University Central Hospital (HUCH). Our aim was to report preoperative diagnostics, surgical excision, and adjuvant therapy in these patients. We also calculated survival rates and evaluated possible prognostic factors for local recurrence.

3 Materials and methods

3.1 MDM2 immunohistochemistry

Permission to use the materials in this thesis was received by The National Institute of Health and Welfare and the ethical committee at HUCH. All cases were chosen from the archives at HUSLAB. After reviewing pathology reports, a total of 75 cases were initially
chosen for this project. The tumours were classified based on the original diagnosis in the pathology reports and consisted of lipomas (n=25), atypical lipomatous tumours (n=25), and grade 1 liposarcomas (n=25).

Samples were retrieved from the pathology archives at HUSLAB. For each case, hematoxylin and eosin-stained slides were carefully reviewed by an experienced soft tissue pathologist (T.B.) and a bachelor of medicine (M.Å.). From the original 75 cases, a total of 63 cases were available for review and initiated for MDM2 immunohistochemical staining. The cases consisted of lipomas (n=25), atypical lipomatous tumours (n=17) and grade 1 liposarcomas (n=21).

Formalin fixed and paraffin embedded blocks were cut 3µm thick with a microtome and mounted on electrostatically charged glass slides. The slides were left to age in a hot air oven at 60 degrees Celsius for one hour.

The MDM2 immunohistochemical staining was performed with the Ventana BenchMark XT staining module (Roche/Ventana, Tucson, Arizona, USA). The slides were deparaffinised and pretreated (CC1, pH 9.0) by this fully automated instrument. The MDM2 antibody used was from Calbiochem (lot D001644452, clone IF2) at a dilution of 1:100 and a concentration of 0.1mg/ml. After the antibody had been applied, the slides were incubated for one hour at 37 degrees Celsius.

After applying the amplifier (Amplifier kit 760-080, Roche/Ventana, Tucson, Arizona, USA) the slides were incubated at 37 degrees Celsius for 16 minutes. Endogenous-peroxidase was blocked with the module’s blocker. Slides were then stained with horseradish peroxidase (HRP) and a multimer based detection system was utilised (Ultraview DAB 760-500, Roche/Ventana, Tucson, Arizona, USA) with a diaminobenzidine (DAB) chromogen (Figure 2). Finally, the slides were counterstained with hematoxylin.
The microscope used to analyse the slides was a Nikon Eclipse E600. The slides were reviewed by both T.B and M.Å. The samples were considered positive if atypical nuclei were positively stained.

The sensitivity for MDM2 staining was calculated by dividing the number of positively stained G1LS and ALT with the total number of cases in each group. MDM2 sensitivity was also calculated for G1LS and ALT as a combined group. The specificity for MDM2 staining was calculated as the number of unstained lipomas divided by the total number of lipomas.

### 3.2 Clinical review

Sixty-eight patients had a final diagnosis of G1LS during 1995-2007. Only tumours located in the extremities, trunk wall, retroperitoneum, or intra-abdominally were included in this thesis. All patients referred for recurrent tumour were excluded, leaving 53 primary
tumours for review. Detailed clinical data were retrieved from patient journals, which were reviewed by an oncologist (M.S.) and a bachelor of medicine (M.Å).

The tumours were defined as touched if fine needle aspiration, core needle biopsies or surgical excisions had been performed on the tumour prior to referral. All other tumours were defined as untouched. Tumour depth was defined as superficial if the tumour was subcutaneous without invading the deep investing fascia. All other tumours were defined as deep. Tumour size was defined as the largest size reported in the original pathology report.

All patients were reviewed for treatment at HUCH. If a preoperative diagnosis had been made, it was based either on fine needle aspiration or a core needle biopsy. The surgical margin was defined as intrallesional when there was microscopic or macroscopic residual tumour tissue, and clear when the surgical margin was microscopically negative.

IBM SPSS\textsuperscript{®} Statistics version 20 (SPSS, Chicago, Illinois, USA) was used for all analysis. Local recurrence free survival rates were estimated using the Kaplan-Meier method. Differences in local control of different subgroups were analysed with the log rank test for discrete variables and with Cox regression analysis for continuous variables. The level of significance was set at $p < 0.050$.

4 Results

4.1 Immunohistochemical staining

No MDM2 immunoreactivity was seen in lipomas (Table 1). ALT were positively stained in 6/17 cases, whereas G1LS were positive in 18/21 cases. The sensitivity was higher for G1LS at 86\% compared to ALT at 35\%. The sensitivity for G1LS and ALT combined was
63%. The specificity of MDM2 immunohistochemistry in distinguishing G1LS and ALT from lipomas was 100%.

<table>
<thead>
<tr>
<th>Tumour type</th>
<th>Number of cases</th>
<th>Positive cases</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoma</td>
<td>25</td>
<td>0</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>ALT</td>
<td>17</td>
<td>6</td>
<td>35%</td>
<td>100%</td>
</tr>
<tr>
<td>G1LS</td>
<td>21</td>
<td>18</td>
<td>86%</td>
<td>63%</td>
</tr>
<tr>
<td>G1LS and ALT</td>
<td>38</td>
<td>24</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

G1LS: Grade 1 Liposarcoma, ALT: Atypical lipomatous tumour

The intensity of the MDM2 staining was weak in many of the positive slides and strong positivity was only found in G1LS (Figure 3).

Figure 3. Grade 1 liposarcoma with positive immunohistochemical staining for MDM2
4.2 Clinical review

The mean age of the 53 patients included in this thesis was 62.0 years (Table 2). The majority of patients (79%) were referred to the sarcoma group at HUCH for tumours that were untouched. Fine needle aspiration had been performed on four tumours, whereas core needle biopsy had been performed on two tumours prior to referral. Five patients had undergone surgery at some other hospital before being referred to the sarcoma group at HUCH.

Table 2. Patient, tumour and treatment characteristics of primary tumours and 5-year local recurrence free survival (LRFS)

<table>
<thead>
<tr>
<th>Variable</th>
<th>n (%)</th>
<th>5-year LRFS (%)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>23 (43)</td>
<td>90</td>
<td>0.046</td>
</tr>
<tr>
<td>Female</td>
<td>30 (57)</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>62.0 (12.4)</td>
<td></td>
<td>0.989</td>
</tr>
<tr>
<td>Primary Tumour Referred(^1)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Untouched</td>
<td>41 (79)</td>
<td>81</td>
<td>0.481</td>
</tr>
<tr>
<td>Touched</td>
<td>11 (21)</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>Tumour Site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extremity or trunk wall</td>
<td>46 (87)</td>
<td>83</td>
<td>0.001</td>
</tr>
<tr>
<td>Intra-abdominal or retroperitoneum</td>
<td>7 (13)</td>
<td>57</td>
<td></td>
</tr>
<tr>
<td>Tumour Depth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Superficial</td>
<td>8 (15)</td>
<td>100</td>
<td>0.273</td>
</tr>
<tr>
<td>Deep</td>
<td>45 (85)</td>
<td>76</td>
<td></td>
</tr>
<tr>
<td>Tumour Size (cm)(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>17.6 (10.3)</td>
<td></td>
<td>0.079</td>
</tr>
<tr>
<td>Preoperative Diagnosis(^3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None or fine needle aspiration</td>
<td>10 (19)</td>
<td>80</td>
<td>0.509</td>
</tr>
<tr>
<td>Core needle biopsy</td>
<td>42 (81)</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Surgical Margin(^4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intralesional</td>
<td>6 (12)</td>
<td>83</td>
<td>0.029</td>
</tr>
<tr>
<td>Clear</td>
<td>46 (88)</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>Adjuvant Therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>52 (98)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Radiation</td>
<td>1 (2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^1\) Missing data for one patient.  \(^2\) Tumour size not recorded in 7 cases.  \(^3\) Data missing for one patient.

\(^4\) Surgical margin not recorded for one patient.
The mean size of tumours was 17.6 cm. Tumours were most often located in either the extremities or the trunk wall. Core needle biopsy was the most common method for preoperative diagnosis of primary tumours at HUCH, and was used in 42 (81%) cases. Surgical margins were microscopically negative in 88% of all tumours excised at HUCH.

Only one patient (2%) with primary G1LS received adjuvant radiation therapy. Marginal resection was recorded as the reason.

The mean (SD) follow-up time for survivals was 8.7 (3.5) years. The follow-up range was 1.1-18.2 years. Sixteen of the 53 patients developed local recurrence during follow-up. The 5-year local recurrence-free survival (LRFS) for all 53 patients was 80% and the 10-year LRFS was 68% (Figure 4).

![Figure 4. Local recurrence-free survival for whole population](image-url)
Seven patients died during follow-up. One death was due to a treatment complication: the patient suffered bleeding and perforation during resection of a second recurrence. He later developed a lethal pneumonia. One death was tumour related: the G1LS had evolved into grade II and was inoperable due to its multifocal intra-abdominal location. Five deaths were unrelated to the tumour.

In univariate analysis margin, sex and site were statistically significant for LRFS (Table 2). Only tumour site retained its significance in multivariate analysis for local recurrence (Table 3).

Table 3. Multivariate analysis for local recurrence

<table>
<thead>
<tr>
<th>Variable</th>
<th>Hazard ratio</th>
<th>95% CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Margin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intralesional</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear</td>
<td>0.665</td>
<td>0.163 - 2.626</td>
<td>0.550</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.294</td>
<td>0.078 - 1.104</td>
<td>0.070</td>
</tr>
<tr>
<td>Tumour Site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extremity or trunk wall</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intra-abdominal or retroperitoneum</td>
<td>4.224</td>
<td>1.065 - 16.760</td>
<td>0.040</td>
</tr>
</tbody>
</table>

5 Discussion

In this thesis, we showed that MDM2 immunohistochemistry was 100% specific and relatively sensitive in differentiating atypical lipomatous tumours (ALT) and grade 1 liposarcomas (G1LS) from benign lipomas. It is therefore a good and affordable adjunct that can be used daily by pathologists when dealing with lipomatous tumours.

We showed that tumour site was a strong prognostic factor for G1LS. The risk for a local recurrence was 4.224 times greater for tumours located in the retroperitoneum or intra-abdominally compared to tumours in the extremity or trunk wall.
Surprisingly, we found that nearly one third (16/53) of the patients included in this study had a local recurrence during follow-up. However, only one patient died during follow-up due to a G1LS.

When analysing the slides for MDM2 positivity, the reviewers were not blinded for the original diagnosis. This could have had a negative impact on our results. When using a microscope, there is naturally a risk of human error, and the interpretation of MDM2 positivity was further complicated by the various amount of positively stained endogenous peroxidase from mast cells.

Combining MDM2 immunohistochemistry with other markers could improve the overall strength of the method when differentiating ALT/WDLS from benign lipomas. Binh et al. showed that CDK4 was a more specific marker than MDM2 (25), while Thway et al. reported that p16 was a more sensitive and specific marker than both MDM2 and CDK4 (24).

Liposarcomas are relatively rare tumours, and in this thesis we only included tumours referred to the sarcoma group in Helsinki. The small sample could have affected our conclusions negatively. The overall power of this thesis also suffered due to the small sample with many of our variables not being statistically significant. Our study was also done retrospectively, which can be considered a weakness.

The specificity in distinguishing ALT/WDLS from benign lipomas was excellent in our study at 100%, exceeding the results of many other studies (Table 4). However, the sensitivity was inferior compared to studies of similar sample size, mainly due to the low sensitivity in recognising ALT in our study, being only 35%.
Table 4. Comparison of sensitivity and specificity of MDM2 immunohistochemical staining in lipomatous tumours

<table>
<thead>
<tr>
<th>Studies</th>
<th>Sensitivity %</th>
<th>Specificity %</th>
<th>ALT/WDLS (n)</th>
<th>Lipomas (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Binh et al. (25)</td>
<td>100</td>
<td>96</td>
<td>44</td>
<td>49</td>
</tr>
<tr>
<td>Aleixo et al. (26)</td>
<td>100</td>
<td>58.8</td>
<td>19</td>
<td>17</td>
</tr>
<tr>
<td>Sirvent et al. (36)</td>
<td>80</td>
<td>93</td>
<td>36</td>
<td>50</td>
</tr>
<tr>
<td>Our study</td>
<td>63</td>
<td>100</td>
<td>38</td>
<td>25</td>
</tr>
</tbody>
</table>

ALT/WDLS: Atypical lipomatous tumours/well-differentiated liposarcomas

Immunohistochemistry is both a less sensitive and specific method compared to fluorescence in situ hybridisation (FISH) when investigating MDM2 overexpression (14). However, immunohistochemistry has been shown to be a faster and less expensive method compared to FISH (48) and could therefore be more applicable for daily use by pathologists.

Our results confirm other reports of tumour site affecting the risk for local recurrence (15). We were however not able to show that surgery with clear margins has a better prognosis compared to intralesion surgery, which has been reported in the literature (46). The mortality due to G1LS in our study was lower compared to similar studies. Smith et al. reviewed 1266 WDLS with a 5-year disease specific survival (DSS) of 98% for extremity tumours and 86.6% for retroperitoneal and intra-abdominal tumours (49). Singer et al. reported a 3-year and 5-year disease specific survival of 92% and 83%, respectively for 99 retroperitoneal WDLS (50).

The actual sensitivity and specificity of MDM2 immunohistochemistry could further be investigated by comparing MDM2 immunohistochemistry with FISH for MDM2 in lipomatous tumours. Another subject of future research would be investigating if the amount of MDM2 positivity in ALT or G1LS affects the prognosis. It would also be interesting to study the risk of G1LS dedifferentiating into more malignant tumours.

MDM2 immunohistochemistry is a useful method in the diagnostics of lipomatous tumours, and our results have in fact led to HUSLAB now using MDM2 immunohistochemistry as a routine stain. The low sensitivity in staining ALT could
indicate that these tumours were in fact some other lipomatous tumour. However, the overall intensity of the MDM2 stain was low and by adjusting the intensity, the sensitivity for ALT could improve. Although G1LS are classified as intermediately malignant tumours and have a relatively high risk of local recurrence, the mortality of these tumours is low, and patients are more likely to die from some other cause than from G1LS.
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